Australian Government

Australian Pesticides and Veterinary Medicines Authority

CHEMICAL REVIEW PROGRAM

REVIEW OF THE MAMMALIAN TOXICOLOGY

AND

METABOLISM/TOXICOKINETICS

OF

METHIOCARB

This Report was prepared for the APVMA by

Office of Chemical Safety

of the

Department of Health and Ageing

Office of Chemical Safety

Canberra

May 2013

ISBN: 978-1-922188-35-6 (electronic)

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This publication is available from the APVMA website: www.apvma.gov.au.

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ABBREVIATIONS

Time

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

Weight

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

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Dosing

i.d.	Intradermal
i.m.	Intramuscular
inh	Inhalation
i.p.	Intraperitoneal
i.v.	Intravenous
p.o.	Oral
s.c.	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

Volume

L	Litre
mL	Millilitre
µL	Microlitre

Concentration

M	Molar
ppb	Parts per billion
ppm	Parts per million

Clinical chemistry, haematology

A/G	Albumin/globulin ratio
ALT	Alanine aminotransferase (SGPT)
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
GGT	Gamma-glutamyl transpeptidase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
T3	iodothyronine
T4	thyroxine
WBC	White blood cell/leucocyte

Chemistry

DMSO	Dimethyl sulfoxide
HPLC	High pressure liquid chromatography
LSC	Liquid scintillation counting
TLC	Thin layer chromatography

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
DFR	Dislodgeable Foliar Residue
GLP	Good Laboratory Practice
LOEL	Lowest Observed Effect Level
MOE	Margin of Exposure
MRL	Maximum Residue Limit or Level
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
OHS	Occupational Health and Safety
OP	Organophosphate pesticide
PHED	Pesticide Handlers Exposure Database
PPE	Personal Protective Equipment
REI	Re-entry interval
RHI	Re-handling interval
SD	Sprague Dawley (rats)
SPF	Specific pathogen free
WHP	Withholding period

Organisations & publications

ACCS	Advisory Committee on Chemicals Scheduling
APVMA	Australian Pesticides and Veterinary Medicines Authority
DoHA	Department of Health and Aging
FAO	Food and Agriculture Organisation of the UN
FAISD	First Aid Instructions & Safety Directions
IPCS	International Programme on Chemical Safety
JMPR	Joint Meeting on Pesticide Residues
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
OCS	Office of Chemical Safety and Environmental Health
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of absorption	Oral: rapid and extensive in rats, moderate to variable in dogs.
Distribution	Highest tissue concentrations were found in the kidney and the spleen.
Potential for accumulation	No evidence of accumulation.
Rate and extent of excretion	Rapid and excreted extensively in urine.
Metabolism	Well metabolised forming at least 3 major metabolites in urine.
Toxicologically significant metabolites	Methiocarb sulfoxide

Acute toxicity

Lowest rat oral LD_{50} (mg/kg bw)	9 (PEG vehicle)
Worst oral LD_{50} in other species (mg/kg-bw)	12.2 in guinea pigs (ethanol and propylene glycol vehicle)
Lowest rat dermal LD_{50} (mg/kg bw)	350-400 (isopropanol vehicle)
Worst dermal LD_{50} in other species (mg/kg bw)	>2000 in rabbits
Rat inhalation LC_{50} (mg/m^3)	585/433 (Males/Females) (unknown vehicle)
Worst inhalation LC_{50} in other species (mg/m^3)	>39 in mice (ethanol vehicle)
Eye irritation	non-irritant in rabbits
Skin irritation	non-irritant in rabbits
Skin sensitisation	non-sensitiser in guinea pigs

Acute toxicity of metabolites - Rat oral LD_{50} (mg/kg bw) values

Methiocarb sulfoxide	6 - 9 (PEG 400 vehicle)
Methiocarb phenol	>1000 (various vehicles)
Methiocarb phenol	>112 (carbowax vehicle)
N-hydroxymethyl methiocarb	>112 (carbowax vehicle)
N-hydroxymethyl methiocarb sulfone	>160 (carbowax vehicle)
Methiocarb sulfone	>1000 (PEG 400 vehicle)
Methiocarb sulfone	>1000 (various vehicles)
Methiocarb phenol sulfone	>1000 (various vehicles)

Short-term toxicity

Target/critical effect	Plasma ChE inhibition
Lowest relevant oral NOEL (mg/kg bw/day)	3 (Wistar rats exposed via gavage in PEG vehicle for 4 weeks)
Lowest relevant dermal NOEL (mg/kg bw/day)	60 (NZW rabbits exposed under occluded conditions 6h per day for 21 days)
Lowest relevant inhalation NOEL (mg/m^3)	6/23 (Males/Females) (Wistar rats with nose-only exposure daily for 6 h/day for 15 work days within a 3 week period) .

Genotoxicity	Non-genotoxic

Long-term toxicity and carcinogenicity

Target/critical effect	Plasma ChE inhibition
Lowest relevant NOEL (mg/kg bw/day)	0.2 (2-year study in dogs)
Carcinogenicity	No evidence of oncogenic potential

Reproductive toxicity

Reproduction target/critical effect	No treatment-related effects in rats
Developmental target/critical effect	Pale areas on the foetal liver at maternotoxic doses (in rabbits)
Lowest relevant developmental NOEL (rabbits)	Maternal NOEL = 3.0 mg/kg bw/day (based on body weight loss and cholinergic effects) Foetal NOEL = 3.0 mg/kg bw/day (based on liver effects)

Delayed neurotoxicity	No delayed neurotoxicity
Immunotoxicity	Inhibits T-cell proliferation (*in vitro*)
Dermal absorption	No data available

Summary	**NOEL**	**Study**	**Safety Factor**
Current ADI (0.002 mg/kg bw/day based on plasma ChE inhibition)	0.2 (mg/kg bw/day)	2-year study in dogs	100
Amendments to the current ADI	None		
Acute RfD was set at 0.03 mg/kg bw/day based on clinical signs observed in two developmental studies conducted in rats and rabbits.	3.0 (mg/kg bw/day)	Two developmental studies (in rats and rabbits)	100
Amendments to the current ARfD	None		

SUMMARY

Introduction

Methiocarb, also known as mercaptodimethur, BAY 37344 or Mesurol, is a insecticidal carbamate of the thio- sub class with a broad range of non-systemic insecticidal and acaricidal activity. It is a molluscicide with neurotoxic action. Methiocarb is used in a wide range of agricultural and suburban situations to control slugs and snails, mites, thrips, aphids, leaf-hoppers, fruit flies and biting insects including some soil insects of field crops. It is also used as a bird repellent. In Australia, methiocarb has been used for over 30 years and at the current time was found in 4 products with different approved uses these being 2 snail and slug baits, a 750 g/L 'bird repellent and snail and slug spray' and a 500g/L SC insecticide. Maximum residue limits have been established for methiocarb in fruits, vegetables and wine. At present, methiocarb is listed in Schedule 7 of the SUSMP, based on high acute oral and inhalational toxicity as indicated in the original version of this report (2000), with a cut-off to Schedule 6 for preparations containing 20 percent or less of methiocarb, or Schedule 5 for pelleted preparations containing 2% or less of methiocarb. The current Australian Acceptable Daily Intake (ADI) of 0.002 mg/kg bw/day was set by the TGA in March 2000 based on the NOEL of 0.2 mg/kg bw/day for plasma ChE depression and reduced food consumption in a 2-year dog study and applying a 100 fold safety factor.

Methiocarb has been reviewed by the Joint FAO/WHO Meeting of Pesticide Residues (JMPR) in 1981, 1983, 1984, 1985, 1987 and 1998. In its 1981 evaluation, the JMPR established an ADI of 0.001 mg/kg bw/day for methiocarb on the basis of the NOEL of 5 ppm from a 2-year dog study and a safety factor of 100. This ADI remained unchanged up until 1998. In the most recent review in 1998, the JMPR amended the ADI to 0.02 mg/kg bw/day, based on a revised NOAEL of 60 ppm (equivalent to 1.5 mg/kg bw/day) from the same 2-year dog study and a safety factor of 100.

This report presents an evaluation of previously submitted toxicology data together with the data from two new submissions to Chemical Review and International Harmonisation Section of the Therapeutic Goods Administration under the Existing Chemicals Review Program (ECRP). In preparation for the 2011 revision of this report a thorough search of the publicly available scientific literature and publications of overseas agencies was made. No new toxicity data was submitted to the OCS for evaluation and inclusion in this report.

Toxicokinetics and Metabolism

Studies on biotransformation and excretion of methiocarb have been performed using both *in vivo* and *in vitro* systems. *In vivo* studies have used rats, dogs and a dairy cow, the routes of administration being intra-peritoneal injection in one rat study and oral in the remaining studies. In addition, several *in vitro* studies have been conducted with methiocarb using rat liver microsomal preparations under both activated and non-activated conditions and foetal and maternal rat tissue preparations.

In vivo studies

Intra-peritoneal Administration

In a study performed to determine and compare detoxification and elimination mechanisms and the metabolic fate of the radiolabel from ten differentially labelled methyl and dimethyl insecticides, [^{14}C]-carbonyl labelled methiocarb in 2-methoxyethanol was administered i.p. to male SD rats. The rats were sacrificed at 48 h post treatment and the radioactive content of the expired CO_2, urine and faeces and in a range of tissues was determined. Of the radiolabel administered, 66.1% was present in the expired air as $^{14}CO_2$. About 20% and 2.2% of ^{14}C radiolabel were recovered in 0–24 h and 24–48 h post-treatment urine samples respectively, with 2.5% radiolabel recovered in faeces. A further 8.9% of the administered radiolabel remained within body tissues 48 h post administration with highest residue levels in the spleen, liver, heart and kidney. The study authors hypothesised that the expired radioactivity originated from hydrolysis of the administered compound or its metabolites to yield carbonate which was subsequently expired as $^{14}CO_2$. The hydrolytic cleavage of the ester group of methiocarb by rat liver microsomes was identified as the probable rate limiting step in the production of $^{14}CO_2$. However, except for $^{14}CO_2$ no other metabolites were characterised in this study and hence only partial interpretation of the nature of the metabolites was possible (Krishna & Casida, 1966).

A metabolism study undertaken by Wheeler and Strother (1974a) examined the quantitative excretion, maternal tissue distribution, placental transfer, and foetal disposition of carbonyl ^{14}C labelled methiocarb following i.p. administration to 18 or 19 days pregnant SD rats. A range of maternal and foetal tissues including amniotic fluid were studied. Methiocarb and its metabolites in tissues were ether extracted and resolved using TLC and GLC techniques. Rapid placental transfer of radioactivity was observed. Pregnant animals showed a slower rate of elimination of $^{14}CO_2$ compared to non-pregnant rats. Methiocarb metabolism kinetics appeared complex because of a rebound elevation of radioactivity seen in all maternal tissues except RBCs and several foetal tissues at 4 h post treatment. Methiocarb was detected at higher levels and appeared to remain longer in the foetal tissues compared to the maternal tissues. Foetal kidney retained the highest concentration of radioactivity in the foetal tissues, whereas in dams the highest concentration of radioactivity 8 h post treatment was found in the liver and the lowest in the muscle and bone. A large proportion of methiocarb metabolites were not organo-soluble and remained in the aqueous phase, suggesting that methiocarb was extensively metabolised to form water soluble metabolites. The major ether extractable metabolite identified in this study was methiocarb sulfoxide.

Oral Administration

Van Hoof and Heyndrickx (1975) examined the urinary excretion of 4 insecticidal carbamates (including methiocarb) and their phenolic metabolites following oral administration of parent compounds to rats. Up to 2.3% of the administered dose of methiocarb was excreted in urine unchanged within 72 h. The only methiocarb metabolite identified in urine was methiocarb phenol accounting for 2.3% of the administered dose.

Metabolism and excretion of metabolites in urine was investigated by Stanley and Johnson, (1976) following a single oral gavage administration of ring-1-^{14}C labelled methiocarb in ethanol to rats at 0.25 or 20 mg/kg bw. Urinary excretion amounted to 95% of administered radioactivity at 20 mg/kg bw and 79/82% in males/females at 0.25 mg/kg bw. The major chloroform extractable metabolites found in urine at 20 mg/kg bw were methiocarb phenol (5%), methiocarb sulfoxide phenol (6%) and an unidentified metabolite (6%) that might correspond to N-hydroxymethyl methiocarb sulfoxide. Methiocarb sulfoxide was detected in

trace quantities (about 1%). The major metabolites found in the aqueous fraction were methiocarb sulfoxide phenol (23%), methiocarb phenol (8%) and methiocarb sulfone phenol in trace quantities (about 1%). At 0.25 mg/kg bw, about 15% of the administered radioactivity was recovered in the chloroform extract while 59% remained in the aqueous phase. However, the proportion of organo-soluble and water-soluble radioactivity and percentages of major metabolites found in urine were independent of the administered dose. Between 57-72% of the radioactivity present in the aqueous phase was rendered organo-soluble by enzyme incubation. No major difference was seen between sexes.

In a study conducted to evaluate the metabolic fate of methiocarb in dogs (Bell, 1974), a single oral dose of ring-UL-^{14}C-methiocarb in gelatin capsules was administered to overnight fasted dogs at 2 mg/kg bw. The dogs were sacrificed at 24, 48, 96 and 144 h intervals post treatment and the radioactivity in a range of tissues and blood was determined. Radioactivity in the blood reached its maximum within one hour after administration. Half-lives of the total radioactivity in plasma or whole blood were estimated to be about 75-76 h. The total radioactivity recovered in urine ranged from 26-66% of the administered dose; methiocarb phenol sulfone and methiocarb phenol sulfoxide being the two major metabolites found at a ratio of about 3:1. The proportion of unchanged methiocarb excreted in urine was not given. Of the administered dose, between 10-56% was excreted in faecal matter as methiocarb suggesting incomplete gastrointestinal absorption and lack of degradation by intestinal flora, or possible secretion of absorbed but unchanged methiocarb in bile. The highest tissue residue level at 144 h post dosing was found in the kidney.

The metabolic fate and urinary excretion of methiocarb in a dairy cow has been studied (Minor & Murphy, 1977) by administering a single dose of ring-1-^{14}C-methiocarb at 0.14 mg/kg in a gelatin capsule orally. The general physical condition of the cow and the milk production were unaffected by treatment. The peak blood radioactivity was noted between 2.5-3 h post treatment, and of the administered radioactivity, 96% was excreted in urine by 144 h post treatment. Faecal matter and milk samples collected during 144 h contained 1% and <1% of the administered radioactivity respectively. Only about 1% of the metabolites in urine were chloroform extractable. Following enzyme and acid hydrolysis, approximately 78-85% of the urine radioactivity became chloroform extractable suggesting that the primary metabolites of methiocarb were in conjugated form. TLC analysis revealed the presence of three major metabolites: methiocarb phenol (25-29%), methiocarb sulfoxide phenol (22-32%) and methiocarb sulfone phenol (20-23%) with trace quantities of methiocarb sulfoxide, methiocarb sulfone and some unidentified components (<1%). The proportion of parent compound excreted in urine was not given, and about 14-21% of the administered radioactivity in urine remained as aqueous residues after chloroform extraction steps.

In vitro studies

In a study conducted to determine the metabolic fate of various methyl and dimethylcarbamate insecticides (Oonithan & Casida, 1966), carbonyl-^{14}C labelled methiocarb was incubated with rat liver microsome fraction, or microsome plus the soluble fraction for 4 h with NADP, $NADPH_2$, NAD, $NADH_2$ or without any of these chemicals. The extent of metabolism of methiocarb was generally higher in the incubation mixture containing the microsomes plus soluble fraction and NADP. About 8% of methiocarb was metabolised to products, about 43% of which were water soluble. Two hydroxylated metabolites of the s-alkyl group were identified: 4-methylsulfinyl-3,5-xylyl methyl-carbamate (sulfoxide) and 4-methylsulfonyl-3, 5-xylyl methyl carbamate (sulfone). One further metabolite was not identified. It was reasoned that the unidentified metabolite may have formed due to hydroxylation reactions at different sites on the substrate. The sulfoxide metabolite was found

to possess plasma ChE inhibitory properties. Neither the metabolites formed by hydrolysis at the carbamic ester site nor those at the origin of the TLC plates were identified.

Wheeler and Strother (1971) conducted a comparative study of the metabolism of Zectran (4-dimethylamino-3,5-xylyl methylcarbamate) and Mesurol (methiocarb) to characterise and identify the metabolites, routes of biotransformation and the extent of biodegradability of the parent compounds using 15000 g supernatant fractions of the liver and the kidney homogenates, and blood of dogs and rats. Two major metabolites of methiocarb formed by the dog and rat liver and the dog kidney were identified. They were 4-methylsulfynyl-3,5-xylyl N-methyl carbamate (methiocarb sulfoxide) and 4-methylthio-3,5-xylyl N-hydroxymethylcarbamate (M-NOHME). Methiocarb metabolites following incubation with serum or whole blood were not identified. The greater part of added methiocarb was found to be bound with plasma proteins when added to plasma. However, when the red cells were present, plasma protein binding was diminished by about 2-fold, demonstrating the ability of methiocarb to bind with RBCs. This binding however, appeared rather weak as the majority of the radioactivity was found in the supernatant fractions obtained after trichloroacetic acid (TCA) precipitation and organo-soluble fractions.

A comparative *in vitro* metabolism study of five methylcarbamate insecticides including methiocarb was undertaken by Strother (1972) using human and rat liver fractions. Rat or human liver 15000 g supernatant fractions were incubated with carbonyl ^{14}C-labelled methiocarb technical for 3 h at 37° C in the presence of NADP. The reaction mixture was then ether extracted and analysed by TLC and GC. With human and rat liver preparations, approximately 45% of the added radioactivity was noted in the aqueous phase, suggesting that *in vitro* metabolism produced more water soluble metabolites than organo-soluble products. Two major metabolites in organo-soluble fractions were identified: 4-methylsulfinyl-3,5-xylyl methylcarbamate (methiocarb sulfoxide, 13% and 16% for human and rat liver respectively), and 4-methylthio-3,5-xylyl N-hydroxymethyl carbamate (8% and 6% for human and rat liver respectively). Both products retained the OC(O)NC functional group considered necessary for ChE inhibition. Twelve further metabolites produced by human liver, and five formed by rat liver preparations were not identified.

Wheeler and Strother (1974b) investigated the ether extractable metabolites produced from *in vitro* metabolism of three carbamate pesticides including carbonyl ^{14}C-labelled methiocarb following incubation with foetal and maternal tissue preparations. Maternal and foetal brain, liver, and placental tissues were obtained from 18 or 19 days pregnant SD rats. The supernatant fractions were fortified with $NADP^+$ and incubated with 0.5 µmol of methiocarb for 2 h. Sulfoxidation was the major pathway of methiocarb metabolism. Foetal and maternal liver converted 23 and 12% respectively of methiocarb to methiocarb sulfoxide. However, other metabolic pathways were also active in the maternal liver. The most prominent of these, accounting for approximately 8% of the added radioactivity, was hydroxylation of the N-methyl carbon to form N-OH methiocarb. Rat placenta had some limited ability to sulfoxidate methiocarb, but the foetal and maternal brain had no measurable metabolic activity towards methiocarb.

The role of flavin adenine dinucleotide (FAD)-dependant monooxygenase in the oxidation of 39 thioether-containing organophosphate and carbamate pesticides including methiocarb, and the structure-activity relationships of these pesticides were studied by Hajjar and Hodgson, (1982). Methiocarb was incubated in the presence of NADPH and FAD-dependant monooxygenase at 37º C for 30-60 seconds. Methiocarb was oxidised by FAD-dependent monooxygenase, purified from pig liver microsomes. The stoichiometric relationship between NADPH and standard substrates during the course of the oxidation reaction was 1:1. The rate

of metabolism of methiocarb was relatively low compared to phosphorodithioates, and was equivalent to about 2.82 ± 0.03 nmoles of NADPH/min/nmole of enzyme. However, the metabolic products of methiocarb metabolism were not identified nor was their optical activity measured.

A study was undertaken to evaluate the enzyme systems involved in microsomal sulfoxidation of methiocarb (MeS), the product enantioselectivity of the reaction and cholinesterase inhibitory properties of the two methiocarb sulfoxide (MeSO) enantiomers by Buronfosse *et al*, (1995) using microsomes prepared from the livers of either control, 3-methylcholanthrene (3MC), dexamethasone (DEX) or pyrazole (PYR) treated male rats. Microsomal preparations were incubated with MeS at pH 7.4 in the presence of $NADP^+$, glucose-6-phosphate and glucose-6-phophate dehydrogenase. The major metabolite identified was MeSO. Based on the order of elution from the chiral column, two MeSO enantiomers (A and B) were identified and their relative proportions were established. FMO dependant sulfoxidation showed high stereoselectivity with an enantiomeric excess of 88% in favour of the A enantiomer. No methiocarb sulfone ($MeSO_2$) in the incubation mixture was detected. Based on the comparative ChE inhibition kinetic data, MeS and its metabolites were arranged according to the order of increasing inhibition: $MeSO_2$, A-MeSO, racemic MeSO, MeS and B-MeSO.

Metabolism and urinary excretion of methiocarb metabolites

Methiocarb phenol

The metabolic fate and urinary excretion of orally administered methiocarb phenol in male rats was studied by Stanley and Johnson (1985). Ring-1-^{14}C labelled methiocarb phenol in ethanol/water (1:1) was administered orally to rats. Of the radioactivity administered, 77-81% was excreted in urine within 48 h and of that about 3-4% was organo-soluble, while 73-78% remained in the aqueous phase. About 45% of the radioactivity excreted in urine was identified and three compounds were detected: unchanged methiocarb phenol (29-35%), methiocarb sulfoxide phenol (3-5%) and methiocarb sulfoxide (5%). The study authors stated that methiocarb phenol contained about 2% of methiocarb sulfoxide phenol and about 1% of unchanged methiocarb phenol can be converted to methiocarb sulfoxide phenol by incubating in pH 5 buffer for about 16 h. The 3-5% methiocarb sulfoxide phenol fraction found in urine therefore could be attributable to impurities in the dosing solution and artefact formation during enzyme hydrolysis. The fraction of methiocarb sulfoxide (5%) identified in urine could be an *in vivo* metabolic product. Apparently, rats did not readily convert methiocarb phenol to methiocarb sulfoxide phenol.

Acute Toxicity

The acute toxicity of methiocarb technical in mammals is high when administered by the oral route. The oral LD_{50} value in rats ranged from 9.0 to 135 mg/kg bw in a variety of vehicles, and was 52.3 mg/kg bw in mice. The oral LD_{50} in guinea pigs ranged from 12.2 to 100 mg/kg bw, and has been shown to be less than 25 mg/kg bw in the Beagle dog. By the i.p. route, the LD_{50} values for mice and rats ranged from 5.5 to 6.0 and 25 to 100 mg/kg bw respectively, and in guinea pigs, it was 17 mg/kg bw.

Methiocarb is moderately toxic in rats by the inhalation route. In a head only 4 h exposure situation, the LC_{50} in rats ranged from 433-1208 mg/m^3.

Generally, the acute dermal toxicity in rats and rabbits was low with LD_{50} values being in excess of 2000 mg/kg bw. However, an early dermal toxicity study conducted in rats using isopropanol as the vehicle, and in which the applied material was not removed after exposure to the chemical for an unspecified duration, reported a dermal LD_{50} of 350-400 mg/kg bw. In

contrast, three further dermal studies conducted in rats using polyethylene glycol 400, saline or unspecified type of oil as vehicles reported LD_{50} values in excess of 500 mg/kg bw. Therefore, the worst dermal LD_{50} of 350-400 mg/kg bw may perhaps be due to the isopropanol vehicle used in that study.

In acute toxicity studies (oral, inhalational and intraperitoneal), cholinergic effects such as diarrhoea, salivation, lacrimation and vomiting (muscarinic effects), muscular tremors and paralysis (nicotinic effects), and restlessness, ataxia and convulsions (CNS effects) have been observed in experimental animals. The signs of acute toxicity appear to be similar to those seen following intoxication with other carbamates.

Methiocarb technical was not an eye or skin irritant in rabbits. Similarly, there was no evidence of skin sensitisation in studies conducted using guinea pigs.

The acute oral toxicity of methiocarb formulations was generally dependent on the concentration of methiocarb, although the formulation type also affects the acute toxicity. LD_{50} values in rats ranged from 23-140 mg/kg bw for formulations containing 75% methiocarb; acute oral LD_{50} of products containing 4% methiocarb ranged from 848 to 945 mg/kg bw, and the value for pellets containing 2% methiocarb was in excess of 2648 mg/kg bw in rats. The formulations were of moderate to low dermal toxicity. Although technical grade methiocarb was not an eye or skin irritant or a dermal sensitiser, some products have been found to cause slight to severe ocular irritation in rabbits and one product was identified as a skin sensitiser. The products which caused severe eye irritation or skin sensitisation were not registered in Australia at the commencement of this review.

Antidote studies

The antidotal effects of pralidoxime (PAM), atropine sulfate or obidoxime chloride (BH6) have been studied in rats when each chemical was administered alone or in combination after administration of a single oral dose of methiocarb (Kimmerle, 1966). Antidotal chemicals were administered by i.p. injection before the appearance of cholinergic signs. The LD_{50} value of methiocarb without antidotes was 67 mg/kg bw. Treatment with atropine sulfate alone increased this value by about 7-fold, PAM alone by about 2.8-fold, and BH6 alone by about 3.3-fold. The combined effect of atropine sulfate and PAM or atropine sulfate and BH6 was slightly higher than atropine sulfate alone, the increases being approximately 7.4- and 7.6-fold respectively. Thus, treatment with atropine sulfate alone appears to be more effective as an antidote against methiocarb compared to PAM or BH6 alone. The effect of atropine sulfate was only slightly increased when combined with PAM or BH6 administration.

Kimmerle (1971) investigated the antidotal effects of tetraethylammonium chloride (TEAC) and atropine sulfate when each was administered alone or in combination after administration of a single oral dose of methiocarb to rats when cholinergic signs of toxicity were evident. The LD_{50} values were: 104.5, 415, 643 and 580 mg/kg bw for the animals receiving no antidote, TEAC, atropine sulfate, and TEAC and atropine sulfate combination respectively. Thus, treatment with atropine sulfate alone produced a 6-fold increase in the LD_{50} value, and appears more effective as an antidote against methiocarb compared to TEAC alone or TEAC and atropine sulfate in combination.

Effects on acetylcholinesterase activity

Baron *et al* (1964) examined the comparative liver and brain esterase inhibiting properties of methiocarb in mice. Female mice were treated by i.p. injection with 16 mg/kg bw of methiocarb in corn oil. An inhibition of liver esterase activity was seen against acetylcholine and other substrates within 30-60 minutes of administration. The study authors claimed that

the enzyme activity was reversed by 24 h post treatment, but no supporting data were provided. No inhibition of brain esterase activity was said to have occurred at or after 1 h post treatment. The absence of some useful supporting data, together with vehicle induced inhibition of liver esterase(s) noted at 24 h post treatment reduced the value of the study findings, and made independent evaluation difficult.

The effects of dermally administered methiocarb and its two plant foliar residue components, methiocarb sulfoxide and methiocarb sulfone, on erythrocyte (RBC) ChE activity in rats were investigated by Knaak *et al* (1980). Each chemical was applied on the shaven intact skin in 1 mL of acetone. The quantities applied were approximately 22, 44, 87, 174 and 348 µg/kg bw, 17, 35, 70 and 139 µg/kg bw, and 22, 44, 87, 109, 217 and 435 µg/kg bw for methiocarb, methiocarb sulfoxide and methiocarb sulfone respectively. Methiocarb and methiocarb sulfoxide caused biologically significant inhibition in RBC ChE activity (>20%) at 87 and 70 µg/kg bw and above respectively. Methiocarb sulfone did not produce any depression in RBC ChE activity when applied at levels as high as 435 µg/kg bw for a period of 24 h.

Short-Term Repeat-Dose Studies

Short term repeat dose toxicity studies have been conducted using rats, dogs and rabbits. The routes of administration were oral, intraperitoneal or inhalational in the rat, oral in the dog, and dermal in the rabbit studies. Synopses of these studies are given in the following sections.

Oral

In the study of Kimmerle (1960), methiocarb was administered by oral gavage to rats at 3 mg/kg bw/day for three days, and at 4 mg/kg bw/day for the next 24 days. Three animals/group were killed every week and the RBC ChE activity was determined. The RBC ChE activity was depressed to about 80% and 50% of the pre-treatment values after 14 days and at termination respectively. It was stated that the recovery of the enzyme activity was slow during the subsequent observation period and did not return to normal values up until 42 days after the completion of the study. No cholinergic signs were observed and the body weight gain of the animals was normal, but no supporting data for any of these study parameters were provided. No NOEL was established as treatment-related effects were observed at the only dose tested.

In a 4-week study (Eben & Kimmerle, 1973), methiocarb in polyethylene glycol 400 was administered by oral gavage to rats at 1, 3 or 10 mg/kg bw/day. Cholinesterase activity in plasma and RBC was determined at 20 minutes post treatment on days 4, 8, 14, 21 and 28, and additionally, 5 h after the last dose. The brain ChE activity was determined 2 h after the final administration. The animals receiving methiocarb at 10 mg/kg bw/day exhibited brief cholinergic signs but details on the type, onset and the duration of such manifestations were not provided. Biologically significant (>20%), consistent plasma and RBC ChE inhibition was seen at 10 mg/kg bw/day in both sexes at the majority of the sampling times. Similarly, the depression in brain ChE activity noted in rats of both sexes at the same dose level was biologically significant. The NOEL for plasma, RBC and brain ChE inhibition was set at 3 mg/kg bw/day based on biologically significant inhibition in plasma ChE at 10 mg/kg bw/day.

A study of the effects of methiocarb or methiocarb sulfoxide on cholinesterase activity in rats was undertaken by Hixson (1981). The test chemicals were administered by oral gavage to groups of female rats at 0.5 or 2.0 mg/kg bw/day in Carbowax, 5 days/week for 4 weeks. A concurrent vehicle control group received Carbowax at 0.5 mL/100 g bw. Sporadic tremors were seen in 6/15 rats receiving methiocarb sulfoxide at 2.0 mg/kg during the first five days.

In animals at 0.5 mg/kg bw, a biologically significant (≥20%) plasma ChE inhibition was observed at 30 minutes post treatment at week 1 only. A trend towards decreased depression of the plasma ChE activity with time was seen. In rats receiving methiocarb at 2.0 mg/kg bw, plasma and RBC ChE activities were reduced at 30 min post treatment, achieving biological significance during the first 3 weeks and at week 1 respectively. No statistically or biologically significant inhibition of plasma or RBC ChE was seen at 4 h after dosing in rats receiving methiocarb at either dosage. With methiocarb sulfoxide, biologically significant inhibition was observed at 0.5 or 2.0 mg/kg in plasma ChE from weeks 1-4, and RBC ChE during all but second week. RBC ChE was slow to recover, showing biologically significant inhibition at 0.5 and 2.0 mg/kg during two of the 4 weeks on study. Both test compounds demonstrated an apparent dose response relationship with respect to ChE inhibition at 30 minutes but not at 4 h post treatment. A NOEL for methiocarb sulfoxide was not observed due to biologically significant inhibition in plasma and RBC ChE activity at 0.5 and 2.0 mg/kg bw/day. The NOEL of methiocarb for RBC ChE inhibition was set at 0.5 mg/kg bw/day. No NOEL of methiocarb for plasma ChE inhibition was established due to the enzyme inhibition seen at 0.5 mg/kg bw/day at week one.

Intra peritoneal (ip) injection)

A study was undertaken by Dubois and Raymund (1961), in which female SD rats were treated with methiocarb in 20% ethanol and 80% propylene glycol by i.p. injection at 0, 5, 10 or 15 mg/kg bw/day, daily for 60 days. No mortalities were observed in the control and 5 mg/kg bw/day groups. However, the survival rates at 10 and 15 mg/kg bw/day were low, being 40% and 0% respectively. It was claimed that there was no treatment-related effect on weight gain of rats at 5 mg/kg bw/day and a "slight gain" in the body weight was seen at 10 mg/kg bw/day. However, no individual data on body weights were provided nor were details of the statistical procedures used supplied to support these claims. The brain, submaxillary gland and serum ChE activity in rats at 5 mg/kg bw/day was unaffected by treatment. The NOEL for this study was established at 5 mg/kg bw/day. However, the validity of the findings is markedly reduced due to methodological deficiencies, lack of absolute data on several useful study parameters and information on clinical observations.

Inhalation

Kimmerle (1960) conducted an inhalation toxicity study in which 400 mg of methiocarb of unstated purity in ethanol was sprayed using a "Flury type atomiser" into a chamber of 400 litre capacity containing 1 rabbit, 1 guinea pig, 2 rats and 4 mice for 1 h/day, for five consecutive days. Two mice died 4 days after the completion of the study. The study author stated that the animals were "observed to suffer from slight irritation of mucous membrane" during the first 4 days. Muscular spasms were observed in the rats and mice on the fifth day. It was stated that the surviving animals recovered soon after treatment. No "poisoning symptoms" except for slight mucous membrane irritation were observed when half of the above dose (ie 200 mg/400 litres) was administered to a different group of animals comprising 1 cat, 1 rabbit, 1 guinea pig, 2 rats and 4 mice for 1 h/day, for five consecutive days. Because of deficiencies including: lack of a control group, information on experimental animals and clinical observations; this study was of limited regulatory value.

Thyssen and Mohr (1983) exposed rats to aerosols of methiocarb technical at concentrations equivalent to 6, 23 or 96 mg/m^3 daily for 6 h/day for 15 work days within three weeks. Animals in the negative and solvent control groups received air only or 20 mL of the solvent respectively. There were no mortalities. Muscular tremors were observed in animals at 96 mg/m^3 from day 5 persisting until termination of the study. Significant reductions ($p \leq 0.05$) in group mean body weight were noted in males at 96 mg/m^3 after 5 days of exposure compared to the negative controls. Group mean body weight of females in the same dose group was significantly depressed compared to the solvent controls after 15 days. No treatment-related changes were seen in haematological parameters. Significant reductions in plasma ChE activities were observed in males at 23 mg/m^3 after exposure for 5 days [32% ($p \leq 0.01$)] and 10 days [31% ($p \leq 0.05$)]. Percent reductions in plasma ChE activity in male rats at 96 mg/m^3 after 5, 10 or 15 days of exposure were 55% ($p \leq 0.01$), 37% ($p \leq 0.05$) and 52% ($p \leq 0.01$) respectively. In females, significant depression of plasma ChE activity was observed at 96 mg/m^3 after 5, 10 and 15 days of exposure, percent inhibitions being 56%, 60% and 61% ($p \leq 0.01$) respectively. RBC ChE activity in males at 96 mg/m^3 was depressed by about 18% ($p \leq 0.01$) compared to the solvent controls after 5 days of exposure. Brain ChE activity was significantly reduced in male rats exposed to 23 and 96 mg/m^3 after 15 days exposure (35 and 39% respectively, $p \leq 0.01$) compared to the solvent controls. In females, brain ChE inhibition (26%, $p \leq 0.01$) was noted only at 96 mg/m^3, on treatment day 15. No histopathological changes attributable to treatment were seen. Based on statistically and biologically significant inhibition of ChE activity in the plasma and brain at higher exposure levels, the NOEL for male rats was 6 mg/m^3, and the NOEL for females was 23 mg/m^3.

Dermal

Technical grade methiocarb applied to shaved flanks of adult white rabbits at 500 mg/kg bw/day for 14 consecutive days did not result in any mortalities, behavioural changes or toxicological symptoms (Kimmerle, 1969c). Weight gain, haematology, and liver and kidney function of treated animals were unaffected by treatment. In addition, urinalysis did not reveal any treatment-related variations in the measured parameters. Dermal application of methiocarb at 500 mg/kg bw/day for 14 days appears to have not produced any treatment-related effects in rabbits. However, the reliability of the study is reduced due to the absence of any pathological examination.

A 21-day dermal toxicity study of technical grade methiocarb was conducted by Procter (1988). Methiocarb was applied to the shaven intact skin of NZW rabbits at 0, 60, 150 or 375 mg/kg bw for 6 h/day under occluded conditions. Two animals at 60 mg/kg bw/day displayed decreased faecal output, weight loss and decreased motor activity, and died prematurely. Similar symptoms were also observed in three other animals; one each at 60, 150 and 375 mg/kg bw/day. No skin reaction to the test substance was evident. Food consumption in males at 375 mg/kg bw/day was depressed and reached statistical significance ($p \leq 0.05$) on days 9 and 15. The total amount of food consumed by this group during the study was about 13% less than the controls. Likewise, the food consumption in 375 mg/kg bw/day females was reduced, to a biologically significant (≥20%) extent on days 7 and 9. Further reductions in food consumption were noted in females at 150 mg/kg bw/day during the second week. Consequently, biologically significant deficits in weight gain occurred in both sexes at 375 mg/kg bw/day and in 150 mg/kg bw/day females. Group mean plasma ChE activity in males was depressed in a dose related manner at 375 mg/kg bw/day, achieving significance at 6 h post treatment on days 14 ($p \leq 0.05$) and 21 ($p \leq 0.01$). Biologically significant reductions in plasma ChE activity were evident in males on day 7 at 375 mg/kg bw/day and on day 14 at 150 mg/kg bw/day at 6 h post treatment. No gross or microscopic tissue changes attributable to the treatment were observed. Based on decreased food consumption and weight gain, and plasma ChE inhibition at 150 mg/kg bw/day, the NOEL for this study was 60 mg/kg bw/day.

Procter (1989) conducted another study in which technical grade methiocarb was applied to the shaven intact skin of female NZW rabbits at 500 mg/kg bw, 6 h/day for 21 days under occluded conditions. No mortalities were observed. Two animals that removed their dressings on treatment day 10 and ingested some test material exhibited clinical signs of cholinergic poisoning which reversed by the next day. Group mean food consumption was depressed by about 13% in males and 10% in females compared to the controls achieving significance ($p \leq 0.05$) during study days 13-15 in males and 19-21 in female rabbits. The total weight gained by the males during the study was about 63% less compared to the controls. Similarly, treated females were always lighter than the controls and showed statistically significant reductions ($p \leq 0.05$) in group mean body weights and gained about 30% less weight during the study. A statistically significant reduction in plasma ChE activity was noted in treated females on day 14 at 6 h post treatment compared to the controls. RBC ChE and brain ChE activities were unaffected by treatment. No inter-group differences in absolute and relative organ weights of the treated animals were seen nor were any gross or microscopic tissue changes attributable to the test chemical observed. The reduced food consumption and body weight gain seen in the treated animals in this study is consistent with the findings of the previous report (Procter, 1988), but ChE inhibition appears to be unexpectedly slight and inconsistent. A NOEL could not be established, as treatment-related effects were observed at the only dose tested.

Dogs

In a 12-week study (Root *et al*, 1963), technical grade methiocarb was administered to Beagle dogs at 0, 50, 100, or 250 ppm in the diet (equivalent to approximately 0.75, 1.25 or 3.75 mg/kg bw/day). Growth rate of the animals was unaffected by treatment. No inhibition in the weekly serum or erythrocyte ChE activity was noted. Food and water consumption, haematology or clinical chemistry parameters were not examined. Because of the small experimental group size adopted, and lack of clinical observations and statistical analysis of data, this study is of limited regulatory use.

Hayes (1981) investigated the effects of technical grade methiocarb or methiocarb sulfoxide on ChE activity in Beagle dogs. The test chemicals were administered orally at 0, 0.05 or

0.5 mg/kg bw/day in gelatin capsules for 29 days. Occasional slight to heavy salivation, and vomiting were observed in dogs receiving either test compound at 0.5 mg/kg bw/day and in one female receiving methiocarb sulfoxide at 0.05 mg/kg bw/day. Generally, both test compounds throughout the study showed a dose relationship with respect to ChE inhibition. The maximum ChE inhibition usually occurred between 0 and 3 h post dosing at 0.5 mg/kg bw/day with either test compound in both sexes. Plasma ChE inhibition did not reach biological significance at the low dose. Twenty percent depression of the RBC ChE activity was noted with methiocarb at 0.05 mg/kg bw/day at week 1 in females and at week 5 in males. Methiocarb sulfoxide was a more potent inhibitor than methiocarb. Greater than 20% inhibition was seen on both plasma and RBC ChE activity with methiocarb sulfoxide at 0.05 mg/kg bw/day in both sexes on several occasions. Plasma ChE depression was slightly more pronounced than RBC ChE depression. Regardless of sex, both plasma and RBC ChE were depressed to biologically significant levels at 0.5 mg/kg bw/day by both test compounds at most of the sampling times. Plasma and RBC ChE activities were generally normal by 6 h post treatment. Although 20% inhibition of RBC ChE activity was seen on two isolated occasions with methiocarb at 0.05 mg/kg bw/day, this was not accepted as a true LOEL because the occurrence was sporadic. However, this was not accepted as a NOEL either, because the data are considered unreliable due to the small numbers of dogs/group. Due to treatment-related inhibition of either plasma and/or RBC ChE enzyme activity seen in both sexes with methiocarb sulfoxide at both dose levels, no NOEL was established for this compound. This study was not considered adequate for regulatory purposes due to the small experimental group size.

Formulations

Flucke and Kimmerle (1977) investigated the toxicity (including erythrocyte (RBC) ChE activity) of orally administered Mesurol slug pellets containing 4% methiocarb in female Chinchilla rabbits at 100 mg/kg bw, twice a day for 5 consecutive days. No mortalities were reported. Treated animals showed a slight loss of body weight at the end of the treatment period. However, they regained weight by 9 days after cessation of treatment. RBC ChE activity was slightly depressed at 5 h after the first daily dosing compared to the reference value (2-7%). However, the RBC ChE data at 12 h after the second daily dosing were comparable to the reference value and showed the recovery of the enzyme activity within 12 h post treatment. Plasma ChE activity was depressed by about 25-35% at 5 h after the first daily dosing and was biologically significant on all treatment days. Plasma ChE activity at 12 h after second daily dosing also remained depressed (12-17%) throughout the study and appeared slower to recover. The validity of the study findings, however, is reduced due to small experimental group size, lack of controls, clinical observations, and statistical analyses. No NOEL could be established as treatment-related effects were observed at the only dose tested.

An inhalation toxicity study was conducted by Groning and Kimmerle (1975) using rats. The animals were exposed to aerosols of methiocarb 50% wettable powder at concentrations of 20.2, 31.5 or 188 mg/m^3, for 4 h/day for 5 days. There was no control group in the study. There were no mortalities, but the authors stated that the general health of the animals at 31.5 and 188 mg/m^3 was affected from the first day of exposure onwards. ChE inhibition was reported in the animals at 188 mg/m^3, but no supporting data were provided. The animals at 20.2 mg/m^3 showed unspecified changes in general health on the second and third day on study. Clinical signs in animals persisted for 1 to 3 days after the 5 day exposure period. Lack of a control group and inadequate information on clinical signs and ChE depression rendered this study inappropriate for regulatory purposes.

In a 3-week dermal study (Dubois et al, 1968) methiocarb 50% wettable powder in water was applied to shaved, abraded skin of rats at 200 mg/kg bw/day, 5 days/week for 3 weeks. There were no mortalities. An inhibition of the growth rate was seen in all animals, which was more pronounced in females. Brain ChE activity was unaffected by treatment, but cholinergic signs were noted after each treatment, particularly at the beginning of the daily treatment period. No gross pathologic changes attributable to treatment were seen. The data showed the potential of the test substance to cause cholinergic effects by repeat administration. However, due to of lack of information on clinical signs, and with no effect seen on brain ChE activity, the ChE inhibitory potential of the formulation at the dose level used cannot be fully explained. The validity of the study findings is reduced due to methodological deficiencies, debatable sensitivity of the ChE assay used, and insufficient use of statistical procedures to analyse the results.

Subchronic Toxicity

There were only two subchronic toxicity studies in the methiocarb toxicology database. The two studies have been conducted in rats. Both studies, however, were found to be inappropriate for regulatory purposes.

In the dietary study of Doull *et al* (1962), methiocarb technical was fed to rats at 0, 5, 10 or 50 ppm (equivalent to approximately 0, 0.5, 1.0 or 5.0 mg/kg bw/day) for 16 weeks. No treatment-related effect on mortality was evident. Food consumption and the growth rate of the animals were unaffected by treatment. None of the treated rats exhibited any cholinergic or other toxic symptoms. Biologically significant (≥20%) inhibition of the serum ChE activity was seen in both males (21%) and females (28%) at 5 mg/kg bw/day. Submaxillary gland ChE in females was inhibited by 23-33% in all treatment groups compared to the controls, with a clear dose-response relationship. RBC and brain ChE activity were slightly inhibited in 5 mg/kg bw/day males. No data on necropsy, organ weights, histopathology or clinical observations were provided. The validity of the study findings is reduced due to lack of justification for dose selection, statistical analyses, clinical observations, and data limitations. A NOEL could not be established, given the evidence of ChE inhibition in the submaxillary gland at 0.5 mg/kg bw/day.

A 24-week dietary study was carried out by Löser (1969) in which methiocarb technical was fed to rats at 0, 30, 100 or 300 ppm (equivalent to approximately 0, 3, 10 or 30 mg/kg bw/day). The rats were participating in a concurrent single generation reproduction study and the studies reported here were performed in animals of F_0 generation at the end of the preliminary treatment period, and after their second litter had been reared. Modest changes in white blood cell counts were seen in both sexes. Perturbations in haematology were noted at 10 weeks at 30 mg/kg bw/day, and not thereafter. The serum AST and ALT levels were higher in animals of both sexes at 30 mg/kg bw/day, 10 weeks after initiation of the study and not thereafter. No clinical observations, data on food and water consumption or body weights of the animals were provided. The modest changes in haematological and clinical chemistry parameters occurred in treated animals appear to be physiological adaptations and were not suggestive of any disease process. However, due to the limitations of the data and lack of statistical analyses, the reliability of the findings is reduced. The data could be considered as supplementary to other long term toxicity studies.

Chronic Toxicity

A chronic study was undertaken by Kroetlinger and Janda (1983) in which methiocarb technical was fed to mice in the diet at 0, 67, 200 or 600 ppm (equal to 0, 14.6, 42.8 and

132 mg/kg bw/day for the males and 0, 19.8, 57.0 and 173 mg/kg bw/day for the females) for 2 years. Mortality among the test groups and controls was high. The body weights at 600 ppm were depressed by about 5% during the first year ($p \leq 0.01$ or 0.05). Consistent with biologically significant elevations in leucocyte counts (42% and 67% at 200 and 600 ppm respectively) seen at 12 months, statistically significant increases occurred in females at 24 months ($p \leq 0.01$ or 0.05) and appeared to be treatment-related at all doses. The ALT activity in both sexes at 200 and 600 ppm was significantly elevated ($p \leq 0.01$ or 0.05) at termination. Moreover, at 12 months, the enzyme activity was elevated by 26-38% in all treated groups compared to the corresponding controls, however, no statistical significance was achieved at this time point. Statistically or biologically significant ($p \leq 0.01$ or 0.05) inhibition of plasma ChE was noted at 200 and 600 ppm in both sexes at one month but not thereafter. The brain ChE activity was unaffected. Statistically ($p < 0.05$) or biologically significant reductions in absolute (33-42%) and relative (32-44%) spleen weight noted at 200 and 600 ppm at termination may have been attributed to the test compound. The absolute and relative liver weights were elevated at 600 ppm in both sexes at termination. Because of statistically and/or biologically significant perturbations seen in haematological parameters and ALT activity in treated animals at all doses at both sampling times, a NOEL for this study was not established.

Methiocarb technical in the diet was fed to rats at 0, 25, 50 or 100 ppm (equivalent to approximately 0, 2, 5 or 10 mg/kg bw/day) for about 80 weeks (Doull *et al*, 1967). Survival was poor, but mortality appeared to be unrelated to treatment as most of the animals appear to have been distressed during the study due to respiratory tract and renal infections. Food consumption and the growth rate of the animals were unaffected by treatment. No cholinergic or other toxic symptoms were observed. Biologically significant (≥20%) inhibition of the serum (22%) and submaxillary gland (24%) ChE activity was noted in females at 100 ppm. The NOEL for ChE inhibition was 50 ppm. The validity of the study however, is reduced due to lack of justification for dose selection, statistical analyses, clinical observations and data limitations most significantly relating to ChE activity in males. Therefore, the findings of this study were of limited regulatory value.

Methiocarb technical in the diet was fed to rats at 0, 67, 200 or 600 ppm (equivalent to 0, 3.27, 9.3 and 29 mg/kg bw/day for the males and 0, 4.98, 13.9 and 42 mg/kg bw/day for the females) for 2 years (Kroetlinger *et al*, 1981, Kroetlinger, 1990). Food consumption in males at 600 ppm was slightly reduced (5%) during the second year. Between weeks 4 and 19 on study, the 200 ppm males showed a statistically significant ($p \leq 0.05$ or 0.01), consistent decrease in body weight (3-8%). At 600 ppm, body weights were significantly depressed ($p \leq 0.05$ or 0.01) in both sexes throughout the study. A statistically significant increase ($p \leq 0.05$ or 0.01) in reticulocyte count and a depressed erythrocyte count were seen in females, at months 3 and 6 at 200 and 600 ppm. Significant increases ($p \leq 0.05$) in plasma urea levels were seen in 600 ppm females at 12 months and termination. Plasma ChE activity in both sexes at 600 ppm was inhibited with either statistical ($p \leq 0.05$ or 0.01) or biological significance (>20%). A reduction of plasma ChE activity in 200 ppm males at termination was biologically significant. RBC ChE activity at 600 ppm showed slight depression achieving statistical significance ($p \leq 0.05$ or 0.01). Significant reductions also occurred at 200 ppm ($p \leq 0.05$ or 0.01). No inhibition in the brain ChE activity was seen. Relative and/or absolute spleen weights in both sexes at 600 ppm were depressed by about 10-18%. In males, relative testes weights were increased by 7% ($p \leq 0.05$) at 600 ppm. In histopathology, about 25% of the animals had evidence of parasitic infection in the bowel, suggesting poor hygiene in the study laboratory. This finding increases the uncertainty surrounding the findings of this study, and therefore this study is considered to be of reduced regulatory value. Based on

transient depression in body weight in 200 ppm males, elevation in reticulocyte counts in 200 ppm females and plasma and RBC ChE inhibition at 200 ppm, the NOEL was established at 67 ppm (3.27 and 4.98 mg/kg bw/day for males and females respectively).

Technical grade methiocarb in the diet was fed to Beagle dogs at 0, 50, 100 or 250 ppm (equivalent to approximately 0, 1.25, 2.5 or 6.25 mg/kg bw/day) for 2 years (Doull *et al.,* 1968). No mortalities or clinical signs were reported, though reporting was limited. Food consumption was unaffected by treatment. The average body weight of the animals at 250 ppm was depressed by about 10% from week 32 to 80 and during the same period it was about 10% greater at 100 ppm compared to the controls. The serum ChE activity was inhibited by up to 20% during weeks 15 to 32, in the animals at 100 and 250 ppm. The RBC ChE activity was variable in all dose groups, but rarely inhibited by more than 20%. The brain and liver ChE activities were similar to controls. The absolute and relative liver weights of dogs at 250 ppm were slightly elevated. Due to lack of clinical observations, limitations of the study data and the low number of dogs/group used, this study is not considered appropriate for regulatory purposes.

A long term toxicity study was conducted in the Beagle dog by Hoffman and Schilde (1980). Technical grade methiocarb in the diet was fed to Beagle dogs at 0, 15 (during the first 15 days), 5 (from week 3 to 104), 60 or 240 ppm (equivalent to approximately 0.6, 0.2, 2.4 and 9.6 mg/kg bw/day respectively) for 104 weeks. Clinical signs such as occasional mild weakness of the hind limbs accompanied by trembling, lameness and infrequent decreased alertness were seen in 5/8 dogs at 240 ppm during the first 14 weeks. Occasional vomiting was seen in all groups, but the incidence was higher at 240 ppm. Food consumption was reduced slightly (5-7%) in 60 ppm females and in both sexes at 240 ppm (12%) during the second year. The body weights, weight gain, and the nutritional state of the animals were unaffected by treatment. Dose related and biologically significant (>20%) depression of the plasma ChE activity was seen at 60 and 240 ppm, 2 h post treatment. At 60 ppm, the percentage decrease in plasma cholinesterase activity ranged from 34-66% at the 2 h time point, over the first year and from 8-55% over the second year of treatment. The statistical significance of this effect was not determined. The ChE inhibition at 240 ppm pre-treatment was near or above 20% at most of the sampling times, and was more pronounced in the males than in the females, suggesting slow recovery of the enzyme activity. RBC ChE inhibition was variable and inhibition did not reach biological significance at any dose at any of the sampling times. The brain ChE activity was unaffected by treatment. Based on biologically significant plasma ChE inhibition in both sexes and reduced food consumption in females observed at 60 ppm, the NOEL was established at 5 ppm (0.2 mg/kg bw/day).

Reproductive Toxicity

A reproductive toxicity study was undertaken by Löser and Newman (1970). Technical grade methiocarb was administered to rats at 0, 30, 100, or 300 ppm (equivalent to approximately 0, 3, 10, and 30 mg/kg bw/day) in the diet for three parental generations and their offspring. Each generation was mated twice. The offspring of each of the second matings were used to produce the next generation. Sporadic changes in some reproductive parameters and neonate data were observed in different generations of animals but did not reveal any consistent, statistically or biologically significant treatment-related effects in all generations. No gross or histopathological changes attributable to the treatment were noted nor were any treatment-related malformations observed in any generation at birth or during lactation. A reproduction NOEL was established at the highest dose of 30 mg/kg bw/day, as no treatment-related effects were observed at this dose. The validity of the findings, however, is reduced due to data limitations.

Developmental Toxicity

Lorke (1971) undertook a study of teratology and embryotoxicity in rats. Methiocarb was administered once daily by oral gavage to mated female rats at 0, 1, 3 or 10 mg/kg bw/day on days 6-15 post-coitum. No mortalities or premature abortions were recorded during the treatment nor were any clinical signs noted. Food consumption and appearance of the animals were unaffected. The average weight gain during pregnancy was depressed by about 10% at 10 mg/kg bw/day. Maternal reproductive indices were unaffected, and no significant group differences in foetal weights, resorptions, and foetal skeletal development were observed compared to the controls. Further, no treatment-related visceral or skeletal malformations were observed (Renhof, 1988). Based on reduced weight gain at the highest dose, the NOEL for maternal toxicity was set at 3 mg/kg bw/day. There were no effects on foetal survival, development or growth at the highest dose of 10 mg/kg bw/day.

In a preliminary dose range finding study (Tesh and Ross, 1981), methiocarb technical was administered by oral gavage to artificially inseminated NZW rabbits at 0, 1, 3 or 10 mg/kg bw/day on days 6 through 18 post insemination. No mortalities were recorded. The animals at 10 mg/kg bw/day showed a marked loss of body weight during the first half of the treatment period compared to the controls. Thereafter the group mean body weight of this group increased and was comparable to that of the controls at termination. Post treatment cholinergic responses such as loss of muscular control, muscular tremors and polypnea of about 3 h duration were noted in all animals at 10 mg/kg bw/day commencing from 15 minutes post dosing. The litter responses were unaffected by treatment. Examination of foetuses at terminal necropsy revealed several anomalies. However, the group incidence of these anomalies did not show any consistent indication of an association with treatment. The study authors concluded that, dose levels of methiocarb up to 10 mg/kg bw/day would be suitable for use in a main teratology study.

In a teratology study methiocarb technical was administered by oral gavage to artificially inseminated NZW rabbits at 0, 1, 3 or 10 mg/kg bw/day on days 6 through 18 post insemination (Tesh *et al*, 1981). There was no treatment-related maternal mortality. Post-mortem examinations of animals either found dead or sacrificed during the experiment revealed evidence of respiratory tract infection and/or gastro-intestinal tract disorder or accidental tracheal intubation. The animals at 10 mg/kg bw/day showed a marked loss of body weight during the first two days of treatment. Consequently, their overall body weight gain was decreased during the remaining test period achieving statistical significance ($p \leq 0.01$) on day 18 of gestation compared to the controls. Post-treatment cholinergic signs were noted at

10 mg/kg bw/day. The incidence of pale areas on the foetal liver was increased at 10 mg/kg/day by about 3.5-fold and 17-fold compared to mean concurrent and historical control data respectively. Reproductive indices were comparable among groups. No evidence of teratogenicity of methiocarb was reported. However, maternotoxicity characterised by cholinergic signs and weight loss was evident at 10 mg/kg bw/day. Therefore, a maternotoxicity NOEL was established at 3 mg/kg bw/day. Based on the effects seen in the foetal liver, the embryo/foetotoxicity NOEL was also set at 3 mg/kg bw/day.

In the dose range finding embryotoxicity and teratogenicity study of Dotti and Biedermann (1993), methiocarb technical was applied dermally to shaved, occluded skin of the backs of rabbits at 0, 250, 500 or 750 mg/kg bw, for 6 h/day from days 6 through 18 post coitum. No mortalities, clinical signs or skin reactions related to treatment were noted. The mean food consumption was markedly depressed in all groups during treatment compared to the controls, achieving statistical significance at 250 and 750 mg/kg bw/day. Overall, the does at 750 mg/kg bw/day consumed about 27% less food compared to the controls. Non-statistically significant moderate loss in body weight was noticed in all groups from days 6 through 12 post coitum. The trend was similar to that observed for food consumption, being most marked at 250 and 750 mg/kg bw/day. Group mean foetal body weights were depressed by about 20% at 750 mg/kg bw/day and by 12% at 500 mg/kg bw/day. One foetus at 500 mg/kg bw/day and all foetuses of one doe at 750 mg/kg bw/day were of less than 19 g body weight. No information on skeletal abnormalities was provided. Because of effects seen in maternal food consumption and body weight gain, and foetal findings at 500 and 750 mg/kg bw/day, dose levels of 10, 50, and 250 mg/kg bw/day were selected for the main embryotoxicity study.

A study of the teratogenic and embryotoxic potential of methiocarb was undertaken by Dotti and Beidermann (1992). Methiocarb technical was applied dermally to the shaved, occluded skin of the backs of rabbits at 0, 10, 50 or 250 mg/kg bw, 6 h/day from days 6 through 18 post coitum. The mean food consumption was depressed at 250 mg/kg bw/day during days 6-11, 11-15 and 15-19 by about 6%, 19.5% and 7.3% respectively. Overall, the does at 250 mg/kg bw/day consumed about 4.5% less food compared to the controls. Weight loss at 250 mg/kg bw/day was distinct during days 6 through 22, being significant on days 13 and 16 ($p \leq 0.01$). The mean foetal body weights were depressed by about 4% at 250 mg/kg bw/day. Some statistically significant increases in the incidence of incomplete or non-ossification of phalangeal nuclei seen at 10 and 50 mg/kg bw/day on a per foetus basis was not noticeable when the data were examined on a per litter basis. Hence, the effects seen in phalangeal nuclei at the 2 lower dose levels were considered to be of limited significance. However, the incidence of some statistically significant increases in incompletely ossified hind limb phalangeal nuclei at 250 mg/kg bw/day appear to lie outside the historical data range, suggesting a slight retardation of the ossification process which could be attributed to the test material. Because of reduced food consumption in does at 250 mg/kg bw/day, and the weight loss during days 6 through 22 post coitum, the maternotoxicity NOEL was established at 50 mg/kg bw/day. Owing to reduced mean foetal body weight and retarded ossification of hind limb phalanges seen at 250 mg/kg bw, the foetotoxicity NOEL was also set at 50 mg/kg bw/day.

Genotoxicity

The genotoxicity of methiocarb has been examined using a battery of *in vitro* and *in vivo* tests. The studies include *in vitro* gene mutation, DNA damage and repair, unscheduled DNA synthesis, chromosomal aberration, micronucleus formation and *in vivo* dominant lethal mutation assays. Eight out of 9 genotoxicity studies reviewed, produced negative results indicating by weight of evidence that methiocarb is not genotoxic. The *in vitro* study of Murli (1990) performed using Chinese hamster ovary (CHO) cells was the only study showing positive results for inducing chromosomal aberrations at the dose levels tested under both activated and non-activated assay conditions.

Neurotoxicity

The neurotoxicity of methiocarb has not been studied extensively. The results of two studies conducted on hens are summarised in the following sections.

The neurotoxicity of methiocarb in relation to its demyelinating potential was studied in hens by Ives (1965). In this study, hens were fed with diets containing either 0, 200, 400 or 800 ppm (equivalent to approximately 25, 50 and 100 mg/kg bw/day respectively) of methiocarb *ad libitum* daily, for 30 days. All birds survived the experimental period. No treatment-related effects on body weight were noted and nor were any histopathological evidence of myelin degeneration or clinical signs of cholinesterase inhibition noticed in any of the treated birds. No other clinical observations or methodological information were provided. However, when age and source of the study, lack of detailed methodology, and the data limitations are considered, the findings of this study are of limited regulatory value.

Thyssen and Schilde (1978) conducted a neurotoxicity study in hens. Methiocarb was administered twice at 380 mg/kg bw (equivalent to LD_{50}) to hens orally at an interval of 3 weeks. The birds were treated with 50 mg/kg bw of atropine sulphate by i.m. injection prior to each treatment. Following the first treatment with methiocarb, the birds manifested unspecified light behavioural changes of brief duration and lethargy on the first day. Two methiocarb treated hens died after an unspecified period. Similar "symptoms" were noted after the second treatment, following which 2 further mortalities occurred after an unspecified period. No delayed neurotoxic effects of methiocarb were observed in the central or peripheral nervous system. The positive control, tri-ortho-cresyl-phosphate produced the classical signs of delayed polyneuropathy. In histopathology, 9/10 methiocarb treated hens showed occasional very minimum to minimum peri-vascular round cell infiltration in one or several of the nerve tissues examined. Four out of 5 positive controls showed "minimal" degeneration of individual fibres in the sciatic nerve, vacuolar distension of myelin sheaths, Schwann cell proliferation, presence of eosinophilic particles and occasional peri-vascular round cell infiltration. The validity of the findings of the study however, was reduced due to lack of negative control data.

Immunotoxicity

Casale *et al* (1993) performed an *in vitro* study using mouse CTTL2 cells. Plates containing CTLL2 cells in a growth medium supplemented with human recombinant IL2 were incubated in the presence of 100 µL of either 0, 0.5, 5.0 or 50 µM methiocarb in 0.2 M acetone for 16 h. IL2 dependent cell proliferation was evaluated by measuring the [^{3}H]-thymidine uptake. Under the study conditions, *in vitro* T cell proliferation was inhibited by methiocarb at 50 µM by about 80% compared to the untreated cells, in the absence of metabolic activation. No inhibition was noticed at other concentration levels. However, no reference to the cytotoxicity of methiocarb was made. It was stated that the potency to produce acute cholinergic toxicity by the tested chemicals did not predict the potency to inhibit T cell proliferation.

Human Studies

Dermal irritation

In the study of Dubois and Raymund (1961), cotton wool compresses containing an unidentified quantity of methiocarb of unstated purity in dry form, moistened with either an unidentified type of oil or with water were applied to the forearm of 8 persons for 8 and 24 h respectively. In some cases symptoms of irritation were noticeable at the site of application after 8 h. Inflammation and swelling were observed at application sites of all test persons after 24 h. Based on the information provided, methiocarb was an irritant to the human skin. However, it is not possible to comment on the severity of irritation or influence of the vehicle ("oil") on the skin reaction observed, with the limited information provided.

Occupational exposure

A dermatological effect ascribed to methiocarb was reported by Willems *et al* (1997) in a published case study. A 35 year old carnation grower developed acute severe hand eczema, who continued his work in spite of this dermatological condition. Though several topical corticosteroids were used, they did not bring about any therapeutic benefit. A patch test conducted with a methiocarb based product yielded a positive result. However, it is unclear whether the allergic reaction occurred in response to methiocarb or non-active constituents in the formulation.

About 250 employees in two methiocarb manufacturing plants were subjected to yearly medical examinations including assay of whole blood ChE activity for more than 20 years (Faul, 1993). The medical tests also included an examination of the work and health history, measurement of the height and weight, a detailed clinical examination and laboratory tests to determine blood sedimentation rate (BSR), blood count, urinalysis, AST and ALT levels. An X-ray examination of the thoracic organs was conducted at 2-3 year intervals. The study authors stated that, under the conditions prevailed in the plants, no adverse health effects related to methiocarb were noted in any of the employees nor were changes in any of the laboratory parameters observed.

DISCUSSION

Metabolism and Toxicokinetics

According to absorption, metabolism and excretion studies in rats, dogs and cattle, methiocarb is reasonably well absorbed when administered orally. It has been demonstrated that more than 75% of the administered dose was excreted in urine by rats within 48 h, mostly as phenolic derivatives. The findings of the *in vivo* studies suggest that methiocarb is extensively metabolised to form a range of metabolic products such as methiocarb phenol, methiocarb sulfoxide phenol, and methiocarb sulfone phenol. In some studies, the formation of N-hydroxymethyl methiocarb sulfoxide, and trace quantities of methiocarb sulfoxide have also been reported. Both of these products retained the OC(O)NC functional group that is necessary for cholinesterase inhibition. Initial hydroxylation of the ester bond followed by sulfoxidation appeared to be the primary steps involved in the formation of methiocarb sulfoxide phenol. However, in the formation of methiocarb sulfoxide, the metabolite which possessed significant anticholinesterase activity, the sulfoxidation reaction preceded the hydrolysis of the ester bond.

One of the major *in vivo* metabolic pathways for many carbamates is hydrolysis of the ester bond and release of the resultant carbonate in exhaled air as CO_2. *In vivo* studies with carbonyl-^{14}C labelled methiocarb have shown elimination of about 66% of the administered dose in the expired air as CO_2 following metabolism of methiocarb or its metabolites by microsomal enzymes. However, under *in vitro* conditions with hepatocytes, the formation of CO_2 during metabolism is low, generally accounting for about 2% of the administered radioactivity. It is possible that under *in vivo* conditions physiological and biochemical processes other than the hepatic microsomal enzymes are involved in methiocarb metabolism and elimination.

A large proportion of methiocarb metabolites formed under *in vivo* conditions and excreted in urine appeared to be water soluble metabolites, while the faeces contained largely unchanged methiocarb. The major metabolites excreted in urine by rats and cattle following oral administration are methiocarb phenol, methiocarb sulfoxide phenol and methiocarb phenol sulfone, whereas in dogs, the primary urinary metabolites are methiocarb sulfoxide phenol and methiocarb phenol sulfone.

The major metabolic products formed under *in vitro* conditions were methiocarb sulfoxide, methiocarb sulfone and N-hydroxymethyl methiocarb sulfoxide. A recent study conducted using rat liver microsomes, however, revealed only the presence of methiocarb sulfoxide in the incubation mixture with no methiocarb sulfone being detected. In general, sulfoxidation or N-methyl oxidation appeared to be the main routes of methiocarb metabolism under *in vitro* conditions. Methiocarb was oxidised relatively slowly by the dog liver or kidney supernatant fractions compared to the rat liver supernatant.

Studies conducted in pregnant animals show rapid metabolism, placental transfer and excretion of the compound following i.p. administration, without producing any gross teratogenic effects in the foetus. As revealed in elimination and tissue distribution studies, foetal tissues appear to retain elevated levels of methiocarb for longer compared to maternal tissues. Although these levels did not cause maternotoxicity or gross teratogenic lesions in the foetus, there may be potential for alterations in enzyme systems such as ChE. Additionally, foetal uptake and retention may perhaps slow the rate at which the chemical could be metabolised by the maternal tissues.

Acute Toxicity

The acute toxicological profile of methiocarb is characterised by cholinergic effects which are similar to other carbamate and organophosphate ChE-inhibiting pesticides. The clinical signs commonly observed in experimental animals following acute exposure were salivation, lacrimation, vomiting, diarrhoea, muscular tremors, restlessness, convulsions, and paralysis in some animals. The acute oral toxicity of methiocarb is high. It was noted that more than 60% of the acute oral toxicity studies reported LD_{50} values ranging from 9.0 to 50 mg/kg bw, while the remainder presented values in excess of 50 mg/kg bw for rats (range 9-135 mg/kg bw). A credible explanation for the wide variability of the acute oral LD_{50} value in rats could not be found from the data provided for individual studies, although the choice of vehicle appeared to make some difference to the result. Methiocarb is of moderate inhalation toxicity, and the lowest acute inhalation LC_{50} in rats was 433 mg/m^3. Methiocarb acute toxicity was not substantially increased by i.p. administration, for which the rat LD_{50} ranged from 25 to 100 mg/kg bw. The acute dermal toxicity in rats and rabbits is generally low, with LD_{50} values being in excess of 2000 mg/kg bw.

Methiocarb technical was not an eye or skin irritant in rabbits. Similarly, there was no evidence of skin sensitisation in studies conducted using guinea pigs.

Amongst methiocarb metabolites, methiocarb sulfoxide appears to be toxicologically significant with oral LD_{50} in rats ranging from 6.0 to 9.0 mg/kg bw. Clinical signs observed in rats and dogs treated with methiocarb sulfoxide by oral gavage (2.0 and 0.5 mg/kg bw respectively) were similar to those observed in methiocarb acute toxicity studies. The rat oral LD_{50} values of N-hydroxymethyl derivatives of methiocarb, methiocarb sulfone and methiocarb sulfoxide were greater than 112 mg/kg bw whilst those of methiocarb phenol, methiocarb sulfone, methiocarb phenol sulfoxide and methiocarb phenol sulfone were in excess of 1000 mg/kg bw.

The LD_{50} values that have been reported for end-use products containing methiocarb are generally representative of the percentage of active ingredient present in the formulation.

Cholinesterase Inhibition

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

Summary of doses (mg/kg bw/day or mg/m^3) at which no inhibition of ChE activity following methiocarb administration was seen

Species	Duration	Route	Plasma	Erythrocyte	Brain	E:P ratio
Mice	2 years	Oral	14.6	ND	132/173 (M/F)	-
Rat	4 weeks	Oral	3.0	3.0	3.0	1.0
	4 weeks	Oral	<0.5	0.5	ND	-
	24 days	Oral	ND	<3.0	ND	-
	2 months	IP	5.0	ND	5	-
	3 weeks	Inhalation	6.0	>23.0	6.0	3.8
Rat	16 weeks	Oral	1.0	ND	ND	-
	80 weeks	Oral	5.0	>10	>10	>2.0
	2 years	Oral	3.27	3.27	>29/42 (M/F)	1.0
Rabbit	3 weeks	Dermal	60.0	>375.0	>375.0	>6.25
Dog	29 days	Oral	<0.05	<0.05	ND	-
	2 years	Oral	1.25	>6.25	>6.25	>5.0
	2 years	Oral	0.2	>9.6	>9.6	>48

ND = not determined. E:P ratio = erythrocyte:plasma no-effect level ratios

The data presented in the Table above indicate that the inhibition of ChE in plasma occurs at relatively low dose levels, and that, in the rat, the LOELs for plasma and brain cholinesterase inhibition were often similar. Due to wide intra- and inter- species variability observed and significant data limitations, no appropriate short-term repeat dose toxicity study could be selected for establishment of an acute reference dose (ARfD); instead, the developmental toxicity study was used to establish the ARfD (see below). The 2-year toxicity study in dogs conducted by Hoffman and Schilde (1980), which yielded the lowest NOEL of 0.2 mg/kg bw/day based on plasma ChE inhibition and related acute clinical signs, was chosen to establish the current Australian ADI.

Repeat-dose effects

In general the repeat-dose toxicity of methiocarb was characteristic of ChE inhibition. These include clinical signs such as muscular tremors/spasms/weakness, and reduced food consumption which was sometimes associated with decreased body weight. Following chronic administration in rats increases were seen in liver weights and decreases in spleen weight, along with increased ALT and increased reticulocyte and leucocyte counts.

Reproduction and Development

A three-generation reproduction study in rats conducted in 1970 did not reveal any consistent statistically or biologically significant treatment-related effects of methiocarb on fertility, litter size, pup birth weight and survival, or lactation in any generation. Histopathological examination of the pups did not reveal any treatment-related abnormalities. This study, however, failed to provide clear individual animal data on several useful study parameters

such as maternal body weights, post cull survival, pup sex ratio and ChE activity, and therefore falls short of the standard that would be expected in a modern reproduction study.

The teratogenicity of methiocarb has been investigated in one rat study, and two rabbit studies following administration of the chemical to the pregnant animal during the period of organogenesis. In the rat study, based on reduced weight gain of the dams at the highest dose, the NOEL for maternotoxicity was established at 3 mg/kg bw/day, and because there were no effects on foetal survival, development or growth at the highest dose tested, the NOEL for foetotoxicity was 10 mg/kg bw/day.

When methiocarb was administered to pregnant rabbits at doses up to 10 mg/kg bw/day by oral gavage, maternotoxicity characterised by cholinergic signs and weight loss, and embryo/foetotoxicity as manifested by the occurrence of pale areas in the liver were evident at the highest dose. The NOEL for maternotoxicity and embryo/foetotoxicity was set at 3 mg/kg bw/day by the oral route. In the remaining rabbit study, methiocarb was administered at 0, 10, 50 or 250 mg/kg bw/day dermally. Because of reduced food consumption and weight loss seen in does at the highest dose tested in this study, the maternotoxicity NOEL was 50 mg/kg bw/day. In addition, a depression in mean foetal body weights and retarded ossification of hind limb phalanges at 250 mg/kg bw/day was noted. These findings were relied on to establish the foetotoxicity NOEL at 50 mg/kg bw/day. However, no teratogenic effect of methiocarb was observed in any of these studies.

Neurotoxicity studies

The neurotoxic potential of methiocarb has not been studied extensively. Nevertheless, in two early studies conducted in hens, methiocarb did not produce delayed polyneuropathy of the organophosphate type. However, both these studies provided limited methodological information and/or data for independent evaluation, and hence were found to be of limited regulatory value.

The antidotal studies conducted in rats using chemicals such as atropine sulfate, pralidoxime (PAM), obidoxime chloride (BH6) and tetraethylammonium chloride (TEAC) indicate that atropine sulfate alone is more effective as an antidote against methiocarb acute toxicity compared to treatment with PAM, BH6 or TEAC alone. The effect of atropine sulfate was only slightly increased when it was combined with any of these chemicals. Further, the effectiveness of the procedures that have been adopted by veterinarians to treat accidentally poisoned animals are consistent with this finding.

Genotoxicity and Carcinogenicity

Methiocarb has been evaluated for genotoxicity using a battery of tests under both *in vivo* and *in vitro* conditions using various end points such as gene mutation, sister chromatid exchange, unscheduled DNA synthesis, micronucleus formation and dominant lethal assay. The weight of evidence indicates that methiocarb is not mutagenic. In an *in vitro* chromosomal effect assay, methiocarb has been found to cause chromosomal aberrations under both activated and non-activated conditions in the absence of cytotoxicity. However, this study used a higher dose range compared to the doses used in an *in vitro* sister chromatid exchange assay which yielded negative results, and an *in vivo* mouse micronucleus assay was negative. Moreover, there was no evidence of carcinogenicity from long term studies in rats and mice.

Human Studies

Only one human study was found in the methiocarb toxicology database. This study investigated the effects of technical grade methiocarb following application of an unidentified quantity of the chemical to the forearm. Based on the information provided, methiocarb was

classified as an irritant to the human skin, but it is not possible to grade the skin irritation and quantify the irritant potential of the chemical due to lack of experimental details. A single report exists of sensitisation to a methiocarb-based product, but it is unclear whether the allergy was caused by the active constituent or an excipient.

Accidental poisoning in animals

Although there were no reports on human poisoning incidents involving methiocarb in the toxicology database provided, poisoning of domestic cats and dogs following ingestion of snail and slug baits containing 2% methiocarb has been reported in Australia and several other countries. A survey conducted in Australia found that snail and slug bait to be the most common cause of poisoning in dogs and cats, accounting for about 43% of accidental poisoning (Studdert, 1985). The incidence of poisoning and fatality rates were independent of body size of the animals. Similar incidents involving poultry, sheep, cattle and horses have also been reported. However, the snail and slug bait products registered in Australia at present contain a non-active ingredient denatonium benzoate (Bitrex) which is a pet deterrent. Published case reports of methiocarb poisoning in dogs and sheep indicated rapid appearance of typical cholinergic signs and successful treatment with atropine sulfate in cases accurately diagnosed in time.

NOEL considerations

In order to establish the lowest NOEL for methiocarb, a summary of the NOELs determined in those studies deemed appropriate for regulatory purposes, and which are relevant to establishing health values for dietary intake are presented below in the following table.

Study Type	NOEL (mg/kg bw/day)	LOEL and Toxic Effects
Mice 2-year dietary	Not established	14.6 mg/kg bw/day in males and 19.8 mg/kg bw/day in females. Dose related increase in leucocyte counts in females at 24 months and increased ALT in both sexes at 12 months .
Rats 2-year dietary	3.27 for males and 4.98 for females	9.3 mg/kg bw/day in males and 13.9 mg/kg bw/day in females. Transient depression in body weight in males, elevation of reticulocyte counts in females and plasma ChE inhibition in females . This study is of reduced regulatory value.
Beagle dog 2-year dietary	0.2	2.4 mg/kg bw/day in both sexes. Plasma ChE inhibition in both sexes and reduced food consumption in females.
FB strain rats Oral gavage, teratology	3.0 (maternal)	**Maternal**: 10 mg/kg bw/d. **Foetal**: No foetotoxicity observed at highest dose level tested. Reduced maternal weight gain at 10 mg/kg bw/day.
NZW rabbit Oral gavage, teratology	3.0 (maternal and foetal)	Maternal cholinergic signs and weight loss, and pale areas in the foetal liver at 10 mg/kg bw/day.

Determination of Public Health Standards

Acceptable Daily Intake

The ADI for humans is the level of intake (via food or water) of an agricultural or veterinary chemical expressed on a body weight basis that can be ingested daily over an entire lifetime without appreciable risk to health on the basis of all the known facts at the time of evaluation. It is calculated by dividing the overall NOEL for the most sensitive toxicological endpoint from a suitable study (typically an animal study) by an appropriate safety factor.

The current acceptable daily intake (ADI) is 0.002 mg/kg bw/day which was derived by applying a 100-fold safety factor to a NOEL of 0.2 mg/kg bw/day (5 ppm), based on plasma ChE depression and reduced food consumption observed in a 2-year dietary dog study at the next highest dose tested (60 ppm).

In the 1998 JMPR review, this same 2-year dog toxicity study was used to set an ADI of 0.02 mg/kg bw/day. The JMPR ADI is 10-fold higher than the Australian value because the JMPR reviewer established a NOEL at the mid-dose of 1.5 mg/kg bw/d (60 ppm). Although the JMPR reviewer noted that at the mid dose there was reduced feed intake in females (without a significant affect on bodyweight), and decreased plasma cholinesterase inhibition, the JMPR established the NOEL at this dose, based on clinical signs at the high dose. Again, a safety factor of 100 was used.

No change to the current Australian ADI is proposed, as this review has not identified any other study that is more suitable for setting the pivotal NOEL, and it is considered that the decreased plasma cholinesterase and reduced feed intake are appropriate toxicological endpoints on which to base the NOEL. The 100-fold safety factor is considered appropriate and does not require revision.

Acute Reference Dose (ARfD)

The acute reference dose (ARfD) is an estimate of the amount of a chemical in food or water, expressed on a body weight basis, that can be ingested over a short period of time, usually during a meal or in one day, without any appreciable health risk to the consumer on the basis of all the known facts at the time of evaluation. The studies usually considered appropriate to estimate this value are short-term dietary repeat dose studies as acute studies only report a very limited number of end-points. The current ARfD for methiocarb was established in December 2001 by applying a safety factor of 100 to the NOEL of 3.0 mg/kg bw/d for clinical signs in two developmental studies conducted in rats and rabbits, respectively, yielding a value of 0.03 mg/kg bw.

Poisons Scheduling

At present (May 2013), methiocarb is in Schedule 7 of the SUSMP, with cut-off to Schedule 6 (for preparations containing 20 percent or less of methiocarb) and Schedules 5 (for pelleted preparations containing 2 percent or less of methiocarb). The registered 75% wettable powder product and the 50 % suspension concentrate product are covered by the S7 classification, while the two 20 g/kg bait products are covered by the S5 classification.

The current Schedule 7 classification for methiocarb was established on the recommendations of the original version of this report, as the previous S6 classification had been established prior to the submission of numerous acute oral toxicity studies in rats. Over half of these acute oral toxicity studies demonstrated LD_{50} values of 50 mg/kg bw or less (the lowest being 9 mg/kg bw). Examination of the data did not reveal any aspect of the experimental methods used which would account for the wide range (9-135 mg/kg bw) of oral LD_{50} values in rats. Consequently, there was no basis upon which to discount any of these studies from being used for regulatory purposes. Given that the acute oral toxicity profile of methiocarb had been underestimated, and the worst inhalation 4h LC_{50} for methiocarb (433 mg/m^3/4h in female

rats) also lay within the classification criteria for Schedule 7, a recommendation to revise the methiocarb from Schedule 6 to Schedule 7 was put forward for consideration at the 27th meeting of the National Drugs and Poisons Scheduling Committee (NDPSC, 16-18 May 2000). On the basis of this recommendation the Scheduling of methiocarb was amended, and the current scheduling was confirmed at the 52nd meeting of the NDPSC in 19-20 February 2008.

No toxicology studies have been performed with the Australian registered 750 g/kg WP product, but the worst acute rat oral and inhalation LD_{50} and 4h LC_{50} values for 75% methiocarb test formulations were 23 mg/kg bw and 403 mg/m^3, respectively. Therefore the data would not justify a cut-off to Schedule 6 for the 750 mg/kg WP product.

Similarly, the only experimental acute toxicity data available for 20 g/kg pellets is derived from a test formulation that differs from the Australian registered product. However, the toxicology profile of this methiocarb test formulation at 20 g/kg is consistent with the existing Schedule 5 listing for methiocarb.

Public exposure— Domestic Use

Australian-registered domestic use products are intended for control of snails, slugs, slaters and millipedes. When applying the product in the domestic setting, the user may be exposed to methiocarb dermally when handling the pellets and also through the inhalation of product dust. The OCS notes that product labels indicate the bait should be sprinkled directly from the packet onto the ground, however, it is possible that some manual handling of the bait takes place. In order to address the acute risks arising from this exposure, safety directions will be established for the home garden products containing methiocarb (see below).

Potential for Accidental Poisoning

The Ag MORAG Guidelines state that, "household, home garden and domestic animal pesticide products must be relatively harmless or capable of causing only mild illness if accidental poisoning occurs" (Section 3.6).

The OCS (or its predecessors) have not evaluated toxicity testing of the products currently available for home garden use. However, a related product was evaluated in the first version of this report. This evaluation indicated that the acute toxicity of the product is low via both the oral and dermal routes (i.e. >1500 mg/kg bw). The OCS notes that the home garden product also contains an embittering agent (Bitrex) to discourage children and pets from consuming pellets. The product is considered appropriate for home garden use.

Post-application dermal exposure

Post-application dermal exposure to methiocarb is possible when re-entering the garden and working/playing in areas where baits have not dissipated and/or by coming into contact with soil on which bait was previously applied.

A re-entry interval has been recommended by the OCS for commercial packs (> 1 kg) of methiocarb bait products in the 2007 revision of the OHS assessment. This re-entry interval was established to address the risks to nursery workers during re-potting activities. No re-entry interval is considered necessary for home garden products due to the differences in application rate and frequency of use.

Safety Directions

Currently, there are four methiocarb-containing products registered in Australia.

Mesurol 750 Bird Repellent and Snail and Slug Spray

Mesurol 750 Bird Repellent and Snail and Slug Spray is a 750 g/kg wettable powder formulation sold as a commercial 400 g pack. It is used for the control of snails, slugs, hibiscus flower beetle, garden weevil and glasshouse sciarids (fungus gnats) and for repelling birds on ornamental plants. The product is applied by spray to grapevines, oranges, ornamentals and hibiscus at a dilution from 100 to 200 g/100 L, or to poppies at 5.5 kg/ha, and to ornamentals as a soil drench at 300 g/100 L. The spraymix is prepared by mixing the product with water in a bucket before addition to the partly filled spray tank under agitation. Label directions recommend use of boom spray or air mist equipment for application. The most probable route of exposure to mixer/loaders would be dermal contact with the powder or concentrated premix, whereas spray operators would be exposed to the dilute spray mix by inhalation and dermal contact.

No toxicology studies have been performed with the Australian registered product Mesurol 750 Bird Repellent and Snail and Slug Spray (Mesurol 750).

Two 750 g/kg methiocarb powder formulations have been subjected to acute toxicity studies. Both of these formulations contain non-active constituents that differ from those present in Mesurol 750. Methiocarb 75% Concentrate yielded an oral LD_{50} of 82 mg/kg bw in male rats and 23 mg/kg in females, while Methiocarb 75% WP/Seed Treatment demonstrated values of 100-130 and 60-140 mg/kg bw in male and female rats, respectively. Both formulations caused slight eye irritation in rabbits. Methiocarb 75% concentrate was not a skin sensitiser in guinea pigs. In these respects, the toxicological characteristics of the two test formulations are consistent with those extrapolated for Mesurol 750. Both test formulations were non-irritating to the rabbit skin.

Although Methiocarb 75% Concentrate had a worst acute dermal LD_{50} of 704 mg/kg bw in female rabbits and an inhalation LC_{50} of 403 mg/m^3 (4 h) in female rats (consistent with those estimated for Mesurol 750), markedly lower dermal and inhalation toxicity was observed with Methiocarb 75% WP/Seed Treatment. These were, respectively, >5000 mg/kg bw in rabbits and >20000 mg/m^3 (1 h) in rats. Differences between the non-active constituents do not explain these discrepancies between the two test formulations. Droplet/particle sizes were not measured in the inhalation toxicity study with Methiocarb 75% WP/Seed Treatment, and so methodological deficiencies may account for the apparently high LC_{50} value obtained. In contrast, droplet sizes were measured in the corresponding study with Methiocarb 75% Concentrate, and so its results are considered as being more reliable. There is no explanation as to why Methiocarb 75% Concentrate had markedly greater dermal toxicity than Methiocarb 75% WP/Seed Treatment. Given that the dermal toxicity study on Methiocarb 75%

Concentrate was GLP-compliant and much more recent than those performed with Methiocarb 75% WP/Seed Treatment (1988 vs 1972-79), its results are preferred.

Therefore, taking into account both the studies conducted on similar products, together with extrapolation from the individual acute toxicity profiles of methiocarb and the non-active constituents present in the product, it is anticipated that this product would have high to moderate acute oral toxicity (based on a 75% methiocarb content and taking into account the full range of its rat LD_{50} values), moderate acute dermal toxicity, and moderate acute inhalation toxicity. The product would also be expected to cause slight eye and skin irritation and it is unlikely to cause dermal sensitisation, though relevant data on some of the non-active constituents are lacking. Safety directions have been established assuming this acute toxicological profile.

The principal hazards of Mesurol 750 to the operator are expected to be dermal and inhalation toxicity when opening the container and preparing the spraymix. Given that dilution rates of 333-fold or greater are used, the toxicological hazard posed by the spray mixture is expected to be low.

The current safety directions for Mesurol 750 are shown in the Table below:

WP 750 g/kg or less	
Product is poisonous if absorbed by skin contact or swallowed	120 130 131 133
Avoid contact with eyes and skin	210 211
Do not inhale dust or spray mist	220 221 223
When preparing wear elbow-length PVC gloves face shield	279 281 290 294 296
If product on skin, immediately wash area with soap and water	340 342
After use and before eating, drinking or smoking wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and face shield	360 361 362

Given that the product is expected to be of high to moderate acute oral toxicity, addition of the 100 ("Very dangerous") statement is warranted. The addition of statement 132 ("inhaled") as part of the hazard statement regarding the poisonous nature of the product ("Poisonous if absorbed by…") is also considered appropriate due to expected moderate acute inhalational toxicity. A 160 162 164 statement ("May irritate the eyes and skin") should be added in view of the anticipated slight dermal and ocular irritancy of the product.

The revised safety directions for Mesurol 750 Bird Repellent and Snail and Slug Spray, based on toxicological hazard alone, are as follows.

Acute Hazard	Safety Direction	Code
	HAZARDS	
High oral toxicity Moderate dermal toxicity Moderate inhalational toxicity	Very dangerous. Poisonous if absorbed by skin contact, inhaled or swallowed	100 130 131 132 133
Slight skin irritant Slight eye irritant	May irritate the eyes and skin	160 162 164
	PRECAUTIONS	
Slight skin irritant Slight eye irritant	Avoid contact with eyes and skin	210 211
Moderate dermal toxicity	If product on skin, immediately wash area with soap and water	340 342
Moderate inhalational toxicity	Do not inhale dust or spray mist	220 221 223
	MIXING OR USING	
Moderate dermal toxicity Moderate inhalational toxicity	When opening the container and preparing the spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow length chemical resistant gloves and disposable face mask covering mouth and nose.	279 280 281 290 292b 294c 306
	AFTER USE	
Anticholinesterase compound	After use, and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350

Combined with the OHS assessment from 2007 the amended entry for methiocarb WP 750 g/kg or less in the FAISD Handbook will read as follows:

		Statement Codes
Methiocarb	WP 750g/kg or less	100 130 131 132 133 160 162 164 210 211 340 342 220 221 223 279 280 281 290 292b 294c 306 279 282 290 292b 294c 296 289 290 291b 294c 296 350 360 361 362 366

The statement codes translate into the following safety directions:

Very dangerous. Poisonous if absorbed by skin contact, inhaled or swallowed. May irritate the eyes and skin. Avoid contact with eyes and skin. If product on skin, immediately wash area with soap and water. Do not inhale dust or spray mist. When opening the container and preparing the spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow length chemical resistant gloves and disposable face mask covering mouth and nose. When using the prepared spray wear cotton overalls buttoned to the neck and wrist, elbow length chemical resistant gloves and face shield. If applying by hand wear chemical resistant clothing buttoned to the neck and wrist and a washable hat and elbow length chemical resistant gloves and face shield. After use, and before eating, drinking or

smoking, wash hands, arms and face thoroughly with soap and water. After each day's use wash gloves, contaminated clothing and face shield.

Mesurol Snail and Slug Bait

Mesurol Snail and Slug Bait is a 20 g/kg pellet formulation available in commercial packs of 5 to 150 kg. It is used for the control of snails and slugs in berry crops, cereals, gardens, nurseries, oilseed crops, orchards, pastures and vegetable crops, and for false wireworm beetle on sunflowers. The bait is to be scattered evenly on the ground at 5.5 or 11-22 kg/ha except on sunflowers, which require a 2.5 kg/ha application rate. The product may be applied by hand or using equipment such as fertiliser spinners, combines or sod seeders. No preparation is required. The most probable route of exposure is by dermal contact, although if dusts are formed, there may also be some potential for inhalation exposure and ocular contact.

No toxicology studies have been performed with Mesurol Snail and Slug Bait. A 2% methiocarb slug and snail pellet test formulation was of low oral toxicity in rats, low dermal toxicity in rabbits, did not cause any deaths in rats when inhaled at 835 mg/m^3, was a slight eye irritant in rabbits but did not irritate rabbit skin. A dermal sensitisation study was not performed. These results are consistent with the anticipated toxicological characteristics of Mesurol Slug and Snail Bait.

By extrapolation from the toxicity of methiocarb in the product, it may be of moderate to low acute oral toxicity (taking into account the full range of LD_{50} values for methiocarb). The other acute hazards arising from the product, obtained by extrapolation, closely match the toxicity studies on the similar product described above. Dermal sensitisation data are lacking on most of the non-active constituents, except for Bitrex (denatonium benzoate), which is a sensitiser at 10% w/v in guinea pigs. However this agent is present in the product at a very low concentration, and is therefore unlikely to pose a significant sensitisation hazard.

The principal hazards to the user from the product would therefore be expected to arise from skin or eye contact with any dusts that may be generated during loading and application. Eye irritation is considered to be the most probable hazard.

The current safety directions for Mesurol Snail and Slug Bait (20g/kg or less, 5-150 kg packs) are presented in the following Table.

BA 20 g/kg or less	
Poisonous if swallowed	130 133
Avoid contact with eyes and skin	210 211
If product on skin, immediately wash area with soap and water	340 342
After use and before eating, drinking or smoking wash hands, arms and face thoroughly with soap and water	350
Obtain an emergency supply of atropine tablets 0.6 mg	373

Given that Mesurol Snail and Slug Bait is expected to be a slight eye irritant, a 160 162 ("May irritate the eyes") statement should be added. Otherwise, the existing safety directions for the product are considered appropriate, from a toxicological viewpoint. The safety direction 373 is no longer used, and the use of antidotal atropine is covered by the first aid instructions.

The revised safety directions for Mesurol Snail and Slug Bait, based on toxicological hazards alone, are as follows.

Acute Hazard	Safety Direction	Code
	HAZARDS	
Low oral toxicity (>500 but <2000 mg/kg bw)	Harmful if swallowed.	129 133
Slight eye irritant	May irritate the eyes.	160 162
	PRECAUTIONS	
Low dermal toxicity Slight eye irritant	Avoid contact with eyes and skin.	210 211
Bait presentation	Do not touch bait. If on skin and after each baiting, wash thoroughly with soap and water	250 252
	MIXING OR USING	
Low inhalational toxicity	If dust is present wear a disposable face mask covering mouth and nose	310 290 306
	AFTER USE	
Anticholinesterase compound	After use, and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350

Combined with the OHS assessment from 2007 the amended entry for methiocarb in the FAISD Handbook will read as follows:

		Statement Codes
Methiocarb	BA 20g/kg or less	129 133 160 162 210 211 250 252 310 290 306 289 290 294c 350 360 361

The statement codes translate into the following safety directions:

Harmful if swallowed. May irritate the eyes. Avoid contact with eyes and skin. Do not touch bait. If on skin and after each baiting, wash thoroughly with soap and water. If dust is present wear a disposable face mask covering mouth and nose. If applying by hand wear chemical resistant gloves. After use, and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use wash gloves.

Baysol Snail and Slug Bait

Baysol Snail and Slug Bait is a 20 g/kg pellet home garden product sold in pack sizes of 250 g to 1 kg, intended for control of snails, slugs, slaters and millipedes. The pellets are to be sprinkled onto the ground at the rate of 100 pellets/m^2. Label directions indicate the pellets are to be applied direct from the container without manual contact or preparation. It is nevertheless possible that some users may experience limited exposure via the dermal route.

Safety directions are required for these home garden products to address the risks following human exposure. Although these home garden products are the same as Mesurol Snail and Slug Bait, no safety directions are currently specified for Baysol Snail and Slug Bait, the safety directions will differ slightly, consistent with the hazards associated with smaller pack sizes (≤1 kg) and taking into consideration that domestic users will be treating smaller areas for shorter durations than agricultural users (hence the potential for exposure will be lower).

The safety directions recommended in the present review for Baysol Snail and Slug Bait for domestic use are as follows.

Acute Hazard	Safety Direction	Code
	HAZARDS	
Low oral toxicity (>500 but <2000 mg/kg bw)	Harmful if swallowed.	129 133
Slight eye irritant	May irritate the eyes.	160 162
	PRECAUTIONS	
Low dermal toxicity Slight eye irritant	Avoid contact with eyes and skin.	210 211
Bait presentation	Do not touch bait. If on skin and after each baiting, wash thoroughly with soap and water	250 252
	MIXING OR USING	
Low inhalational toxicity	If dust is present wear a disposable face mask covering mouth and nose	310 290 306
	AFTER USE	
Anticholinesterase compound	Wash hands after use	351

The amended entry in the FAISD Handbook will read:

		Statement Codes
Methiocarb	BA HG 20g/kg 1kg pack or less	129 133 160 162 210 211 250 252 310 290 306 351

The statement codes translate into the following safety directions:

Harmful if swallowed. May irritate the eyes. Avoid contact with eyes and skin. Do not touch bait. If on skin and after each baiting, wash thoroughly with soap and water. If dust is present wear a disposable face mask covering mouth and nose. Wash hands after use.

First Aid Instructions

In the edition current to March 2011, the following standard statements for methiocarb are specified in the FAISD Handbook – *Handbook of First Aid Instructions, Safety Directions, Warning Statements and General Safety Precautions for Agricultural and Veterinary Chemicals*.

Methiocarb m

m If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.

The APVMA Agricultural Manual of Requirements and Guidelines (MORAG) states that "There should be appropriate directions for first aid measures to be taken, should poisoning occur in the household. Household, home garden and domestic animal products should not require specific antidotes or aggressive first aid measures". Based on this MORAG requirement, a product with the First Aid Instruction "m" would not be appropriate for home garden use, as a requirement for a specific antidote is included on the label.

On the basis of the estimated acute toxicity, the First Aid Instruction "m" may not be necessary for the home garden baits products. These pelleted preparations containing 2% or less of methiocarb are in Schedule 5 of the SUSMP on the basis of their low acute toxicity profile.

Therefore, it is recommended that a new First Aid Instruction be specified for pelleted preparations containing 2% or less of methiocarb.

Methiocarb · in pelleted preparations containing 2% or less	a
Methiocarb · in other preparations	m

a If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126, New Zealand 0800 764 766.

m If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.

First Aid Instructions should appear on product labels when the substance is present in concentrations at which they would be scheduled as poisons in the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP).

9

RECOMMENDATIONS FOR PUBLIC HEALTH STANDARDS

1. Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.002 mg/kg bw/day. This ADI was derived by applying a 100-fold safety factor to a NOEL of 0.2 mg/kg bw/day, based on plasma ChE depression and reduced food consumption observed at the next highest dose in a 2-year dog study. This review does not recommend any amendments to the current ADI.

2. Acute Reference Dose (ARfD)

The ARfD was estimated by applying a safety factor of 100 to the NOEL of 3.0 mg/kg bw/d for clinical signs in 2 developmental studies conducted in rats and rabbits, respectively, yielding a value of 0.03 mg/kg bw/d.

3. Poisons Scheduling

No further changes to the scheduling of methiocarb are recommended at this time.

4. Safety Directions

The following amended safety directions are recommended:

For methiocarb formulated products containing WP 750 g/kg or less:

		Statement Codes
Methiocarb	WP 750g/kg or less	100 130 131 132 133 160 162 164 210 211 340 342 220 221 223 279 280 281 290 292b 294c 306 279 282 290 292b 294c 296 289 290 291b 294c 296 350 360 361 362 366

The statement codes translate into the following safety directions:

Very dangerous. Poisonous if absorbed by skin contact, inhaled or swallowed. May irritate the eyes and skin. Avoid contact with eyes and skin. If product on skin, immediately wash area with soap and water. Do not inhale dust or spray mist. When opening the container and preparing the spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow length chemical resistant gloves and disposable face mask covering mouth and nose. When using the prepared spray wear cotton overalls buttoned to the neck and wrist, elbow length chemical resistant gloves and face shield. If applying by hand wear chemical resistant clothing buttoned to the neck and wrist and a washable hat and elbow length chemical resistant gloves and face shield. After use, and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use wash gloves, contaminated clothing and face shield.

For methiocarb formulated products containing BA 20 g/kg or less (NOT for domestic use):

		Statement Codes
Methiocarb	BA 20g/kg or less	129 133 160 162 210 211 250 252 310 290 306 289 290 294c 350 360 361

The statement codes translate into the following safety directions:

Harmful if swallowed. May irritate the eyes. Avoid contact with eyes and skin. Do not touch bait. If on skin and after each baiting, wash thoroughly with soap and water. If dust is present

wear a disposable face mask covering mouth and nose. If applying by hand wear chemical resistant gloves. After use, and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use wash gloves.

For methiocarb formulated products containing BA 20 g/kg or less for domestic use:

		Statement Codes
Methiocarb	BA HG 20g/kg 1kg pack or less	129 133 160 162 210 211 250 252 310 290 306 351

The statement codes translate into the following safety directions:

Poisonous if swallowed. May irritate the eyes. Avoid contact with eyes and skin. Do not touch bait. If on skin and after each baiting, wash thoroughly with soap and water. When opening the container and using the product wear a disposable face mask covering mouth and nose. If applying by hand wear disposable gloves. Wash hands after use.

5. First Aid Instructions

The following amended First Aid Instruction are recommended:

Methiocarb · in pelleted preparations containing 2% or less	a
Methiocarb · in other preparations	m

a If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126, New Zealand 0800 764 766.

m If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.

6. Approval Status

No change is recommended to the approval status of methiocarb TGAC.

7. Additional Data

The principal limitations to the toxicology database for methiocarb are the age of the existing multi-generation reproduction studies in rats, and the absence of studies on developmental neurotoxicity and dermal absorption. It would be desirable for the sponsor to submit modern multi-generation reproduction and developmental studies in rats and percutaneous absorption studies, performed in accordance with GLP and the relevant OECD Guidelines.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

1.1 Regulatory History of Health Considerations in Australia

Methiocarb [3,5-dimethyl-4-(methyl thio)phenyl methyl carbamate] is an insecticidal carbamate of the thio sub class. Its insecticidal properties were first reported by G. Unterstenhöfer in 1962, and it was introduced by Bayer AG under the code numbers of 'Bayer 37344' and 'H 321'. It was first introduced to Australia in 1971, as pelleted preparations containing 2% or less of the active ingredient for the control of snails and slugs. It is a non-systemic acaricide with contact and stomach action, a molluscicide with neurotoxic action, and a bird repellent when used as a seed treatment. At present, products containing methiocarb are registered for the control of snails and slugs in the home garden, nurseries, green houses and on pasture lands as well as an insecticide and a bird repellent in a wide range of agricultural situations. As of May 2013 there are four methiocarb containing products with a range of approved uses in Australia.

In Australia, public health standards for agricultural and veterinary chemicals including the poisons schedule, first aid and safety directions, and acceptable daily intake (ADI) are recommended by staff within the Department of Health and Ageing. Poisons schedules for chemicals are determined by a delegate of the Department of Health and Ageing in conjunction with advice from the Department and the Advisory Committee on Chemicals Scheduling (ACCS). Previously this was the responsibility of the National Drugs and Poisons Schedule Committee (NDPSC).

MRLs were formerly established by the Pesticide and Agricultural Chemicals Committee (PACC) of the NHMRC, with the Department of Health subsequently becoming directly responsible for them in 1992. However, this function was subsequently transferred to the National Registration Authority for Agricultural and Veterinary Chemicals (NRA), now known as the Australian Pesticides and Veterinary Medicines Authority (APVMA) in June 1994. Maximum Residue Limits for methiocarb were first established in 1981.

The ADI value in Australia was initially set at 0.05 mg/kg bw/day based on a NOEL of 6.25 mg/kg bw/day from a chronic dog study, consistent with the 1981 JMPR evaluation. In 1983, the Australian ADI was subsequently amended to 0.001 mg/kg bw/day in 1983, on the basis of the NOEL of 0.125 mg/kg bw/day for plasma ChE inhibition in a subsequent chronic dog study; this value remained unchanged until 1986. This ADI was then increased to 0.002 mg/kg bw/day in 1986, employing a revised NOEL of 0.2 mg/kg bw/day, calculated on the actual (as opposed to estimated) food consumption in the chronic dog study. A safety factor of 100 was used to encompass intra- and inter species variability.

The current ARfD value of 0.03 mg/kg bw was established in the original version of this report and is based on a NOEL of 3 mg/kg bw/d from developmental studies conducted on both rats and rabbits.

The regulatory history of public health considerations of methiocarb by Australian regulatory committees is summarised below.

History of Public Health Consideration of Methiocarb in Australia

Date	Decision
November 1971	NDPSC: New Schedule 5 entry: METHIOCARB in pelleted preparations containing 2% or less of methiocarb when labelled and packed for the control of snails and slugs.
December 1971	PACC: Denied company request to set tolerances for garden products as they are only set for commercial products. Use is not within Committee's terms of reference.
August 1974	PACC: Further data on use, residues and toxicity required.
August 1977	NDPSC: Amend Schedule 5 entry to read: Methiocarb in pelleted preparations containing 2% or less of methiocarb.
February 1981	PACC: Provisional MRLs recommended for berry fruit, pending JMPR evaluation.
May 1981	PACC: MRLs recommended for stone fruit and vegetables
February 1982	PACC: Council to adopt the following: Delete: 15 mg/kg entry for cherries
November 1983	PACC: Amended NEL to 0.125 mg/kg and the ADI to 0.001 mg/kg/day.
December 1985	PACC: MRL recommended for stone fruit, berry fruit and grapes. Provisional to May 1986 pending residue data and outstanding toxicology data. Concern at potential misuse resulting in ADI being exceeded.
May 1986	PACC: Current MRL not suitable for table grapes; methiocarb should not be used on table grapes.
November 1986	PACC: MRL recommended for wine. Foreshadow deletion of entry for grapes pending comment on action.
February 1987	PACC: Use as a bird repellent on grapes should be withdrawn. Data for use as a snail and slug spray required. Full details of the residues in cherries and data for residues in apricots to be requested.
June 1987	PACC: MRL recommended for oranges, berry fruits, grapes, stone fruits and fruit. Provisional to Aug 88 pending residue data from new use patterns to meet the MRL.
August 1988	PACC: MRL recommended for grapes, citrus fruits, and fruit except grapes and citrus fruit. Extended prov. MRL to Aug 1989.
August 1991	PACC: Agreement was given to the clearance for the technical grade active constituent (TGAC).
November 1995	NDPSC: A summary was considered of the background to scheduling in Australia, toxicological data, and a comparison of the scheduling between New Zealand and Australia for methiocarb. The Committee agreed that a proposal should be developed – the toxicological values indicated that some harmonisation would be achievable.
May 2000	NDPSC: The draft review of methiocarb (the original version of this document) was considered and the committee made the decision that methiocarb met the criteria for Schedule 7. Cut-offs to Schedules 5 and 6 were established based on the available toxicity information.

ACPH – Advisory Committee on Pesticides and Health; PACC - Pesticide and Agricultural Chemicals Committee; DPSC - Drugs and Poisons Scheduling Committee; SCOT - Standing Committee on Toxicity; NDPSC - National Drugs and Poisons Scheduling Committee.

Health Standards

NOEL/ADI/ARfD

The current acceptable daily intake (ADI) is 0.002 mg/kg bw/day. This ADI was derived from a NOEL of 0.2 mg/kg bw/day, based on plasma ChE inhibition observed in a 2-year dog study.

The current acute reference dose (ARfD) is 0.03 mg/kg bw. This ARfD was derived from a NOEL of 3 mg/kg bw/day, based on clinical signs observed in developmental toxicity studies in rats and rabbits.

Poisons Scheduling

At present, methiocarb is listed in Schedule 7 of the SUSMP, with a cut-off to Schedule 6 for preparations containing 20 percent or less and an additional cut-off to Schedule 5 for pelleted preparations containing 2% or less of the active ingredient.

Existing Chemicals Review Program (ECRP)

Methiocarb is one of 80 agricultural and veterinary chemicals identified as candidates for priority review under the ECRP. The review of methiocarb was initiated in 1995. Following data call-in processes, two additional submissions on toxicology were received from the methiocarb producer. These data, together with all previously submitted data and reports found in the published literature, have been evaluated and are detailed in this report.

1.2 International Toxicology Assessments

Joint FAO/WHO Meeting of Pesticides and Residues (JMPR)

Methiocarb has been reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1981, 1983, 1984, 1985, 1987 and 1998. In its 1981 evaluation, the JMPR established an ADI of 0.001 mg/kg bw/day for methiocarb on the basis of the NOEL of 0.125 mg/kg bw/day from a 2-year dog study and a safety factor of 100. This ADI remained unchanged up until 1998. In its most recent review in 1998, the JMPR amended the ADI to 0.02 mg/kg bw/day, by applying a safety factor of 100 to a NOEL of 1.5 mg/kg bw/day based on the clinical signs (mild weakness of the hind limbs, trembling, reduced alertness and some vomiting) observed at the next highest dose (6.0 mg/kg bw/day) in the same 2-year dog study. The reduction in food consumption in bitches, and biologically significant plasma ChE inhibition in both sexes at 1.5 mg/kg bw/day were not considered as toxicologically relevant end points by the JMPR in establishing this ADI. The JMPR also used this same NOEL to establish the ARfD at 0.02 mg/kg bw. In addition, the following NOELs have been established by the JMPR.

Mouse: No NOEL. LOEL: 15 mg/kg bw/day (2-year study; Haematological changes at all dose levels)

Rat: NOEL of 3.3 mg/kg bw/day (2-year study; Haematological changes at the next highest dose)

Rabbit: NOEL of 3.0 mg/kg bw/day (Maternal toxicity characterised by weight loss at the highest dose in a developmental toxicity study).

Dog: NOEL of 1.5 mg/kg bw/day (2-year study; Clinical signs of mild weakness of the hind limbs, trembling, reduced alertness and some vomiting observed at the highest dose).

International Program on Chemical Safety (IPCS)

The International Program on Chemical Safety (IPCS) conducted a general review of all available information on carbamate group of pesticides in 1986, using well known carbamates such as carbaryl and benomyl. Although a detailed evaluation of methiocarb has not been undertaken, physico-chemical properties and the acute oral and dermal toxicity data of methiocarb were compared with a range of other carbamate pesticides.

United States Environmental Protection Agency (US EPA)

Methiocarb was first registered as a pesticide in the USA in 1972.

A Reregistration Eligibility Decision (RED) document issued in 1994 indicates that the producers of methiocarb have deleted all the food uses from their product labels between 1989-1992. According to this document, the USEPA has found that the uses of methiocarb on residential and commercial ornamentals (except large pack size products for use by home owners on ornamentals), by home owners around building foundations, in green houses, on commercially grown turfgrass and on ginseng are eligible for reregistration. Further, these products will be reregistered once the required confirmatory generic and product specific data, and revised labelling are provided and accepted by the USEPA.

However, the USEPA did not make a reregistration eligibility decision regarding large pack size methiocarb products and turf use of methiocarb until appropriate post-application re-entry exposure, ecological effects and environmental fate data were submitted and evaluated. Similarly, the agency did not make a reregistration eligibility decision regarding large pack size methiocarb products marketed in about 9-11 kg bags for use by home owners on ornamentals, until soil dissipation and dermal exposure data were received and evaluated.

Parts of the US EPA RED that are considered relevant to the present review include:

- The US EPA has classified methiocarb as Group D: Not Classifiable as to Human Carcinogenicity (due to a lack of information)
- Methiocarb was classified as a developmental toxicant based on a dermal exposure study. The NOEL was 50 mg/kg bw/d
- Coveralls and a dust mask must be worn during mixing/loading of wettable powder formulations. A respirator is also required during "ventilation activities".
- Methiocarb is classified as a restricted use pesticide for all outdoor uses (except home garden use)
- Home owner use did not require PPE.

Methiocarb is currently undergoing a review of its registration status in the USA (as at April 2011).

International Agency for Research on Cancer (IARC)

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

European Food Safety Authority (EFSA)

Methiocarb is authorised for use in the EU under Directive 91/414/EEC. Methiocarb was added to Annex I of the directive (for approved substances) in September 2006 following a review by the rapporteur Member State the United Kingdom.

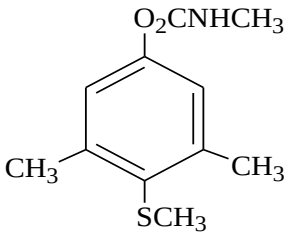
The scientific report (Conclusion on the peer review of methiocarb) contains the following information which is relevant to this assessment:

- The recommended classification of methiocarb was T+ Very toxic, and the risk phrases R28 Very toxic if swallowed and R23 Toxic on inhalation were assigned.

- The ADI, ARfD and Acceptable Operator Exposure Level (AOEL) of 0.013 mg/kg bw/d were proposed
- Under aerobic conditions at 20°C methiocarb is degraded in soil in less than 2 days
- A 90 day study conducted in dogs and dermal absorption studies were submitted by the registrant, which were not available to OCS for evaluation.
- A default 100% dermal absorption factor was applied to the bait formulation – this meant occupational exposure exceeded the AOEL

1.3 Identification

Common name:	Methiocarb
Chemical name:	3,5-dimethyl-4-(methylthio)phenyl methylcarbamate (CAS) 4-methylthio-3,5-xylyl methylcarbamate (IUPAC)
CAS Registry Number:	2032-65-7
Empirical formula:	$C_{11}H_{15}NO_2S$
Molecular Weight:	225.3
Chemical Structure:	



Chemical and physical properties (TGAC)

Colour:	Colourless
Odour:	Phenol-like
Physical state:	Crystals
Melting point:	119^0C
Density (20^0C):	1.236
n-Octanol/water partition coefficient: (log Kow)	3.34
Vapour pressure:	0.015 mPa (20^0C); 0.036 mPa (25^0C)
Solubility: in water:	27 mg/L (20^0C)
in organic solvents:	In dichloromethane >200, isopropanol 50-100, toluene 20-50, hexane 1-2 (all in g/L, 20^0C).
Stability:	Unstable in highly alkaline media. Hydrolysis DT_{50} (22^0C) >1 y (pH 4), ≤35 d (pH 7), 6 h (pH 9). Photodegradation contributes to the overall elimination of methiocarb from the environment; DT_{50} 6-16 d.

1.4 End Use Products

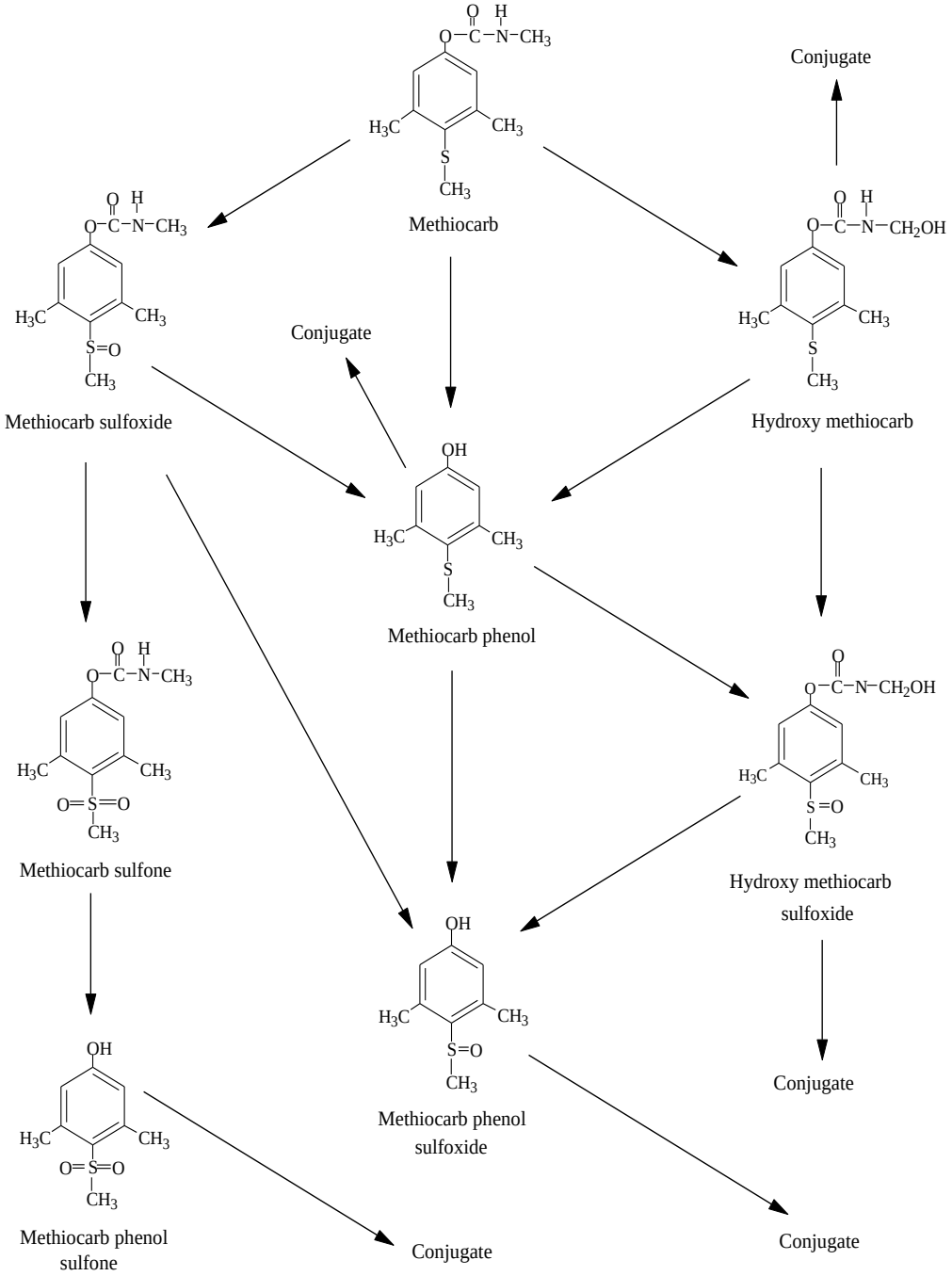
At the initiation of this review, there were four methiocarb containing products registered in Australia, of which three were pelleted snail and slug bait products, and a bird repellent and snail and slug spray which was marketed as a wettable powder.

2. METABOLISM AND TOXICOKINETICS

Studies on biotransformation, excretion and tissue distribution of methiocarb have been conducted using both in vivo and in vitro systems. A generalised metabolic pathway for methiocarb in mammals, developed using the experimental data described in the following evaluated studies is shown in Figure 1.

Figure 1.

METABOLISM



2.1 Absorption, distribution, metabolism and excretion

In vivo studies

Intraperitoneal administration

Krishna JG & Casida JE (1966) Fate in rats of the radiocarbon from ten variously labelled methyl- and dimethyl-carbamate-^{14}C insecticide chemicals and their hydrolysis products. Division of Entomology and Acarology, University of California, Berkeley, CA, USA. J Agr Food Chem 14: (2) 98-105.

Study & Observations: This study was performed to determine and compare the detoxification and elimination mechanisms as well as the metabolic fate of the radiolabel from ten differentially labelled methyl and dimethyl insecticides (including methiocarb). [^{14}C]-carbonyl-labelled methiocarb (specific activity: 1.0 µCi/mmol, radiochemical purity>99%) in 2-methoxyethanol was administered by i.p. injection to male SD rats (1 rat/study; Berkeley Pacific Laboratories, Berkeley, CA, USA, 160-170 g bw) at 7.5 µmoles/kg bw (0.25 mL/rat) under ether anaesthesia. Two replicate studies were performed with duplicate analysis on each sample. Following injections, the rats were housed individually in metabolic cages under standard laboratory conditions for 48 h and provided with food and water *ad libitum*. During this time the radioactive content of the expired CO_2 (0-5 h or 5-9 h) urine (24 h and 24-48 h) and faeces (48 h) were determined. The rats were sacrificed at 48 h post treatment (method unspecified) and the radioactive content of blood cells and plasma (heart blood), bone, brain, fat, heart, kidney, liver, lungs, muscle, spleen and testicular tissues was determined.

Findings: The mean total recovery of administered radiolabel was 99.8%, 66.1% of which was present in the expired air as $^{14}CO_2$. About 20% and 2.2% of ^{14}C were recovered in 0–24 h and 24–48 h post treatment urine samples respectively, with 2.5% recovered in faeces (48 h sample). The radioactivity remaining in the body at 48 h post treatment accounted for approximately 8.9% of the administered dose. At 48 h post treatment 2.75 and 0.65 µmoles of radiolabel/kg tissue were recovered in the blood cells and plasma respectively. The levels of administered ^{14}C-radiolabel found in the tissues were 1.68 (spleen), 1.32 (liver), 1.19 (heart), 1.0 (kidney), 0.95 (lungs), 0.58 (brain), 0.54 (muscle), 0.49 (fat), 0.47 (bone) and 0.34 (testes) µmoles/kg of fresh tissue. The study authors hypothesised that the expired radioactivity originated from hydrolysis of the administered compound or its metabolites to yield carbonate which was subsequently expired as $^{14}CO_2$. Further, the hydrolytic cleavage of the ester group of methiocarb by rat liver microsomes was identified as the probable rate limiting step in the production of $^{14}CO_2$. Besides $^{14}CO_2$, no other metabolites were characterised and hence only partial interpretation of the nature of the metabolites is possible.

Conclusions: Hydrolysis of carbonyl-^{14}C labelled methiocarb or its metabolites by microsomal enzymes yielded $^{14}CO_2$ in the expired air accounting for about 66.1% of the administered dose. At 48 h post treatment, the tissues that appear to retain higher levels of methiocarb or its metabolites were the spleen, liver, heart, kidney and lungs.

Wheeler L & Strother A (1974a) Placental transfer, excretion and disposition of [^{14}C]Zectran and [^{14}C]Mesurol in maternal and foetal rat tissue. Toxicol Appl Pharmacol 30: 163-174.

Study & Observations:

Tissue distribution: The study examined the quantitative excretion, maternal tissue distribution, placental transfer, and foetal disposition of two ^{14}C labelled carbamate pesticides, Zectran (4-dimethylamino-3,5-xylylmethylcarbamate) or Mesurol (methiocarb) following i.p. administration to pregnant rats. Only the data pertaining to methiocarb were evaluated and discussed in this report. [^{14}C]-carbonyl-labelled methiocarb (5.5 µCi/kg, purity > 99%) was administered by i.p. injection to 18 or 19 days pregnant or non-pregnant SD rats (mated prior to treatment, age and group sizes unstated, bw at arrival: 220 and 240 g, Simonsen Laboratories, Gilroy, CA, USA). Following injection, the animals were placed in clear plastic boxes which were modified to accommodate CO_2 absorber towers (containing 20 mL of a 2:1 mixture of 2-methoxyethanol and monoethanolamine) to collect exhaled $^{14}CO_2$. The absorber mixture was changed hourly until the animals were sacrificed by cervical dislocation at 30 min, 1, 2, 4, or 8 h post treatment. After dissection, maternal blood samples were obtained by cardiac puncture and the following tissues were sampled for radiolabel analysis: brain, heart, lung, fat, kidney, spleen, liver, muscle and bone. Urine and faeces were collected, sampled and radioactivity content was determined. Amniotic fluid was collected and the following foetal tissues were sampled after dissection for radiolabel analysis: brain, liver, kidney, intestine and stomach, lung and heart. The pooled foetal tissues were considered as one experiment. Both foetal and maternal tissues were macerated and dried over phosphorous pentoxide and the radioactivity in the dried samples was determined using a wet combustion technique. Based on the total $^{14}CO_2$ collected, disintegrations/min/g of dried tissue were quantified.

Isolation and Identification of Extractable ^{14}C metabolites: Further experiments were conducted using extracts of whole foetus, foetal brain and liver, maternal brain and liver, amniotic fluid, plasma and placental tissues to clarify the structural identity of ^{14}C-compounds present in these tissues. For these experiments, 10 µCi of methiocarb/kg was injected i.p. into one pregnant SD rat, which was sacrificed by cervical dislocation 30 minutes later. Tissue homogenates were prepared with 0.25 M sucrose, and methiocarb and its metabolites were ether extracted. The extracts were dried and resuspended in absolute ethanol. The ^{14}C-containing compounds in the extracts were resolved using TLC and GLC techniques. Elimination constants were calculated by extrapolating back to zero time with semi-logarithmic plots of ^{14}C content vs time. The $T_{1/2}$ was 50% of the zero-time value. Elimination constants were calculated by the method of Notari (1971). For methiocarb, the efficiency of ^{14}C recovery was about 95%. No correction was made for any radioactivity losses.

Findings: Of the radioactivity administered, about 75% was exhaled by non-pregnant rats as $^{14}CO_2$. The amount of $^{14}CO_2$ exhaled by pregnant rats was about 65%. The slow rate of elimination noted in the pregnant animal may be due to the placental transfer, foetal uptake and other pregnancy-related maternal physiological factors. There was no difference in the 8 h urinary excretion of ^{14}C by pregnant and non-pregnant rats (10.7%). Faecal excretion of ^{14}C-radiolabel was less than 1% of the administered dose. Based on the urinary and pulmonary excretion data it appeared that about 25% of the administered radioactivity was retained in the pregnant animal at 8 h post treatment. In non-pregnant rats, the amount retained at 8 h was about 15%.

In the line graphs provided, the placental transfer of methiocarb appeared rapid. Loss of radioactivity from the placenta appeared slower than from the foetus, but both tissue samples had approximately the same radioactivity level at 8 h post treatment. Maternal plasma demonstrated a similar radioactivity elimination profile to that of the whole foetus, but the elimination phase was longer (by about 2 h). According to the graphs, the radioactivity in the maternal brain declined rapidly in a non-linear fashion. Maternal liver appeared to have a short elimination phase (about 1 h). Maternal kidney, lung, heart and fat showed a plateau or irregular trend in radioactivity levels during the first 1-2 h with an apparent increase in concentration occurring between 2-4 h post treatment. By 8 h, however, the radioactivity in these tissues had declined by about 25-40% compared to the 4 h data (see Table below).

In the whole foetus, the elimination of methiocarb was relatively fast ($T_{1/2}$=11.3 min). However, commencing from 2 h post treatment, the radioactivity content in the whole foetus slightly increased up until 4 h, and then declined slowly. This was similar to the trend seen in dams and suggests that the kinetics of methiocarb are somewhat complex. The elimination of methiocarb from the foetal brain appeared to have a biphasic decay curve with very short elimination phase of about 1 h. At 8 h post treatment, the radioactivity content in the foetal brain was about twice that of maternal brain. The ^{14}C content of selected foetal and maternal tissues following i.p. administration of methiocarb is presented in the following table.

^{14}C content[a] of foetal and maternal tissues following intraperitoneal administration of labelled methiocarb to pregnant rats

Tissue	Time (min)				
	30	**60**	**120**	**240**	**480**
Maternal					
Kidney	28.8 ± 0.5	28.9 ± 2.5	17.9 ± 1.1	26.9 ± 1.8	18.7 ± 2.3
Lung	24.9 ± 3.9	26.7 ± 4.2	15.2 ± 0.3	19.7 ± 1.1	13.9 ± 1.8
Heart	27.9 ± 3.4	28.5 ± 2.6	15.2 ± 0.5	19.1 ± 1.2	14.2 ± 0.9
Fat	3.7 ± 1.5	9.8 ± 3.8	4.4 ± 0.9	10.5 ± 1.9	6.2 ± 2.2
Red blood cells	50.4 ± 2.3	68.9 ± 4.0	40.6 ± 3.7	38.1 ± 3.9	26.0 ± 4.5
Foetal					
Kidney	78.2 ± 10.8	55.7 ± 3.5	43.8 ± 2.1	60.1 ± 7.1	46.7 ± 9.2
Lung	38.5 ± 4.2	35.6 ± 3.1	26.6 ± 2.5	26.3 ± 11.9	24.2 ± 0.5
Heart	54.1 ± 5.0	53.1 ± 3.6	40.0 ± 4.8	41.5 ± 1.4	41.8 ± 8.5
Intestine	43.5 ± 3.6	37.4 ± 3.5	30.3 ± 2.6	24.9 ± 7.1	26.7 ± 3.9

[a]Data are expressed as disintegrations/min/g of dried tissue (mean ± SE of 3-6 experiments).

Although the foetal kidney and heart showed an increase in the radioactivity content from 2 to 4 h post treatment, other foetal tissues showed either a slow decline or a plateau level of radioactivity. Foetal kidney had the highest concentration of administered radiolabel compared to the other tissues. The presence of methiocarb sulfoxide in the foetal liver preparations could be due to both foetal metabolism and placental transfer. The study authors stated that the highest concentration of radioactivity was found in the maternal liver and the lowest in muscle and bone, but the individual data were not provided. Methiocarb seems to remain longer in the foetal tissues compared to the maternal tissues. This may have been due to low rate of clearance in the foetus.

The following table presents data on the percent radioactivity remaining in the aqueous phase after ether extraction and proportions of ether extractable compounds in various tissues.

Percent radioactivity remaining in the aqueous phase after ether extraction and proportions of ether extractable compounds in various tissues

Tissue	N	Aqueous	Compound (% in ether extract)		
			Methiocarb[a]	M sulfoxide	Spot C[b]
Maternal					
Liver	3	73.6	38.6	37.5	41.5
Plasma	2	72.9	35.0	37.4	16.9
Brain	2	58.0	19.8	26.8	37.4
Placenta	2	78.0	2.6	75.1	2.6
Whole foetus	3	55.3	-	-	-
Foetal					
Amniotic fluid	1	65.6	-	-	-
Brain	2	58.7	25.2	35.1	34.7
Liver	2	80.7	30.2	32.7	28.9
Carcass	2	73.4	8.7	75.6	0

N= number of experiments.
[a] Methiocarb identified by TLC only. Percentages are based on ^{14}C spotted on TLC plates.
[b]Unidentified metabolite.

The study authors stated that isolation and characterisation of methiocarb metabolites proved to be difficult because of the presence of interfering residues. The data indicate that a large proportion of the administered radioactivity remained in the aqueous phase (about 58-78% and 55-80% for maternal and foetal tissues respectively). The metabolites contributing to the detected radioactivity were not characterised. The only ether extractable methiocarb metabolite identified was methiocarb sulfoxide. Although a further radioactive area was noted in TLC plates, this area was also not identified (Spot C). The study authors hypothesised that spontaneous conversion of methiocarb to the sulfoxide accounts for some of the latter in various tissues, but noted that only 1% of such conversion occurred during TLC.

Conclusions: Following i.p. administration of [^{14}C]-carbonyl-methiocarb at 5.5 µCi/kg to pregnant rats, rapid placental transfer of radioactivity was observed. Pregnant animals showed a slower rate of $^{14}CO_2$ elimination compared to non-pregnant rats. The kinetics of methiocarb appeared complex because of a rebound elevation in radioactivity seen in all maternal tissues (except RBCs) and several foetal tissues at 4 h post treatment. In addition, methiocarb concentrations peak at higher levels and remain longer in the foetal tissues compared to maternal tissues, probably due to low rates of clearance. The data suggest that methiocarb was extensively metabolised to form water soluble metabolites. The major ether extractable metabolite identified was methiocarb sulfoxide. A further ether extractable metabolite and the water soluble metabolites were not identified.

The elevated levels of methiocarb in foetal tissues compared with maternal tissues may be of some toxicological importance, as they could possibly cause foeto-toxicity at doses that do not cause maternal toxicity.

Oral administration

Van Hoof F & Heyndrickx A (1975) The excretion in urine of four insecticidal carbamates and their phenolic metabolites after oral administration to rats. Arch Toxicol 34: 81-88.

Study & Observations: This study was performed to investigate the urinary excretion of 4 insecticidal carbamates including methiocarb, and their phenolic metabolites following oral administration of the parent compounds to rats. Methiocarb of unstated purity (source unstated) was administered to Wistar rats (age, bw, sex or source unspecified, 5.0 mg for one rat and 5.8 mg for 2 rats) in maize oil as a single oral dose. Following dosing, the test animals were kept in metabolism cages, and urine samples from each rat were collected at 24 h intervals over 5 days for the isolation and quantitative identification of the parent compound. The samples were partitioned using diethyl ether and benzene to extract the "unchanged compounds". To extract methiocarb metabolites excreted in urine, the aqueous phase of each of the first 24 h urine extracts was incubated with 5 mL of acetic acid-sodium acetate buffer of pH 5.2 and 0.5 mL of enzyme solution (800,000 IU glucuronidase and 100,000 IU sulphatase) for 24 h at 37° C. The incubated samples were again partitioned with diethyl ether. The extracts were then concentrated, and the parent compound and the metabolite, methiocarb phenol were resolved by Gas Liquid Chromatography (GLC) and Mass Spectrometry. In quantitative assay of methiocarb metabolites, 5 mL of urine samples which had previously been ether partitioned were acid digested in the presence of 1 mL of concentrated HCl and 5 mL of distilled water. The metabolites in these samples were extracted using 12N NaOH and pentane, and resolved by GLC. Percent recovery of the parent compound or phenolic metabolites in urine was assessed by including 1-naphthyl N-methyl-carbamate or 4-dimethylamino, 3,5-dimethylphenol respectively, in the corresponding ether extract as an internal standard. No further information on experimental methods was provided.

Findings: According to studies with blank urine samples fortified with 100 µg of methiocarb, the efficiency of recovery of the parent compound was about 76% (SD=5.7). The efficiency of recovery of methiocarb phenol (4-methylthio, 3,5-dimethylphenol) added to blank urine samples was about 92% (SD=1.7).

The treated rats excreted between 0.58 and 2.3% of administered methiocarb via the urine in the form of the parent compound. Urinary excretion occurred over a 24-72 h period. The urine from one animal treated at 5.8 mg was assayed for the metabolite methiocarb phenol. Approximately 2.3% of the administered dose appeared as methiocarb phenol, all during the first 24 h post treatment. The remaining 95-97% of radioactivity administered to the rats was not accounted for, possibly because there was no assay for metabolites other than methiocarb phenol. Although the aqueous phase metabolite concentrations of two other carbamates [Moban (4-benzothienyl N-methyl carbamate) and Zectran (3,5-dimethylphenyl N-methyl carbamate)] in urine after dosing were monitored by analysing 2 consecutive 24 h urine samples, this procedure was not adopted for the samples of methiocarb treated animals.

Conclusions: Following administration of 5.0 to 5.8 mg methiocarb orally to rats, up to 2.3% of the administered dose was excreted in urine unchanged within 72 h. The only methiocarb metabolite identified in urine was methiocarb phenol accounting for 2.3% of the administered dose. The marked contrast to the rapid and extensive urinary excretion of methiocarb and its metabolites observed in the following and subsequent studies possibly arises from the lack of assay for metabolites of methiocarb other than methiocarb phenol.

Stanley CW & Johnson GA (1976) Metabolites of ®Mesurol in rat urine. Study No. not stated. Lab: Chemagro Agricultural Division, Mobay Chemical Corporation. Sponsor: Farbenfabriken Bayer GmbH, Leverkusen, Germany. Study duration: not stated. Report No. 50732.

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

Study & Observations: Excretion of metabolites in urine was investigated following a single oral gavage administration of 0.25 (3/sex, 14.5 mCi/mmol) or 20 (3 females, 4.8 mCi/mmol) mg/kg bw ring-1-^{14}C labelled Mesurol (methiocarb, purity >97%, source not stated) in ethanol to rats (Strain, source not stated, 204-217 and 200-258 g bw for males and females respectively). After dosing, the animals were housed individually in modified plastic metabolic cages and urine was collected under conditions which ensured minimal degradation of metabolic products. The animals were provided with feed and water *ad libitum*. A 48 h composite urine sample was collected from each rat and chloroform partitioned to extract the organo-soluble metabolites. The aqueous phase of each urine sample was incubated with maltase for 16 or 24 h, and again partitioned with chloroform to extract organo-soluble metabolites. After concentration and assay for radioactivity, the metabolites were resolved using one or two dimensional TLC.

Findings: Urinary excretion by rats amounted to 95% of administered radioactivity at 20 mg/kg bw and 79/82% in males/females at 0.25 mg/kg bw. Based on percent of administered radioactivity, the major non-conjugated metabolites found in the chloroform extract at 20 mg/kg bw was methiocarb phenol (5%), methiocarb sulfoxide phenol (6%) and an unidentified metabolite (6%). The study authors stated that the unknown component might correspond to a previously reported N-hydroxymethyl methiocarb sulfoxide. Methiocarb sulfoxide was detected in trace quantities (about 1%). The major metabolites found in the aqueous fraction were methiocarb sulfoxide phenol (23%), methiocarb phenol (8%) and methiocarb sulfone phenol in trace quantities (about 1%).

At 0.25 mg/kg bw, about 15% of the administered radioactivity was recovered in the chloroform extract while 59% remained in the aqueous phase except in one male rat which was injured and believed to have metabolised the test compound differently than the other rats. However, the proportion of organo-soluble and water soluble radioactivity and percentages of major metabolites found in urine were independent of the administered dose. Between 57-72% of the radioactivity present in the aqueous phase was rendered organo-soluble by enzyme incubation. No major difference was seen between sexes.

Conclusions: When ^{14}C-Mesurol was administered orally to rats at 0.25 or 20 mg/kg bw (single gavage dose), 79-94% percent of the total administered radiolabel was excreted in urine by 48 h after administration. Methiocarb phenol, methiocarb sulfoxide phenol and methiocarb sulfoxide were major metabolites found in urine together with an unidentified metabolite. There were no major differences in the metabolites identified or their relative amounts seen at the two dose levels. No difference in elimination patterns was noted between sexes.

Bell RL (1974) The metabolic fate in vivo of Mesurol [3,5-dimethyl-4-(methylthio) phenol methylcarbamate] in dogs. Study No. not stated. Lab: Research and Development Department, Chemagro Division of Baychem Corporation. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 39243. Report date: January 11, 1974.

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

Study & Observations: This study was conducted to evaluate the *in vivo* metabolic fate of Mesurol (methiocarb) in dogs. A single oral dose of ring-UL-^{14}C-Mesurol (source, batch and purity not stated) in gelatin capsules was administered to overnight fasted dogs [(2/sex/breed), 2 Beagle females of 14.5 and 11.8 kg bw (age, source not stated), 2 Mongrel males of 11.0 and 12.8 kg bw, Chemagro Research Farm, Stanley, KA, USA, age not stated] at 2 mg/kg bw (specific activity 0.6-1.02 mCi/mM). After dosing the dogs were housed individually in

stainless steel metabolism cages and offered food (Purina Dog Chow) about 4 h later. Urine and faecal material were collected during the experiment as samples became available and the radioactivity of each sample was determined. The dogs were sacrificed (by i.v. overdose of phenobarbital) at 24, 48, 96 and 144 h post treatment. Liver, heart, muscle, fat, kidney, brain and skin were collected for radioactivity determination. Blood samples were collected at sacrifice from three dogs, and from the fourth animal (from the saphenous vein) at 1, 2, 4, 24, 48, 72 and 96 h post dosing for blood cell and plasma radioactivity determination. Chloroform, acetone and methanol and acetone partitioning methods were used to extract methiocarb and related metabolites from urine, faeces and tissues respectively. The metabolites in the aqueous phase of urine were characterised following either maltase, sulfatase or β-glucuronidase hydrolysis. Thick and thin layer chromatography (TLC) and column chromatography were used to characterise and identify the metabolites.

Findings: Radioactivity in the blood reached its maximum within one hour following oral administration of the labelled compound. Half lives of the total radioactivity in plasma or whole blood were estimated to be about 75-76 h. The total radioactivity recovered in urine ranged from 26-66% of the administered dose; methiocarb phenol sulfone and methiocarb phenol sulfoxide being the two major metabolites found at a ratio of about 3:1. Nearly 6% of the radioactivity found in urine was chloroform extractable. Hydrolysis by maltase rendered approximately 75% of aqueous urinary metabolites organo-soluble. The proportion of unchanged methiocarb excreted in urine was not given. Of the administered dose, about 10-56% was excreted in faecal matter as methiocarb suggesting incomplete gastrointestinal absorption, possible secretion of absorbed but unchanged methiocarb in bile, or lack of degradation by intestinal flora.

The combined methanol acetone extraction removed about 80-90% of the tissue radioactivity. Residue levels found in the tissues at 144 h post dosing were about 0.27 (kidney), 0.12 (liver), 0.20 (skin), 0.11 (heart), 0.08 (muscle), 0.02 (fat), and 0.02 (brain) ppm methiocarb equivalents. The major metabolites identified in the kidney and muscle tissue were similar to those found in urine (no individual data were provided). Other sampled tissues were not tested for metabolites and radioactivity in the expired air was not determined.

Conclusions: When ^{14}C-Mesurol was administered to dogs at 2 mg/kg bw, about 64-92% of the administered dose was recovered in urine and faeces. The major metabolites identified in urine were methiocarb phenol sulfone and methiocarb phenol sulfoxide at a ratio of 3:1. Faecal radioactivity was attributable to the parent compound possibly indicating incomplete gastrointestinal absorption and degradation of methiocarb, or secretion of absorbed but unchanged methiocarb in bile. The highest tissue residue level at 144 h post dosing was observed in the kidney.

Minor RG & Murphy JJ (1977) Metabolism and excretion of ®Mesurol by a dairy cow. Study No. not stated. Lab: Research and Development Department, Chemagro Agricultural Division of Mobay Chemical Corporation. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 51144. Report date: July 01, 1977.

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

Study & Observations: This study was performed to investigate the metabolic fate and urinary excretion of orally administered Mesurol (methiocarb) in a dairy cow. A single dose of ring-1-^{14}C labelled methiocarb (purity, batch not stated. specific activity 4.83 mCi/mmol, prepared in β-lactose) in a gelatin capsule was administered orally to a cow (breed, age and source not stated; weight: 511 kg) at 0.14 mg/kg bw. Blood was collected (method unspecified) at 30 min intervals for 4 h and then at 5, 8 and 24 h post treatment. Urine was collected by a

catheter and samples were assayed for radioactivity hourly for the first 8 h and at 24 h intervals thereafter until study termination. Faeces was collected as they became available and the radioactivity of the samples were determined. Milk was collected in the morning and evening, weighed and radioassayed. Blood, milk, urine and faeces samples collected prior to treatment served as controls.

The radioactivity in blood, milk and urine samples was determined by direct liquid scintillation counting. Faeces samples were combusted to form $^{14}CO_2$, which was subsequently collected in ethanolamine and radioassayed. Urine samples (4, 8, 24 and 48 h) were chloroform partitioned and the radioactivity in chloroform was determined. The aqueous phase was enzyme (with sulfatase-glucuronidase, 2 mg/mL for 18 h) and acid (with 2N HCl for 2 h) hydrolysed in succession and chloroform partitioned. The chloroform extracts were concentrated, radioassayed and the metabolites in each fraction were resolved by TLC. No tissue residue levels were assayed, and radioactivity in the expired air was not determined.

Findings: The general physical condition of the cow and the milk production were unaffected by treatment. The peak blood radioactivity was noted between 2.5-3 h post treatment. Of the administered radioactivity, 96% was excreted in urine by 144 h post treatment. Faecal matter and milk samples collected over 144 h contained 1% and <1% of the administered radioactivity respectively and therefore these samples were not examined further. Only about 1% of the metabolites in urine were chloroform extractable. Following enzyme and acid hydrolysis approximately 78-85% of the urine radioactivity became chloroform extractable suggesting that the primary metabolites of methiocarb in urine were in conjugated form. Based on percent distribution of the radioactivity, the major metabolites identified in urine were methiocarb phenol (25-29%), methiocarb sulfoxide phenol (22-32%) and methiocarb sulfone phenol (20-23%) with trace quantities of methiocarb sulfoxide, methiocarb sulfone and some unidentified components (<1%). The proportion of parent compound excreted in urine was not given. About 14-21% of the radioactivity in urine remained as aqueous residues.

Conclusions: When ring-1-^{14}C labelled methiocarb was administered orally to a dairy cow at 0.14 mg/kg bw, about 96% of the administered dose was excreted in urine, 1% in faeces and <1% in milk after 144 h. Three major metabolites in urine were identified: methiocarb phenol (25-29%), methiocarb sulfoxide phenol (22-32%) and methiocarb sulfone phenol (20-23%), with methiocarb sulfoxide, methiocarb sulfone and some unidentified components (<1%) present in trace quantities. About 14-21% of the radioactivity in urine remained as aqueous residues.

In vitro studies

Oonnithan ES & Casida JE (1966) Metabolites of methyl- and dimethylcarbamate insecticide chemicals as formed by rat liver microsomes. Division of Entomology and Acarology, University of California, Berkeley, CA, USA. Bull Environ Contam Toxicol 1: (2) 59-69.

Study & Observations: This study was conducted to determine the metabolic fate of various methyl and dimethylcarbamate insecticides including carbonyl-^{14}C labelled Mesurol (purity 99%, batch, source not stated). Rat (strain not stated) liver microsome fraction or microsome plus the soluble fraction (equivalent to 200 mg of liver) was incubated for 4 h with 2 µmoles of labelled Mesurol and 2 µmoles of either NADP, $NADPH_2$, NAD or $NADH_2$, or without any of these co-factors. The parent compound and metabolites were recovered by ether extraction and resolved by TLC and autoradiography. Co-chromatography and autoradiography were used to identify the metabolites. For localisation and assay of ChE

inhibitory properties of the metabolites, a qualitative colorimetric assay was performed by spraying the TLC plates containing resolved metabolites with undiluted human plasma and acetylcholine bromide. Emergence of red spots in a yellow background in 30 minutes indicated the presence of ChE inhibitors. The coloured spots were then compared with autoradiographs of TLC plates for identification of metabolites.

Findings: For Mesurol, the extent of metabolism of the labelled parent compound was generally higher in the incubation mixture containing the microsomes plus soluble fraction and NADP than in mixtures containing microsomes plus other co-factors. Under these conditions, 8% of Mesurol was metabolised to products, of which about 43% were water soluble. Two main hydroxylated metabolites of the s-alkyl group were identified: 4-methylsulfinyl-3,5-xylyl methyl-carbamate (sulfoxide) and 4-methylsulfonyl-3,5-xylyl methyl carbamate (sulfone). One further metabolite was not identified. It was reasoned that the unidentified metabolite may have formed due to hydroxylation reactions at different sites on the substrate. The sulfoxide metabolite was found to possess plasma ChE inhibitory properties. The metabolites formed by hydrolysis at the carbamic ester site or those at the origin of the TLC plates were not identified.

Conclusions: In an *in vitro* metabolism assay using rat liver microsomes, two major metabolites of ^{14}C labelled Mesurol were identified and characterised [4-methylsulfinyl-3,5-xylyl methyl-carbamate (sulfoxide) and 4-methylsulfonyl-3,5-xylylmethyl carbamate (sulfone)]. Among the three metabolites detected (one unidentified), the sulfoxide showed ChE inhibitory properties.

Wheeler L & Strother A (1971) In vitro metabolism of the N-methylcarbamates, Zectran and Mesurol by liver, kidney and blood of dogs and rats. J Pharm Exp Therap 178 (2): 371-382.

Study & Observations: This comparative *in vitro* study of the metabolism of Zectran (4-dimethylamino-3,5-xylyl methylcarbamate) and Mesurol (methiocarb) was conducted to characterise and identify the metabolites, routes of biotransformation and to understand the extent of biodegradability of the parent compounds. Livers were obtained from male SD rats weighing 250-350 g (age, group size and source unspecified), and liver and kidney specimens were obtained from mongrel dogs (sex, age, bw, sample size unspecified). Tissues were removed following sacrifice either by cervical dislocation or ether anaesthesia (rats), and from dogs after anaesthetising the animal with phenobarbital and making a high median abdominal incision exposing the liver and the kidney. The tissue specimens were cut into small pieces, weighed and homogenised in sufficient volumes of 0.25 M sucrose to obtain 20% tissue homogenates. Each homogenate was centrifuged at 15,000 g for 30 minutes and the supernatant was used as the enzyme source. One mL of the supernatant was incubated with 1-2 µM of carbonyl ^{14}C-labelled methiocarb technical (adjusted specific activity 1 mCi/mmole, radiochemical purity >99%, New England Nuclear Co, Boston, USA) in 0.1 mL of propylene glycol for 2 h at 37° C in Erlenmeyer flasks in the presence of 1 µmole of NADP, 0.1 M phosphate buffer of pH 7.4, 1 µmole of glucose-6-phosphate, 25 µmoles of $MgCl_2$, and 0.5 units of glucose 6-phosphate dehydrogenase. The final volume of the incubation mixture was 3.0 mL. An aliquot of the labelled methiocarb was counted for radioactivity to determine the total radioactivity added to the incubation flasks. After incubation, aliquots were taken to determine the radioactivity loss during incubation.

In studies with blood samples (rat blood was obtained from the aortic artery after sacrifice, for the dog blood the source of extraction was unspecified), the incubation mixture contained 2.5 mL of whole blood or serum, 0.1 mL of propylene glycol, and 1-2 µM of carbonyl ^{14}C-

labelled methiocarb technical. Control flasks consisted of 0.1 M $KHPO_4$ of pH 7.4 in the same volume as that occupied by whole blood or serum. The incubation mixtures were incubated for 2 h at 37° C.

Organo-soluble compounds in the incubation mixtures were ether partitioned, concentrated, and the radioactivity in the ether extract and the aqueous phase (for the liver and kidney) was determined by liquid scintillation counting. Non-ether extractable radioactivity in whole blood or serum mixtures was extracted by trichloroacetic acid (TCA). Further experiments were conducted using TCA precipitates and supernatant fractions to determine the effect of whole blood on plasma protein-methiocarb binding using rat plasma and plasma derived from rat whole blood. Metabolites in the ether extracts were resolved by TLC and identified by X-ray autoradiography, GLC and chromogenic spraying. Substractive and derivative chromatography were performed using sodium borohydride and bis (trimethylsilyl) trifluoracetamide to identify specific functional groups of the compounds analysed by GLC. Carbon dioxide liberated from the incubation mixtures was collected and the radioactivity in the samples was determined. The total recoverable [^{14}C]-radiolabel from all constituents was taken as the 100% value.

Findings: Although TLC data indicated the presence of several methiocarb metabolites, only two major metabolites were identified. They were 4-methylsulfynyl-3,5-xylyl N-methyl carbamate (methiocarb sulfoxide) and 4-methylthio-3,5-xylyl N-hydroxymethylcarbamate (M-NOHME). The study authors stated that the radioactivity losses during extraction and incubation ranged from 2 to 29%. The data on radioactivity distribution in various methiocarb metabolites are presented in the following Table.

Distribution of radioactive carbon in different methiocarb metabolites following incubation with dog and rat liver and dog kidney supernatants

Metabolite	**Supernatant source** (percentage of added radioactivity)		
	Dog liver	Dog kidney	Rat liver
A	0.12	0.15	0.10
2A	0.51	-	-
C	0.19	-	-
D	0.61		0.30
B: 4-methylsulfynyl-3,5-xylyl N-methyl carbamate (methiocarb sulfoxide)	16.8	2.44	26.0
E	-	-	0.32
F: 4-methylthio-3,5-xylyl N-hydroxymethylcarbamate (M-NOHME)	2.54	-	9.5
L	0.21	-	0.41
H	0.42	-	-
Methiocarb	8.15	88.97	1.9
Aqueous phase	77.14	6.59	57.7

According to the data, the rat liver supernatant fractions appeared to be more active (about 26% added radioactivity) in metabolising methiocarb to form methiocarb sulfoxide compared to the dog liver (about 16% of the added radioactivity) and kidney (about 2.4% of the added radioactivity). The rat liver also appeared about 3.7-fold more active in forming the metabolite M-NOHME compared to the dog liver. Formation of M-NOHME was not seen with the dog kidney supernatants and in these incubation mixtures about 89% of the added radioactivity was found as unchanged methiocarb. With the dog and rat liver fractions, a

decreased amount of ether extractable radioactivity was recovered from incubation mixtures, with about 1.5 to 2.5-fold more of the added radioactivity remaining in the aqueous phase.

Percentages of the radioactivity remaining in the aqueous phases following incubation of methiocarb with dog/rat whole blood, plasma and in the controls were 93/89, 99/99 and 6.5/6.5 respectively. The results of the plasma or whole blood-methiocarb binding studies are presented in the table below.

Influence of whole blood on the binding of methiocarb to plasma proteins in the rat

Fraction	**Percent radioactivity**	
	Plasma	Whole blood[d]
SN1[a] + SN2[b]	13	18
Organo-soluble	24	52
Plasma protein[c]	51	29
Total recovery	88	99

[a]Primary supernatant fraction obtained by adding TCA to serum.

[b]Supernatant obtained by adding TCA to the precipitate of the above.

[c]TCA precipitate.

[d]Following incubation the blood was centrifuged at 3100 g for 50 minutes and plasma was used in the analyses.

The data indicated that when added to plasma, the majority of the radioactivity was associated with plasma proteins. However, when red cells were present, this association was diminished by about 2-fold, demonstrating the capacity of methiocarb to bind with RBCs. RBC-methiocarb binding appeared rather weak as the majority of the radioactivity was released in the SN1, SN2 and organo-soluble fractions. Methiocarb metabolites formed following incubation with serum or whole blood were not identified. Further, it was stated that more $^{14}CO_2$ was liberated by the liver during incubation than by blood (about 1-1.5% vs 1% of the added radioactivity), but no further quantitative details were provided.

Conclusions: In this *in vitro* metabolism study, two major metabolites of methiocarb formed by dog and rat liver and dog kidney were identified: 4-methylsulfynyl-3,5-xylyl N-methyl carbamate (methiocarb sulfoxide) and 4-methylthio-3,5-xylyl N-hydroxymethylcarbamate (M-NOHME). Methiocarb metabolites formed following incubation with serum or whole blood were not identified. The majority of added methiocarb was found to be bound with plasma proteins when added to plasma. However, when the red cells were present, the binding was diminished by about 2-fold, demonstrating the ability of methiocarb to bind with RBCs. This binding however, appeared rather weak as the majority of the radioactivity was seen in the SN1, SN2 and organo-soluble fractions.

Strother A (1972) In vitro metabolism of methylcarbamate insecticides by human and rat liver fraction. Toxicol Appl Pharmacol 21: 112-129.

Study & Observations: This comparative study investigated in vitro metabolism of five methylcarbamates including methiocarb by human and rat liver fractions. The data pertaining to methiocarb are included in this evaluation. The rat (derived from male SD rats of 250-350 g bw, age, source not stated) or human liver (biopsy specimens obtained from the Department of Surgery, Loma Linda School of Medicine, CA, USA) supernatant fractions (equivalent to 200 mg of liver) were incubated with carbonyl ^{14}C-labelled methiocarb technical (adjusted specific activity 1 mCi/mmole) for 3 h at 37° C in Erlenmeyer flasks in the presence of 2 µmoles of NADP in 0.1 M phosphate buffer of pH 7.4, 20 µmoles of glucose-6-phosphate, 25 µmoles of $MgCl_2$, and 0.1 mL of propylene glycol in a final volume of 3 mL. Control flasks contained supernatant fractions from boiled liver homogenate, buffer and substrate. The $^{14}CO_2$ released during metabolism was collected in a 2:1 mixture of 2-methoxyethanol and monoethanolamine for radioactivity detection. Following incubation, methiocarb and its metabolic products were three times ether extracted, concentrated and subsequently resolved by TLC and GC. Autoradiographs were prepared by exposing the TLC plates for a period of 2-6 days to medical no-screen X-ray films. The metabolites were identified by co-chromatography using non-labelled standards and various chromogenic and functional group reagents. The aqueous phase of each incubation mixture was sampled and its radioactivity was determined. The data obtained from 3-6 experiments (in triplicates) were provided.

Findings: Average recovery of the parent compound from the controls following incubation was about 88%. Average total recovery of the added radioactivity from TLC through the quantitation procedure was about 75%. The study authors stated that, in relation to losses during extraction, more variation was encountered with methiocarb in comparison to the other carbamates studied. Further, it was noted that in general less than 2% of administered radiolabel was metabolised to $^{14}CO_2$ in preliminary in vitro studies, although no additional data on this parameter were provided. The distribution of radioactivity in organo-soluble and aqueous phases of the incubation mixture and the radioactivity losses during the studies are given in the table below.

Distribution of radioactivity in organo-soluble and aqueous phases following *in vitro* metabolism by human and rat liver fractions

Phase	**Percent of radioactivity recovered from liver fractions**	
	Human	Rat
Ether extractable		
Methiocarb	12	2
Metabolites	26	31
Aqueous	45	44
Losses	16	24

As the data indicate, in both human and rat liver studies, approximately 45% of the added radioactivity was detected in the aqueous phase. In addition, in organo-soluble fractions from both human and rat liver studies, the parent compound was found in smaller quantities (about 12% and 2% for human and rat liver fractions respectively) than metabolites. Thus, methiocarb appeared to have been metabolised to a large extent by both human and rat liver fractions to form water soluble products. When compared with the other carbamates tested, the study authors stated that methiocarb produced the largest amount of water soluble metabolites.

Following incubation, two major metabolites with human and rat liver preparations were identified: 4-methylsulfinyl-3,5-xylyl methylcarbamate (methiocarb sulfoxide, 13% and 16% for human and rat liver respectively), and 4-methylthio-3,5-xylyl N-hydroxymethyl carbamate (8% and 6% for human and rat liver respectively). Twelve and five further unidentified metabolites were produced by human and rat liver preparations respectively and found in low quantities (16 metabolites accounted for <1% of administered radioactivity and one rat liver metabolite equivalent to 7% added radioactivity). The study authors stated that the major metabolites of methiocarb identified in this study retained the OC(O)NC group, which is necessary for ChE inhibition.

Conclusions: In both human and rat liver studies, approximately 45% of the added radioactivity was noted in the aqueous phase, suggesting that in vitro metabolism produced more water soluble metabolites than organo-soluble products. Two major metabolites in organo-soluble fractions were identified: 4-methylsulfinyl-3,5-xylyl methylcarbamate (methiocarb sulfoxide; 13% and 16% of administered radioactivity for human and rat liver assays respectively), and 4-methylthio-3,5-xylyl N-hydroxymethyl carbamate (8% and 6% for human and rat liver assays respectively). Both products retained the OC(O)NC group necessary for ChE inhibition. Twelve and five further metabolites produced by human and rat liver preparations respectively were not identified.

Wheeler L & Strother A (1974b) In vitro metabolism of ^{14}C-Pesticidal carbamates by foetal and maternal brain, liver, and placenta of the rat. Drug Metabolism and Disposition. 2(6): 533-538.

Study & Observations: This study investigated the ether extractable metabolites produced from *in vitro* metabolism of three carbamate pesticides including carbonyl-^{14}C-labelled methiocarb following incubation with foetal and maternal tissue preparations. Maternal and foetal brain, liver, and placental tissues were obtained from 18 or 19 days pregnant SD rats (age, bw, group size, source not stated) following sacrifice of the animals by cervical dislocation and blood sampling by cardiac puncture. Liver, brain and placenta samples were weighed, then processed to form enzyme-containing supernatant fractions for metabolism experiments Subsequently, the supernatant fractions were fortified with 3.6 µmol of $NADP^+$, 12.5 µmol of glucose-6-phosphate, 15 µmol of $MgCl_2$, and 0.1 M phosphate buffer of pH 7.4 and incubated with 0.5 µmol of methiocarb (specific activity: 1 mCi/mmol, purity > 99%) for 2 h. The final volume of the incubation mixture was 2.5 mL. The control incubation flasks contained boiled homogenates, buffer, and 0.5 µmol of methiocarb. Following incubation, the metabolites were ether extracted, evaporated to dryness and the residue was dissolved in 0.12 mL of absolute ethanol and sampled to determine the ether extractable ^{14}C fraction. The metabolites were resolved by TLC and GLC.

Findings: No significant loss in metabolic activity was noted after freezing and storage of the homogenates overnight. Loss of radioactivity due to experimental and unknown causes ranged from 17.5% to 24.5%. About 72.4% of the administered radiolabel was recovered from TLC plates. Incubation mixtures containing maternal liver supernatant produced 6 radioactive metabolites from methiocarb, two of which were identified. They were 4-methylthiosulfinyl-3,5-xylyl methylcarbamate (M-sulfoxide) and 4-methylthio-3,5-xylyl N-hydroxymethyl carbamate (N-OHM). The proportions of each quantified methiocarb metabolite in the ether extractable fraction following incubation with various tissue preparations are given in the table.

Based upon the data on ether extractable metabolites with maternal liver, sulfoxidation appears to be one of the major pathways of methiocarb metabolism. Of the radioactivity

added, about 48.5% remained in the aqueous fraction as water soluble metabolites. Another metabolic pathway forming organo-soluble metabolites in the maternal liver was hydroxylation of the N-methyl carbon to form N-OHM (about 7.9%). Relative to the maternal liver, the foetal liver preparations produced more organo-soluble sulfoxide from administered methiocarb (23.1% vs 12.3%). No other individual metabolite was present at detectable levels in the foetal liver preparation. Of the radioactivity added about 36% was found as water soluble metabolites in the aqueous fraction.

Proportions of radioactive metabolites of methiocarb found in the ether extractable and aqueous fractions following incubation with maternal and foetal rat tissue preparations

Source of ^{14}C	**Percentage distribution of radioactivity in various tissues[a]**				
	Foetal brain	Maternal brain	Foetal liver	Maternal liver	Placenta
Ether extractable methiocarb	77.6 ± 5.7	84.1 ± 0.1	17.5 ± 4.1	3.3 ± 1.5	71.2 ± 4.8
Metabolites[b]					
A				1.7± 0.2	
B (M-sulfoxide)	0.5 ± 0.4		23.1± 2.6	12.3 ± 0.9	3.2 ± 0.5
D				0.4 ± 0.1	
E				1.8 ± 1.1	
F (N-OHM)				7.9 ± 1.8	
O				0.2 ± 0.1	
Total	0.5	0	23.1	24.3	3.2
Aqueous fraction[c]	3.5 ± 0.4	3.5 ± 0.6	36.0 ± 3.2	48.5 ± 0.7	5.4 ± 0.4
Break down products[d]	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9± 0.1	0.9 ± 0.1
Losses	17.5	11.5	22.5	23.0	19.3

[a]Mean percentage ± SE of 4 experiments

[b]Letters refer to the metabolite designations as indicated on the representative radioautograms.

[c]Radioactivity remaining after ether extraction.

[d]the total percent radioactive metabolites found in the controls.

Although the proportion was rather small, the data indicate that rat placenta also has some ability to metabolise methiocarb (about 3.2% of the added radioactivity was metabolised). However, no measurable metabolic activities above that of controls were noted for foetal and maternal brain tissues. The radioactivity in CO_2 was not determined.

Conclusions: Under the conditions of the study, sulfoxidation was the major pathway of methiocarb metabolism. Foetal and maternal liver converted 23 and 12%, respectively, of methiocarb to methiocarb sulfoxide. However, other metabolic pathways were also active in maternal liver only. The most prominent of these accounting for approximately 8% of the added radioactivity was hydroxylation of the N-methyl carbon to form N-OH methiocarb. Rat placenta had some limited ability to sulfoxidate methiocarb, but the foetal and maternal brain had no measurable methiocarb metabolic activity.

Hajjar NP & Hodgson E (1982) Sulfoxidation of thioether-containing pesticides by the flavin-adenine dinucleotide dependant monooxygenase of pig liver microsomes. Biochem Pharmacol 31(5): 745-752.

Study & Observations: This *in vitro* study was performed to investigate the role of flavin-adenine-dinucleotide (FAD)-dependant monooxygenase in the oxidation of 39 thioether containing organophosphate and carbamate pesticides including methiocarb (purity unstated, US EPA, Research Triangle Park, NC, USA), and the structure-activity relationships of these pesticides. The reaction mixtures contained 0.05 M potassium phosphate buffer of pH 7.4 and 0.1 mM NADPH in a total volume of 3.0 mL which was incubated at 37º C with continuous stirring. Following an initial 3 minute incubation period, the reaction was initiated by the addition of the enzyme solution (purified to homogeneity from pig liver, provided by Professor DM Ziegler, University of Texas, Austin, USA) to a final concentration of about 1 µM. The endogenous oxidation of NADPH was then recorded for 30 seconds. Then, 25 and/or 50 µM of the substrate in 5 µL of acetone was added and the oxidation of NADPH was recorded for an additional 30-60 seconds. The sulfoxidation of methiocarb by the FAD-dependant monooxygenase was determined by following the substrate dependent oxidation of NADPH using a spectrophotometric method. Reaction velocity was calculated from the oxidation reaction data during the first 10-30 seconds.

The stoichiometric relationship between NADPH and the substrate during the course of the reaction was evaluated using methylene [^{14}C]-phorate (O,O-diethyl S-ethylthiomethyl phosphorodithioate) as the standard. In this assay, the labelled substrate (specific activity 9.7 µCi/µmole) was incubated with the enzyme mixture at a final concentration of 50 µM. The oxidation reaction was monitored as indicated above until the rate returned to the initial endogenous rate. The reaction was then stopped (method unspecified), and the products were recovered by chloroform extraction. The chloroform extracts were concentrated, and the products were located by means of UV fluorescence and autoradiography. Similar experiments were performed using unlabelled disulfoton (O,O-diethyl S-2-ethylthioethyl phosphorodithioate) and phorate at a final concentration of 100 µM (five replicates each). In these studies, the compounds in the reaction mixture were resolved by TLC and identified by co-chromatography and comparison of R_f values with those of standards. The optical activity of disulfoton sulfoxide was measured by optical rotary dispersion spectroscopy.

Findings: According to the line graphs presented, metabolism of both disulfoton and phorate was complete in 2-3 minutes. The K_m and V_{max} values of these two substrates were 21.3 and 28.0 µM and 394 and 334 nmoles NADPH/min/mg of protein respectively. Further, in both cases, the reaction stoichiometry was 1:1, the ratio of which remained unchanged over a 10-fold range of substrate concentration (5-50 µM). The metabolic product identified with labelled phorate was its corresponding sulfoxide (radioactivity recovery from the samples and controls were >94%). Similar results were obtained for unlabelled disulfoton and phorate. No product was found in the absence of NADPH in the incubation mixture. The data on optical rotation studies indicated that the disulfoton sulfoxide was optically active suggesting the reaction was at least in part stereospecific.

Studies on structure-activity relationships revealed that the carbamate pesticides tested were generally not as good substrates as the phosphorodithioates (eg. Thiofanox = 3,3-dimethyl-1-methylthiobutanone O-methylcarbamoyloxime) for the monooxygenase. The rate of metabolism of methiocarb was equivalent to 2.82 ± 0.03 nmoles of NADPH/min/nmole of enzyme. The rates of metabolism of other substrates tested varied from 26.4 to 0.3 nmoles of NADPH/min/nmole of enzyme; the highest rate being observed for disulfoton and phorate. No metabolic products of methiocarb were identified nor was their optical activity measured.

Under the study conditions, no further oxidation of pesticide sulfoxides was observed at concentrations up to 100 µM.

Conclusions: In the presence of NADPH and under aerobic conditions, methiocarb was oxidised by FAD-dependent monooxygenase, purified from pig liver microsomes. The stoichiometric relationship between NADPH and standard substrates during the course of the oxidation reaction was 1:1. The rate of metabolism of methiocarb was relatively low compared to phosphorodithioates, and was equivalent to about 2.82 ± 0.03 nmoles of NADPH/min/nmole of enzyme. The metabolic products of methiocarb were not identified, nor was its optical activity measured.

Buronfosse T, Moroni P, Benoit E & Reviere JL (1995) Stereoselective sulfoxidation of the pesticide methiocarb by flavin-containing monooxygenase and cytochrome P450-dependent monooxygenases of rat liver microsomes. Anticholinesterase activity of the two sulfoxide enantiomers. J Biochem Toxicol 10 (4): 179-189.

Study & Observations: This study investigated the enzyme systems involved in *in vitro* microsomal sulfoxidation of methiocarb (MeS), the product enantioselectivity of the reaction, and cholinesterase inhibitory properties of the two methiocarb sulfoxide (MeSO) enantiomers. Microsomes prepared from the livers of either control (phosphate buffer in isotonic saline), 3-methylcholanthrene (3MC), dexamethasone (DEX) or pyrazole (PYR) treated male rats (IOPS-OFA, IFFA CREDO, St Germaine sur l' Arbresle, France, 4-6 rats/group, 180-200 g bw, age not stated) were incubated with methiocarb (Source: Bayer AG, Leverkusen, Germany. purity 99%, batch not stated) at pH 7.4 in the presence of $NADP^+$, glucose-6-phosphate and glucose-6-phophate dehydrogenase. After incubating for 10 minutes, 10 µL of propoxur in methanol (150 µg/mL) was added to the incubation mixture as an internal standard and the reaction was stopped by adding 4 mL of dichloromethane. Methiocarb and its metabolites were extracted with dichloromethane and resolved by reverse phase HPLC. The eluate corresponding to the chromatographic peak of MeSO was collected, concentrated and subsequently analysed by HPLC on a chiral stationary phase in an isocratic elution mode for separation of the two enantiomers. The relative proportions of the two enantiomers were determined and they were referred to as A and B, based on the order of elution from the chiral column. Results were expressed as enantiomeric excess (e.e = concentration of A-MeSO – concentration of B-MeSO/concentration of MeSO).

The effect of substrate concentration on the ratio of sulfoxide formation and stereoselectivity of the reaction was evaluated over a concentration range of MeS 7.5 to 500 µm in the presence of microsomes from a pool of four untreated rats. Inhibition of flavin containing monooxygenase (FMO) and NADPH cytochrome P450 reductase (NCR) dependant oxidation of MeS was studied either by adding methimazole and clotrimazole to the incubation mixture respectively, by thermal inactivation and combined chemical and thermal treatment of microsomes. Specific immuno-inhibition studies were performed using antibodies directed against rat liver FMO1 and NCR. Activities of the following specific P450 enzymes were assayed using appropriate substrates to evaluate the enzyme systems: ethoxy and pentoxyresorufin O-dealkylases, ethoxycoumarin O-deethylase, methimazole S-oxidase and S-oxidation of toltrazuril sulfoxide (HPLC method). Methiocarb metabolites were analysed for the presence of the hydrolysed sulfoxide and other enantiomers as previously described. *In vitro* inhibition of bovine RBC ChE by MeS and its metabolites was evaluated by incubating the diluted HPLC extracts with the enzyme for 1 minute followed by the colorimetric assay of Ellman *et al* (1961) with modifications according to Johnson and Wallace (1987). Statistical significance of the results of duplicate or triplicate analyses was assessed using the Student's t-test at $p \leq 0.05$ level.

Findings: Under the conditions of the study, the major metabolite identified was MeSO. The efficiency of extraction of MeSO and propoxur from the incubation mixtures was 96% and 97% respectively. The rate of sulfoxidation noted for different microsomal samples ranged from 3 to 6 nmol/min/mg of microsomal protein. A product derived from the hydrolysis of MeSO in the incubation mixture was identified but was not fully characterised. This hydrolytic product of MeSO represented about 3% of the total MeSO formed. No *in vitro* metabolism of sulfoxide (to form sulfone) by microsomal enzymes in the presence of NADPH was demonstrated.

The enzymatic metabolism of MeS by sulfoxidase formed 2 enantiomers of MeSO; A-MeSO and B-MeSO. The k_m values for the production of MeSO, A-MeSO and B-MeSO were 45, 47 and 179 µM respectively. During chromatographic analysis, no significant differences in the ratio of the two sulfoxide enantiomers were observed for the given substrate concentration range. FMO dependant sulfoxidation showed high stereoselectivity with an e.e of 88% in favour of A enantiomer. The A-MeSO fraction contained less than 1% of B-MeSO and the B-MeSO fraction contained less than 6% of A-MeSO.

Methiocarb sulfoxidase activity was modified by thermal and chemical treatments and inhibitors (reduced by 45% and 50-55% by clotrimazole and combined thermal and methimazole treatment respectively and lowered by combined thermal and clotrimazole treatment to 6% of the total activity). In the presence of antibodies against NCR, sulfoxidation was reduced by about 30-39% and when the antibodies against FMO were present in the incubation mixture, the uninhibited activity was further reduced from about 59% to 38%. Methimazole S-oxidation was completely inhibited by MeS.

Pretreatment with PB, 3MC and PYR reduced the total sulfoxidase activity to 67%, 50% and 29% of the control values respectively while DEX did not produce a significant effect. All inducers decreased the NCR activity by 38-51%. FMO activity was more variable representing 24-168% of the activity in untreated rats. When compared on the basis of their relative proportions, the FMO activity was about 4.6 times greater than the NCR activity in DEX induced livers while it was about 2.2 and 1.4 fold higher in PB and 3MC induced preparations respectively. Pretreatment with PYR resulted in a NCR activity greater than the FMO activity. These results indicate that the changes in the relative involvement of FMO and P450 in sulfoxidase activity in pretreated animals modified the stereoselectivity sulfoxidation. In further analysis, only the FMO mediated metabolism was used.

Based on the comparative ChE inhibition kinetic data, MeS and its metabolites were arranged according to the order of increasing inhibition: methiocarb sulfone ($MeSO_2$), A-MeSO, racemic MeSO, MeS and B-MeSO (k_i values were 0.03, 0.05, 0.22, 0.34 and 0.50 µM/minute respectively). According to the reported k_i values, B-MeSO appears to be a more potent ChE inhibitor (10 fold stronger) than A-MeSO. The rates of formation of B-MeSO in PB, 3MC, DEX and PYR pretreated animals were 109%, 76%, 190% and 25% respectively of the rates found in untreated animals showing about an 8 fold difference between the lowest and the highest rates of formation.

Conclusions: In an *in vitro* assay using rat liver microsomes, stereoselective metabolism of MeS by FMO and NCR and anticholinesterase activity of the two MeSO enantiomers (A-MeSO and B-MeSO) were studied. Under the conditions of the study, the major metabolite identified was MeSO. FMO dependant sulfoxidation showed high stereoselectivity with an e.e of 88% in favour of A enantiomer. No $MeSO_2$ was detected in the incubation mixture. Based on the comparative ChE inhibition kinetic data, MeS and its metabolites were arranged

according to the order of increasing inhibition: $MeSO_2$, A-MeSO, racemic MeSO, MeS and B-MeSO.

Metabolism and urinary excretion of methiocarb metabolites

Stanley CW & Johnson GA (1985) ®Metabolism of Mesurol phenol by rats. Study No. not stated. Lab: Research and Development Department, Agricultural Chemicals Division, Mobay Chemical Corporation. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 88899. Report date: January 23, 1985.

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

Study & Observations: This study was performed to investigate the metabolic fate and urinary excretion of orally administered Mesurol (methiocarb) phenol in male rats. Ring-1-[^{14}C]-labelled methiocarb phenol (purity>97%, specific activity 14.5 mCi/mmol) in ethanol/water (1:1) was administered by oral gavage to rats (3 rats, strain, age, source not stated, 280-300 g bw) at 0.19 mg/kg bw. After dosing, the animals were housed individually in modified plastic metabolic cages and urine was collected under conditions which ensured minimal degradation of metabolic products. The rats were given feed and water *ad libitum*. A 48 h composite urine sample was collected from each rat and assayed for radioactivity. A portion of each sample was chloroform partitioned, and the radioactivity in chloroform and the aqueous phase was determined. Aliquots of the aqueous phase was subsequently incubated with maltase for 24 h and chloroform partitioned to extract the organo-soluble metabolites. After concentration and radioassay, the metabolites in each chloroform extract were resolved by two dimensional TLC. No further information on methodology was provided.

Findings: Of the radioactivity administered, 77-81% was excreted in urine within 48 h. Chloroform extracted metabolites comprised about 3-4% of detected radioactivity in urine, while 73-78% of radioactivity remained in the aqueous phase. About 45% of the radioactivity excreted in urine was identified and three compounds were detected: unchanged methiocarb phenol (29-35%), methiocarb sulfoxide phenol (3-5%) and methiocarb sulfoxide (5%). The study authors stated that methiocarb phenol contained about 2% of methiocarb sulfoxide phenol and about 1% of methiocarb phenol can be converted to methiocarb sulfoxide phenol by incubating in pH 5 buffer for about 16 h. Therefore, the 3-5% methiocarb sulfoxide phenol fraction found in urine could be attributable to impurities in the dosing solution and artefact formation during enzyme hydrolysis. The fraction of methiocarb sulfoxide (5%) identified in urine could be an *in vivo* metabolic product of administered methiocarb phenol.

Conclusions: Under the conditions of the study, male rats did not readily convert methiocarb phenol to methiocarb sulfoxide phenol.

3. ACUTE TOXICITY

3.1 Technical Grade Active Constituent

3.1.1 Median Lethal Dose Studies

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

Methiocarb

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Mouse [NS]	M	15	Water & unspecified organic solvents	98.2	25, 35, 50, 75	52.3	Kimmerle (1972)
Rat [SD]	M/F	4/sex	Ethanol & propylene glycol	99	36, 43.2, 51.8, 62.2 (non-fasted) and 15, 30, 60, 120 (fasted)	46 (M: non-fasted) 47 (F: non-fasted) 30 (M/F: fasted)	Crawford & Anderson (1973)
Rat [SD]	M/F	NS	Ethanol & propylene glycol	NS	NS	130 (M) 135 (F)	Dubois & Raymund (1961)
Rat [SD]	F	25	Ethanol & propylene glycol	NS	NS	~100	Dubois & Raymund (1962)
Rat [NS]	M/F	10/sex	Lutrol (PEG)	NS	17.5, 20, 25, 30, 35 (M) 17.5, 20, 25, 30 (F)	22 (M), fasted 24 (F), fasted	Flucke (1978)
Rat [NS]	M	10	Lutrol (PEG)	98.3	15, 20, 25, 30, 35	22.1 (fasted)	Flucke (1980)

NS=Not stated; SD=Sprague-Dawley; Lutrol (PEG) = polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral (continued)							
Rat [NS]	M	5	Lutrol (PEG)	98.2	10, 14, 15, 16, 20, 25	19 (fasted)	Flucke (1988)
			Cremophor EL/ water		10, 16, 18, 20, 25, 31.5	26 (fasted)	
Rat [Sherman]	M/F	NS	Peanut oil	NS	NS	70 (M) 60 (F)	Gaines (1969)
Rat [NS]	M	5 or 10/group	Cremophor EL/ water	98.6	15, 16, 17, 18, 20, 25	17 (fasted)	Heimann (1983)
Rat [NS]	M	1 or 3/group	Water & tragacanth	NS	5, 10, 25, 50, 100, 250, 500	100	Kimmerle (1960)
Rat [Wistar-CFN]	M	10	Tween 80-Tylose	NS	25, 50, 75, 100, 125, 150	87	Klimmer (1963)
Rat [SD]	F	5	Lutrol (PEG)	NS	20, 40, 80, 160	49 (fasted)	Lamb & Matzkanin (1975b)
	F	5	Lutrol (PEG)	NS	2.5, 5, 10, 20	9 (fasted)	
	F	5	Ethanol & propylene glycol	NS	10, 20, 40, 80	22 (fasted)	
	F	5	Ethanol & propylene glycol	NS	5, 10, 20, 40	16 (fasted)	

NS=Not stated; SD=Sprague-Dawley; Lutrol (PEG) = polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral (continued)							
Rat [SD]	F	5	Lutrol (PEG)	NS	20, 40, 80, 160	57 (fasted)	Lamb & Matzkanin (1975c)
	F	5	Lutrol (PEG)	NS	5, 10, 20, 40	16 (fasted)	
	F	5	Ethanol & propylene glycol	NS	10, 20, 40, 80	31 (fasted)	
	F	5	Ethanol & propylene glycol	NS	5, 10, 20, 40	14 (fasted)	
Rat [SD]	M/F	5/sex	Lutrol (PEG)	NS	5, 10, 20, 40 (M) 10, 20, 40, 80 (F)	15 (M) 31 (F)	Lamb & Matzkanin (1976a)
Rat [SD]	M/F	10/sex	Lutrol (PEG)	NS	5, 10, 20, 40 (M) 10, 20, 40, 80 (F)	13 (M) 32 (F)	Lamb & Matzkanin (1976b)
Rat [SD]	M/F	10/sex	Lutrol (PEG)	NS	10.6, 13.8, 17.9, 23.3	14 (M) fasted 16 (F) fasted	Lamb & Matzkanin (1977)
					51.1, 66.5, 86.4 (M/F) and 39.3 (M), 112.3 (F)	51 (M) non-fasted 79 (F) non-fasted	
Rat [SD]	M/F	10/sex	Carbowax	98	15, 36, 86.4, 207.4 (M/F) and 6.3 (M)	33 (M) 47 (F)	Nelson (1979b)
Rat [NS]	M/F	10/sex	Lutrol (PEG)	98.6	25, 50, 75, 100, 150, 200	82.8 (M) 94.9 (F)	Thyssen (1975)

NS=Not stated; SD=Sprague-Dawley; Lutrol (PEG) = polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral (continued)							
Rat [SD]	M/F	5 or 10/sex	Lutrol (PEG)	98.7	25, 30.25, 39.33, 51.13, 66.47, 86.41, 112.33, 146.03 [dose volume: 0.25 mL/100 g bw]	74.56 (M) non-fasted 75.71 (F) non-fasted	Thyssen (1977a)
					3.71, 4.82, 6.27, 8.15, 10.59, 13.77, 17.9, 25 (M/F), 23.27 (F) [dose volume: 0.25 mL/100 g bw]	10 (M) fasted 10.85 (F) fasted	
					25, 30.25, 39.33, 51.13, 66.47, 86.41, 112.33, 146.03 [dose volume: 0.5 mL/100 g bw]	50.79 (M) non-fasted 64.72 (F) non-fasted	
					3.71, 4.82, 6.27, 8.15, 10.59, 13.77, 17.9, 23.27, 25 (M/F) [dose volume: 0.5 mL/100 g bw]	10.75 (M) fasted 14.14 (F) fasted	
					25, 30.25, 39.33, 51.13, 66.47, 86.41, 112.33, 146.03 [dose volume: 1 mL/100 g bw]	42.55 (M) non-fasted 41.3 (F) non-fasted	

SD=Sprague-Dawley; Lutrol (PEG) = polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral (continued)							
Rat [SD]	M/F	5 or 10/sex	Lutrol (PEG)	98.7	3.71, 4.82, 6.27, 8.15, 10.59, 13.77, 17.9, 23.27, 25 (M/F) [dose volume: 1 mL/100 g bw]	13.15 (M) fasted 10.84 (F) fasted	Thyssen (1977a)
Rat [NS]	M	10	Lutrol (PEG)	NS	17.5, 20, 25, 30, 40, 50	33.2 (fasted)	Thyssen (1977b)
					17.5, 20, 25, 30, 40	30.6 (fasted)	
					17.5, 20, 25, 30, 40, 50	35 (fasted)	
					17.5, 20, 25, 30, 40	28 (fasted)	
					25, 30, 40, 50	35.1 (fasted)	
Rat [NS]	M	1, 3 or 5/group	Water tragacanth suspension	NS	5, 10, 25, 50, 100, 250, 500	100	Kimmerle (1960)
Guniea Pig [NS]	M	4	Ethanol & propylene glycol	NS	5, 10, 20, 40	14.12	Crawford & Anderson (1972a)
Guinea Pig [NS]	M	4	Ethanol & propylene glycol	NS	5, 10, 20, 40	12.19	Crawford & Anderson (1972b)
Guinea Pig [NS]	M	25	Ethanol: propylene glycol	NS	NS	40	Dubois & Raymund (1961)
Guinea Pig [NS]	F	5	Emulsifier W	NS	25, 50, 100, 250	50 –100 (2/5 deaths at 50 mg/kg; 4/5 deaths at 100 mg/kg; 5/5 deaths at 250 mg/kg)	Kimmerle (1969a)
Rabbit [NS]	NS	2	NS	NS	25	>25 (0/2 deaths)	Kimmerle (1960)

NS=Not stated; SD=Sprague-Dawley; Lutrol (PEG) = polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral (continued)							
Dog [Beagle]	F	1 or 2/group	Emulsifier W)	NS	1, 5, 10, 25	≤ 25 (10; 0/2 deaths 25; 1/1 deaths)	Kimmerle (1969b)
Dog [Mongrel]	M/F	2/sex	Gelatin capsules	NS	5, 7.5, 11.25, 16.9, 25.4	~25.4	Lamb & Matzkanin (1975a)

NS=Not stated; SD=Sprague-Dawley; Lutrol (PEG) = polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Dermal (non-abraded)							
Rat [SD]	M	10	Ethanol: Propylene glycol	NS	100, 200	>200 (0 deaths)	Dubois & Raymund (1961)
Rat [SD]	F	10	Ethanol	NS	NS	>300 (deaths not stated)	Dubois & Raymund (1962)
Rat [Sherman]	M/F	NS	Xylene	NS	NS	>2000 (M/F) (0 deaths)	Gaines (1969)
Rat [Wistar-CFN]	M	5	Isopropanol	NS	100, 200, 400, 800 (without occlusion, and the applied material was not removed)	350 – 400 (0/5 deaths at 200 mg/kg; 3/5 deaths at 400 mg/kg; 5/5 deaths at 800 mg/kg)	Klimmer (1963)
Rat [NS]	M	5	Lutrol (PEG)	NS	500 (occluded, applied material was not removed)	>500 (0/5 deaths)	Solmecke (1969)
Rat [NS]	NS	NS	Oil	NS	1000 (2 h, occluded, applied material was removed) 1000 (4 h, occluded, applied material was removed)	>1000 (0 deaths)	Kimmerle (1960)

NS=Not stated; SD=Sprague-Dawley; PEG= Polyethylene glycol 400.

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Dermal (non-abraded, continued)							
Rat [Wistar]	M/F	5/sex	Lutrol (PEG)	98.1	100, 1000, 5000, 24 h	>5000 (M/F) (0/5 deaths/sex)	Thyssen (1977c)
Rabbit [NZW]	M/F	4/sex	Saline	NS	2000 (abraded; 24h, occluded, applied material was removed)	>2000 (M/F) (1/4 deaths/sex)	Crawford & Anderson (1972c)

Species (strain)	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Intraperitoneal							
Mouse [NS]	M/F	40/sex	Ethanol: Propylene glycol	NS	NS	6 (M) 5.5 (F)	Dubois & Raymund (1961)
Rat [SD]						35 (M) 30(F)	
Rat [SD]	F	NS	Ethanol & propylene glycol	NS	NS	25	Dubois & Raymund (1962)
Rat [NS]	NS	3	Water & tragacanth	NS	5, 10, 25, 50, 100, 250	100	Kimmerle (1960)
Rat [Wistar-CFN]	M	5	Tween 80-Tylose	NS	15, 20, 25, 30, 40, 50, 60, 70, 80	43	Klimmer (1963)
Rat [NS]	NS	3 or 5/group	Water & tragacanth	NS	5, 10, 25, 50, 100, 250	100	Wademeyer (1960)
Guinea Pig [NS]	M	30	NS	NS	NS	17	Dubois & Raymund (1961)

NS=Not stated; SD=Sprague-Dawley; NZW=New Zealand White, PEG= Polyethylene glycol 400.

NS=Not stated; SD=Sprague-Dawley

Species (strain)	Sex	Mode	Group Size	Vehicle	Purity (%)	Dose Tested (mg/m^3)	LC50 (mg/m^3)	Reference
Inhalation								
Mouse [Carworth]	F	NS	10	Ethanol	100	22, 270, 34.4, 39 (50% droplets ≤3μm)	>39 (1/10 deaths)	Dilley & Doull (1962)
Rat [SD] (1 h)	M/F	NS	4/sex	NS	NS	2000, 20000 (droplet sizes not given)	>20000 (0/4 deaths)	Crawford & Anderson (1972a)
Rat [SD]	F	NS	10	Ethanol	100	22, 27, 34.4, 39 (50% droplets ≤3μm)	>39 (0/10 deaths)	Dilley & Doull (1962)
Rat [NS] (1 h)	M	NS	20	1:1 alcohol: Lutrol (PEG 400)	NS	85, 280, 450 (droplet sizes not given)	>450 (0/20 deaths)	Kimmerle (1966)
Rat [NS] (4 h)						45, 86, 160, 308, 397 (droplet sizes not given)	>397 (6/20 deaths) approx. 535	
Rat [SD] (4 h)	M/F	Head only	10/sex	NS	98.8	199, 550, 799, 1443 (100% droplets ≤5.4μm)	585 (M) 433 (F)	Shiotsuka (1987a) [GLP]
Rat [SD] (1 h)	M/F	Head only	10/sex	NS	98.8	380, 927, 1388, 2037, 2374 (100% droplets ≤5.5μm)	1208 (M) 1144 (F)	Shiotsuka (1987b) [GLP]
Rat [Wistar]	M/F	Head only	10/sex	Ethanol: Lutrol (PEG 400) (1:1)	97.9	68, 100, 237, 322 (droplet sizes not given)	> 322 (M/F) (0/10 deaths)	Thyssen (1982)

NS=Not stated; SD=Sprague-Dawley

Clinical signs in acute toxicity studies were in general similar for all species tested, and predominantly consisted of muscarinic effects that are similar to those noted for other carbamates. These included: diarrhoea, salivation, lacrimation, vomiting, muscular tremors, paralysis, ataxia and convulsions.

3.1.1.1 Metabolites of methiocarb

Median lethal dose studies

Methiocarb sulfoxide

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [NS]	NS	10	PEG	NS	25, 30, 40, 50, 60, 1000	42.9	Institute of Toxicology (1970b)
Rat [SD]	M/F	5/sex	Lutrol (PEG 400)	NS	2.5, 5, 10, 20	9 (M) 7 (F)	Lamb & Matzkanin (1976a)
Rat [SD]	M/F	10/sex	Lutrol (PEG 400)	NS	2.5, 5, 10, 20	6 (M) 8 (F)	Lamb & Matzkanin (1976b)

SD=Sprague-Dawley; NS=Not stated

Methiocarb phenol

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [NS]	NS	10	PEG	NS	100, 250, 500, 1000	>1000 (0/10 deaths)	Solmecke (1970)
Rat [SD]	F	4	PEG/ Ethanol	NS	500, 1000	>1000 (0 deaths)	Dubois (1963)
Rat [NS]	M	4	Propylene glycol	NS	1000	>1000	Dubois (1964)
Dermal							
Rat [SD]	M	4	Xylene	NS	500, 1000	>1000	Dubois (1963)
Rat [NS]	M	4	Xylene	NS	1000	>1000	Dubois (1964)
Rat [SD]	M	4	Ethanol/ PEG	NS	500, 1000	>1000 (0 deaths)	Dubois (1964)

NS=Not stated; PEG=Polyethylene glycol 400.

N-hydroxymethyl methiocarb

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [SD]	M/F	10/sex	Carbowax	98	10.6, 13.8, 17.9, 23.3, 39.4, 66.7, 112.7	>112 (M/F) (0/10 deaths/sex)	Nelson (1979b)

Methiocarb sulfone

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [NS]	NS	10	PEG 400	NS	100, 250, 500, 1000	>1000	Institute of Toxicology (1970c)

NS=Not stated

N-hydroxymethyl methiocarb sulfone

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [SD]	M/F	10/sex	Carbowax	98	23.3, 39.4, 66.6, 112.2	>112 (M/F) (0/10 deaths/sex)	Nelson (1979b)

N-hydroxymethyl methiocarb sulfoxide

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [SD]	M/F	10/sex	Carbowax	98	2.5, 5, 10, 20, 40, 80, 160	>160 (M/F) (0/10 deaths/sex)	Nelson (1979b)

Methiocarb phenol sulfoxide

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [NS]	M	4	Ethanol/ PEG	NS	1000	>1000 (0 deaths)	Dubois (1964)
Rat [NS]	NS	10	PEG	NS	100, 250, 500, 1000	>1000 (0 deaths)	Institute of Toxicology (1974)
Dermal							
Rat [NS]	M	4	Xylene	NS	1000	>1000 (0 deaths)	Dubois (1964)

NS=Not stated

Methiocarb phenol sulfone

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Oral							
Rat [NS]	M	4	Ethanol/ PEG	NS	1000	>1000 (0 deaths)	Dubois (1964)
Rat [NS]	NS	10	PEG	NS	100, 250, 500, 1000	>1000	Institute of Toxicology (1970a)
Dermal							
Rat [NS]	M	4	Xylene	NS	1000	>1000 (0 deaths)	Dubois (1964)

NS=Not stated

3.1.2 Eye and Dermal Irritancy & Sensitisation Studies

A summary of the findings of eye and dermal acute irritancy and sensitisation studies are shown in the Table below.

Summary of the findings of eye and skin irritation and skin sensitisation studies

Route	Species	Sex	Group size	Method	Findings	Reference
Ocular	Rabbit [NZW]	-	6	100 mg in the right eye	Non irritant	Crawford & Anderson (1970)¶
Dermal	Rat	-	2	1.0g/kg bw to the shaved abdominal skin Occlusive	Non irritant	Dubois & Raymund (1961)
	Rabbit	-	-	Intact skin of the ear Occlusive	Non irritant	Dubois & Raymund (1961)
	Rabbit [NZW]	-	6	500 mg on intact and abraded skin Occlusive	Non irritant	Crawford & Anderson (1970)
Skin sensitisation	Guinea pig	M	20	Induction applications	Non sensitiser	Mihail (1984)
			30			David (1988)§

¶method modified from US FIFRA Guidelines. §US EPA and OECD Guidelines.

Crawford CR & Anderson RH (1970) The skin and Eye Irritating properties of BAY 37344 Technical to Rabbits. Research Department, Chemagro Corporation, Germany. Report Date: October 15, 1970.

Pre GLP non quality assured study. Method modified from Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) guidelines was used.

Study & Observations: A quantity of 100 mg of BAY 37344 technical (methiocarb, Control No. 8059504), was applied to right eye of mature NZW rabbits (6 animals, no source, age, sex or weight stated). The left eye served as a control. Eyes were examined 24, 48 and 72 h after application for signs of irritation using the method described in "Illustrated guide for Grading Eye Irritation by Hazardous Substances" by the U.S. Department of Health, Education and Welfare, Food and Drug Administration. Eyes were examined for corneal injury under UV light after placing 2% fluorescein sodium ophthalmic solution on the eye. No further description on methodology or any non-ocular effects was provided.

Findings & Conclusions: Under the conditions of the study, methiocarb was not an eye irritant in rabbits.

Dubois KP & Raymund AB (1961) The acute toxicity of Bayer 37344 to mammals. Department of Pharmacology, University of Chicago, U.S.A. Report date: March 25, 1960.

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

Rat

Study & Observations: H 321 (methiocarb, E 37344, Production No: 2410) suspended in oil was applied at a dosage of 1.0 g/kg bw to the shaved abdominal skin of rats (2 animals) (source, sex, age, weight not stated) fastened down on their backs. After 2 h exposure period the test substance was removed. No further information was provided.

Findings & Conclusion: No signs of skin irritation were observed. Similarly, no dermal irritation was reported when the same dosage of the test chemical was left on the abdominal skin for a 4 h period. But the treated animals were observed to suffer from muscular spasms which started about 2 h after the removal of chemical. The animals later recovered and methiocarb was not a skin irritant.

Rabbit

Study & Observations: Cotton wool compresses containing Bayer 37344 (methiocarb, Production No: 2410, source, purity not stated) in dry form or moistened with oil or water were placed inside a rabbit's ear (no source, sex, age, weight stated). Compresses containing the test substance were kept in place for 24 h using adhesive bandages. No further information on methodology or clinical observations was provided.

Findings & Conclusions: Under the conditions of the study, no symptoms of irritation were observed. Methiocarb was not a skin irritant to rabbits.

Crawford CR & Anderson RH (1970). The Skin and Eye Irritating properties of BAY 37344 Technical to Rabbits. Research Department, Chemagro Corporation, Germany. Report Date: October 15, 1970.

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

Study & Observations: Technical grade methiocarb (Control No. 8059504, purity not stated) was applied to the shaved, abraded and intact skin in aliquots of 500 mg to mature New Zealand White rabbits (6 animals, no source, age, sex or weight stated). Two test sites on each animal (1 abraded and 1 intact) were used. A one inch square of gauze was placed over each test site and then an unspecified volume of acetone was applied to each patch. To prevent shifting and to retard evaporation, a piece of rubber glove of about 5^2 cm was placed over the compound and patch. The trunk of each animal was then wrapped with elastoplast. The wrapping was removed after 24 h and the treated areas were examined at this time, and again at 72 h post treatment. No further methodological information was provided and no signs of toxicity were reported.

Findings & Conclusions: No evidence of erythema or oedema was observed in any of the test animals under the test conditions of the study. Methiocarb technical was not a skin irritant to rabbits.

Skin sensitisation

Mihail F (1984) Study for skin sensitising effect on Guinea Pigs. Institute of toxicology, Bayer AG, Germany. Report Date: December12, 1984.

Quality assured study conformed to the OECD principles of Good Laboratory Practice (GLP, Bundesanzeiger 35: 3-16. March 2, 1983). No test guidelines were cited.

Study & Observations: Methiocarb (batch No. 234 302 629, purity 97.8%) was administered intradermally in 0.1 mL aliquots to paired skin sites (cranial, medial, caudal) on the flanks of 20 male Guinea pigs (BOR: DHPW, Borchen, 280-365 g bw, animal acclimatisation period was not stated). The skin was shorn 24 h before injection and the interval between injection sites was approximately 1-2 cm. The three pairs of sites were injected with either (a) Freund's complete adjuvant diluted 1:1 with demineralised water, (b) 1% methiocarb formulated in 0.9% NaCl solution using 2% Cremophor EL V/V or (c) 1% methiocarb formulated in 0.9% NaCl solution using 2% Cremophor EL v/v and Freund's complete adjuvant diluted 1:1 with demineralised water.

Two control groups of 10 animals were treated similarly to the test group but formulations for sites b and c did not contain any test compound. No positive control was used.

One animal in the test compound group died after intra-dermal induction exposure (time, cause of death, autopsy or histopathological findings were not provided).

One week after the intra-dermal injections, the sites were topically induced either with methiocarb 25% formulated in 0.9% NaCl solution using 2% Cremophor EL V/V (test group) or formulation vehicles but without the test compound (control groups). Test animals and the animals in the first control group were challenged (2 and 3 weeks after topical and intra-dermal induction respectively) using a hypoallergenic dressing soaked with 25% test compound formulated using the same vehicle. Each animal was also treated with a second dressing soaked with the vehicle alone. The treated skin areas were grossly appraised 24 and 48 h after removal of the dressing. "To eliminate the concentration related effects", animals in the test compound group and the second control group were rechallenged (four and five weeks after topical and intra-dermal induction respectively) with 12.5% test compound formulation (same vehicle) and with the vehicle alone.

Findings: Seven animals in the test compound group reacted positively (grade 1 skin redness) to the compound and control dressing after first challenge application (0% difference). However, there were three more animals in the test compound group reacting positively to the compound dressing after the second challenge compared to the control dressing (13 and 10 positive reactions respectively, 15.8% difference). No differences in positive reactions were observed in any of the animals in two control groups after first (2 positive reactions, each for compound and control dressings) or second challenge (6 positive reactions for compound and control dressings). Results of the study as provided by the authors are presented in the table below.

Study for skin sensitising effect of methiocarb on guinea pigs (adjusted group results)

Test compound (n=19)		**Control group 1 (n=10)**	
Methiocarb	Control	Methiocarb	Control
First challenge			
7	7	2	2
Adjusted value			
0 (0%)	-	0 (0%)	-
Second challenge			
13	10	6	6
Adjusted value			
3 (15.8%)	-	0 (0%)	-

n=number of animals tested

Percent difference in positive reactions shown by animals of the test compound group observed after second challenge however, was not considered significant by the study authors.

Conclusions: Under the conditions of the study, methiocarb did not induce skin sensitisation in guinea pigs.

David RM (1988) EPA/OECD Dermal Sensitisation test of Mesurol technical in guinea pigs. Project No: G-7081.245, Lab: Microbiological Associates, Inc., 5221 River Road, Bethesda, MD 20816-1493, USA. Sponsor: Mobay Corporation, Health, Environment and Safety, 17745 South Metcalf, Stllwell, KS 66085, USA. Study duration: December 9, 1987 to January 9, 1988. Report No: 1015, Report date: April 15, 1988.

GLP, quality assured study conducted according to US EPA and OECD guidelines.

Study: Methiocarb technical (Technical lot #86I004, 99.2%, stability 7 years at room temperature) was administered dermally in 0.5 g quantity (per animal/day, moistened with saline) to shaven backs (4 cm^2 area, shaved 1-3 h prior to induction or 24 h prior to subsequent challenge) of male Hartley guinea pigs (Hazelton Research Products, Denver, PA, 5 wks old, 360-462 g initial bw) once weekly for three weeks (induction exposures) followed by a challenge dose after a two-week rest period. The animals were quarantined for approximately two weeks before the commencement of the study. Test animals were housed two/cage during quarantine and then individually and provided with food (Purina certified guinea pig chow) and water *ad libitum*. The 3 concurrent common control groups consisted of 5 guinea pigs/group. The complete study design is outlined below.

Study design

Treatment Group	**Treatment**	**Number of animals**	**Exposure regimen**
1	Test chemical (neat)	15	Once weekly for 3 wk, challenge dose 2 wk later
2	Test chemical (neat)	5	Once at time of challenge dose
3	DNCB0.05%)	5	Once weekly for 3 wk, challenge dose 2 wk later
4	DNCB (0.05%)	5	Once at time of challenge dose

The dosage of 0.5 g was selected, based on the information gathered from a preliminary range finding study.

Range-finding study: Five guinea pigs received single 24 h exposures of 0.5 mL of 12.5%, 25%, 50% (w/v) Mesurol technical in 80% reagent alcohol (90% ethanol + 5% methanol +

5% isopropyl alcohol) in water and the neat chemical (0.5 g moistened with saline) each at a different site. The chemical was introduced on a 25 mm diameter Hilltop chamber and by placing it (without a gauze pad for the neat chemical) on the shaved area. The chamber was then secured in place with elastic and adhesive tapes. After 48 and 72 h of dosing the sites were examined for irritation/sensitisation and scored according to the method of Ritz and Buehler (1980).

Slight erythema was noticed in one animal exposed to 12.5% and the neat chemical. No reactions were observed at any other dose levels. Because the reaction was only slight erythema, the neat chemical was chosen as the non-irritant dose for the main study.

Main study: The test compound was introduced to the animals according to the procedure described above. The chamber containing the chemical was left in place for approximately 6 h and 24 h for induction and challenge exposures respectively. The animals in the positive control group received (group 3) 0.5 mL of dinitrochlorobenzene (DNCB, 0.05% in reagent alcohol) by the same procedure. After the last induction dose, the test animals received a two week rest period at the end of which groups 1 and 3 were administered a challenge dose using a Hilltop chamber on a site different from area used for induction. The primary challenge control groups (groups 2 & 4) were treated with a single dermal dose of either 0.5 g of Mesurol technical or 0.5 mL of 0.05% DNCB as described above. The application sites were observed for signs of irritation/sensitisation approximately 24 and 48 h following each induction exposure and approximately 48 and 72 h following the challenge according to the method of Ritz and Buehler (1980). To facilitate scoring, the application sites were depilated 24 h after the challenge dose.

The study data were analysed by calculating the "incidence index" (the number of animals showing a response grade of one or greater at either 48 or 72 h out of the total number of animals of that group) and "severity index" (by dividing the sum total of grades in a given treatment group by the total number of animals in that group for both 48 and 72 h response readings). In addition, the body weights were recorded on day 1 of the test for all treatment groups and on the last day of the study for all test animals. Data on food and water consumption were not provided.

Findings: Four animals in the induction group died (two within 24 h and two within 48 h) after the challenge dose without clinical signs being observed. Body weights of the animals were not affected by treatment.

No skin reactions were noticed either at 24 or 48 h following both first and second inductions. Following the third induction exposure, slight erythema was noticed in one animal. After the challenge dose slight erythema was observed in a different animal in the induction group at 72 h. The skin reactions of animals in the induced positive (DNCB) control group (group 3) varied from no erythema after first induction to slight irritation following second and third induction doses. Four animals showed slight erythema while one developed moderate erythema after challenge DNCB exposure. Similar skin reactions were observed in non-induced animals in group 4 following DNCB challenge whilst no responses were noted in animals in group 2 which received only a challenge dose of the test chemical. Response data are summarised in the following table.

Skin responses of treated animals

Treatment group	Observation time	Number of animals responding				Incidence index	Severity index
		Response grade					
		0	1	2	3		
1. Methiocarb technical	48 h	11	0	0	0	0/11	0
	72 h	10	1	0	0	1/11	0.1
2. Methiocarb technical challenge only	48 h	5	0	0	0	0/5	0
	72 h	5	0	0	0	0/5	0
3. 0.05% DNCB	48 h	1	3	1	0	4/5	1.0
	72 h	0	4	1	0	5/5	1.2
4. 0.05% DNCB challenge only	48 h	4	1	0	0	1/5	0.2
	72 h	1	3	1	0	4/5	1.0

The animals that died during the experiment were necropsied but the findings of only two out of the four animals were provided. The reports revealed that one animal had excessive salivation (soiling around mouth), an indication of carbamate toxicity and both had intussusceptions of the intestine.

Conclusions: Under the conditions of the study, methiocarb technical caused slight skin irritation in treated animals. However, as assessed by using the incidence and severity indices, methiocarb technical was not considered a dermal sensitiser. Further, the available necropsy findings and mortality data in dermal toxicity studies with rabbits suggest that the mortality in guinea pigs in the present study (4/15 animals) is an effect related to treatment.

3.2 Formulations

A summary of findings of acute dose studies with methiocarb 75% concentrate formulation is shown in the Table below. In all studies, doses quoted refer to the weight of the formulation.

3.2.1 Mesurol 75% 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 (mg/m^3)	Reference
Oral	Rat [SD]	M/F	5/sex	12.5, 25, 50, 100 (M/F) plus 150, 200 (M), 75, 125 (F)	82 (M) 23 (F)	Eigenberg (1988a) [GLP]
Dermal	Rabbit [NZW]	M/F	5/sex	500, 1000, 2000 (M/F) plus 250 (M) (24 h, with occlusion and the applied material was removed)	805 (M) 704 (F)	Eigenberg (1988b) [GLP]
Inhalation (4 h, head only)	Rat [SD]	M/F	6/sex	348, 529 (M/F) plus 433 (M), 234 (F) (100% droplets ≤4.9μm)	479 (M) 403 (F)	Shiotsuka (1988) [GLP]

SD=Sprague-Dawley; NZW=New Zealand White

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbit [NZW], M/F	3/sex	100 mg conjunctival sac, unrinsed	Slight irritant	Eigenberg (1988)¶
Dermal	Rabbit [NZW], M/F	3/sex	500 mg intact, occlusive	Non-irritant	
Skin Sensiti-sation	Guinea pigs, M	15/group 5/group x 3	500 mg, induction exposure	Non-sensitiser	David (1988)§

¶US EPA& FIFRA guidelines. §US EPA & OECD guidelines.

Eigenberg DA (1988) Primary eye irritation of Mesurol 75% concentrate in albino rabbits. Project No. 87-323-05, Lab: Mobay Corporation, Health, Environment and Safety, Corporate Toxicology Department, 17745 South Metcalf, Stilwell, KS 66085-9104. Sponsor: Mobay Corporation, Agricultural Chemical Division, Box 4913, Hawthorn Road, Kansas City, Missouri 64120-0013 USA. Study duration: September 28, 1987 to October 2, 1987. Report No. 978, Report Date: January 27, 1988.

GLP, quality assured study. Conducted according to the US EPA-FIFRA Pesticide Assessment Guidelines of November, 1984 and the US EPA-TOSCA Health Effects Test Guidelines of September, 1985.

Study & Observations: The eye irritancy potential of Mesurol 75% concentrate (methiocarb technical content 77.1%, Batch No. 7035135, stability: indefinite at freezer conditions, solubility: unspecified, formulation details: provided, appearance: solid off-white powder) was examined in adult NZW rabbits (3/sex, bw, age not stated, Small Stock Industries, Pea Ridge, Arkansas, USA). Lesions of the cornea, iris and conjunctiva following instillation of 100 mg of the formulation into the conjunctival sac of the left eye of each rabbit were examined and scored at 1, 24, 48, 72 h post treatment. The right eye served as a control. The

animals were housed individually under conventional laboratory conditions and provided with food and water *ad libitum*.

Findings: No corneal or iridial changes were observed in any of the test animals. Conjunctival redness was seen up until 24 h in three rabbits, 48 h in one rabbit (grade 1) and 72 h in the two remaining animals (grade 1-2). The mean irritation grades at 24, 48 and 72 h were 0.5, 0.3 and 0 respectively. Grade 1 conjunctival swelling was seen in two rabbits at 1 h post treatment and not thereafter. Grade 3 ocular discharge was observed in two rabbits at 1h post treatment and not afterwards. Constriction of the pupil of the treated eye was noted at 1 h post treatment in two rabbits but was reversed by 24 h. No other information on clinical observations was provided.

Conclusions: On the basis of these findings, methiocarb 75% concentrate was considered an eye irritant in rabbits by the study authors. Under Australian guidelines, the formulation is classified as a slight eye irritant.

Eigenberg DA (1988) Primary dermal irritation of ®Mesurol 75% concentrate in albino rabbits. Project No. 87-323-04, Lab: Mobay Corporation, Health, Environment and Safety, Corporate Toxicology Department, 17745 South Metcalf, Stilwell, KS 66085-9104. Sponsor: Mobay Corporation, Agricultural Chemical Division, Box 4913, Hawthorn Road, Kansas City, Missouri 64120-0013 USA. Study duration: September 28, 1987 to October 2, 1987. Report No. 978, Report Date: January 27, 1988.

GLP, quality assured study. Conducted according to the US EPA-FIFRA Pesticide Assessment Guidelines of November, 1984 and the US EPA-TOSCA Health Effects Test Guidelines of September, 1985.

Study: Mesurol 75% concentrate (methiocarb technical content 77.1%, 0.5 g/animal, batch: 7035135, formula number: 011142, stability: indefinite at freezer conditions) was applied to a 6 cm^2 area of shaven skin (the backs and sides) of six adult NZW rabbits (3/sex, Small Stock Industries, Pea Ridge, Arkansas, age and bw not stated). The test animals were acclimatised to the test conditions for at least 6 days prior to the initiation of the study. After application of the test formulation the sites were covered with a gauze patch secured with hypoallergenic tape which was later covered with a square of plastic and held in position with the use of adhesive bandage. Plastic collars were used on animals to prevent access to the treatment sites. They were housed individually in stainless steel cages and had access to food and water ad libitum. After 4 h of exposure the patch, tape, plastic square, adhesive material and the collar were removed. Any remaining test material on the site was then cleaned by gently wiping with a paper towel moistened with tap water.

The treatment sites were examined for erythema and oedema within 0.5-1 and at 24, 48, and 72 h after removal of the patch and graded according to the scoring system described in the followed test guidelines. The adjacent untreated skin was used as a control. The treatment sites were shaved 24 h prior to scoring for irritation. Other skin lesions and toxic responses were also noted. The "Individual Irritation Index" for each rabbit was calculated by adding the erythema and oedema scores of each animal at 0.5-1, 24, 48 and 72 h time points and dividing it by 4. The individual values were then averaged to calculate the "Primary Irritation Index".

Findings & Conclusions: Under the conditions of the study, no erythema or oedema was noted in treated animals. Based on the Primary Irritation Index of 0.0 observed in this study, methiocarb 75% concentrate was not a primary skin irritant in adult, male and female NZW rabbits.

Skin sensitisation

David RM (1988) EPA/OECD Dermal Sensitisation test of Mesurol 75% concentrate in guinea pigs. Project No: G-7080.245, Lab: Microbiological Associates Inc., 5221 River Road, Bethesda, MD 20816-1493, USA. Sponsor: Mobay Corporation, Health, Environment and Safety, 17745 South Metcalf, Stillwell, KS 66085, USA. Study duration: December 9, 1987 to January 9, 1988. Report No: 1010, Report date: April 14, 1988.

GLP, quality assured study conducted according to US EPA and OECD guidelines.

Study: Mesurol 75% concentrate (methiocarb technical content 77.1%, Lot #7035135, stability: 2 yrs at room temperature) was administered dermally in 0.5 g quantity (per animal/day, moistened with saline) to shaven backs (4 cm^2 area, shaved 1-3 h prior to induction or 24 h prior to subsequent challenge) of male Hartley guinea pigs (Hazelton Research Products, Denver, PA, 5 wks old, 350-460 g initial bw) once weekly for three weeks (induction exposures) followed by a challenge dose after a two-week rest period. The animals were quarantined for approximately two weeks before the commencement of the study. Test animals were housed two per cage during quarantine and individually afterwards and provided with food (Purina certified guinea pig chow) and water *ad libitum*. The 3 concurrent common control groups consisted of 5 guinea pigs/group. The study design is outlined in the table below.

Study design

Treatment Group	Treatment	Number of animals	Exposure regimen
1	Test chemical (neat)	15	Once weekly for 3 weeks, challenge dose 2 weeks later
2	Test chemical (neat)	5	Once at time of challenge dose
3	DNCB*(0.05%) (positive control)	5	Once weekly for 3 weeks, challenge dose 2 weeks later
4	DNCB*(0.05%)	5	Once at time of challenge dose

The dosage of 0.5 g was selected based on the information gathered from a preliminary range finding study.

Range-finding study: Five guinea pigs received a single 24 h exposure of 0.5 mL of 12.5%, 25%, 50% (w/v) Mesurol 75% concentrate in 80% reagent alcohol (90% ethanol + 5% methanol + 5% isopropyl alcohol) in water and the neat formulation (0.5 g moistened with saline) each at a different site. The chemical was introduced on a 25 mm diameter Hilltop chamber and by placing it (without a gauze pad for the formulation) on the shaved area. The chamber was then secured in place with Coban elastic and adhesive tapes. After 48 and 72 h of dosing the sites were examined for irritation/sensitisation and scored according to the method of Ritz and Buehler (1980).

Slight erythema was noticed in one animal exposed to 50% solution after 48 h. No reactions were observed at any other dose levels. The reaction at 50% level was not considered as meaningful by the study authors and hence, the neat chemical was chosen for the main study.

Main study: The test compound was introduced to the animals according to the procedure described above. The chamber containing the test formulation was left in place for approximately 6 and 24 h for induction and challenge exposures respectively. The animals in the positive control group received (group 3) 0.5 mL of dinitrochlorobenzene (DNCB, 0.05% in reagent alcohol) by the same procedure. After the last induction dose, the test animals received a two week rest period at the end of which groups 1 and 3 were given a challenge

dose using a Hilltop chamber on a site different from the area used for induction. The primary challenge control groups (groups 2 & 4) were treated with a single dermal dose of either 0.5 g of Mesurol 75% concentrate or 0.5 mL of 0.05% DNCB as described above. The application sites were observed and graded for signs of irritation/sensitisation approximately 24 and 48 h following each induction and 48 and 72 h following the challenge exposure, according to the method of Ritz and Buehler (1980). To facilitate scoring, the application sites were depilated 24 h after the challenge dose.

The study data were analysed by calculating the "incidence index" (the number of animals showing a response grade of one or greater at either 48 or 72 h out of the total number of animals of that group) and "severity index" (by dividing the sum total of grades in a given treatment group by the total number of animals in that group for both 48 and 72 h response readings). In addition, the body weights of all animals were recorded on day one and on the last day of the study.

Findings: Body weights of the animals were not affected by the treatment. No skin reactions were observed either at 24 or 48 h scoring times following either the primary, secondary or tertiary inductions. Similarly, no skin reactions were observed in groups 1 or 2 after the challenge dose. The response of the animals in the induced positive (DNCB) control group (group 3) varied from no erythema after first induction to slight irritation following second and third induction doses. Four animals showed slight erythema while one developed moderate erythema after challenge DNCB exposure. Similar skin reactions were observed upon challenge in non-induced positive controls (group 4). No other information on clinical observations was provided.

Conclusions: Under the conditions of the study and based on the results observed, methiocarb 75% concentrate was not considered a dermal sensitiser in male guinea pigs.

3.2.2 Mesurol 75% 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
Oral	Rat [Hz]	M/F	4/sex	50, 75, 100, 140, 160, 200	130 (M) 140 (F)	DuBois (1970a)
Oral	Rat [SD]	M/F	4/sex	36, 47, 60, 78, 100 (M/F) plus 130, 170 (M), 28 (F)	100 (M) 60 (F)	Lamb & Matzkanin (1975e)
Dermal	Rabbit	NS	2	1000 (24 h, occluded)	>1000 (0/2 deaths)	DuBois (1970a)
Dermal	Rabbit [NZW]	M/F	2/sex	2000 (abraded; 24 h, occluded and the applied material was removed)	>2000 (M/F) (0/2 deaths/sex)	Crawford & Anderson (1972c)
Dermal	Rabbit [NZW]	M/F	2/sex	5000 (abraded; 24h occluded and the applied material was removed)	>5000 (M/F) (0/2 deaths)	Nelson (1979a)
Inhalation (1 h,whole body)	Rat [SD]	M/F	4/sex	2000, 5000, 20000 (particle sizes not stated)	>20000 (M/F) (0/4 deaths/sex)	Crawford et al (1970)

SD=Sprague-Dawley; NZW=New Zealand White; Hz=Hotzman

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	6	100 mg, unrinsed	Slight irritant	Crawford & Anderson (1971)¶
Dermal			200 mg paste intact, abraded, occlusive	Non-irritant	

¶Method modified from US FIFRA guidelines.

Crawford CR & Anderson RH (1971) The skin and eye irritation properties of Mesurol 75% WP to rabbits. Reference: 70-189, Lab: Research Department, Chemagro Corporation. Sponsor: Bayer AG, Chemagro Corporation. Study duration: Not stated. Report No. 29624, Report date: March 9, 1971.

Pre GLP, non-quality-assured study.

Eye irritation

A modification of the method for skin and eye irritating properties recommended by the Agricultural Research Service for Revision of Interpretation 19, Revision II of the Federal Insecticide, Fungicide and Rodenticide Act (of the USA) was used.

Study & Observations: One hundred mg of Mesurol 75% WP (methiocarb technical content 78%, control no. 0050428, solubility and stability data not provided) was placed in the left eye of each of six mature NZW rabbits (age, bw, sex and source not stated). The right eye served as a control. After 24 h, the eyes were examined for signs of irritation according to procedures described in the "Illustrated Guide for Grading Eye Irritation by Hazardous Substances" of the US FDA. The treated eyes were also examined under UV for corneal injury after placing a drop of 2% fluorescein ophthalmic solution in the eye. Further observations were made at 48 and 72 h post treatment.

Findings: Slight erythema in the iris was seen in 2/6 rabbits at 24 h post treatment. This ocular response was cleared 48 h after the treatment.

Conclusions: Under the condition of the study, methiocarb 75% WP was not classified by the study authors an ocular irritant in rabbits. However, under Australian guidelines, the formulation is considered to be a slight eye irritant.

Skin irritation

A modification of the method for skin and eye irritating properties recommended by the Agricultural Research Service for Revision of Interpretation 19, Revision II of the Federal Insecticide, Fungicide and Rodenticide Act (of the USA) was used.

Study & Observations: Mesurol 75% WP (methiocarb technical content 78%, Control No. 0050428, solubility and stability data not provided.) was applied to clipped, abraded and intact skin areas on the back of six rabbits (age, sex, bw, source not specified). The formulation was mixed with water (400 mg/mL) to make a paste and 0.5 mL of the mixture was then applied on about a 2.5 cm^2 gauze patch. A piece of rubber glove (approximately 24 cm^2) was placed over the patch to prevent shifting and evaporation, and the trunk of the animal was then wrapped with Elastoplast bandage. The treated areas were examined without rinsing upon removal of the patch after 24 h, and again at 72 h. No further information on methodology or clinical observations was provided.

Findings & Conclusions: No erythema or oedema was noted during the 72 h observation period. Under the conditions of the study, methiocarb 75% WP was not a primary skin irritant in rabbits. However, the study authors appear to have used 200 mg of the test substance per animal in the study as opposed to the dose of 500 mg per animal as specified in the current OECD guidelines.

3.2.3 Mesurol 75% Seed treatment

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Inhalation (1 h, whole body)	Rat [SD]	M/F	4/sex	2000, 5000, 20000 (droplet sizes not stated)	>20000 (M/F) (0/4 deaths/sex)	Crawford et al (1970)
	Rat [SD]	M/F	10/sex	20000 (droplet sizes not stated)	>20000 (M/F) (0/10 deaths/sex)	Lamb & Anderson (1977b)

SD=Sprague-Dawley

Eye and dermal irritancy studies

Study	Species/ Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	9	50 mg, rinsed (3) unrinsed (6)	Irritant	Nelson & Burke (1977)
Dermal		6	500 mg intact, abraded occlusive	Non-irritant	

NZW=New Zealand White

Nelson DL & Burke MA (1977) Eye and dermal irritancy of Mesurol 75% seed treater. Reference: 77-163, Lab: Research and Development, Chemagro Agricultural Division, Mobay Chemical Corporation. Sponsor: Bayer AG, Germany. Study duration: Not stated, Report No. 54140, Report date: December 22, 1977.

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

Eye irritation

Study & Observations: A quantity of 50 mg of Mesurol 75% seed treater (Batch No. 7030061, Formula No. 1254, methiocarb technical content: 78%, solubility and stability data not provided) was applied into the left eye of each of 9 adult, NZW rabbits (age, sex, bw, source not stated). The right eye of the animal served as a control. Three rabbits had their eyes washed with 200 mL lukewarm water 45 seconds after the test compound application (group A) and the eyes of the remaining 6 rabbits were not rinsed (group B). Evidence of ocular irritation (responses in the cornea, iris, conjunctivae) was assessed on days 1, 2, 3, 4 and 7 post treatment.

Findings: Slight erythema (score of 1) was seen in 1 animal in group A up until day 2 post treatment. In group B, two treated rabbits exhibited slight erythema up until day 3, and 2 up until day 2 while in the fourth animal the condition was seen only on day 1. No other information on clinical observations was provided. The mean scores for erythema in group B animals at day 1 and 3 were 0.7 and 0.2 respectively.

Conclusions: Based on the above observations, methiocarb 75% seed treater was not considered an eye irritant in rabbits by the study authors, but would be classified as a slight eye irritant under Australian guidelines.

Skin irritation

Study & Observations: A quantity of 0.5 g (per site) Mesurol 75% seed treater (Batch No. 7030061, Formula No. 1254, methiocarb technical content: 78%, solubility and stability data not provided) was applied to shaved abraded and intact skin on the back and sides (area not specified) of 6 adult, NZW rabbits (age, sex, bw, source not stated) under a gauze patch. The patches were secured in place by wrapping the trunk of the animal with a sheet of plastic and adhesive tape. Animal holders were used to restrain the rabbits. After 24 h the patches were removed and the skin sites were evaluated. The test substance on the site was removed by washing the backs of the animals with acetone. The next evaluation was done 2 days later and at both times the degrees of erythema/eschar and oedema formation were graded.

Findings & Conclusions: No skin reactions were noted in any of the test animals either at 24 or 72 h following the test formulation application. No other information on clinical observations was provided. Under the conditions of the study, methiocarb 75% seed treater was not a skin irritant in rabbits.

3.2.4 Methiocarb 70% WP

According to the letter of Bayer Australia dated November 19, 1999, the actual concentration of methiocarb technical in this formulation is 75%.

Median lethal dose studies

Route	Species	Sex	Group Size	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral	Rat [Hz]	M/F	4/sex	50, 100, 120, 140, 160, 180, 200	160 (M/F)	DuBois (1970b)
Dermal	Rabbit	NS	2	1000 (24h, with occlusion)	>1000 (0/2 deaths)	

Hz=Holtzman

3.2.5 Mesurol 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
Oral	Mouse [NMRI]	M	15	5, 10, 25, 50, 75, 100, 125, 150	84.5	Gröning & Kimmerle (1975)
		F		5, 10, 15, 20, 25, 35, 50, 60	29.7	
	Rat [Wistar]	M/F	15/sex	5, 10, 25, 50, 75, 100, 150, 200, 250 (M/F) plus 125, 500 (F)	139 (M) 155 (F)	
Oral	Rat [Wistar]	M/F	5/sex	20, 50, 200	>50 (M/F: 0/5 deaths) <200 (M:4/5 deaths; F: 5/5 deaths)	Bomann (1995a) [GLP]
Oral	Rat [SD]	F	NS	NS	65	Dubois & Raymund (1962)
Oral	Rat [NS]	M/F	10/sex	60, 70, 80, 100, 120 (M/F) plus 130 (M), 140 (F)	94 (M) 98 (F)	Flucke (1978)
Dermal	Rat [Wistar]	M/F	5/sex	5000 (24 h, occluded, applied material was removed)	>5000 (M/F) (0/5 deaths/sex)	Bomann (1995b)
Dermal	Rat [SD]	F	NS	NS	>500 (deaths not stated)	Dubois & Raymund (1962)
Dermal	Rat [Wistar]	M/F	NS	500 (24 h, occluded, applied material was removed)	>500 (0 deaths)	Gröning & Kimmerle (1975)
Inhalation (1 or 4 h, head only)			10/sex	31, 60, 212 (1 h) 19, 76, 194 (4 h) (droplet sizes not stated)	>212 (1 h) (M/F) >194 (4 h) (M/F)	
Inhalation (4 h, nose only)	Rat [Wistar]	M/F	5/sex	64, 675, 863, 1035, 5043 (50% droplets <3µm)	798 (M) 899 (F)	Martins (1996) [GLP]
Intraperit--oneal	Rat [SD]	F	NS	NS	62.5	Dubois & Raymund (1962)
Intraperit--oneal	Rat [Wistar]	M	15	2.5, 5, 10, 25, 40, 50, 75, 100	56.8 (M)	Gröning & Kimmerle

		F		2.5, 5, 10, 25, 35, 50, 75, 100, 125	75.5 (F)	(1975)

NS=Not stated; SD=Sprague-Dawley

Eye and dermal irritancy and sensitisation studies

Study	Species/ Sex	Formulation	Group size	Method	Result	Reference
Ocular	Rabbit	Mesurol 50% WP	2	50 mg, conjunctival sac, unrinsed	Non-irritant	Groning & Kimmerle (1975)
	Rabbit [NZW], F	H321 50WP 0430/0363	3	25 mg, conjunctival sac, rinsed	Slight irritant	Krotlinger (1995)¶§
Dermal	Rabbit	Mesurol 50%WP	2	500 mg intact, occlusive	Non-irritant	Groning & Kimmerle (1975)
	Rabbit [NZW], F	H321 50WP 0430/0363	3	500 mg intact, semi-occlusive	Non-irritant	Krotlinger (1995)¶§
Skin sensiti-sation	Guinea pigs, M	H321 50WP00430/ 0363	20/group 10/group x 2	500 mg, induction exposure	Sensitiser	Vohr (1995)¶

¶OECD Guidelines.§EEC Directives. NZW=New Zealand White.

Eye irritation

Groning P & Kimmerle G (1975). Study of acute oral intraperitoneal and dermal toxicity, acute and subacute inhalation toxicity and skin and mucous membrane tolerance. Institute of Toxicology, Bayer AG, Germany, Report date: February 12, 1975.

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

Study: Approximately 50 mg of Mesurol 50 WP (methiocarb, Batch No. X0001A, in the form of whitish yellow powder), was placed in the conjunctival sac of the right eye of each of two albino rabbits (Source: Winkelmann and Gierlich; Bochum, 3.4 - 4.5 bw, sex, age not stated). No irritation of the conjunctivae or alterations of the cornea were observed in the treated eyes. No further information on methodology (clinical observations, observation times and scoring) or non-ocular effects were provided.

Findings & Conclusions: Under the conditions of this study methiocarb 50 WP was not an eye irritant.

Krotlinger F (1995) H 321 50 WP 0430/0363 (c.n.: Methiocarb): Study for skin and eye irritation/corrosion in rabbits. Study No. T5058363, Lab: Institute of Toxicology, Agrochemicals, Fachbereich Toxikologie, Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333. Sponsor: Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333, Germany. Study duration: November 08 – 22, 1994. Report No. 23672. Report date: January 25, 1994.

QA study conforms with OECD GLP and guidelines Nos. 404 and 405 for Testing of chemicals and EEC directive 92/69/EEC Part B, No.B.4.

Study & Observations: A quantity of 25 mg pulverised H 321 50 WP 0430/0363 [Formulation No. 0426 based on Form No: 00430/0363, methiocarb technical content 50.1%, appearance: light grey powder, pH 9.3 (2% in 0.1% saline)] was placed into the conjunctival sac of one eye of each of three adult female albino rabbits (HC: NZW, Interfauna, U.K. Ltd., Wyton, Huntington, England, 3.5-4.1 kg bw, age not stated). The other eye served as a control. The rabbits were individually housed under conventional laboratory conditions and provided with

Ssniff K4 diet (100-120 g per animal/day) and water *ad libitum*. Twenty four hours after the treatment, the eyes were rinsed with normal saline. The ocular responses [cornea (opacity), iris (hyperaemia), conjunctivae (erythema and chemosis), aqueous humour (opacity) and discharges] were examined and the eye irritation was scored at 1, 24, 48, 72 h and on days 7 and 14 post treatment according to the methods described by Draize (1985) and McDonald and Shadduck (1987). Furthermore, 24 h post treatment, a drop of 1% fluorescein solution was placed on the cornea of each of the treated eye which was 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. The individual Draize scores recorded at 24, 48 and 72 h were added and the total was divided by three to obtain the mean "irritation index". Because only three animals were used in the present study, the interpretation was based on individual irritation indices of the two most sensitive animals.

Findings: Slight erythema (Draize score of 1) was observed in all three rabbits up until 24 h post treatment but not thereafter. In addition swelling of the conjunctivae along with an ocular discharge was seen in one animal 1 h after the treatment. An "individual irritation index" of 0.3 was observed for all three animals. No further information on clinical observations was provided.

Conclusions: Based on these results, methiocarb 50 WP 0430/0363 was not considered an eye irritant in rabbits by the study authors, but is classified as a slight eye irritant under Australian guidelines.

Skin irritation

Groning P & Kimmerle G (1975). Study of acute oral, intraperitoneal and dermal toxicity, acute and subacute inhalation toxicity and skin and mucous membrane tolerance. Institute of Toxicology, Bayer AG, Germany, Report date: February 12, 1975.

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

Study & Observations: A cellulose pad of 1.5 cm^2 containing 500 mg Mesurol 50 WP (methiocarb, Batch No. X0001A, in the form of whitish yellow powder), was placed on the hairless inside section of one ear of each of 2 albino rabbits (source: Winkelmann and Gierlich; Bochum, 3.4-4.5 bw, sex, age not stated). The pads were placed in position by means of plaster bandages. The treated sites were observed for signs of irritation after 24 h (upon removal of pads) and during the 1 week of post-treatment period. No further information on methodology or clinical observations was provided.

Findings & Conclusions: Under the conditions of this study, methiocarb 50 WP was not a skin irritant.

Krotlinger F (1995) H 321 50 WP 0430/0363 (c.n.: Methiocarb): Study for skin and eye irritation/corrosion in rabbits. Study No. T5058363, Lab: Institute of Toxicology, Agrochemicals, Fachbereich Toxikologie, Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333. Sponsor: Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333, Germany. Study duration: November 08 – 22, 1994. Report No. 23672. Report date: January 25, 1994.

Quality assured study conforms with OECD GLP and guidelines Nos. 404 and 405 for Testing of chemicals and EEC directive 92/69/EEC Part B, No.B.4.

Study & Observations: A 500 mg quantity of pulverised H 321 50 WP 0430/0363 [Formulation No. 0426 based on Form No: 00430/0363, methiocarb technical content 50.1%,

Stability: study duration, Storage: room temperature, appearance: light grey powder, pH 9.3 (2% in 0.1% saline)] moistened with deionised water was applied on the shaven intact skin of the dorso-lateral area (6 cm^2) of each of three adult female albino rabbits (HC: NZW, Interfauna, U.K. Ltd., Wyton, Huntington, England, 3.1-3.6 kg bw, age not stated) on a hypoallergenic Hansamed patch (Beiersdorf No. 2342 PV2). A further patch moistened with water was applied on the shaven skin of the opposite dorso-lateral area of the animal and served as a control. Both patches were secured in place by using a semi-occlusive dressing (Fixomull-Strech Klebevlies, Beiersdorf no. 2293) during the exposure period of 4 h. The rabbits were individually housed in stainless steel cages under conventional laboratory conditions and provided with standard diet (Ssniff K4, Ssniff Spezialdiaten GmbH, Soest) 100-120 g per animal/day, once in the morning and water *ad libitum*. The treated skin sites were examined and the degree of dermal irritation was scored at 1, 24, 48, 72 h and on days 7 and 14 after termination of the exposure according to the Draize method. The Draize scores of individual animals at 24, 48 and 72 h were summed and divided by 3 to obtain the "individual irritation index" (separately for erythema/eschar and oedema formation).

Findings: All treated rabbits displayed grade 1-2 erythema combined with grade 1 oedema in 1/3 animals, with all symptoms resolving by day 14 post treatment. The results are presented in the following table.

Draize irritation indices (individual) of rabbits exposed (4 h) to 500 mg H 321 50 WP 0430/0363

Animal	Irritation index after												Irritation Index	
	1 h		24 h		48 h		72 h		7 d		14 d			
	e	o	e	o	e	o	e	o	e	o	e	o	e	o
1	2	1	2	1	1	0	1	0	1	0	0	0	1.3	0.3
2	1	0	1	0	0	0	0	0	0	0	-	-	0.3	0.0
3	1	0	2	0	1	0	1	0	0	0	-	-	1.3	0.0

Abbreviations: - = not examined, h = hour, d = day, e = erythema/eschar, o= oedema

Conclusions: Based on these results, methiocarb 50 WP 0430/0363 was a slight skin irritant in rabbits.

Skin sensitisation

Vohr HW (1995) H321 50 WP 00430/0363: Study for skin sensitising effect in guinea pigs (Buehler Patch Test). Study No. T 8058357, Lab: Institute of Toxicology, Agro-Chemicals, Bayer AG, Fachbereich Toxicologie in Wuppertal, Friedrich-Ebert-Str. 217-333, Germany. Sponsor: Bayer AG, Werk Elberfeld, Institute of Toxicology, Friedrich-Eber-Str. 217-333, D-42096 Wuppertal, Germany. Study duration: November 8 – December 9, 1994. Report No. 23724, Report date: February 10, 1995.

GLP, quality assured study conducted according to OECD guidelines for testing of Chemicals of 1992, the EC guidelines for Classification, Packaging and Labelling of Hazardous Materials, "Skin Sensitisation" Method B.6 of 1992 and the US EPA Pesticide Assessment Guidelines of 1984.

Study: H 321 50 WP 00430/0363 (Formulation No. 0426 based on formulation 00430/0363, methiocarb technical content: 50.1%) was applied dermally at 0.5 g/animal/day, (mixed to a paste with 0.4 mL of saline, 55.6% w/v) on 2 x 4 cm skin area on shaven left flanks of male guinea pigs (SPF-bred, Hsd Win:DH, Harlan Winkelmann GmbH Laboratory Animal Breeders, D-33176 Borchen, initial bw 368-407 g, 5-7 weeks old) once weekly for three

weeks (induction exposures) followed by a challenge dose (0.5 g paste and 25% formulation in saline, w/v) after a two weeks rest period. The animals were quarantined for at least a week before the commencement of the study. Test animals were housed five/group during quarantine, and in groups of two or three during the study in type IV Makrolon cages under standard laboratory conditions and provided with food (Altromin 3020 Maintenance Diet for Guinea Pigs, Altromin GmbH, Lage) and water ad libitum. The 2 concurrent control groups consisted of 20 animals (10/group). The study design is outlined in the following table.

Study design

Treatment Group	Treatment	Number of animals	Exposure regimen
1 Test	Test chemical in 0.4 mL of saline	20	Once weekly for 3 wk, challenge dose 2 wk later
2 Control	Hypoallergenic patch moistened with saline (0.5 mL)	10	Once weekly for 3 wk, challenge dose 2 wk later
3 Reserve group	Hypoallergenic patch moistened with saline (0.5 mL)	10	Once weekly for 3 wk, challenge dose 2 wk later

Prior to the range-finding and main study, the sensitivity of the animal model was validated using alpha-hexylcinnamaldehyde formulated in polyethylene glycol 400 (25% formulation, 125 mg/animal) in female guinea pigs (bw and age not specified). Following first challenge with 12% formulation, 70% of the animals showed dermal reactions (unspecified) whereas 60% of the animals showed reactions after the second challenge with 6% formulation. No dermal reactions were noted in control animals.

A range-finding study was conducted to select the suitable dose levels for the main study.

Range-finding study: Five guinea pigs (specified above) received single 6 h exposures to 0.5 mL of 6%, 12%, 25% formulation (w/v, in saline) and 0.5 g paste (on an aluminium foil and mixed in 0.4 mL saline) of the test chemical on four patches (area not specified) applied to each animal under occlusive conditions. The patches were held in position with a "Fermoflex" adhesive plaster. At the end of the exposure period the patches were removed and the sites were cleaned with tap water to remove residual test chemical. After 21 h the skin sites were depilated with Pilca® cream and assessed for irritation/sensitisation at 30, 54 and 78 h following exposure initiation (3, 27 and 51 h after depilation). No challenge dose was administered.

As no skin reactions were noticed in any of the test animals at any concentrations, a dose of 0.5 g/animal (paste) was chosen for the main study.

Main study: The test formulation was applied (0.5 g/animal as a paste) to the left flanks of the animals according to the procedure described above. The animals in the two control groups were treated with hypoallergenic patches moistened with saline (0.5 mL/animal) applied and secured on to the left flanks of the animal similar manner. The patches were removed after a 6 h exposure period. Any remaining test substance on the site was cleaned using tap water. The skin reactions were graded two weeks after the last induction exposure and the animals in the test formulation and the first control groups were challenged with 0.5 g/animal paste (on aluminium foil) and a hypoallergenic patch with 25% test chemical (0.5 mL) applied on the shaven right flanks according to the procedures described previously. The relative position of the patch and the aluminium foil, either cranial or caudal, was alternated from one animal to the next. Further, as a control two hypoallergenic patches treated with physiological saline (0.5 mL) were applied on the left flanks of the animals in the similar manner. After 6 h of exposure the patches were removed and the sites were cleaned with tap water. Twenty one

hours later the sites were depilated with Pilca® cream and graded (30 h after induction and 30, 54, and 78 h after challenge) according to the scoring system described in the guidelines followed. The criterion used was the occurrence of skin reactions in test substance treated animals at a higher incidence and greater intensity compared to controls. In addition, the body weights of the animals were recorded on day 1 of the study and its conclusion on day 31. The animals were observed for clinical signs at least once daily.

Findings: There were no treatment-related effects of H 32150 WP 00430/0363 on the body weights, appearance and behaviour of the animals. No skin reactions could be observed following primary, secondary or tertiary inductions due to the brown colour in the treatment site. Eight out of 20 (40%) of the induced animals showed reactions ranging from "slight localised" to "moderate confluent" skin redness upon challenge with 0.5 g/animal paste. No skin reactions were noticed in control animals. None of the animals in either the control or test formulation group showed any skin reactions following challenge with 25% test substance. No other information on clinical observations or doubtful skin reactions was provided.

Conclusions: Under the conditions of the study, methiocarb 50 WP 00430/0363 exhibits skin sensitising potential in male guinea pigs.

3.2.6 Mesurol 500 FS

Median lethal dose studies

Route	Species	Sex	Group Size	Doses Tested (mg/kg bw) or (mg/m^3)	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Oral	Rat [Wistar]	M/F	5 or 10/sex	1, 5, 25, 50, 80, 100 (M/F) plus 40, 112 (M), 56, 71 (F)	53 (M) 60 (F)	Mihail & Pauluhn (1983)
Dermal	Rat [Wistar]	M/F	5/sex	1000, 2500, 5000 (24 h, with occlusion and the applied material was removed)	>5000 (M/F) (0/5 deaths/sex)	
Inhalation (7 h, whole body)				13464 (M), 14121 (F) – nominal concentrations. (droplet sizes not stated)	>13764 (M: 0/5 deaths) >14121 (F: 0/5 deaths)	

Eye and dermal irritancy studies

Study	Species/ Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	3	100 µL, conjunctival sac, rinsed	Slight irritant	Mihail & Pauluhn (1983)¶
Dermal		3	500 mg intact, occlusive	Non-irritant	

¶OECD guidelines.

Mihail F & Pauluhn (1983) Mesurol 500 FS (c.n. Methiocarb) Studies on formulation toxicity. Study No. T 2015989. Lab: Bayer AG Institute of Toxicology, Wuppertal-Elberfeld Facility. Sponsor: Bayer AG, Germany. Study duration: June to July, 1983. Report No. 12088. Report date: September 21, 1983.

Pre GLP, non quality assured study. Study conducted according to appropriate OECD guidelines.

Eye irritation

Study & Observations: One hundred µL of Mesurol 500 FS [H 321 500 FS 038 A (034), methiocarb technical content, batch, stability and solubility unspecified] was instilled into the conjunctival sac of one eyelid of each of 3 albino rabbits (HC: NZW, Hacking & Churchill Ltd, Huntington, U.K., 3.0-3.9 kg bw, sex, 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. At 24 h, 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. The animals were housed individually under conventional laboratory conditions and provided with standard food and water *ad libitum*. The Draize scores 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.

Findings: In one animal, grade 2 conjunctival redness was seen at 1 h along with swelling and tear flow (grade 1) which resolved by 24 h (irritation grade of 0.0) post treatment. In the two remaining rabbits, conjunctival erythema (grade 1) was observed at 1h in one animal and in the other up until 24 h (irritation grades of 0 and 0.3 respectively) and not thereafter. Both of these animals also displayed grade 1 conjunctival swelling at 1 h post treatment and not afterwards.

Conclusions: Based on these results, methiocarb 500 FS was not considered to be an eye irritant in rabbits by the study authors. The formulation is classified as a slight eye irritant under Australian guidelines.

Skin irritation

Study & Observations: Five hundred µL of Mesurol 500 FS [H 321 500 FS 038 A (034), methiocarb technical content, batch, stability and solubility unspecified] was applied on shaven, intact skin (6.25 cm^2) on the flanks of each of 3 albino rabbits (HC: NZW, Hacking & Churchill Ltd., Huntington, U.K., 3.5-3.9 kg bw, sex, age unspecified). The test substance was administered on a cellulose dressing. A further patch moistened with water was placed on the clipped intact skin of the opposite flank of the animal and served as a control. Both patches were held in place by using elastic adhesive tape during the exposure period. The rabbits were individually housed under conventional laboratory conditions and provided with standard diet (Ssniff K, Versuchstierdiaten GmbH, Soest, Westphalia) and water ad libitum. 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 (as specified in the Draize method) 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". No information on any clinical observations was provided.

Findings & Conclusions: No skin reactions were observed in any of the test animals at any of the assessment times. Under the conditions of the study, methiocarb 500 FS was not a primary skin irritant in rabbits.

3.2.7 H 321 500 SC

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
Oral	Rat [Wistar]	M/F	5/sex	50, 63 (M/F) plus 1, 25 (M), 2, 20 (F)	>25 - <50 (M: 25: 0/5 deaths; 50: 3/5 deaths) 56 (F)	Bomann (1993a) [GLP]
Dermal	Rat [Wistar]	M/F	5/sex	200, 1000, 2000, 5000 (24 h, with occlusion and the applied material was removed)	>5000 (0/ deaths/sex)	Bomann (1993b) [GLP]
Inhalation (4 h, nose-only)	Rat [Wistar Bor: WISW]	M/F	5/sex	15, 84, 405 (50% droplets <3µm)	>405 (M/F) (0/5 deaths/sex)	Martins (1995) [GLP]

Eye and dermal irritancy studies

Study	Species/ Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW], F	3	100 µL, conjunctival sac, rinsed	Slight irritant	Krotlinger (1993)¶
Dermal	Rabbit [NZW], F	3	500 mg intact, semi-occlusive	Non-irritant	

¶OECD guidelines Nos. 404 and 405.

Krotlinger F (1993) H 321500 SC 02373/0028 A: Study for skin and eye irritation/corrosion in rabbits. Study No. T5050000, Lab: Institute of Toxicology, Agrochemicals, Fachbereich Toxikologie, Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333. Sponsor: Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333, Germany. Study duration: January 26, 1993-February 09, 1993. Report No. 22180. Report date: April 14, 1993.

QA study conforms with OECD GLP and OECD Test Guidelines Nos. 404 and 405.

Eye irritation

Study: A volume of 100 µL of H 321 500 SC 02373/0028 A (Formulation No.: 030 based on Form: No. 02373/0028 A, methiocarb technical content: 503.1 g/L, appearance: beige suspension) was instilled into the conjunctival sac of one eye of each of 3 adult female albino rabbits (HC: NZW, Interfauna, UK Ltd., Wyton, Huntington, England, 3.0-3.4 kg bw, age not stated). The other eye served as a control. The rabbits were individually housed under conventional laboratory conditions and provided with Ssniff K4 diet (100-120 g per animal/day) and water *ad libitum*. Twenty four hours after the treatment, the eyes were rinsed with normal saline. The ocular responses [cornea (opacity), iris (hyperaemia), conjunctivae (erythema and chemosis), aqueous humour (opacity) and discharges] were examined and the eye irritation was scored at 1, 24, 48, 72 h and on days 7 and 14 post treatment according to the methods described by Draize (1985) and McDonald and Shadduck (1987). In addition, at 24 h post treatment, a drop of 1% fluorescein solution was placed on the cornea of each of the treated eye which was 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 the similar procedure was repeated at the later observation times. Only those effects

persisting for more than 24 h were included in the evaluation. The individual Draize scores recorded at 24, 48 and 72 h were added and the total was divided by three to obtain the mean "irritation index". As only three animals were used in the present study, the interpretation was based on individual irritation indices of the two most sensitive animals.

Findings: Slight erythema of the conjunctivae (Draize score of 1) was noticed in all three animals together with swelling of the conjunctivae in 1 animal at 1 h post treatment but not thereafter. No other information on clinical observations was provided.

Conclusions: Based on these results, methiocarb 500 SC 02373/0028 A was not considered an eye irritant in rabbits by the study authors but is classified as a slight eye irritant under Australian guidelines.

Skin irritation

Study: An aliquot of 500 µL/animal of H 321500 SC 02373/0028 A (methiocarb technical content: 503.1 g/L, Stability: study duration, appearance: beige suspension) was applied on shaven, intact dorso-lateral skin area (6 cm^2) of the trunks of 3 adult, female albino rabbits (HC: NZW, Interfauna, U.K. Ltd., Wyton, Huntington, England, 3.2 kg bw, age not stated). The test substance was administered on a hypoallergenic ®Hansamed patch (Beiersdorf no. 2342 PV3). A further patch moistened with deionised water was placed on the clipped intact skin of the opposite dorso-lateral area of the animal and served as a control. Both patches were held in place by using a semi-occlusive dressing (Fixomull-Strech Klebevlies, Beiersdorf no. 2293) during the exposure period. The rabbits were individually housed under conventional laboratory conditions and provided with standard diet (Ssniff K4, Ssniff Spezialdiaten GmbH, Soest) 100-120 g per animal/day and water *ad libitum*. After 4 h of exposure, the dressing was removed and the exposed sites were washed with water. The skin sites were evaluated and scored for erythema/eschar and oedema formation (as specified in the Draize method) at 1, 24, 48 and 72 h and on days 7 and 14 after patch removal. The individual scores (separately for erythema/eschar and oedema formation) at 24, 48 and 72 h were added and the total was divided by three to obtain the mean "irritation index". As only three animals were used, the interpretation was based on individual irritation indices of the two most sensitive animals. Other skin lesions and toxic responses were also recorded. No other information on clinical observations was provided.

Findings: Slight erythema (barely perceptible, Draize score of 1) was noticed in 1 rabbit at 1 h and in 2 rabbits at 24 h after patch removal but not thereafter.

Conclusions: Based on these results, methiocarb 500 SC 02373/0028 A was not considered a primary skin irritant in rabbits.

3.2.8 Mesurol 50% Hopper box

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
Oral	Rat [NS]	M/F	10/sex	110, 136, 169, 210, 260 (M): 58, 72, 89, 110, 136, 169, 260 (F)	209 (M) 163 (F)	Nelson (1978)
Dermal	Rabbit [NZW]	M/F	5/sex	5000 (abraded; 24 h, occluded, applied material	>5000 (M: 0/5 deaths; F:1/5 deaths)	Lamb et al. (1977)

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
				was removed)		
Inhalation (1 h, whole body)	Rat [SD]	M/F	10/sex	20000 (droplet sizes not given)	>20000 (M/F) (0/10 deaths/sex)	Lamb & Anderson (1977a)

SD=Sprague-Dawley; NZW=New Zealand White

Eye irritancy studies

Study	Species/Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	9	50 mg, rinsed (3), unrinsed (6)	Slight irritant	Lamb, Matzkanin & Burke (1977)

NZW=New Zealand White

Lamb DW, Matzkanin CS & Burke MA (1977) Eye irritancy of Mesurol 50% Hopper Box. Reference. 76-282, Lab: Research & Development, Chemagro Agricultural Division, Mobay Chemical Corporation. Sponsor: Bayer AG, Study duration: Not stated. Report No. 53293, Report date: June 9, 1977.

Pre GLP, non-quality assured study. Testing and scoring method described by JH Draize in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics', edited and published by the editorial committee of the Association of Food and Drugs, Officials of the USA was used.

Study & Observations: The eye irritancy potential of Mesurol 50% Hopper Box (methiocarb, batch no. 7035072, solubility, stability, formulation details not provided) was examined in 9 NZW rabbits (age, bw, sex, source not stated) using the above procedure. A 50 mg quantity of the formulation (physical form not specified) was placed in the left eye of each animal. The right eye served as a control. The eyes of three rabbits were rinsed with 200 mL of lukewarm water 45 seconds after the treatment. The treated eyes of the remaining six rabbits were not rinsed and all were examined for ocular reactions (opacity, iritis, erythema, chemosis and discharges) on days 1, 2, 3, 4, and 7 post treatment.

Findings: Slight erythema (Grade 1) was observed in rabbits whose eyes were rinsed after the treatment. In this group, the ocular response of one animal lasted for 2 days and in the other two rabbits the condition prevailed for 24 h after rinsing. All symptoms were resolved by day 3 post treatment. Likewise, for those with un-rinsed eyes, slight erythema (Grade 1) was noticed in 5 animals where the condition lasted for 48 h in 4 animals and for 72 h in one animal and all eyes were cleared by day 4 post treatment. One animal in this group did not show any ocular reactions. The mean score for erythema at day 3 was 0.2.

Conclusions: Under the conditions of the study and based on the results observed, it was concluded that, methiocarb 50% Hopper Box was not an eye irritant in rabbits. However, given that most treated eyes exhibited an erythematous response, the formulation is classified as a slight eye irritant under Australian guidelines.

3.2.9 H 321 4GR

Eye and dermal irritancy studies

Study	Species/Sex	Group size	Method	Result	Reference

Study	Species/Sex	Group size	Method	Result	Reference
Ocular	Rabbit Himalayan, M	3	100 mg conjunctival sac, unrinsed	Non-irritant	Leuschner (1998)¶
Dermal		3	500 mg intact, semi-occlusive	Non-irritant	

¶ OECD and the EC Guidelines of 1992.

Leuschner J (1998) Acute eye irritation study of H 321 4 GR 00313/1967 by instillation into the conjunctival sac of rabbits. Project No: 9301/261/95. Lab: LPT Laboratory of Pharmacology and Toxicology, Redderweg 8, D-21147 Hamburg, Germany. Sponsor: Bayer AG, Werk Elberfeld, Institute of Toxicology, Friedrich-Ebert-Str. 217-333, D-42096 Wuppertal, Germany. Study duration: April 27 to May 01, 1998. Report No. R 7160 Report date: June 2, 1998.

GLP, Quality assured study. Conducted in compliance with the OECD Guidelines for testing of chemicals of 1992 and the EC Guidelines of 1992. OECD-Series on Principles of GLP and Compliance Monitoring and GLP of Japan regulations were considered.

Study: H 321 4 GR 00313/1967 (development no. 0218556, formulation no. 1970 based on 00313/1967, methiocarb technical content 4.08%) in 100 mg quantity/animal was applied into the conjunctival sac of the right eye of each of 3 male Himalayan rabbits (Chr. Fred Leuschner & Co. D-24601 Londorf/Post Wankendorf, 4 months old, 2.1-2.2 kg bw). The left eye served as a control. The test animals were acclimatised to the test conditions for at least 20 days before the test. After application of the test substance the animals were kept for 8 h individually in restrainers that allowed free movement of the head but prevented complete turning of the body and wiping of the eyes by the paws. They were provided with food (Altromin, GmbH, D-32791 Lage/Lippe) and water ad libitum and were individually housed before and after the exposure period.

The eyes were not rinsed and the cornea, iris and conjunctivae were examined opthalmoscopically with a slit lamp at 1, 24, 48 and 72 h after application for opacity, redness and chemosis. Twenty four hours after application the eyes were also treated with fluorescein and examined. Reactions were graded according to the OECD guideline. No other clinical signs were observed.

Findings: No irritation (Draize score 0) was observed during the observation period.

Conclusions: Under the test conditions no ocular reaction was observed in any of the test animals up to 72 h after instillation. Methiocarb 4 GR 00313/1967 (4.08%) was a not an eye irritant in rabbits.

Leuschner J (1998) Acute skin irritation test (patch test) of H 321 4 GR 00313/1967 in rabbits. Project No:9300/261/95, Lab: LPT Laboratory of Pharmacology and Toxicology, Redderweg 8, D-21147 Hamburg, Germany. Sponsor: Bayer AG, Werk Elberfeld, Institute of Toxicology, Friedrich-Eber-Str. 217-333, D-42096 Wuppertal, Germany. Study duration: April 23 to 26, 1998. Report date: May 25, 1998.

GLP, Quality assured study. Conducted in compliance with the OECD Guidelines for testing of chemicals of 1992 and the EC Guidelines of 1992. OECD-Series on Principles of GLP and Compliance Monitoring and GLP of Japan regulations were considered.

Study: Three male Himalayan rabbits (Chr. Fred Leuschner & Co. D-24601 Londorf/Post Wankendorf, 4.5 months old, 2.1-2.8 kg bw) were treated with 500 mg H 321 4 GR 00313/1967 (methiocarb technical content 4.08%, formulation details were not provided),

prepared as a paste with water. The paste was applied on the intact dorsal skin (patch of approx: 6 cm2 area) of the trunk of the animals under semi-occlusive dressing for 4 h. The test animals were acclimatised to the test conditions for at least 20 days before the test. They were kept in restrainers during the exposure that allowed free movement of the head but prevented a complete body turn. The animals were provided with food (Altromin, GmbH, D-32791 Lage/Lippe) and water ad libitum and were individually housed before and after the exposure period. The patch was held in contact with the skin by means of semi-occlusive dressing and the surrounding untreated skin area served as the control.

After 4 h of exposure the dressing was removed and the skin was evaluated for erythema and eschar and oedema formation 1, 24, 48 and 72 h post treatment. Observations were continued daily for up to 14 days to determine the reversibility of the reactions. No other clinical observations were provided.

Findings: No irritation (Draize score 0) was observed during the observation period.

Conclusions: Under the test conditions no signs of skin irritation were observed in any of the test animals. Methiocarb 4 GR 00313/1967 (4.08%) was not a skin irritant to rabbits.

3.2.10 H 321 4 RB 00313/0679

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
Dermal	Rat [Wistar]	M/F	5/sex	5000 (24 h, with occlusion and the applied material was removed)	>5000 (M/F) (0/5 deaths/sex)	Bomann (1988)
Inhalation (4 h, head only)	Rat [Wistar]	M/F	5/sex	224 (40% droplets ≤5µm)	>224 (M/F) (0/5 deaths/sex)	Pauluhn (1988) [GLP]

Eye and dermal irritancy studies

Study	Species/ Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW], M	3	100 µL, conjunctival sac, rinsed	Slight irritant	Martins (1988)¶
Dermal		3	500 mg intact, occlusive	Non-irritant	

¶OECD guidelines Nos. 404 and 405. NZW=New Zealand White.

Martins T (1988) H 321 4 RB 00313/0679 (c.n. Methiocarb): Study for irritation/corrosion potential to skin and eye (rabbit) according to OECD Guidelines Nos. 404 and 405. Project No. T6029681, Lab: Institute of Toxicology, Agrochemicals, Fachbereich Toxicologie, Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-313 Germany. Sponsor: Bayer AG, Germany, Study duration: May 5 - 10, 1988. Report No. 17020, Report date: August 10, 1988.

Quality assured study conforms with OECD GLP and Test Guidelines Nos. 404 and 405.

Eye irritation

Study: One hundred µL (from a stock solution equivalent to 40 mg of applied mass) of H 321 4 RB 00313/0679 [Formulation No.: 703 P "in accordance with 00313/0679", methiocarb technical content: 3.93%, appearance: red-violet pellets, pH 6.2 (2% in 0.1% aqueous NaCl solution), formulation details were not provided] was instilled into the conjunctival sac of one eye of each of 3 adult male albino rabbits (HC: NZW, Interfauna, U.K. Ltd., 2.9-3.1 kg bw, age not stated). The untreated eye served as a control. The rabbits were individually housed under conventional laboratory conditions and provided with standard Ssniff K4 diet (100-120 g per animal/day) water *ad libitum*. Twenty four hours after treatment, treated eyes were rinsed with normal saline. The ocular responses to the treatment [cornea (opacity), iris (hyperaemia and reaction to light), conjunctivae (erythema and chemosis), and discharges] were examined and the degree of eye irritation was scored at 1, 24, 48, 72 h and on days 7, 14 and 21 post treatment according to the guidelines used and McDonald and Shadduck (1987). In addition, at 24 h post treatment a drop of 1% fluorescein solution was placed on the cornea of each of the treated eye which was 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.

Findings: Slight erythema of the conjunctivae (Draize score of 1) was observed in two rabbits 24 h after the treatment but not at later assessment times. Swelling of the conjunctivae (Draize score of 1) was seen in one of these animals at 1 h post treatment and not thereafter. An irritation grade of 0.3 was noted for both animals while the third animal did not exhibit any ocular response. No other information on clinical observations was provided.

Conclusions: Based on these results, methiocarb 4 RB 00313/0679 was not considered to be an eye irritant in rabbits but is classified as a slight eye irritant under Australian guidelines.

Skin irritation

Study: A 0.5 g quantity of H 321 4 RB 00313/0679 [Formulation No.: 703 P "in accordance with 00313/0679", methiocarb technical content: 3.93%, appearance: red-violet pellets, pH 6.2 (2% in 0.1% aqueous NaCl solution), formulation details were not provided] mixed to a paste with water and spread on a Hansamed hypoallergenic dressing was applied on shaven intact skin (6 cm^2) of the flank of each of 3 adult male albino rabbits (HC: NZW, Interfauna, U.K. Ltd., 3.0-3.3 kg bw, age not stated). A similar dressing moistened with water was applied on the shaven, intact skin of the opposite flank of the animal and served as a control. Both dressings were fastened with elastic adhesive. The rabbits were individually housed under conventional laboratory conditions and provided with Ssniff K4 diet (100-120 g per animal/day) and water *ad libitum*. Four hours after the treatment, the dressings were removed and the sites were rinsed with water. The skin response (erythema/escharosis and oedema formation) was examined at 1, 24, 48, 72 h and on days 7, 14 and 21 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.

Findings & Conclusions: No skin responses were noted at any of the assessment times in any of the treated animals. Under the conditions of the study, methiocarb 4 RB 00313/0679 was not a primary skin irritant in rabbits.

3.2.11 4% Methiocarb: H 321 4 GR 00313/1967

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD_{50} mg/kg bw	Reference
Oral	Rat [Wistar]	M/F	3/sex	500 (M/F) plus 200 (M)	>500 - <1000 (M: 1/3 deaths; F: 0/3 deaths)	Andrews (1998a) [GLP]
Oral	Rat [NS]	M/F	10/sex	750, 850, 1000, 1100, 1200 (M/F) plus 350, 500 (M)	848 (M) 945 (F)	Flucke (1978)
Dermal	Rat [Wistar]	M/F	5/sex	2000 (24 h, with occlusion and the applied material was removed)	>2000 (M/F) (0/5 deaths/sex)	Andrews (1998b) [GLP]
Dermal	Rat [Wistar]	M/F	5/sex	5000 (24 h, with occlusion and the applied material was removed)	>5000 (M/F) (0/5 deaths/sex)	Bomann (1988)

3.2.12 Bayer 37344 Bran Bait (4% methiocarb)

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD_{50}	Reference
Oral	Dog [Beagle]	M	1	10, 25, 50	>50 (0/1 death)	Doull & Root (1963)

3.2.13 H 321 3RB 0589

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
Oral	Rat [Wistar]	M/F	5 or 10/ sex	10, 100, 2500, 5000 (M/F) plus 2000, 2240 (M), 1000, 3550 (F)	3305 (M) 3645 (F)	Krotlinger (1987)
Inhalation (4 h) (head-only)	Rat [Wistar Bor:WISW]	M/F	5/sex	181 (31% droplets ≤5μm)	>181 (M/F) (0/ deaths/sex)	Pauluhn (1986)

Eye and dermal irritancy studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbit [NZW]	3 M/F	100 μL, conjunctival sac, rinsed	Slight irritant	Ruf (1986)¶
Dermal		3 F	500 mg, intact, occlusive	Non-irritant	

¶OECD guidelines. NZW=New Zealand White.

Ruf J (1986) H 321 3 RB 0589 (common name: methiocarb) Tests for dermal and ocular irritancy/corrosivity (rabbits). Study No. T9023365, Lab: Institute of toxicology agrochemicals, Department of Toxicology, Bayer AG, Wuppertal, Friedrich-Ebert-Str 217-333, Germany. Sponsor: Bayer AG, Wuppertal, Friedrich-Ebert-Str 217-333, Germany. Study duration: in July, 1986. Report No. 15005, Report date: August 28, 1986.

Non-quality assured study conducted according to OECD guidelines Nos. 404 and 405. No GLP statement provided.

Eye irritation

Study: The eye irritancy potential of H 321 3 RB 0589 (Sample No. 225/4, methiocarb technical content 3%, solubility and stability not specified) was examined in three adult NZW rabbits weighing between 2.7 and 3.3 kg bw (1 male and 2 females, Interfauna UK Ltd. age not stated). Any ocular response (opacity, hyperaemia, reaction to light, erythema and chemosis, and lacrimation) following instillation of 100 μL (equivalent to 65 mg) of the formulation into the conjunctival sac of one eye of each rabbit was 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. At 24 h, 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. The untreated eye served as a control. The animals were housed individually in type III wire cages under conventional laboratory conditions and provided with standard food and water *ad libitum*. The Draize scores recorded at 24, 48 and 72 h post treatment (separately for opacity and chemosis) were used to calculate the individual "irritation values". The interpretation was based on the "individual irritation values" of the two most sensitive animals.

Findings: In one female, grade 1 conjunctival redness was seen for 48 h along with swelling of the conjunctiva which resolved by 24 h (mean irritation value of 0.7) following treatment. In the two remaining rabbits, conjunctival erythema (grade 1) was observed up until 24 h

together with swelling of the conjunctivae at 1 h post treatment (mean irritation value of 0.3) but not thereafter. No other information on clinical observations was provided.

Conclusions: Based on these results, methiocarb 3 RB 0589 was not considered an eye irritant in rabbits by the study authors, but is classified as a slight eye irritant under Australian guidelines.

Skin irritation

Study: Three female NZW rabbits weighing between 3.2 and 3.4 kg bw (Interfauna UK Ltd.) received 0.5 g/animal of H 321 3 RB 0589 (Sample No. 225/4, methiocarb technical content 3%, solubility and stability not specified). The test formulation was mixed with water to form a paste and applied in a Hansamed hypoallergenic adhesive plaster to an area (6 cm^2) of shaven intact skin of one flank of each animal. Another plaster moistened with water was applied on the shaven intact skin of the opposite flank of each animal and served as a control. Both plasters were held in place by using Fixomull-Stretch, Klebevlies elastic adhesive dressing. The animals were housed individually in type III wire cages under conventional laboratory conditions and provided with standard food and water *ad libitum*. After 4 h of exposure, the dressings and the plasters were removed and the exposed skin sites were washed with water. The application sites were examined after 1, 24, 48, 72 h and on days 7 and 14 following patch removal and the degree of erythema/eschar and/or oedema was scored. All other noticeable findings were also recorded. The individual Draize scores which were recorded separately for erythema and oedema at 24, 48 and 72 h post treatment were used to calculate individual "irritation values". Because 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.

Findings & Conclusions: No skin reactions were evident at any time in any of the treated rabbits. Based on these results, methiocarb 3 RB 0589 was not considered a primary skin irritant in rabbits.

3.2.14 2% Slug and Snail Pellets

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
Oral	Rat [SD]	M/F	10/sex	2648 (M), 3097 (F)	>2648 (M: 0/10 deaths) >3097 (F: 0/10 deaths)	Lamb et al. (1981a)
Oral	Dog [NS]	M/F	2/sex	600, 900	900 (M/F)	Lamb & Matzkanin (1975d)
Dermal	Rabbit [NZW]	M/F	5/sex	2000 (abraded; 24 h with occlusion and the applied material was removed)	>2000 (M/F) (0/5 deaths/sex)	Lamb et al. (1981b)
Inhalation (4 h, head only)	Rat [SD]	M/F	11/M 9/F	835 (50% particles ≤3µm)	>835 (M: 0/11 deaths) (F: 0/9 deaths)	Sangha (1981)

SD=Sprague-Dawley; NZW=New Zealand White.

Eye and dermal irritancy studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbit [NZW]	9	100 mg, rinsed (3) unrinsed (6)	Slight irritant	Lamb, Hixson & English (1981c)
Dermal		6	500 mg intact, abraded, occlusive	Non-irritant	

NZW=New Zealand White.

Lamb DW, Hixson EJ & English TD (1981c) Eye and dermal irritation of Mesurol 2% slug and snail pellets. Study No. 81-333-05. Lab: Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Center, 17745 South Metcalf, Stilwell, KA, 66085 USA. Bayer AG, Germany. Study duration: February 24 to March 19, 1981. Report No. 173. Report date: April 13, 1981.

Pre GLP, non-quality assured study. Testing and scoring method of JH Draize as found in "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" of 1956 was used.

Eye irritation

Study & Observations: The eye irritancy potential of Mesurol 2% slug and snail pellets (active ingredient content 2%, batch no. 0030133, solubility, stability unspecified, formula no. 12501, methiocarb technical content 2.2%) was examined in 9 NZW rabbits (Small Stock Inc., Pea Ridge, AK, USA, age, bw not stated) using the above assessment procedure. A 100 mg quantity of the formulation (ground to a powder using a ball mill) was placed in the left eye of each animal. The right eye served as a control. The eyes of three rabbits were rinsed with 200 mL of lukewarm water 45 seconds after the treatment. The treated eyes of the remaining six rabbits were not rinsed and all were examined for ocular reactions (corneal defects and opacity, iritis, erythema, chemosis and discharges) on days 1, 2, 3, 4, and 7 post treatment. All animals were individually housed and provided with food and water *ad libitum*.

Findings: No corneal or iridial changes were observed in any of the test animals. The mean grades recorded for other ocular responses at different observation times are presented in the following Table. Ocular changes in all animals were reversed within 7 days.

Ocular responses of rabbits treated with 2% Mesurol slug and snail pellets

Parameter	Mean grade			
	Observation time (day)			
	1	2	3	4
Eyes washed after 45 seconds (3 rabbits; 1M+2F)				
Conjunctival erythema	2.0	0.3	0.3	0
Ocular discharge	1.0	0.6	0.3	0
Total score (conjunctiva)	6.6	2	1.3	0
Eyes unwashed (6 rabbits; 2M+4F)				
Conjunctival erythema	2.5	2.1	1.3	0.8
Chemosis	3.0	2.3	1.5	0.6
Ocular discharge	2.8	2.3	1.8	1.0
Total score (conjunctiva)	16.6	13.6	9.3	5.0

Conclusions: On the basis of these findings, 2% methiocarb slug and snail pellets was considered to be an eye irritant in rabbits by the study authors. Under Australian guidelines, the formulation is classified as a slight eye irritant.

Skin irritation

Study & Observations: A quantity of 500 mg of powdered (using a ball mill) 2% Mesurol slug and snail pellets (batch no. 0030133, methiocarb technical content 2.2%) mixed with physiological saline was applied under a one inch gauze patch on shaven intact (2 sites/animal) and abraded (2 sites/animal) skin of the backs and sides of each of 6 NZW rabbits (Small Stock Inc., Pea Ridge, AK, USA, age, sex, bw unspecified). The trunks of the animals were then wrapped with a plastic sheet and secured in place with adhesive tape. A plastic collar was placed on each animal to prevent oral ingestion of the test substance. The animals were individually housed under conventional laboratory conditions and offered food and water *ad libitum*. After 24 h of exposure, the patches were removed and wiping with a damp cloth cleaned the exposed skin sites. The exposed sites were examined and the skin responses were scored at that time and 48 h later.

Findings: Grade 1 erythema was observed on both abraded skin sites of 1 rabbit and 1 abraded site in another rabbit at 24 h. No other skin responses were noted in any animals at other observation times.

Conclusions: Based on these results, 2% methiocarb slug and snail pellets was not considered to be a skin irritant in rabbits.

3.2.15 Imidacloprid/Methiocarb

Eye and dermal irritancy studies

Study	Species/ Sex	Group Size	Method	Result	Reference
Ocular	Rabbit [NZW] F	3	500 µL, conjunctival sac, rinsed	Severe irritant	Krotlinger (1995) OECD,EEC
Dermal		3	500 mg intact, semi-occlusive	Slight irritant	

NZW=New Zealand White.

Krotlinger F (1995) NTN 33893 0,062 VL 02911/0659 (05428/0000) & H 321 0,125 [c.n.: Imidacloprid (proposed); Methiocarb). Study for skin and eye irritation/corrosion in rabbits. Study No. T8058159. Lab: Institute of Toxicology Agrochemicals, Fachbereich Toxicologie, Bayer AG, Wuppertal, Friedrich-Ebert-Str. 217-333. Germany. Sponsor: Bayer AG, Germany. Study duration: July 19 to August 16, 1994. Report No. 23593. Report date: January 04, 1995.

Quality assured study conforms with OECD GLP and guidelines Nos. 404 and 405 for Testing of chemicals and EEC directive 92/69/EEC Part B, No.B.4.

Eye irritation

Study & Observations: Five hundred µL of the test substance [NTN 33893 0,062 VL 02911/0659 (05428/0000) & H 321 0,125, Formulation No. 0003 based on Form No: 02911/0659 (05428/0000), methiocarb technical content 0.12%, Imidacloprid content: 0.064%, Stability: study duration, Storage: room temperature] was placed into the conjunctival sac of one eye of each of three adult female albino rabbits (HC: NZW, Interfauna, U.K. Ltd., Wyton, Huntington, England, 3.2-4.0 kg bw, age not stated). The untreated eye served as a control. The rabbits were individually housed under conventional laboratory conditions and provided with Ssniff K4 diet (100-120 g per animal/day) and water *ad libitum*. Twenty four hours after treatment, the treated eyes were rinsed with normal saline. The ocular responses [cornea (opacity), iris (hyperaemia), conjunctivae of bulbus, lids and nictitating membrane (erythema and chemosis) and discharges] were examined and the eye

irritation was scored at 1, 24, 48, 72 h and on days 7 and 14 post treatment according to the Draize method. 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 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. The individual Draize scores recorded at 24, 48 and 72 h were added and the total was divided by three to obtain the mean "irritation index". As only three animals were used in the present study, the interpretation was based on individual irritation indices of the two most sensitive animals.

Findings: The mean Draize scores recorded for all ocular responses are presented in the following table.

Mean draize grades recorded for various ocular responses in rabbits following treatment with formulation NTN 33893 0, 062 VL 02911/0659 (05428/0000) & H 321 0, 125

Parameter	Mean Draize grade					
	Observation time					
	1h	24 h	48 h	72 h	7 days	14 days
Cornea						
Opacity	1.0	1.0	0.6	0.6	0.3	0
area affected	3.6	3.3	2.3	2.3	0.6	0
Defects (fluorescein)	-	1.0	0.6	0.6	0	-
area affected	-	3.3	2.3	2.0	0	-
Iris	0	0.6	0.3	0	0	0
Conjunctiva						
Erythema	1.3	2.0	2.3	2.3	1.3	0.3
Chemosis	2.0	1.3	1.6	1.6	1.3	1.0
Ocular discharge	2.3	2.0	2.0	0.6	0.6	0

As the data indicate, the test substance caused a range of ocular changes including corneal opacity, iritis and conjunctival erythema and swelling following treatment. With the exception of the observation of peri-orbital loss of hair in one animal at day 21, all other ocular signs were reversed by that day. No other information on clinical observations was provided.

Conclusions: On the basis of these results NTN 33893 0,062 VL 02911/0659 (05428/0000) & methiocarb 0,125 is classified as a severe eye irritant in rabbits.

Neither imidacloprid nor methiocarb technical are eye irritants in rabbits. Therefore the observed ocular responses in the present study may have occurred due to the constituents in the formulation.

Skin irritation

Study & Observations: A volume of 500 µL of the test substance [NTN 33893 0,062 VL 02911/0659 (05428/0000) & H 321 0,125, Formulation No. 0003 based on Form No: 02911/0659 (05428/0000), methiocarb technical content: 0.12%, Imidacloprid content: 0.064%, Stability: study duration, Storage: room temperature] was applied on the shaven intact skin of the dorso-lateral area (6 cm^2) of the trunk of each of three adult female albino rabbits (HC: NZW, Interfauna, U.K. Ltd., Wyton, Huntington, England, 3.2-4.1 kg bw, age

not stated) on a hypoallergenic ®Hansamed patch (Beiersdorf No. 2342 PV3). A further patch moistened with water was applied on the shaven skin of the opposite dorso-lateral area of the animal and served as a control. One animal was tested initially due to the expected irritant potential of the test substance. Two further animals were tested at a later date. Both patches were secured in place by using a semi-occlusive dressing (Fixomull®-Strech Klebevlies, Beiersdorf no. 2293). Hence possible oral and inhalational exposure to the test substance during the 4 h exposure period was prevented. The animal acclimatisation period was about 2 weeks. Following treatment, the animals were individually housed in stainless steel cages under conventional laboratory conditions and provided with standard diet (Ssniff K4, Ssniff Spezialdiaten GmbH, Soest, 100-120 g per animal/day), once in the morning and water *ad libitum*. After patch removal the treated skin sites were rinsed with water and the degree of dermal irritation was scored according to the Draize method at 1, 24, 48, 72 h and on days 7 and 14 after termination of the exposure. The Draize scores of individual animals at 24, 48 and 72 h were summed and divided by 3 to obtain the "irritation index" (separately for erythema/eschar and oedema formation). Interpretation of the results was based on the individual indices obtained from the two most sensitive animals.

Findings: Slight erythema (barely perceptible, grade 1) was noticed in all three rabbits. The mean individual "irritation indices" for the three animals were 0, 0.3 and 1.0 and the signs were resolved by 24, 48 h and 7 days respectively. No other signs of toxicity were observed.

Conclusions: On the basis of these results and under Australian guidelines the test substance NTN 33893 0,062 VL 02911/0659 (05428/0000) & methiocarb 0,125 is classified as a slight skin irritant.

3.3 Antidote Studies

Kimmerle G (1966) Mesurol Active Ingredient (Wedemeyer H 321; Ht. No. 3657) – Antidotal effect. Study No. not stated. Lab: Institute for Toxicology, Wuppertal-Elberfeld. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 34267. Unpublished letter dated August 8, 1966.

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

Study & Observations: This short report presents the data on a study conducted to investigate the antidotal effects of Pralidoxime (PAM), atropine sulfate and Obidoxime chloride (BH6) in rats (strain, age, sex, bw, group size and source not stated) following oral administration of H 321 technical (methiocarb, Wedemeyer H 321; No. 3657, purity not stated) in Lutrol (polyethylene glycol 400). Methiocarb was administered to test animals at 10, 25, 50, 75, 100, 150, 175, 250, 500, 750 and 1000 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, using an unspecified method. Antidotal chemicals were administered to the animals by i.p. injection after administration of methiocarb but before the appearance of cholinergic signs.
Findings & Conclusions: The LD_{50} value of methiocarb without antidotes was 67 mg/kg bw. Treatment with atropine sulfate alone increased this value by about 7-fold, PAM alone by about 2.8 fold and BH6 alone by about 3.3 fold. The combined effect of atropine sulfate and PAM or atropine sulfate and BH6 was slightly greater than atropine sulfate alone, the increases in LD_{50} being approximately 7.4 and 7.6 fold respectively. Thus, treatment with atropine sulfate alone appears to be more effective as an antidote against methiocarb compared to PAM or BH6 alone. The effect of atropine sulfate was only slightly increased when it was combined with PAM or BH6.

Kimmerle G (1971) Comparison of antidotal actions of tetraethylammonium chloride and atropine in acute poisoning of carbamate insecticides in rats. Arch Toxicol 27: 311-314.

Study & Observations: This study investigated the antidotal effects of tetraethylammonium chloride (TEAC) and atropine sulfate in acute poisoning of 9 carbamate insecticides including methiocarb in male Wistar II rats. The animals (170-200 g bw, age, group size, source not stated) were treated orally with methiocarb technical (purity, batch and doses not stated) dissolved in polyethylene glycol (Lutrol®). The LD_{50} values were determined without or with treatment with either 20 mg of TEAC/kg bw, 50 mg of atropine sulfate/kg bw or 20 mg of TEAC and 50 mg of atropine sulfate ip according to the method of Litchfield and Wilcoxon (1949). Atropine sulfate and TEAC in saline were administered to the animals when cholinergic signs of toxicity were evident (within 10 minutes after dosing). After treatment the animals were observed for 14 days.

Findings & Conclusion: The following LD_{50} values were established for methiocarb: 104.5, 415, 643 and 580 mg/kg bw for the animals receiving no antidote, TEAC, atropine sulfate and TEAC and atropine sulfate combination respectively. Thus, treatment with atropine sulfate alone produced a 6-fold amplification in the LD_{50} value, and appears more effective as an antidote against methiocarb compared to TEAC alone or TEAC and atropine sulfate in combination.

3.4 Effects on acetylcholinesterase enzyme activity

Baron RL, Casterline, Jr. JL & Fitzhugh OG (1964) Specificity of carbamate-induced esterase inhibition in mice. Toxicol Appl Pharmacol 6: 402-410.

Study & observations: This study examined the comparative liver and brain esterase inhibiting properties of several aromatic and heterocyclic carbamate esters of N-methyl and N,N-dimethylcarbamic acid including Bay 37344 (methiocarb, source and purity unstated) in mice. Female mice of 20-25 g bw (5-10 animals/sampling time, Dierolf Farms; strain, age and source unstated) were treated with 16 mg/kg bw of methiocarb (approximate LD_{50} dose) in corn oil by i.p. injection. Concentration of the test solution was adjusted for administration of the approximate LD_{50} dose in 10 µL of solvent/g of bw. The study authors stated that in some instances 15% dimethyl formamide was used in the solution as a solubiliser, however, whether it was used to dissolve methiocarb was unstated. The animals were sacrificed at 30 min, 1 h and 24 h following treatment using an unspecified method, and the brain and liver tissues were collected and homogenised immediately at 4°C with a Teflon glass homogeniser. Tissue samples of the solvent controls were analysed simultaneously with those of the treated animals. Analysis of brain and liver homogenates (equivalent to: 80 mg of brain tissue/flask, and 16 mg of liver tissue/flask) was made using a manometric method, based on the ability of liver or brain homogenates to hydrolyse the following substrates: acetylcholine chloride, acetyl-β-methylcholine chloride, propionylcholine *p*-toluene sulfonate, butyrylcholine *p*-toluene sulfonate, benzoylcholine *p*-toluene sulfonate, triacetin, tributyrin and tripropionyn. Hydrolytic activity was calculated from the initial rate of hydrolysis of the substrate and corrected for non-enzymatic hydrolysis and endogenous enzyme activity. The readings at 1 h were combined from 3-5 analyses, whereas those at 30 minutes were derived from "preliminary data" incorporating 1 or 2 analyses only.

Findings: The data on liver esterase activity in the solvent controls at 1 and 24 h post treatment revealed a marked reduction in liver activity ($p \leq 0.01$ or 0.05) against all substrates except acetyl-β-methylcholine and benzoylcholine at 24 h following administration of the solvent. Reduction in activity at 24 h post treatment varied from about 6.0% to 30% compared to the data at 1 h post treatment. The study authors stated that further analyses using various

corn oil plus dimethyl formamide combinations revealed that the reduction in esterase activity was due to corn oil, not to dimethyl formamide. Furthermore, it was claimed the studies conducted with 1 h liver homogenates of solvent control mice gave the same results as found with untreated animals, but no supporting data were provided.

In liver homogenates, esterase activity against acetylcholine and propionylcholine was not inhibited at 30 minutes post treatment when compared with solvent control values. However, 1 h after dosing, the esterase activity against these two substrates was inhibited by 21 and 30% respectively. With the exception of benzoylcholine *p*-toluene sulfonate, for which no data were given, the esterase activity against the remaining substrates was inhibited more rapidly, ranging from 12% (against butrylcholine p-toluene sulfonate) to 69% (against triacetin) at 30 minutes post dosing.

Inhibition of the esterase activity persisted, but without any consistent trend. Partial recovery was evident against acetyl-β-methylcholine chloride, and tripropionyn, whereas the activity against the remainder was as or more marked at 1 h than at 30 minutes. The authors stated that complete recovery was observed at 24 h after methiocarb administration, but no data were shown. Brain esterase activity was claimed not to have been inhibited at either 1 or 24 h, by methiocarb or any of the other test chemicals. Again, no supporting evidence was given.

Conclusions: Methiocarb caused inhibition of liver esterase activity against acetylcholine and other substrates within 30-60 minutes of administration, which, according to the authors, reversed by 24 h. No inhibition of brain esterase activity was said to have occurred at or after 1 h post treatment. The absence of some supporting data, combined with vehicle induced inhibition of liver esterase(s) 24 h post administration, limits the value of this study and makes independent evaluation difficult.

Knaak JB, Ackerman CR, Yee K, Fredrickson AS & Lee P (1980/81) Reentry research: Dermal dose red cell cholinesterase-response curves for methiocarb and alteration products. Study No. not stated. Lab: California Department of Food and Agriculture, 1220 N Street, Sacramento, CA 95814, USA. Sponsor, study duration: not stated. Report No. 69470. Report date: 1980/81.

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

Study & Observations: This study was performed to investigate the effects of dermally administered methiocarb and its two plant foliar residue components, methiocarb sulfoxide and methiocarb sulfone, on erythrocyte (RBC) ChE activity in male albino SD rats (Simonsen, Gilroy, CA, USA, 220-240 g bw, age not stated). Methiocarb was applied on the shaven skin of the backs of each rat at 200, 400, 800 (6 rats/group), 1600 and 3200 (3 rats/group) µg/cm^2 (equivalent to approximately 22, 44, 87, 174 and 348 µg/kg bw). Methiocarb sulfoxide was administered at 160, 320, 640 and 1280 (equivalent to approximately 17, 35, 70 and 139 µg/kg bw) and methiocarb sulfone at 200, 400, 800, 1000, 2000 and 4000 µg/cm^2 (3 rats/group, equivalent to approximately 22, 44, 87, 109, 217 and 435 µg/kg bw). No information on source, purity and batch numbers of any of the chemicals was provided. Each chemical was applied on a 25 cm^2 area in 1 mL of acetone (purity not stated) using a digital pipette and a glass rod to ensure uniform application. Increments of 0.5 mL were applied to the skin inside a rubber template and allowed to dry prior to the application of the second increment. The control group consisted of 23 rats. Test animals wore collars and hence, any oral exposure to the residual test substance was prevented. The method of animal housing was not provided but it was stated that all animals had free access to food (Purina Rat Chow 5012, Ralston Purina Co., MO, USA) and water. During the exposure period of either 24 or 72 h, the rats were observed for signs of toxicity, loose collars

and chewed rubber templates. The animals receiving methiocarb sulfoxide and methiocarb sulfone and 3 rats/group receiving methiocarb at 200, 400 and 800 µg/cm^2 were sacrificed after 24 h of exposure and the remaining animals after 72 h. The animals were sacrificed by decapitation and the blood was assayed colorimetrically for RBC ChE activity together with statistical analysis of the data according to the method of Knaak et al (1980). No other information on methodology was provided.

Findings: Two line graphs illustrating RBC ChE response following 24 h exposure to methiocarb and methiocarb sulfoxide were provided. The study authors stated that the dose response curves for the two chemicals were linear between 400 and 1600 µg/cm^2, and 320 and 1280 µg/cm^2 respectively. However, the curves were extrapolated to calculate the respective ED_{50} values which were established to be 3915 and 2640 µg/cm^2 for methiocarb (slope 1.16) and methiocarb sulfoxide (slope 1.38) respectively. It was stated that methiocarb sulfone did not produce any depression in RBC ChE activity when applied at levels as high as 4000 µg/cm^2 for a period of 24 h (no data were provided). Biologically significant inhibition of RBC ChE (>20%) following 24 h exposure was observed approximately at 800 and 640 µg/cm^2, and beyond for methiocarb and methiocarb sulfoxide respectively. At 200 µg/cm^2 of methiocarb, RBC ChE activity was unaffected. Study authors stated that similar RBC ChE responses were observed following 72 h exposure, but relevant data to support this claim were not provided. No information on clinical observations was provided.

Conclusions: Under the conditions of the study, methiocarb and methiocarb sulfoxide in acetone were absorbed when dermally administered and caused biologically significant inhibition in RBC ChE activity (>20%) at approximately 800 and 640 µg/cm^2 and above respectively. Methiocarb sulfone did not produce any depression in RBC ChE activity when applied at levels as high as 4000 µg/cm^2 for a period of 24 h.

Although, the study presents data to gain some understanding on the relative toxicity of the test chemicals, due to several study deficiencies (e.g. lack of information on purity of the test chemicals, clinical observations and data limitations and experimental methodology), the findings are of limited value in explaining the events leading to RBC ChE inhibition and recovery. Use of acetone as a vehicle may have resulted in enhanced dermal absorption of the test chemicals.

4. SHORT TERM REPEAT DOSE STUDIES

4.1 Technical Grade Active Constituent

4.1.1 Rats

4.1.1.1 Oral

Kimmerle G (1960) Product Dr Wademeyer H 321 (E 37344) Production No. 2410., Toxicological and Industrial Hygiene Laboratory, Bayer AG. Unpublished. March 25, 1960.

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

Study & Observations: H 321 (methiocarb, purity and source unspecified) in tragacanth suspension was administered by gavage to an unspecified number of albino rats (age and bw not stated) at 3 mg/kg bw/day for the first three days and at 4 mg/kg bw/day for the next 24 days. Two groups of three animals were killed every week and the RBC ChE activity was determined (method unspecified). No details were provided for procedures on animal acclimatisation, housing, feeding, the treatment of control animals and method of sacrifice of test animals.

Findings & Conclusions: The RBC ChE activity was depressed to about 80% after 14 days and to 50% by the end of the study. It was stated that the recovery of the enzyme activity was slow during the following observation period and it did not return to normal values up until 42 days after the completion of the study. However, no further data on enzyme inhibition were provided. No cholinergic signs were observed in the treated animals, and the animals gained weight normally, the study authors stated, but no individual data on any of these parameters were provided. No NOEL for RBC ChE inhibition was established as treatment-related effects were identified at the only dose administered in the study.

Eben A & Kimmerle G (1973) Mercaptodimethur: Effect of acute and subacute oral doses on acetylcholinesterase activity in plasma, erythrocytes and brain of rats. Lab: Bayer AG, Institut Fur Toxikologie, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: Not stated. Report No. 4284. Report date: November 9, 1973.

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

Study: Mercaptodimethur (methiocarb, purity 99%, batch 1/73) in Lutrol (polyethylene glycol 400) was administered by gavage to SPF Wistar rats (10/sex/dose, Winklemann breed, Kirchborchen, initial bw 160-180 g, age not stated) at 1, 3 or 10 mg/kg bw/day for 4 weeks. No details were provided for procedures for animal acclimatisation, housing, feeding, the treatment of control animals and method of sacrifice of test animals at termination. Blood samples for the ChE assay were collected from the retro-orbital venous plexus.

Observations: Acetylcholinesterase activity in plasma and RBC (3/sex/group) was determined at 20 minutes post treatment on days 4, 8, 14, 21 and 28, and additionally 5 h after administration of the last dose using a modified colorimetric method. The brain ChE activity (5/sex/group) was determined 2 h after the final administration using a modified spectrophotometric method. No further information on methodology was provided.

Findings: The study authors stated that the animals which received the test compound at 10 mg/kg bw/day exhibited brief cholinergic signs, but details on the type, onset and the duration of such manifestations were not provided. Plasma, RBC and the brain ChE data are presented in the following table.

Plasma, RBC and the brain ChE (µeq acetylcholine) activities in rats treated with methiocarb for up to 4 weeks

Sampling time (day)	Dose (mg/kg bw/day)			
	Control	**1.0**	**3.0**	**10.0**
Plasma: Males				
21 (2 h p.t.)	0.89	0.88	0.84	0.57 (-36%)
28 (5 h p.t.)	0.98	1.11	0.92	0.62 (-36%)
Females				
21 (2 h p.t.)	2.85	2.69	2.86	1.80 (-37%)
28 (5 h p.t.)	2.74	2.90	3.25	2.22 (-19%)
RBC: Males				
21 (2 h p.t.)	5.07	5.00	5.06	3.35 (-34%)
28 (5 h p.t.)	4.49	4.37	5.02	4.00 (-11%)
Females				
21 (2 h p.t.)	5.20	4.60	4.36	3.49 (-33%)
28 (5 h p.t.)	4.91	4.83	5.03	4.19 (-15%)
Brain*: male				
2 h p.t.	181.5	153.4	165.5	124.4 (-31%)

Female				
2 h p.t.	165.2	144.8	146.7	117.4 (-29%)

*µmoles thiocholine/g of brain tissue. 2 or 5 h p.t. = 2 or 5 h post treatment.

Values in parenthesis represent percent change in ChE activity compared with controls.

Biologically significant, consistent plasma (34-54%) and RBC (22-35%) ChE inhibition was seen at 10 mg/kg bw/day in both sexes at the majority of the sampling times and the inhibition appears to be test compound related. Similarly, the depression in brain ChE activity noted in rats of both sexes (31% in males and 29% in females) at the same dose level was biologically significant and was probably attributable to treatment.

Conclusions: Under the conditions of the study, the NOEL for plasma, RBC and brain ChE inhibition was established at 3 mg/kg bw/day based on ≥20% inhibition at 10 mg/kg bw/day.

Hixson EJ (1981) Cholinesterase no-effect level of Mesurol and Mesurol sulfoxide in female rats. Lab: Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Centre, 17745 South Metcalf, Stilwell, Kansas 66085, USA. Sponsor: Bayer AG, Germany. Study duration: February 16 to March 16, 1981. Report No. 199. Report date: August 31, 1981.

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

Study: Methiocarb technical (purity 97%, Batch: 0030058) or methiocarb sulfoxide (purity 95.2%, Batch: Prasad 75-20-132C; Strankowski 79-R-187; Koch 80-R-162-9; Vial # 21) in Carbowax was administered by oral gavage (0.5% of the animal's bw) to groups of 15 female SD rats (Sasco Inc., Omaha, Nebraska, USA, initial bw 192-280 g, age not stated) at doses of 0.5 or 2.0 mg/kg bw/day, 5 days/week for 4 weeks. A concurrent vehicle control group of 15 rats received Carbowax at 0.5 mL/100 g bw/day in an identical dose pattern during the treatment period. For ChE determination, each group was subdivided into three subgroups of five. From the first subgroup, blood samples were collected (method unspecified) before dosing and 30 minutes after dosing on days 0, 7, 14, 21 and 28. Blood samples were collected from the second subgroup before dosing and 4 h after dosing on days 4, 11, 18 and 25. The third subgroup was held in reserve in case anaemia was observed in either of the other two subgroups.

Observations: The rats were observed daily at the time of dosing for general appearance and at 30 minutes, 1 and 4 h after treatment during the first 5 days for cholinergic signs. Thereafter, in addition to daily checks, they were observed for moribundity late in the afternoon on days that the blood samples were collected. The animals were weighed weekly. Plasma and RBC ChE activities were determined (method unspecified) and the percent depression of ChE activity was calculated for each individual animal based on its pre-treatment data. The results were reported as mean ± SD. Haematocrit values were determined during blood fractionation. No further information on methodology was provided.

Findings: One rat treated with methiocarb at 2.0 mg/kg bw/day was found dead on the third day (cause not specified) and another treated at 0.5 mg/kg bw/day died immediately after treatment on day 10, probably due to inadvertent deposition of the test chemical in the respiratory passages. Group mean body weights and body weight gains were not affected by treatment. Sporadic tremors were seen in 6/15 rats receiving methiocarb sulfoxide at 2.0 mg/kg bw/day during the first five days but not thereafter. No anaemia was observed in any group. Plasma and RBC ChE activities observed at 30 minutes post treatment are presented in the following Table.

Mean depression of plasma and RBC ChE activities[a] in rats 30 minutes after treatment with methiocarb and methiocarb sulfoxide

Week	Plasma			RBC		
	Vehicle Control	0.5 mg/kg	2.0 mg/kg	Vehicle control	0.5 mg/kg	2.0 mg/kg
Methiocarb						
1	1 (9)	21 (2)	41 (6)	-7 (7)	12 (8)	29 (9)
2	-4 (9)	12 (5)	28 (6)	6 (9)	2 (8)	15 (3)
3	3 (10)	11 (2)	25 (6)	3 (7)	11 (4)	17 (6)
4	-4 (16)	1 (16)	19 (7)	2 (2)	5 (10)	17 (4)
Methiocarb sulfoxide						
1	1 (9)	34 (20)	49 (7)	-7 (7)	26 (8)	34 (9)
2	-4 (9)	21 (4)	39 (5)	6 (9)	13 (1)	32 (8)
3	3 (10)	39 (10)	62 (5)	3 (7)	31 (10)	46 (5)
4	-4 (16)	36 (6)	52 (7)	2 (2)	22 (2)	37 (6)

[a]Data represent the mean percent inhibition of plasma or RBC ChE with standard deviation in parenthesis. Percent inhibition is based on the pre-treatment enzyme activity. Negative numbers indicate an increase in enzyme activity.

In rats receiving methiocarb at 0.5 mg/kg bw/day, biologically significant (>20%) plasma ChE inhibition was observed 30 min post treatment at week one only. A trend towards decreased depression of the plasma ChE activity with time was observed in these animals. In animals receiving methiocarb at 2.0 mg/kg bw/day, biologically significant plasma ChE inhibition was noted during the first three weeks and RBC ChE inhibition at week one and not thereafter. No statistically or biologically significant inhibition of plasma or RBC ChE was seen at 4 h in rats receiving Mesurol at either dosage (data not shown).

Thirty minutes after treatment with 0.5 or 2.0 mg/kg methiocarb sulfoxide, biologically significant inhibition was observed in plasma ChE (from weeks 1-4) and RBC ChE (during all but second week). At 4 h, plasma ChE activity had returned to its baseline value, irrespective of dose. RBC ChE was slower to recover, showing biologically significant inhibition at 0.5 and 2.0 mg/kg in 2 of the 4 weeks on study.

Mean depression of plasma and RBC ChE activities[a] in rats 4 h after treatment with methiocarb sulfoxide

Week	Plasma			RBC		
	Pre-treatment	0.5 mg/kg	2.0 mg/kg	Pre-treatment	0.5 mg/kg	2.0 mg/kg
1	1 (9)	2 (16)	5 (5)	-7 (7)	10 (10)	20 (11)
2	-4 (9)	-3 (14)	4 (4)	6 (9)	21 (7)	21 (7)
3	3 (10)	10 (16)	5 (13)	3 (7)	25 (5)	18 (3)
4	-4 (16)	-15 (8)	3 (5)	2 (2)	12 (6)	10 (40

[a]Results are shown as mean percent inhibition (with standard deviation in parenthesis). Percent inhibition is based on the pre-treatment enzyme activity. Negative numbers indicate an increase in enzyme activity.

Throughout the study both test compounds demonstrated an apparent dose relationship with respect to ChE inhibition 30 minutes post treatment but not at 4 h.

Conclusions: Under the conditions of the study, a NOEL for methiocarb sulfoxide was not observed due to biologically significant (≥20%) inhibition in plasma and RBC ChE activity at

0.5 and 2.0 mg/kg bw/day. The NOEL of methiocarb for RBC ChE inhibition was established at 0.5 mg/kg bw/day. No NOEL of methiocarb for plasma ChE inhibition was established due to the enzyme inhibition (≥20%) seen at week one of the study at the lowest dose of 0.5 mg/kg bw/day.

4.1.1.2 Intraperitoneal

Dubois KP & Raymund AB (1961) The subacute parenteral toxicity of Bayer 37344 to rats. Project No., Sponsor, Study duration: Not stated, Lab: Department of Pharmacology, University of Chicago, Chicago 37, IL, USA. Report No. 7637. Report date: September 19, 1961.

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

Study: Young, adult female SD rats (5/group, age, bw and source not stated) were treated with daily i.p. injections of Bayer 37344 (methiocarb; batch, purity, source not stated) in 20% ethanol and 80% propylene glycol solution at dose levels of either 0 (control), 5, 10 or 15 mg/kg bw/day for 60 days. The treatment received by the control animals was not specified. Body weights of the animals were measured every five days (data not provided). The mortality among treated animals during the 60-day treatment period is presented in the following table.

Mortality in rats treated with methiocarb i.p. daily for 60 days

Dose	**Days after first injection**				**Mortality**	**%**
(mg/kg bw/day)	**0-5**	**5-10**	**10-30**	**30-60**	**in 60 days**	**Mortality**
Control	0	0	0	0	0/5	0
5.0	0	0	0	0	0/5	0
10.0	0	0	2	1	3/5	60
15.0	4	1	0	0	5/5	100

According to the study authors, no treatment-related effect was seen in weight gain of the animals at 5 mg/kg bw/day dose level compared to the control animals. A "slight" gain in the body weight was noted in rats in the 10 mg/kg bw/day group during the treatment period. However, no absolute data on body weights were provided.

In addition to mortality, "severe" cholinergic symptoms (unspecified) were noted at the two higher dose levels but appeared "completely reversible" within a few hours (time not specified). Further, no treatment-related effect was seen in either the brain, submaxillary gland or serum ChE activity in rats at 5 mg/kg bw/day, but no supporting data for any dose group were provided. No other information on experimental methodology, food and water consumption or clinical observations was provided.

Conclusions: Under the conditions of the study and based on the mortality data provided, a toxicological NOEL could be set at 5 mg/kg bw/day. However, the validity of this finding is markedly reduced due to methodological deficiencies, lack of absolute data on several useful study parameters and information on clinical observations.

4.1.1.3 Inhalation

Kimmerle G (1960) Product Dr. Wedemeyer H 321 (E 37 344) production No. 2410 – Inhalation Tests. Toxicological and Industrial Hygiene, Laboratory Bayer AG. Unpublished. March 25, 1960.

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

Study & observations: In this study, 400 mg of H 321 (methiocarb, purity and source unspecified) in an unstated volume of ethanol was sprayed using a "Flury type atomiser" into a chamber of 400 L capacity containing 1 rabbit, 1 guinea pig, 2 rats and 4 mice (bw, age, sex, source unstated) for 1 h/day, for 5 consecutive days. No details were provided for procedures on animal acclimatisation, housing, feeding, treatment of control animals, or size of the aerosols.

Findings: Two mice died four days after the completion of the study. It was stated that the animals were "observed to suffer from slight irritation of mucous membrane" during the first 4 days. Muscular spasms were observed in the rats and mice on the fifth day. The study authors indicated that the surviving animals showed recovery soon after treatment. No "poisoning symptoms" were observed when half of the above dose (ie 200 mg/400 litres) was administered to a different group of animals involving 1 cat, 1 rabbit, 1 guinea pig, 2 rats, 4 mice (bw, age, sex and source not specified), for 1 h/day, for 5 consecutive days, except for slight mucous membrane irritation.

Conclusions: Because of the lack of a control group, limited information on experimental animals, and no clinical observations or necropsy findings, this study is of limited regulatory value.

Thyssen J & Mohr U (1983) H 321 (Mesurol Active Ingredient) Subacute inhalation study with rats. Project No: T7011555. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Landwirtschaft, Friedrich-Ebert-Stasse 217, D5600, Wuppertal-Elberfeld, Germany. Study duration: July 21 to August 13, 1982. Report No. 12120. Report date: September 30, 1983.

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

Study: Wistar albino rats (Bor.: WISW, SPF/Cpb, Winkelmann, Borchen, initial body weights of animals were 230 and 200 g for males and females respectively, age not stated) were exposed to aerosols of technical H 321 (methiocarb, batch 234002624, purity 97.9%). The test formulations were prepared weekly from a stock solution of 5% H 321 in a 1:1 ethanol:Lutrol® (polyethylene glycol 400) vehicle. Animals were fed with species specific feed and water *ad libitum*. No information on animal acclimatisation was provided. Rats (10/sex/group) were exposed daily for 6 h/day for 15 workdays within three weeks (6 x 5 x 3 h) to targeted concentrations of either 0.1, 0.4 or 1.6% of methiocarb (aerosols, equivalent to 20, 80 or 320 mg/m^3 air respectively). Methiocarb was sprayed dynamically and the test was carried out in a dynamic inhalation apparatus (Kimmerle and Eben, 1973). Animals in negative and solvent control groups received air only and 20 mL of the solvent vehicle respectively. Study authors indicated that the "exposure conditions largely ensured that the animals could only inhale the aerosols and there was virtually no skin contact with the aerosols".

As determined by a spectrophotometric method, the actual mean concentrations of H 321 in air were 6, 23 and 96 mg/m^3 air (mean of 9 double determinations in each case, approximately 30% of the theoretical concentrations).

The mass median diameter (MMAD) were determined four times in each case for the solvent control and each treatment group using a cascade impactor with an extraction speed of 125 cm/s ± 10%. The MMADs of aerosols were 2.27, 2.27, 2.50 and 2.14 μm for solvent control, 6, 23 and 96 mg/m^3 air respectively for exposure groups. Data indicated that over 90% of the particles in the chamber had an aerodynamic diameter < 4.0μm (70% related to mass) and the aerosol populations in the exposure chamber were "monodisperse" with Geometric Standard Deviation (GSD or σ_g) of aerosols ranging from 1.69-1.75 μm.

Observations: Animals were examined daily for appearance and behaviour and the body weights were recorded before commencement of study and at the end of each 5 day exposure period. Upon completion of exposure, haematology, clinical chemistry and urinalysis were performed (5 animals/dose). Haematology parameters determined were Hct, Hb, WBC, RBC, differential WBC counts (WBC-DC), thrombocyte count, MCV, MCH, MCHC and reticulocyte count. Clinical chemistry determinations included, AST, ALT, AP, plasma urea, blood sugar, creatinine and bilirubin. The blood (erythrocyte and plasma) ChE activities were determined before commencement and after each exposure period (5, 10, and 15 days) using blood samples collected from plexus retro-orbitalis, while the brain ChE was assessed at the conclusion of the study using a colorimetric method (modified from Ellman *et al*, 1961). Mean values of study parameters examined were tested for statistical significance using the Wilcoxon rank-sum test.

After completion of treatment, the animals were sacrificed by heart puncture under ether anaesthesia and the internal organs were grossly appraised. The absolute and relative weights of thyroid, heart, lung, liver, spleen, kidneys, adrenals and testes or ovaries were determined. Tissue samples from heart, lung, liver, kidneys, testes, ovaries, spleen, thyroids, adrenals, oesophagus, stomach, bronchial lymph nodes, eyes, trachea, larynx and head (nose-throat area) collected and fixed in 10% buffered formaldehyde solution for histopathological examinations.

No further information on inhalation chamber design, housing of animals in the inhalation chamber, aerosol generation method or any clinical observations on skin, eyes, mucous membranes was provided.

Findings: There were no treatment-related changes in appearance or behaviour observed in animals in the two control groups and at 6 and 23 mg/m^3. Non-specific disturbed behaviour and muscular tremor were observed in animals at 96 mg/m^3 starting from day 5 and 6 and remained apparent until termination (except during weekends) of the study. No mortalities occurred during the study. Data on food and water intake were not provided.

No treatment-related effects of methiocarb were observed on the body weights of animals at 6 and 23 mg/m^3. Significant reductions described by the study authors as "toxicologically relevant" in mean body weights of male animals after 5, 10 and 15 days exposure were noted ($p \leq 0.05$) in the 96 mg/m^3 dose group compared to the mean body weights of animals in the air only (negative) control group. When the same treatment group was compared with the solvent controls the difference noted was not statistically significant. A significant reduction ($p \leq 0.05$) in mean body weight was also noticeable in female rats receiving methiocarb at 96 mg/m^3 after 15 days compared with the solvent control group. Data are presented in the following tables. It is noted that male rats in both negative and solvent control groups and female rats in the solvent control group lost body weight after 5 days exposure and regained weight thereafter. Female rats in the negative control group showed a similar pattern of body weight change but regained their initial weight only after 15 days of exposure. Transient reductions of mean body weights of animals in test and control groups may therefore have been associated with stress related factors.

Mean body weights (g) of male rats

Dose mg/m^3	Exposure Period (week)			
	0	1	2	3
Control (air only)	243	235	239	244
Control (solvent)	243	233	236	241
6	239	230	234	241
23	238	225	233	241
96	235	221*	224*	229*

*Significantly different from air only control group (p≤0.05).

Mean body weights (g) of female rats

Dose mg/m^3	Exposure Period (week)			
	0	1	2	3
Control (air only)	201	197	184	198
Control (solvent)	201	196	198	201
6	195	193	192	194
23	196	194	196	198
96	195	193	196	193*

*Significantly different from solvent control group (p≤0.05).

There was no evidence for any treatment-related changes in any of the haematological parameters tested. Several of the clinical chemistry parameters tested after 15 days treatment showed significant (P≤0.05 or p≤0.01) differences from either the air or solvent control groups. In the majority of cases these differences are not considered to be treatment-related because they did not show any consistent dose-related trend and failed to achieve significance against both control groups. In males, serum AST and AP activities were significantly reduced compared to air and solvent controls (see table below). However, the modest reductions in enzyme activity observed (approximately 13-18%) are not considered to be indicative of any disease process because they lay within the historical control ranges provided by the study authors.

Results of clinical chemistry studies in male rats (Treatment day 15)

Dose mg/m^3	AST u/L	ALT u/L	AP u/L
Control (air only)	58	50	350
Control (solvent)	65	44	314
6	51	42**	322
23	48*	44	326
96	48**	42**	305*

*Significantly different from air control (p≤0.05)
** Significantly different from air control (p≤0.01)

The data on urinalysis did not provide any evidence for impaired renal function or tissue damage.

Significant reductions in plasma ChE activities were observed in male animals in the 23 mg/m^3 dose group after exposure periods of 5 days [32% (p≤0.01)] and 10 days [31% (p≤0.05)]. Although no statistical significance was achieved, the enzyme activity in males at

23 mg/m^3 was inhibited by 21% and showed a trend towards recovery after 15 days' exposure. Reductions in ChE activities in animals at 96 mg/m^3 after 5, 10 and 15 days were 55% (p≤0.01), 37% (p≤0.05) and 52% (p≤0.01) respectively. Mean plasma ChE activities in male rats are presented in the table below.

Plasma ChE activity (u/mL) in male rats

Dose mg/m^3	**Exposure Period**			
	wk 0	**wk 1**	**wk 2**	**wk 3**
Control (solvent)	0.50	0.49	0.51	0.56
6	0.48	0.43	0.43	0.53
23	0.47	0.33** (32)	0.35* (31)	0.44 (21)
96	0.46	0.22**(55)	0.32* (37)	0.27** (52)

Values in parentheses represent percent inhibition
*Significantly different from control (p≤0.05)
**Significantly different from control (p≤0.01)
wk = week.

Mean plasma ChE values for female rats are presented in the following table. Significant depressions of ChE activity were observed in 96 mg/m^3 dose group after 5, 10 and 15 days, where 56%, 60% and 61% (p≤0.01) inhibition occurred respectively.

Plasma ChE activity (u/mL) in female rats

Dose mg/m^3 air	**Exposure Period**			
	wk 0	wk 1	wk 2	wk 3
Control (solvent)	1.98	1.59	1.64	1.71
6	2.25	1.49	1.52	1.72
23	2.26	1.47	1.55	1.72
96	2.11	0.69* *(56)	0.66**(60)	0.67** (61)

Values in parentheses represent percent inhibition
**Significantly different from control (p≤0.01).
wk = week

The erythrocyte ChE activities for male and female rats were not significantly depressed except in male animals in the 96 mg/m^3 dose group, where an 18% reduction (p≤0.01) of enzyme activity was recorded compared to the solvent control group, after 5 days of exposure. The 6 mg/m^3 group showed elevated erythrocyte ChE activity after 15 days' exposure (22% and 25% increases for males and females respectively) compared to the solvent control group. In view of its isolated occurrence, this apparent elevation may have been an experimental artefact.

Brain ChE activity was significantly reduced in male rats at 23 and 96 mg/m^3 after 15 days exposure (35 and 39% respectively, p≤0.01) compared to the solvent controls. In females, ChE inhibition (26%, p≤0.01) was noted only at 96 mg/m^3, again on treatment day 15.

Post-mortem examinations did not reveal any treatment induced changes in any of the organs (specific organs and/or systems, surfaces examined were not identified). The absolute liver weights of male animals at 96 mg/m^3 (p≤0.05) and females at 23 and 96 mg/m^3 (p≤0.01) were decreased (13%, 7% and 14% respectively) in comparison to the negative controls. When treated animals were compared with the solvent controls, a significant reduction (13%) of absolute liver weight was recorded in female rats at 96 mg/m^3 (p≤0.05). A decrease (16%) of

absolute spleen weight occurred in males at 96 mg/m^3 ($p \leq 0.05$) compared with the solvent control group.

Significant reductions in relative liver weights were noted in female rats in both 23 and 96 mg/m^3 dose groups ($p \leq 0.01$) compared to the negative controls. Conversely, the male animals at 96 mg/m^3 showed an increase in relative weight (4%) of the kidney ($p \leq 0.05$) compared to the negative controls. A comparison with the solvent controls revealed a significant reduction in relative liver weight (9%) in female animals, and an increase of kidney weight (6%) in males at 96 mg/m^3 ($p \leq 0.05$). The study authors concluded that, the effect was "toxicologically irrelevant because the changes were within the normal physiological range" for which supportive historic data (for liver and kidney) were provided. There were no histopathological changes that could be attributed to the treatment.

Conclusions: Based on statistically and biologically significant (>20%) inhibition of ChE activity in the plasma and brain at higher exposure levels, the NOEL in male rats was 6 mg/m^3 and the NOEL for females was 23 mg/m^3.

4.1.2 Rabbits

4.1.2.1 Dermal

Kimmerle G (1969c) BAY 37344: Subacute dermal toxicity study on rabbits. Report No. 1291. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: not stated. Report date: April 1, 1969.

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

Study: Technical grade BAY 37344 (methiocarb, purity not stated) was applied to shaved flanks of 10 adult white rabbits (Chinchilla breed, Koospol, Prague, 5/sex/group, bw not stated) at 500 mg/kg bw/day for 14 consecutive days. Ten rabbits served as controls (5/sex). The test chemical was applied as an emulsion (50%) prepared using water and Emulsifier W evenly spread on two small flexible plastic plates (6 x 8 cm) which were held in contact with the skin by a leather sleeve wrapped around the trunk (modified from Draize, 1959). After 24 h of exposure the plastic plates were removed and the next dose was applied without removing the residual test compound present on the skin, and the application procedure was continued daily for the remaining treatment period. The control rabbits were treated similarly with aqueous emulsion without methiocarb. The test animals were housed singly in cages and received food and water *ad libitum*. Food and water intake of test animals during the study was not measured. The application sites were washed with soap and water 24 h after final application and the animals were kept under observation for 2 more weeks. The animals were inspected daily during the experiment and body weights were recorded weekly. No further information was provided on experimental methodology.

Observations: Haematological [Hb, Cyanomethemoglobin, RBC, WBC, Hct, Colour index, & MCV)] liver [(ALT, AST, sorbitol dehydrogenase (SDH)], serum bilirubin and protein content) and kidney function (serum urea concentration) tests and urinalysis (glucose, protein, blood, bile pigments and deposits) were performed on each animal prior to treatment, upon termination of treatment and at the end of the 2 week post treatment observation period.

Findings: There was no premature mortality. No behavioural changes or any toxicological symptoms were observed in any of the treated animals during the treatment or post treatment observation periods compared to control rabbits. There were no effects on weight gain, haematology or liver and kidney function of treated animals. Urinalysis did not reveal any treatment-related variations or significant differences in the measured parameters.

Conclusions: Under the conditions of the study, dermal application of methiocarb at 500 mg/kg bw/day daily for 14 days did not appear to have produced any treatment-related effects in rabbits. However, the reliability of the study is reduced due to the absence of any histopathological examination.

Procter BG (1988) A 21-day dermal toxicity study of Mesurol technical in Albino rabbits. Project No. 51901, Lab: Bio-research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec, H9X 3R3 Canada. Sponsor: Mobay Corporation, Health, Environment and Safety Corporate Toxicology, Stanley Research Centre, 17745 South Metcalf, Stilwell, Kansas 66085-9101, USA. Study duration: May 4, 1988 – November 23, 1988. Report No. 1084, Report date: November 23, 1988.

GLP (US EPA FIFRA 40 CFR, Part 158.340, 82-2), quality assured study. No test guidelines were cited.

Study: Mesurol technical (methiocarb, Control/Batch No. 86I004, Purity: 99.3%) was applied to the shaven intact skin (approximately 10 x 20 cm^2) of NZW rabbits (5/sex/dose, initial bw 2.4-3.3 kg, 12 weeks old, Maple Lane Farm, Clifford, Ontario, Canada) at either 0, 60, 150 or 375 mg/kg bw, 6 h/day for 21 days under occluded conditions. The rabbits were acclimatised to the laboratory conditions at least for 14 days. The test substance was moistened with physiological saline (1.0 and 0.5 mL of 0.9% saline for the lower doses and highest dose respectively), spread evenly on a piece of gauze and then applied on the treatment site of each rabbit. The dosing was based on the animal's most recent body weight. Each treatment site was occluded with an impervious wrapping material and upon its removal after 6 h, the treated area was cleaned with a tap water moistened gauze pad to remove the residual test chemical. Between exposure periods the animals wore (for approximately 18 h) a flexible Elizabethan collar and hence, any oral exposure to the residual test chemical was prevented. The animals in the control group received a patch moistened with 0.5 mL saline solution. The animals were housed individually under conventional laboratory conditions and were provided with standard pelleted diet (Purina Certified Rabbit Chow, No. 5322) and water *ad libitum*. Statistical significance among group mean values of the study observations was tested using appropriate statistical procedures including: Bartlett's test, one-way ANOVA, Dunnett's test and the Kruskal-Wallis test.

Observations:
In-life: Mortality was checked daily and any moribund animal was sacrificed. Clinical signs were observed twice daily and signs of skin irritation were scored using the method of Draize (1965) just prior to the test article application. The animals were weighed immediately prior to the onset of the study and twice weekly during the study including immediately before the terminal kill after an overnight fast. The daily food consumption of each rabbit was measured 3 times weekly during the first 2 weeks and then 4 times during the last week of the study. In addition the following haematology and blood chemistry parameters were determined one week prior to the commencement of the study and pre-terminally: Hct, Hb, RBC, WBC, WBC-DC, reticulocyte and platelet counts, prothrombin time (PT), activated partial thromboplastin time (AAPT), MCV, MCH and MCHC. Serum chemistry parameters examined were: blood urea nitrogen (BUN), total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase (AP), glutamic pyruvic acid transaminase (GPT), glucose, total bilirubin, gamma glutamyl transpeptidase (GGTP), lactic dehydrogenase (LDH), creatinine, cholesterol, triglycerides, phosphorus, chloride, sodium and calcium.

Plasma and RBC ChE levels in all animals were determined using samples (1 mL whole blood/rabbit/occasion) taken once during the treatment period and again immediately after the 6 h exposure period on days 1, 7, 14 and 21 (method not specified). For the animals in the 375 mg/kg bw/day group, the analyses were repeated using blood samples collected approximately 16 h following the end of exposure on days 1,7, 14, and 21.

Terminal: A detailed necropsy was conducted on all animals, either found dead or sacrificed on the day following last treatment using an overdose of pentobarbital sodium (i.v. injection)

followed by exsanguination by incision of the axillary arteries. At necropsy, the following organs were dissected free of fat and weighed: adrenals, kidneys, liver, brain, heart, lungs (bronchi), ovaries/testes, pituitary, spleen, thyroid and parathyroid glands. Following weighing of the entire brain, it was divided into two halves by a median sagittal section. The left half was then weighed and frozen at –20°C while the right half was processed for histopathological examination. Tissue samples from the liver, kidneys, testes/ovaries, skin and macroscopic lesions were processed for histological examination, while a range of other tissues were stored for possible future analysis. The frozen left half of the brain was homogenised and ChE activity of the homogenate was determined (method not specified).

Findings: Two animals (1 male and 1 female) at 60 mg/kg bw/day became progressively ill during the first 2 weeks of treatment and the female animal died prematurely during treatment on study day 10. The male animal was sacrificed *in extremis* on treatment day 14. Prior to death, both animals displayed clinical signs such as decreased faecal output, weight loss and decreased motor activity. Similar symptoms were also observed in three other animals (two females, one each in the low and mid dose groups and a male in the high dose group), but there were no further mortalities.

Food consumption in males receiving the high dose was reduced on days 9, 11, 13 and 15 of the study and achieved statistical significance on days 9 and 15 ($p<0.05$) compared to the concurrent controls. Likewise, the food consumption in the high dose group females was less than the corresponding controls, to a biologically significant extent on days 7 and 9 of the study. Further reductions in the food consumption were noted in female animals in the mid dose group during the second week but the parameter showed a regaining trend thereafter. Overall, the total amount of food consumed by the males in the high dose group during the study was 13% less compared to that of the control animals and the decreases observed at this dose level appear to be treatment-related. Relevant food consumption data are summarised in the following Table.

Food consumption in rabbits treated with methiocarb technical

Observation day	Mean food consumption (g/rabbit/day)			
	Dose (mg/kg bw/day)			
	Control	60	150	375
Males				
9	379	339	374	256[a] (32%)
11	374	308	381	248 (34%)
13	354	327	380	227 (36%)
15	363	382	383	257[a] (29%)
Females				
7	338	311	257 (24%)	360
9	383	319	296 (23%)	384
19	407	372	377	310 (24%)
21	442	377	385	354 (20%)

[a]Significantly different from control values ($p<0.05$)
Values in parenthesis represent the percent reductions compared to the controls.

There were no significant differences seen in the mean group body weight data of treated animals. The data on total weight gained by the treated animals during the study are presented in the following Table.

Total weight gained by the animals during the study

Group	Mean weight gain (g)			
	Dose (mg/kg bw/day)			
	Control	60	150	375
Males	180	200	220	140 (22%)
Females	240	215	140 (42%)	180 (25%)

Values in parenthesis represent the percent reductions compared to the controls.

Compared with controls, biologically significant deficits in weight gain occurred in both sexes at the high dose and in mid dose females.

The study authors stated that no skin reaction (erythema or oedema) to the test substance was evident at any time during the study in any of the test animals, and methiocarb technical was not a skin irritant (data not provided). A few pre-terminal haematological parameters (segmented neutrophils, lymphocytes and MCHC) in males in the low-dose group showed significant differences ($p<0.05$) from the respective controls but the values for the mid- and high-dose groups were comparable to those of corresponding controls. All other haematological data were comparable to those of the corresponding controls.

Compared with the values found at pre-treatment, there was a trend towards depressed serum cholesterol and triglyceride levels in males and bilirubin in females at the pre-terminal analysis. The trend seen in controls and treated groups was not dose related and its magnitude was inconsistent between the various groups. This led to a statistically significant ($p<0.05$) reduction in serum cholesterol in high dose males and a significant ($p<0.05$) increase in serum bilirubin in females at the low and mid doses compared with controls at pre-termination. However, these findings are not considered biologically significant because they were caused by variation in the magnitude of a trend common to both control and treated groups.

Group means of plasma ChE activity in males showed dose related reductions on days 7, 14 and 21 (6 h post treatment) of the study, with the values in the high-dose group achieving significance on days 14 ($p<0.05$) and 21 ($p<0.01$), compared to the concurrent control data (see Table below). Biologically significant reductions in plasma ChE activity were evident in males on day 7 at 375 mg/kg and on day 14 at 150 mg/kg (6 h post treatment) compared with parallel controls.

Plasma ChE activity in male rabbits treated with methiocarb technical

Dose mg/kg bw/day	Plasma ChE activity (U/L, mean ± SD) Observation day				
	Pre-treatment	1	7	14	21
6 h post treatment					
Control	363 ± 43	330 ± 94	320 ± 23	346 ± 55	361 ± 34
60	369 ± 58	360 ± 66	305 ± 77	345 ± 59	339 ± 47
150	331 ± 84	289 ± 38	275 ± 71	261[c] ± 34 (24%)	304 ± 31
375	337 ± 83	305 ± 82	250[c] ± 72 (22%)	239[a] ± 83 (31%)	274[b] ± 44 (24%)
16 h post treatment					
375	-	304 ± 41	258[d] ± 76 (23%)	281 ± 79	288 ± 54

[a]Significantly different from control value ($p<0.05$).

[b]Significantly different from control value ($p<0.01$).

[c]Biologically significant compared to the parallel controls.

[d]Biologically significant compared to the pre-treatment data.

No statistically significant test substance related effects were seen in plasma ChE in female rabbits (see Table). A biologically significant reduction in plasma ChE activity was noticed in females at 150 mg/kg bw/day on day 7 (6 h post treatment) compared with both pre-treatment (23%) and concurrent control (28%) data. However, plasma ChE activity was not reduced to biologically significant levels at 375 mg/kg bw/day.

Plasma ChE activity in female rabbits treated with methiocarb technical

Dose mg/kg bw/day	Plasma ChE activity (u/L, Mean ± SD) Observation day				
	Pre-treatment	1	7	14	21
6 h post treatment					
Control	372 ± 71	322 ± 55	336 ± 35	317± 43	341 ± 39
60	409 ± 79	349 ± 51	302 ± 71	329 ± 38	345 ± 47
150	314 ± 66	264 ± 41	242 ± 86 (28%)	267 ± 58	294 ± 64
375	347 ± 55	323 ± 96	281 ± 83	289 ± 75	317 ± 44
16 h post treatment					
375		354 ± 89	309 ± 83	301 ± 64	307 ± 31

Value in parenthesis is biologically significant compared to the parallel controls.

There was marked inter- and intra-group variation in RBC ChE activity, but statistical or biological significance were not attained. No treatment-related effects were observed in brain ChE activity.

At necropsy, ulceration and haemorrhages on the gall bladder mucosa were observed in one male rabbit at 60 mg/kg bw/day, that was sacrificed on day 14 and mild pneumonia and multifocal intra-alveolar haemorrhage in the lungs in one doe found dead on day 10. In the surviving animals pale areas in the liver and dark and/or depressed areas in the lungs were noted. No microscopic changes attributable to the treatment were observed in tissues except slight to mild (histological grade 1-2) hyperkeratosis, epidermal hyperplasia and mixed cell infiltration in the upper dermis in both the treated and control animals of both sexes, with

incidences of 2/5 and 0/5 (males) and 5/5, 1/5 (females) for the control and high dose groups respectively. Therefore this finding may have been a consequence of the experimental procedure.

The absolute group mean heart weights of treated males were significantly lower both in the low and high-dose groups compared to the control values. This is not considered to be biologically significant, in the absence of a corresponding effect on relative heart weight. Other absolute and relative organ weight data did not reveal any treatment-related intergroup differences.

Conclusions: Based on decreased food consumption and weight gain and plasma ChE inhibition at 150 mg/kg bw/day, the NOEL for this study is set at 60 mg/kg bw/day.

Procter BG (1989) A 21-day dermal toxicity study of Mesurol technical in Albino rabbits. Project No. 51925, Lab: Bio-research Laboratories Ltd., 87 Senneville Road, Senneville, Quebec, Canada H9X 3R3. Sponsor: Mobay Corporation, Health, Environment and Safety Corporate Toxicology, Stanley Research Centre, 17745 South Metcalf, Stilwell, Kansas 66085-9101, USA. Study duration: July 7, 1988 – August 31, 1989. Report No. 1160, Report date: August 31, 1989. Supplemental submission to the US EPA MRID# 40922301.

GLP (US EPA FIFRA 40 CFR, Part 158.340, 82-2), quality assured study. No test guidelines were cited.

Study: Mesurol technical (methiocarb, Control/Batch No. 86I004, Purity: 97.5%) was applied to the shaven intact skin (approximately 10 x 20 cm^2) of NZW rabbits (5/sex, initial bw 2.6-3.1 kg, 12 weeks old, Maple Lane Farm, Clifford, Ontario, Canada) at a single dose level of 0.5 g/kg bw, 6 h/day for 21 days under occluded conditions. The rabbits were acclimatised to the laboratory conditions for 15 days. The test substance was moistened with physiological saline (1.5 mL of 0.9% saline), spread evenly on a piece of gauze and then applied on the treatment site of each rabbit. The dosing was based on the animal's most recent body weight. The control animals (5/sex) were treated similarly and received gauze pads moistened with 1.5 mL of physiological saline. Each treatment site was occluded with an impervious wrapping material and upon its removal after 6 h, the treated area was cleaned with a tap water moistened gauze pad to remove the residual test chemical. Between exposure periods the animals wore a flexible Elizabethan collar and hence, any oral exposure to the residual test chemical was prevented. They were housed individually under conventional laboratory conditions and were provided with standard pelleted diet (Purina Certified Rabbit Chow, No. 5322) and water *ad libitum*. Statistical significance among group mean values of the study observations was tested using appropriate statistical procedures which included: Bartlett's test, one-way ANOVA, Dunnett' test and Kruskal-Wallis test.

Observations:
In-life: Mortality among the test animals was checked daily. Clinical signs were observed twice daily (pre and post dosing) and signs of skin irritation were scored daily prior to treatment and before necropsy using the method of Draize (1965). The animals were weighed immediately prior to the onset of the study and twice weekly during the study including weighing immediately before the terminal kill after an overnight fast. The food consumption of each rabbit was measured every 2 days. The following haematology and blood chemistry parameters were determined using blood samples collected from the auricular artery one week prior to the commencement of the study and pre-terminally: Hct, Hb, RBC, WBC, WBC-DC, reticulocyte and platelet counts, prothrombin time, activated partial thromboplastin time, MCV, MCH and MCHC. Serum chemistry parameters examined were: blood urea nitrogen (BUN), total protein, albumin, globulin, albumin/globulin ratio, alkaline phosphatase (AP),

glutamic pyruvic acid transaminase (GPT), glucose, total bilirubin gamma glutamyl transpeptidase (GGTP), lactic dehydrogenase (LDH), creatinine, cholesterol, triglycerides, phosphorus, chloride, sodium and calcium.

The plasma and red blood cell (RBC) ChE levels of all animals were determined using samples (1 mL whole blood/rabbit/occasion) taken once during the treatment period and again immediately after the 6 h exposure period on days 1, 7, 14 and 21 (method unspecified). For the animals in the test substance group, the same analyses were repeated using blood samples collected approximately 16 h following the end of exposure on days 1, 7, 14, and 21.

Terminal: A detailed necropsy was conducted on all animals, either found dead or sacrificed on the day following last treatment using an overdose of pentobarbital sodium (i.v. injection) followed by exsanguination by incision of the axillary arteries. At necropsy, the following organs were dissected free of fat and weighed: adrenals, kidneys, liver, brain, heart, lungs (bronchi), ovaries/testes, pituitary, spleen, thyroid and parathyroid glands. Following weighing of the entire brain, it was divided into two halves by a median sagittal section. The left half was then weighed and frozen at -20°C while the right half was processed for histopathological examination. Tissue samples from a range of tissues were retained while the samples from the liver, kidneys, testes/ovaries, skin and macroscopic lesions were preserved in 10% formalin for histological examination. The frozen left half of the brain was homogenised and the ChE activity of the homogenate was determined (method unspecified).

Findings: There were no premature mortalities. Two treated animals removed their dressings following application on treatment day 10 and it was suspected that they ingested some test material by grooming. Clinical signs of cholinergic poisoning were observed subsequently in the two animals, which reversed on the following day. The other clinical signs observed were slight to moderate lacrimation in one or both eyes in several animals in both the control and treatment groups and periocular alopecia and focal skin lesions in a few control and treated animals. Dermal erythema and oedema were noted occasionally in both the control and treated animals, particularly during the final study week. The study authors claimed that these findings were "incidental to treatment with methiocarb technical" and the test chemical was not irritant to the rabbit skin (data not provided). No further information on clinical observations was provided.

Group mean food consumption in treated rabbits of both sexes was consistently lower than those of the controls (13% and 10% less for treated males and females respectively). The difference achieved significance ($p<0.05$) during study days 13-15 in males and 19-21 in female rabbits.

No significant intergroup differences in body weight were seen among the treated males. However, the total weight gained by the treated animals during the study period was 63% (60 g) less compared to the gain by the control animals (160 g). Similarly, treated females were always lighter than the controls and showed statistically significant reductions ($p<0.05$) in group mean body weights on days 15, 19 and 21 of the study. The treated female rabbits gained 30% less weight (140 g) during the study compared to the gain of 200 g by the control animals.

No statistically significant group mean differences between the pre-treatment and pre-terminal haematological parameters were noted in the animals of either sex. Clinical chemistry results revealed a slight reduction in pre-terminal serum calcium and phosphorous levels in both males and females in the treated group and controls compared to respective pre-treatment values which may have been a consequence of reduced food consumption. Statistically significant differences in both pre-treatment and pre-terminal AST and ALT enzyme levels

were seen in treated females compared to the parallel controls (see Table). The inter-group differences may have been due to low and declining control values rather than elevated activity in treated rabbits and are not considered to be biologically significant.

Changes in enzyme activity levels in methiocarb treated female rabbits

Dose (mg/kg bw/day)	Enzyme activity (u/L) ± SD			
	Pre-treatment		Pre-terminal[a]	
	AST	ALT	AST	ALT
Control	17.6 ± 4.4	48.4 ± 8.8	14.0 ± 3.5	36.6 ± 7.6
500	22.0 ± 5.7	79.8 ± 13.8**	21.6 ± 5.0*	60.6 ± 14.2*

[a]21 days after initiation of the study.

*Significantly different from corresponding controls ($p<0.05$)

**Significantly different from corresponding controls ($p<0.01$)

Plasma ChE activity (group mean ± SD) in rabbits treated with methiocarb technical

Dose mg/kg bw/day	Plasma ChE activity (u/L, Mean ± SD) Observation day				
	Pre-treatment	1	7	14	21
Males: 6 h post treatment					
Control	508 ± 99	444 ± 95	419 ± 62	482 ± 51	517 ± 62
500	548 ± 189	488 ± 149	428 ± 122	449 ± 137	525 ±135
16 h post treatment					
500	-	458 ± 181	465 ± 114	430 ± 242	536 ± 174
Females: 6 h post treatment					
Control	760 ± 137	521 ± 70	500 ± 57	535 ± 41	575 ± 69
500	730 ± 102	503 ± 65	447 ± 46	460* ± 32 (14%)	561 ± 47
16 h post treatment					
500	-	477 ± 56	480 ± 69	488 ± 69	553 ± 71

*Statistically significant from the corresponding control value ($p<0.05$).

Value in parenthesis represent the percent reduction in comparison to corresponding controls.

Pre-treatment plasma ChE activity was higher in both sexes compared to the values observed at all other sampling points. This may have occurred due to an error in the experimental procedure and/or variable sensitivity of the ChE assay method. A statistically significant reduction in plasma ChE activity was noted in treated females on day 14 (6 h post treatment) compared to the parallel controls. No significant alterations in RBC ChE activity were seen in treated animals, but the parameter appeared variable between individuals. Brain ChE activity was not affected by treatment.

There were no inter-group differences in absolute and relative organ weights of the treated animals, and gross or microscopic tissue abnormalities were not attributable to the test substance.

Conclusions: The present study has been conducted as a supplement to a previously conducted study by the same author using methiocarb technical and the same laboratory model (Procter, 1988, Report No: 1084). However, the primary objective of the present study was not clearly indicated in the report at hand. In the previous study report, there were statistically and/or biologically significant reductions in food consumption, weight gain and plasma ChE activity following exposure to the test substance at 150 and 375 mg/kg bw/day.

The reduced food consumption and body weight gain seen at 500 mg/kg bw/day is therefore consistent with the previous findings but ChE inhibition is unexpectedly slight and inconsistent with findings at 150 and 325 mg/kg bw/day. This may have been caused by experimental variation. No NOEL could be established, as treatment-related findings were reported at the only dose tested.

4.1.3 Dogs

4.1.3.1 Oral

Root M, Doull J & Cowan J (1963) Determination of the safe dietary level of Bayer 37344 for dogs. Report No. 11159, Lab: Department of Pharmacology, University of Chicago, IL, USA. Sponsor & Study duration not stated. Report date: March 23, 1963.

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

Study: Technical grade Bayer 37344 (methiocarb, PF 131, purity not stated) was administered to 16 young (10 wks old) purebred Beagle dogs (2/sex/dose, 4.5-7.5 kg bw) at 0, 50, 100, or 250 ppm in the diet (equivalent to approximately 0.75, 1.25 or 3.75 mg/kg bw/day) for 12 weeks. The dogs were selected from five litters (8/sex) and were assigned to the four experimental groups so that each group contained at least three dogs that had litter-mates on the other dietary levels. The animals were obtained at the age of 10 weeks, vaccinated for distemper and hepatitis and dewormed during the 4 week observation period prior to commencement of the feeding study. Individually housed animals were offered food (Rockland Dog Food) and water *ad libitum*.

The diets were prepared from a single sample of methiocarb biweekly or more often as required. The rationale for dose level selection was not provided. Each animal was examined daily for cholinergic symptoms (time of observation, symptoms observed were not stated) and weighed fortnightly.

Observations: Base line serum and erythrocyte ChE activity of each animal during the two week period prior to initiation of the study and data provided in the report (average of at least 5 determinations ± SD). During the feeding study, weekly blood samples were collected from the saphenous vein for ChE assay using a manometric method which involved measurement of µL of CO_2 produced/50 mg tissue/10 min.

Findings: Food and water consumption, haematology or clinical chemistry parameters were not examined. A line graph on average growth rate of the test animals (absolute values were not provided, presented as average body weight in kg vs weeks on diet) during the feeding experiment did not reveal any reduction of growth rate. Similarly, the two line graphs on weekly serum and erythrocyte ChE activity did not indicate an inhibition of ChE activity. No other information on clinical observations or experimental procedures was provided.

Conclusions: Under the conditions of the study, exposure to dietary levels of up to 3.75 mg of methiocarb/kg bw/day for a period of 12 weeks did not cause an inhibition of serum or erythrocyte ChE activity or produce any cholinergic effects in Beagle dogs. Individual data on absolute body weights, enzyme activity and food and water consumption were not available for assessment. Further, due to the small experimental group size used in the study, and lack of statistical analysis of data, the usefulness of the findings is reduced.

Hayes RH (1981) Cholinesterase evaluation study of Methiocarb and Methiocarb sulfoxide in dogs. Project No. Not stated. Lab: Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Centre, 17745 South Metcalf, Stilwell, Kansas 66085, USA. Sponsor: Bayer AG, Germany. Study duration: November 17 - December 18, 1980. Report No. 202. Report date: September 29, 1981.

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

Study: Methiocarb technical (purity: 97%, Batch: 0030058) or methiocarb sulfoxide (purity: 95.2%, Batch: Prasad 75-20-132C; Strankowski 79-R-187; Koch 80-R-162-9, Vial # 21) was administered orally to Beagle dogs (Midwest Research Institute, Kansas City, Missouri and Theracon, Topeka, Kansas, age and body weight not stated, 1/sex in the control group and 2/sex/treatment group) at 0 (control), 0.05 or 0.5 mg/kg bw/day in gelatin capsules for 29 days. Control animals received empty gelatin capsules. The last dose was administered on the first day of the fifth week of blood sampling. The animals were quarantined for one week before the initiation of the study. The dogs were housed individually in stainless steel metabolic cages under conventional laboratory conditions and provided with food (Field and Farm Dog Meal, Ralston Purina Co.) and water *ad libitum*.

Observations: The test animals were observed twice daily (one hour after dosing and late afternoon) for clinical signs, morbidity and mortality. Blood samples were collected from all dogs without fasting for plasma and RBC ChE assay (collection site and the ChE assay method were not stated) at the sampling times given in the following Table. Because the first week's data indicated that the peak ChE inhibition occurred between dosing and at 3 h, the 1 and 3 h interval blood sampling schedule was replaced by 2 h sampling schedule starting from the second week. The mean percent ChE depression was calculated using the mean pre-treatment ChE value of each week for each group. Body weights, food consumption, haematology and clinical chemistry were not evaluated, and gross or histopathology studies were not conducted.

Blood sampling schedule for plasma and RBC ChE assay

Sampling Week	Sampling times
Week 1	0 h (before day 1 dosing), and 1, 3, 6, and 24 h after day 1 dosing, and 3 h after day 3rd dosing.
Week 2	0 h (before day 1dosing), 2, 6, and 24 h after day 1 dosing, and 2 h after day 2nd dosing.
Weeks 3 & 4	0 h (before day 1 dosing), 2, 6, and 24 h after first dosing and 2 h after 3rd dosing.
Week 5	0 h (before day 1 dosing), 2, 6, and 24 h after day 1 dosing. Dosing discontinued after first day and another blood sample was taken on the third day.

Findings: Occasional slight to heavy salivation (in 3 females and 2 males) and vomiting (in 2 males) were observed at weeks 2, 4 or 5 in 6 animals receiving either of the test compounds at 0.5 mg/kg bw/day and in one female receiving methiocarb sulfoxide at 0.05 mg/kg bw/day. The clinical signs noted may have occurred due to the administration of the test substance. Plasma and RBC ChE activities in treated animals measured at different sampling points are presented in the following Tables.

Mean percent depression of plasma and RBC ChE activity in dogs treated with methiocarb

Observation Time	Plasma		RBC	
	0.05 mg/kg	0.5 mg/kg	0.05 mg/kg	0.5 mg/kg
Week 1, day 1: Males				
1 h	0	48	0	28
3 h	0	22	0	17

6 h	0	0	5	1
Week 1, day 1, Females				
1 h	7	63	13	37
3 h	12	39	11	29
6 h	0	12	8	8
Week 1, day 3: Males				
3 h	0	16	8	12
Week 1, day 3: Females				
3 h	13	26	20	21
Week 5, day 1: Males				
2 h	9	32	20	15
Week 5, day 1: Females				
2 h	16	50	0	23

Generally, both test compounds throughout the study demonstrated a dose relationship with respect to the extent of ChE depression. The maximum ChE depression usually occurred between 0 and 3 h after dosing at 0.5 mg/kg bw/day with either test compound in both sexes. No biologically significant depression of either the plasma or RBC ChE activity was observed at 6 h post treatment with methiocarb in either sex. Twenty percent depression of the RBC ChE enzyme activity was noted at 2-3 h post-treatment with methiocarb technical at 0.05 mg/kg bw/day at week 1 in females and at week 5 in males. However, plasma ChE inhibition did not reach biological significance (≥20%) at the low dose. Methiocarb sulfoxide was a more potent ChE inhibitor than methiocarb. There was a > 20% inhibition in both the plasma and RBC ChE activity at 0.05 mg/kg bw/day methiocarb sulfoxide in both sexes on several occasions. Plasma ChE depression was slightly more pronounced than RBC ChE depression.

Regardless of sex, both the plasma and RBC ChE were depressed to biologically significant levels (21-71%) by both test compounds at most of the sampling times. The samples obtained at 24 h at weeks 2, 3, 4, and 5 and subsequent sampling times at week 5 (data not shown) indicated that both the plasma and RBC ChE activity recovered to near normal levels by about 6 h post-treatment.

Conclusions: The high dose of 0.5 mg/kg bw/day was a clear effect level for methiocarb on plasma and RBC ChE activity. At 0.05 mg/kg bw/day, no treatment-related effect was seen on plasma ChE. Although 20% inhibition of RBC ChE activity was seen on 2 isolated occasions after treatment with 0.05 mg/kg bw/day methiocarb, this cannot be accepted as a true LOEL because the occurrence was sporadic. However, 0.05 mg/kg bw/day cannot be accepted as a NOEL because the data are considered unreliable due to the small numbers of dogs/group.

A treatment-related inhibition of either plasma and/or RBC ChE enzyme activity was seen in both sexes with methiocarb sulfoxide at 0.05 and 0.5 mg/kg bw/day and hence, no NOEL can be established for this compound.

Mean percent depression of plasma and RBC ChE activity in dogs treated with methiocarb sulfoxide

Observation Time	**Plasma**		**RBC**	
	0.05 mg/kg	**0.5 mg/kg**	**0.05 mg/kg**	**0.5 mg/kg**
week 1: Males				
1 h	27	62	23	51
3 h	0	12	20	19
6 h	16	4	17	9

Females				
1 h	41	68	17	48
3 h	13	11	15	15
6 h	1	0	5	0
week 5: Males				
2 h	38	53	21	24
Females				
2 h	31	50	7	21

4.2 Formulations

4.2.1 Rabbit

4.2.1.1 Oral

Flucke W & Kimmerle G (1977) Mesurol slug pellets (4% Mercaptodimethur), Toxicity study with determination of cholinesterase activity. Study No. not stated. Lab: Bayer AG, Institute of Toxicology, Wuppertal – Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: Not stated. Report No. 6913. Report date: July 19, 1977.

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

Study & Observations: This study was performed to investigate the toxicity of orally administered Mesurol slug pellets containing 4% methiocarb with determination of erythrocyte ChE activity in rabbits. The test substance (batch no: illegible, formulation details were not provided) was administered orally (method unspecified) to 4 adult, female chinchilla rabbits (initial mean bw 3.8 kg, age not stated, source illegible) at 100 mg/kg bw, twice a day (in the morning and afternoon) for 5 consecutive days. Blood samples were collected from the ear vein for erythrocyte (RBC) and plasma ChE assay at 5 h after the first dosing and 12 h after the second dosing using the colorimetric method of Ellman et al (1961). Body weights of the animals were recorded pre-treatment, and on days 7 and 14 after commencement of dosing. No control group was used in the study but the data obtained were compared with stated "reference" values. No other information on experimental methods was provided.

Findings: No mortalities were recorded. Treated animals showed a slight loss of body weight (100 g) at the end of the treatment period. However, the animals regained weight (200 g) by day 14 after commencement of treatment and showed signs of recovery. The observed weight loss was attributed to frequent handling, withdrawal of blood and to effects of the test chemical, as stated by the study authors. No further information on clinical observations was provided. Erythrocyte and plasma ChE data are presented in the following Table.

Erythrocyte and plasma ChE activities (mean of 4 animals) in rabbits treated orally with 4% methiocarb slug pellets

Sampling time	**Reference Value**	**ChE activity (Units /mL)**				
		Treatment day				
		1	2	3	4	5
Erythrocytes						
5 h after 1st dose	1.64	1.56	1.59	1.52	1.53	1.60
12 h after 2nd dose		1.64	1.72	1.62	1.60	1.66
Plasma						

5 h after 1st dose	0.40	0.27*	0.30*	0.26*	0.27*	0.26*
12 h after 2nd dose		0.35	0.35	0.35	0.33	0.34

*Biologically significant (>20%) compared to the corresponding reference value.

Erythrocyte (RBC) ChE activity was slightly depressed at 5 h after the first daily dosing compared to the reference value by about 2-7%. However, the RBC ChE data at 12 h after the second daily dosing were comparable to the reference value and demonstrate the recovery of the enzyme activity within 12 h post treatment. Plasma ChE activity was depressed by about 25-35% at 5 h after the first daily dosing compared to its reference value and reached biological significance on all treatment days. Although biological significance was not achieved, plasma ChE activity at 12 h after the second daily dosing also remained depressed (12-17%) throughout the treatment period compared to the reference value and appeared slower to recover to its baseline activity.

Conclusions: Under the conditions of the study, oral administration of Mesurol slug pellets containing 4% methiocarb to adult rabbits at 100 mg/kg bw, twice a day for 5 consecutive days produced a biologically significant depression in plasma ChE activity at 5 h after dosing. However, the validity of the findings of this study is reduced due to small sample size, and lack of controls, clinical observations and statistical analyses. A NOEL cannot be established as treatment-related effects were observed at the single dose tested.

4.2.2 Rat

4.2.2.1 Inhalation

Groning P & Kimmerle (1975) Mesurol 50 WP. Study of acute oral, intraperitoneal and dermal toxicity, acute and subacute inhalational toxicity, and skin and mucous membrane tolerance. Study No. not stated. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 5231. Report date: February 12, 1975.

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

Study & Observations: In this study, Wistar rats of 170-240 g bw (10/sex/group, Winkelmann, age not stated) in a dynamic flow inhalation chamber were exposed (nose only) to Mesurol 50% wettable powder aerosols (methiocarb, Batch X0001A, in a mixture of 1:1 ethanol and polyethylene glycol 400) at concentrations of either 20.2, 31.5 or 188 mg/m^3, 4 h/day for 5 days. There was no control group in the study. Further, no information on animal acclimatisation and inhalational aerosol diameter distribution was provided. The test animals were provided with Altromin R standard diet (Altromin GmbH, Lage/Lippe) and water *ad libitum.* The content of methiocarb in the inhaled air was determined using an indirect colorimetric method. Following the 5 day exposure period, the animals were observed for 14 days for evidence of "poisoning symptoms" (unspecified). No further information on experimental methods was provided.

Findings: No mortalities were observed. The study authors stated that, the general health condition of the animals at 31.5 and 188 mg/m^3 was affected (unspecified) from the first test day onwards. Further, a depression in the ChE activity (unspecified) in the animals at 188 mg/m^3 was seen, but no additional data on this study parameter were provided. The animals at 20.2 mg/m^3 showed unspecified changes in general health on the second and third day on study. The symptoms persisted for 1 to 3 days after the 5 day exposure period.

Conclusions: The study is not considered appropriate for regulatory purposes because of lack of a control group, and inadequate information on clinical signs and ChE depression.

4.2.2.1 Dermal

Dubois KP, Root M, Kinoshita FK, Mesakauskas J & Flynn M (1968) Subacute dermal toxicity of a wettable powder of BAY 37344 to rats. Reprt No. 23067, Lab: Toxicology Laboratory, University of Chicago, USA. Sponsor: Bayer AG, Landwirtschaft, Friedrich-Ebert-Stasse 217, D5600, Wuppertal-Elberfeld, Germany. Study duration: Not stated. Report date: August 21, 1968.

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

Study: BAY 37344 50% wettable powder (methiocarb, Control No. E956A, source not stated) was applied to shaved, abraded skin (by using sandpaper) of young adult (5/sex/dose group) Holtzman rats (source, age and bw not stated) at 200 mg/kg bw/day for 5 days a week for a period of 3 weeks. Five further animals/sex were included in a control group. Information on animal acclimatisation, housing, feeding and treatment procedures on control animals was not provided nor were details on test substance application method given.

The dose applied was determined based on the information obtained from a preliminary acute dermal toxicity study (LD_{50}s were 1363 and 1296 mg/kg bw for males and females respectively). On the basis of these data a preliminary trial with 300 mg/kg was conducted, which resulted in high mortality rates (unspecified) within a few days in experimental animals. Consequently, a 200 mg/kg bw/day dose was chosen. The wettable powder was homogenised in water at 200 mg/mL and was spread over an area equivalent to 10% body surface (site of application, dimensions of the application site or animal restraint procedures used were not discussed).

Observations: The body weights were measured weekly, three times during the exposure period and provided as percent of original body weight (see Table, only the absolute body weights of the animals at terminal kill were provided). At the time of sacrifice, the haematological parameters (Hb, Hct, RBC, WBC, clotting time and prothrombin time) were measured using blood samples obtained from the tail vein and the brain ChE (3/group) was assessed using a manometric method (as measured by µl of CO_2 produced/50 mg of brain tissue/10 minutes).

The study authors stated that "a number of tissues were removed from each surviving animal for gross and microscopic examination" (tissue types, amount and method of preservation not specified). The absolute weights of brain, liver, kidney, spleen, heart, lung, testis, thymus and adrenal glands were measured and relative weights were calculated.

Findings:

Effect of repeated dermal application of methiocarb 50% WP on the body weight of rats

Week	Percent of initial body weight			
	Controls		Treated	
	Males	Females	Males	Females
1	105	102	98	92
2	112	104	101	94
3	122	106	105	98

No mortality was observed during the study. Body weight data indicated that the dermal application of methiocarb 50% WP caused an inhibition of the growth rate in treated animals at all tested time points and was more pronounced in female rats. The significance of growth rate difference between the control animals and treatment group however, was not statistically compared.

Brain ChE activity in treated animals was unaffected. Cholinergic symptoms were noted after each treatment, particularly at the beginning of the treatment period. However, the details on types of symptoms, onset and duration and the rate of their disappearance were not provided. No evidence of any cumulative effects was noticed during the study.

Average absolute weights of the liver, kidney, spleen and thymus in treated male rats were depressed by 15%, 19%, 19% and 26% respectively. These findings are consistent with the depression in weight gain shown by treated males. In contrast, the average absolute weight of the adrenal gland was increased by 20% in treated female rats. When the average relative organ weights were considered, a 31% increase was noted in lung and adrenal glands whilst a 15% reduction was observed in the thymus in treated male rats. Relative weight of the adrenal glands in female rats showed a 23% decrease. Given the inconsistency between the sexes, the adrenal weight variations are not considered to be treatment-related.

Variability in lung weights was noticed within both the control and treated males and was attributed to pulmonary consolidation by the study authors. The condition was noted in 1 and 2 male animals in the control and treatment groups respectively, presumably occurring due to respiratory tract infection and hence, may not be a treatment-related effect. There were no gross pathologic findings reported in relation to treatment.

Conclusions: The results show the potential of the tested 50% WP formulation to cause clinically observable anticholinesterase effects by repeat administration. Because useful information on the cholinergic symptoms observed was not provided, and no effect on brain ChE activity was observed, the effects of the formulation at the dose level used cannot be fully explained. The validity of the data is reduced due to methodological deficiencies, debatable sensitivity of the ChE assay method used and insufficient use of statistical procedures to compare the study results.

5. SUBCHRONIC STUDIES

5.1 Rats

5.1.1 Oral

5.1.1.1 16 week dietary study

Doull J, Cowan J & Root M (1962) Subacute (16 week) oral toxicity of Bayer 37344 to male and female rats. Project No: not stated. Lab: Department of Pharmacology, University of Chicago, Chicago 37, IL, USA. Study duration: not stated. Report No: 9456, Report date: June 12, 1962.

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

Study: BAY 37344 technical (methiocarb, PF 131, purity 100%, stability not specified) was fed to SD rats (weanlings, initial bw 52-85 g, 12/sex/group) at 0 (control), 5, 10 or 50 ppm (equivalent to approximately 0, 0.5, 1.0 or 5.0 mg/kg bw/day) for 16 weeks (112 days) in the diet (Rockland Laboratory Food). A rationale for the dose selection was not provided. The animals were individually housed in an air conditioned room and had constant access to the diets and water throughout the study period. The admixture was prepared weekly or more often if required by mixing the test chemical with the pulverised rat diet to obtain required dietary concentrations. No details on animal acclimatisation were provided.

Observations: Mortality and the symptoms of toxicity were checked daily. The growth rate and food consumption were measured weekly. At the end of the study, 5 rats/sex/group were sacrificed under ether anaesthesia and the tissues (unspecified) were removed, weighed and

processed for histopathology. An additional group (5 rats/sex/group) was sacrificed similarly and the blood (by cardiac puncture), submaxillary glands and the brains were collected for ChE assay using a manometric method. No further information on experimental methodology was provided.

Findings: Two males in the control group died during the treatment period (days unspecified). Additionally, there were 8 deaths in the three experimental groups namely, 4, 2 and 2 for the 0.5, 1.0 and 5.0 mg/kg bw/day groups (days and the sex of the animals were not specified). From these mortality figures, no treatment-related effects of the test substance was evident. Food consumption and the growth rate of the animals were unaffected by treatment (no absolute data were provided). The study authors stated that none of the treated rats exhibited any cholinergic or other toxic symptoms during the study.

Serum, RBC and tissue ChE activity at termination are given in the Table below.

ChE activity in rats fed diets containing methiocarb for 16 weeks

Dose (mg/kg bw/day)	ChE activity[a]			
	Serum	RBC	Submaxillary gland	Brain
Males				
0 (control)	5.7 ± 0.6	9.6 ± 1.0	25.1 ± 4.2	104.8 ± 5.3
0.5	5.1 ± 0.7	8.7 ± 0.7	24.5 ± 1.6	97.7 ± 3.6
1.0	5.1 ± 1.0	8.7 ± 0.3	26.0 ± 1.8	97.6 ± 3.1
5.0	4.5 ± 0.5 (21%)	8.3 ± 0.5	23.3 ± 2.6	92.7 ± 3.3
Females				
0 (control)	18.0 ± 3.8	11.1 ± 1.0	30.8 ± 2.2	95.3 ± 4.1
0.5	17.5 ± 1.7	8.9 ± 0.7	23.8 ± 2.3 (23%)	88.5 ± 2.6
1.0	16.8 ± 1.6	10.5 ± 0.9	22.5 ± 2.3 (27%)	89.3 ± 3.1
5.0	13.0 ± 1.1 (28%)	9.4 ± 0.8	20.8 ± 3.3 (33%)	90.9 ± 8.8

[a]Expressed as µL of CO_2 produced/10 minutes/50 g of wet tissue. Average of duplicate determinations from at least 5 animals/group.

Values in parentheses are % inhibitions, calculated for those inhibitions considered to be biologically significant compared to the corresponding controls.

Biologically significant inhibition of the serum ChE activity was seen in both males (21%) and females (28%) at 5.0 mg/kg bw/day. There was a dose-related inhibition in submaxillary gland ChE in female rats in all treatment groups compared to the parallel controls. The RBC and brain ChE activity appeared to be variable among the females but were inhibited dose-relatedly in males, by up to 14 and 7% respectively at 5 mg/kg bw/day.

No data on necropsy findings, organ weights, histopathology and clinical observations were provided.

Conclusions: The validity of the findings of this study is reduced due to lack of justification for dose selection, statistical analyses and clinical observations and data limitations. A NOEL cannot be established, given evidence of ChE inhibition in the submaxillary gland at the lowest dose of 0.5 mg/kg bw/day.

5.1.1.2 24-Weekdietary study

Lőser E (1969) BAY 37344: Blood analyses, urinalysis and clinical chemistry examinations following oral administration to rats. Farbenfabrik Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld Germany. Study duration: not stated. Report No. 1358. Report date: March 03, 1969.

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

Study: BAY 37 344 (methiocarb) technical of unspecified purity, mixed with the diet (Ssniff pulverised rat diet) was fed to 28-32 days old rats (strain FB 30, Elberfeld, initial bw 45-55 g, 10/sex/dose) at 0 (control), 30, 100 or 300 ppm (equivalent to approximately 0, 3, 10 or 30 mg/kg bw/day) for 24 weeks. The representative animals were taken from groups of rats participating in a concurrent single generation reproduction study and the studies reported here were performed in F_0 generation animals at the end of the preliminary treatment period (at 10 weeks, ie shortly before mating) and after their second litter had been reared (at 24 weeks). Information on the preparation procedure, homogeneity and stability of the admixture was not provided. The animals were held individually in Makrolon cages and provided with food and water *ad libitum*.
Observations: Haematology, urinalysis, renal function and blood glucose tests were performed (5 rats/sex/dose) at 10 and 24 weeks after the commencement of the study. The haematological parameters measured were: Hb, Hct, RBC and WBC counts, relative mean haemoglobin concentration of erythrocytes (Hb_E value), MCV, thrombocyte count, reticulocyte count and differential WBC counts. The following liver function tests were performed: AST, ALT, LDH and Bilirubin and SDH, ALP and total serum protein (at 24 weeks). Urine was tested for glucose, protein, blood (Combi-Uristix, Merck, Darmstadt), bile pigments and microscopic sediments. Cholesterol in blood (5 rats/sex/dose) was measured at 24 weeks after initiation of the study.

Findings: An increase in RBC count (polycythaemia) was seen at 10 weeks in both male and female animals at 30 mg/kg bw/day but no change was observed in the haematocrit value. Modest changes in white blood cell counts were apparent in both sexes. The changes in haematology that were observed in animals at 30 mg/kg bw/day at 10 weeks did not recur at the later sampling point. The serum AST and ALT levels were higher in animals of both sexes at 30 mg/kg bw/day 10 weeks after commencement of the study. However, this elevation was not seen at 24 weeks. The study authors stated that the values observed for all parameters were within the normal range but no supporting historic data were provided. No clinical observations were reported nor were data on food and water consumption and body weights of the animals provided.

Conclusions: Under the conditions of the study, no biologically significant effects attributable to treatment were noted in any parameters at either analysis time. The modest changes in haematological and clinical chemistry parameters that occurred in treated animals appear to be physiological adaptations and are not suggestive of any disease process. Due to the limitations of the data and lack of statistical analyses, the reliability of the findings is reduced but, the data can be considered as supplementary to other long term toxicity data.

6. CHRONIC STUDIES

6.1 Mice

6.1.1 Oral

Kroetlinger & Janda (1983) H 321 (Mercaptodimethur, the active ingredient of ®Mesurol): Chronic toxicity study on mice (2-year feeding experiment). Study No. H 321/004. Lab: Institute of Toxicology, Bayer AG, Wuppertal, Federal Republic of Germany. Sponsor: Bayer AG, Wuppertal, Federal Republic of Germany. Study duration: June 1979 to July 1981. Report No. 11908. Report date: July 4, 1983.

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

Study: H 321 (methiocarb, composite sample from batches 234 702-602, -607, -609 and -611, purity 98.5%) was mixed with pulverised feed (Altromin R, Altromin GmbH, Lage, Federal Republic of Germany) and fed to SPF mice (BOR:CFW1, Winkelmann, Borchen, Federal Republic of Germany, 30-35 days old, initial mean bw 25 and 22 g for males and females respectively, 50 mice/sex/dose) at concentrations of either 0, 67, 200 or 600 ppm (mean amounts of test compound ingested were equal to 0, 14.6, 42.8 and 132 mg/kg bw/day for the males and 0, 19.8, 57.0 and 173 mg/kg bw/day for the females) for 2 years. Information on animal acclimatisation and preparation procedure, homogeneity and stability of the admixture was not provided. The animals were individually housed in type II Makrolon cages under conventional laboratory conditions and provided with feed (supplied fresh once a week) and water *ad libitum*. Statistical significance between the control and the treatment groups was tested using the Mann -Whitney and Wilcoxon tests.

Observations: The animals were inspected twice daily and the clinical signs were recorded. The body weights were recorded weekly during the first 15 weeks and at three weeks intervals thereafter. Feed consumption was measured weekly by weighing back the unconsumed feed. Haematology (RBC, WBC, and thrombocyte counts, Hb, Hct, differential WBC counts, MCH, MCHC, MCV), clinical chemistry (ALT and urea) were conducted on 5 mice/sex/dose at month 12 and on 10 mice/sex/dose (or the mice still surviving) at termination. The ChE activity in plasma was determined at 1 and 12 months from 5 mice/sex/dose, at termination from 10 mice/sex/dose (or the mice still surviving) and in brain at termination using a colorimetric method (Ellman *et al*, 1961). The blood samples for clinical chemistry tests were obtained from the retro-orbital venous plexus under ether anaesthesia. The animals found moribund during the experiment, 10 mice/sex/dose at 12 months and all surviving at termination were anaesthetised with diethyl ether, sacrificed by exsanguination and necropsied. The weights of the following organs were recorded: heart, lungs, liver, spleen, kidneys, testes and ovaries. Samples from the following tissues were processed for histopathology: aorta, eyes, intestine, brain, urinary bladder, heart, testes, pituitary, salivary glands, liver, lungs, lymph nodes, stomach, spleen, epididymis, adrenals, kidneys, oesophagus, ovaries, pancreas, prostate gland, seminal vesicles, thyroid, skeletal muscle with femur and sciatic nerve, sternum, trachea and uterus and the tissues that were found to have changes at gross examination.

Findings: Mortality among the test groups is presented in the following Table.

Cumulative mortality among test groups at termination

Dose (ppm)	No. mice used	No. mice dying[a]	Mortality (%)[b]
Male			
0	49	32 (5)	65.3

67	49	42 (5)	85.7* (31%)
200	49	42 (7)	85.7* (31%)
600	47	34 (8)	72.3 (11%)
Females			
0	51	43 (15)	84.3
67	51	38 (8)	74.5
200	50	35 (9)	70.0
600	52	35 (9)	67.3

*Significantly different from controls ($p<0.05$)

[a]Values in parenthesis represent the number of animals sacrificed *in extremis.*

[b]Values in parenthesis represent percent change from corresponding controls.

No treatment-related inter-group differences in mortality were seen in either sex. However, the percent mortality among the animals appeared high, particularly during the second year on study. At 18 months the cumulative mortalities in males were 26%, 53%, 41% and 34% and in females 31%, 47%, 36% and 31% for the control, 67, 200 and 600 ppm groups respectively. Cumulative mortality among 67 and 200 ppm males was significantly higher ($p<0.05$) compared to the corresponding controls and the 600 ppm group. In addition, only about 16% of the control females survived until the end of study.

The treated animals were not different in their appearance and behaviour from the controls nor were any cholinergic symptoms observed. No inter-group differences in food consumption were observed in either sex. The animals of both sexes at 67 and 200 ppm gained weight comparable to the controls. However, the body weights of the 600 ppm groups were about 5% less than the corresponding controls, exhibiting statistical significance at the majority of sampling times during the first year in males and during the first 7-8 months on study in females ($p<0.01$ or 0.05). At termination, the group mean body weight at 600 ppm males was depressed by about 4% compared to the concurrent controls. No inter-group differences in mean body weights in females were seen at termination

Body weights (g, mean ± SD) of mice fed methiocarb in the diet at selected study weeks

Dose (ppm)	**Study week**					
	0	**9**	**27**	**51**	**87**	**105**
Males						
0	25 ± 2	35 ± 2	42 ± 4	46 ± 5	42 ± 5	38 ± 5
67	25 ± 3	34 ± 2	41 ± 4	45 ± 6	40 ± 5	37 ± 4
200	26 ± 2	35 ± 2	42 ± 4	45 ± 5	44 ± 5	41 ± 5
600	25 ± 2	33 ± 2**	40 ± 4*	44 ± 5*	41 ± 4	37 ± 4
Females						
0	22 ± 2	29 ± 2	33 ± 2	36 ± 4	35 ± 3	32 ± 3
67	23 ± 2	29 ± 2	33 ± 3	37 ± 5	37 ± 5	34 ± 3
200	22 ± 2	27 ± 2	32 ± 2	37 ± 5	36 ± 4	35 ± 4
600	22 ± 2	27 ± 2**	32 ± 2*	36 ± 4	35 ± 3	35 ± 3

* Significantly different from the corresponding controls ($p<0.05$).

** Significantly different from the corresponding controls ($p<0.01$).

Haematology data revealed several statistically significant but intermittent and biologically insignificant changes in some variables at 12 and 24 months. A dose related increase in MCHC values was noted in both sexes achieving statistical significance at all dose levels in males ($p<0.01$ or 0.05) and at 600 ppm in females ($p<0.05$) at 12 months. However, an opposite trend towards decreasing MCHC values was seen at termination. Consistent with

biologically significant elevations (42% and 67% at 200 and 600 ppm respectively) seen at 12 months, a statistically significant increase in leucocyte counts in treated females at 24 months ($p<0.01$ or 0.05) appears to be treatment-related.

Leucocyte count in mice fed methiocarb in the diet

Dose (ppm)	Leucocyte count (10^9/L)	
	12 months	24 months
Males		
0	5.6	6.9
67	6.2	6.3
200	6.6	8.5
600	7.3	7.6
Females		
0	4.9	3.9
67	4.9	6.7*
200	8.2	6.0**
600	7.0	10.3*

** Significantly different from the corresponding controls ($p<0.01$).

* Significantly different from the corresponding controls ($p<0.05$).

In clinical chemistry, significant elevation in ALT activity was seen at termination in both sexes in the 200 and 600 ppm dose groups ($p\leq0.01$ or 0.05) compared to the corresponding controls, exhibiting a noticeable dose response relation in females (see Table). Moreover, at 12 months, the enzyme activity was elevated by 26-38% in all treated groups compared to the corresponding controls, attaining biological significance with the exception of 67 ppm males at 12 months. However, no statistical significance was achieved at this time point.

ALT activity in mice fed methiocarb in the diet for 2 years

Dose (ppm)	ALT activity (u/L)	
	12 Months	24 Months
Males		
0	49.8	56.1
67	46.0	69.9 (24%)
200	67.5 (35%)	101.1 (80%)*
600	68.7 (38%)	80.3 (43%)**
Females		
0	30.2	32.6
67	41.5 (37%)	40.1 (23%)
200	38.1 (26%)	57.9 (78%)**
600	41.5 (37%)	167.0 (412%)**

** Significantly different from the corresponding controls ($p<0.01$).

* Significantly different from the corresponding controls ($p<0.05$).

Values in parenthesis represent percent increase compared to corresponding controls.

Plasma and the brain ChE activity in test animals measured at different sampling times is presented in the following Table. Statistically ($p\leq0.01$ or 0.05) or biologically significant inhibition of the enzyme activity was observed in 200 and 600 ppm males and in all treated female groups compared to the parallel controls at one month. Although plasma ChE activity in all treated females appeared slightly depressed (by about 8-12%) at 12 months, no

statistically or biologically significant group differences were seen at this sampling time or thereafter in either sex. No inhibition in brain ChE activity was observed.

Plasma ChE activity in mice fed methiocarb for 2 years

Dose (ppm)	Plasma ChE activity (u/mL)		
	1 month[a]	12 months	24 months
Males			
0	4.70	4.47	6.72
67	4.70	4.30	6.01
200	2.32* (50%)	4.25	6.45
600	3.12 (33%)	4.70	6.17
Females			
0	7.16	8.30	7.78
67	5.46 (22%)	7.65	9.01
200	4.09**(42%)	7.66	8.20
600	4.74* (34%)	7.32	10.77

*Significantly different from the corresponding controls ($p<0.05$).

** Significantly different from the corresponding controls ($p<0.01$).

[a]Values in parenthesis represent percent change compared to the concurrent controls.

[b]Values in parenthesis represent the number of animals.

Necropsy examination of animals at intercurrent sacrifice at 12 months revealed significantly increased ($p<0.05$) relative heart weight (by about 20%) at 600 ppm females compared to the parallel controls. However, no biological significance is attributed to this finding, given that it was not repeated at 24 months.

Relative organ weights (group means) of mice fed methiocarb for 2 years

Dose (ppm)	12 months		24 months	
	Liver	Spleen	Liver	Spleen
Males				
0	524	204	556	634
67	553	211	551	397
200	601 (15%)	529	585	588
600	576 (10%)	233	732 (32%)	420 (34%)
Females				
0	538	288	646	1003
67	546	327	741	882
200	571 (6%)	261	601	684 (32%)
600	563 (5%)	307	1153 (78%)	557 (44%)

*Significantly different from the corresponding controls ($p<0.05$)

Values in parenthesis represent percent increase or reduction compared to concurrent controls.

A 2-fold increase in absolute and relative spleen weight seen in 200 ppm males at 12 months was attributable to gross enlargement in a single animal. In males, the absolute and relative spleen weight change at 24 months was confined to the 600 ppm group in which a 34% reduction was noted, considered to be biologically significant. The depression in relative spleen weight (about 22%) in 67 ppm males at termination was due to lowest absolute (101 mg) and relative (289) spleen weight data of a single animal in the group of 4 mice. In addition, statistically ($p<0.05$) or biologically significant reductions in absolute (33-42%) and relative (32-44%) spleen weight noted in the 200 and 600 ppm females and in 600 ppm males

at termination may have been attributed to the test compound. Though no statistical significance was achieved, the relative liver weights in both sexes at 200 and 600 ppm at 12 months appeared slightly elevated compared to the corresponding controls. Absolute liver weights at 600 ppm were elevated by about 23% in males, and by about 92% in females. Parallel increases in relative liver weights were seen in the same group with 32% and 78% elevations in males and females respectively. Both theses changes were considered to be biologically significant, and appears to be test compound related. No treatment-related gross tissue abnormalities were observed at necropsy of animals either died or sacrificed *in extremis* during the experiment.

In histopathology, no treatment-related non-neoplastic or neoplastic tissue abnormalities were observed. The reported neoplasia appeared to be age related and hence were not regarded as test compound related.

Conclusions: Because statistically and/or biologically significant perturbations were seen in haematological parameters (elevation of leucocyte counts in females at termination), a NOEL for this study cannot be established.

Note: US EPA Guidelines on Carcinogenicity Studies of 1998 870.42 specify that in mice, the survival in any group should not fall below 50% at 15 months or 25% at 18 months while the OECD guidelines of 1981 stipulate that the study termination may take place when the number of survivors in the lower dose or control group has declined to 25%. According to the mortality data in the present study at 18 months, the study conduct only just meets the US EPA requirements but contravenes the OECD test requirements.

6.2 Rats

6.2.1 Oral

6.2.1.1 80 week dietary study

Doull J, Root M & Meskauskas (1967) Chronic oral toxicity of BAY 37344 to rats. Project No: not stated. Lab: Toxicology Laboratory, University of Chicago, Chicago IL 60637, USA. Sponsor: Bayer AG, Germany. Study duration: Not stated. Report No: 21791, Report date: December 15, 1969.

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

Study: Methiocarb (Control No. PF 131, stability, purity not specified) was fed to SD rats (weanlings, initial bw 80-110 g and 72-98 g for males and females respectively, 24/sex/group) at concentrations of either 0, 25, 50 or 100 ppm (equivalent to approximately 0, 2, 5 or 10 mg/kg bw/day) for about 80 weeks in the diet (Rockland Rat Diet). A rationale for the dose selection was not provided. The animals were individually housed in an air conditioned room and had constant access to the diets and water throughout the study. The admixture was prepared biweekly or more often if required by mixing the test chemical with the pulverised rat diet to obtain required dietary concentrations. No details on animal acclimatisation were provided. Although the exposure duration was initially planned for 96 weeks, the test animals were sacrificed on unspecified days during weeks 68-70 (control males), 78 (treated males) and 81(all females) ensuring that there would at least 5 animals/sex/group be available for the ChE and pathology tests at termination.

Observations: Mortality and the symptoms of toxicity were checked daily. Individual body weights were measured every two weeks and the daily average food consumption was measured weekly or biweekly during the first few months and "infrequently" thereafter. The animals were sacrificed under ether anaesthesia (method unspecified) and the blood (by

cardiac puncture), submaxillary glands and the brain were collected from at least 5 rats/sex/dose for subsequent ChE assay using a manometric method. All sacrificed animals were autopsied and the following tissues were dissected out and fixed in neutral buffered formalin for histopathology: liver, heart, brain, kidney, spleen, lungs, gonads, thymus, adrenal glands, urinary bladder, mesenteric lymph nodes, stomach, duodenum, pancreas, jejunum, ileum and colon. A portion of the sternum was prepared for bone marrow evaluation. No other information on experimental methodology was provided.

Findings: The median survival times of rats in different treatment groups calculated using the group mortality data and "maximum likelihood probit regression analysis" did not reveal any treatment-related changes in male rats. However, the median survival time (on linear time regression basis) in females was slightly reduced at 25 ppm by 13% and at 100 ppm by 10%, with no noticeable dose relationship. From the line graphs provided it was evident that the proportions of male rats surviving at termination were about 20% for the 25 and 100 ppm dose groups and 60% at 50 ppm. Only about 20% of the male control animals survived until weeks 68-70, and all were sacrificed on an unspecified date during that period. Likewise, in female rats, about 62% in the controls, 40% at 25 ppm, 80% at 50 ppm and 40% at 100 ppm were alive when the study terminated on an unspecified day during weeks 78-80. No actual numerical mortality data were provided nor was any statistical comparison of the group median survival times carried out but there does not appear to be an association between mortality and treatment. However, mortality rate appears to be higher in males than in females.

Food consumption and the growth rate of the test animals were unaffected by treatment (no absolute data on either parameter were provided). The study authors stated that, none of the rats fed different levels of methiocarb exhibited any cholinergic or other toxic symptoms during the feeding study and they were not different from the controls in their appearance and physical condition. The blood and tissue ChE data at terminal kill are presented in the Table below.

ChE activity in rats fed diets containing methiocarb for 80 weeks

Dose (ppm)	ChE activity[a]			
	Serum	**RBC**	**Submaxillary gland**	**Brain**
Males				
0	7.5 ± 1.1	8.1± 0.6	22.7 ± 3.8	89.3 ± 4.7
25	8.0 ± 1.0	7.3 ± 1.2	21.4 ± 3.1	85.3 ± 2.4
50	7.3 ± 0.9	7.9 ± 0.2	22.5 ± 2.8	89.6 ± 4.1
100	7.3 ± 0.6	8.0 ± 0.2	24.3 ± 2.0	86.3 ± 8.5
Females				
0	16.1 ± 2.1	9.4 ± 0.5	25.4 ± 1.0	88.1 ± 8.3
25	15.4 ± 1.6	9.4 ± 0.7	23.6 ± 1.6	94.1 ± 1.9
50	15.4 ± 1.7	9.7 ± 0.3	23.2 ± 1.4	91.3 ± 1.5
100	12.5 ± 1.9 (22%)[b]	8.8 ± 1.0	19.4 ± 1.9 (24%)	91.8 ± 3.6

[a]Expressed as µL of CO_2 produced/10 minutes/50 g of wet tissue. Average of duplicate determinations from at least 5 animals/group.

Biologically significant inhibition of the serum (22%) and submaxillary gland (24%) ChE activity was noted in female rats at 100 ppm compared to the concurrent control data. No other intergroup differences in ChE activity were noted. From the experimental methods however, it would appear that the control male ChE data were derived 10 weeks before ChE

data from the treated males, a procedure which is experimentally unsound and prevents direct comparison between treated and control males.

No changes in gross or relative organ weights or in gross abnormalities attributed to treatment were observed at necropsy. Consolidation of the lungs and suppurative pneumonitis associated with oedema and abscess formation were observed in most of the treated and control rats except for the control females. The study authors stated that this condition was due to a viral pneumonia, which appeared endemic to the colony of rats. In histopathology, the condition was identified as bronchopneumonia with incidences of 3/5, 1/5, 3/5 and 4/5 (for males) and 0/5, 2/4, 4/5 and 3/5 (for females) for the control, 25, 50 and 100 ppm dose levels respectively. Further, interstitial nephritis and protein casts were prevalent in most of the animals, both control and treated alike with the exception of females at 100 ppm. This renal condition did not appear to be treatment-related. No other information on clinical observations was provided.

Conclusions: The NOEL for ChE inhibition was 50 ppm (equivalent to approximately 5 mg/kg bw/day). However, the validity of this study is reduced due to lack of statistical analyses, clinical observations and data limitations most significantly relating to ChE activity in males. Further, mortality was high and most of the animals appear to have been distressed during the study because of respiratory tract (viral pneumonia) and renal infections (interstitial nephritis). Therefore, the findings of this study are of limited regulatory value.

6.2.1.2 2-Year rat dietary study

Kroetlinger F, Loeser E & Vogel O (1981) H321 (Mercaptodimethur, the active ingredient of Mesurol) Chronic toxicity study on rats (2 year feeding experiment). Study No. H 321/005, Lab: Institute of Toxicology, Bayer AG, Wuppertal, Federal Republic of Germany. Sponsor: Bayer AG, Wuppertal, Federal Republic of Germany. Study duration: July 1977 to July 1979. Report No. 10039. Report date: July 2, 1981.

Includes: Krotlinger F (1990) Addendum to Bayer report No. 10039 entitled: "H 321 (Mercaptodimethur, the active ingredient of Mesurol®) Study of the chronic toxicity to rats (2 year feeding study). Study No. T9013384, Lab: Institute of Toxicology, Bayer AG, Wuppertal, Federal Republic of Germany. Sponsor: Bayer AG, Wuppertal, Federal Republic of Germany. Study duration: 1987-1989, Report No. 10039A, Report date: February 16, 1990.

The main study was pre GLP, and non-quality assured. The addendum was quality-assured. No test guidelines were cited.

Study: H 321 (methiocarb, Batch 75/76, purity 98.9%) was mixed with pulverised rat feed (Altromin R, Altromin GmbH, Lage, Federal Republic of Germany) and fed to SPF rats (Wistar TNO W.74, Winkelmann, Borchen, Federal Republic of Germany, 40-45 days old, initial mean bw 92 g and 81 g for males and females respectively, 60 rats/sex/dose) at concentrations of either 0, 67, 200 or 600 ppm (mean amounts of test compound ingested were equivalent to: 0, 3.27, 9.3 or 29 mg/kg bw/day for the males and 0, 4.98, 13.9 or 42 mg/kg bw/day for the females) for 2 years. Information on animal acclimatisation and preparation procedure, homogeneity and stability of the admixture was not provided. The animals were held under conventional laboratory conditions and provided with feed and water *ad libitum*. In the main study, statistical significances between the control and the treatment groups were tested using Mann-Whitney and Wilcoxon tests. The addendum included a survival analysis using the Breslow test.

Observations: The animals were inspected daily and the clinical signs were recorded. The body weights were recorded weekly during the first 26 weeks and biweekly thereafter. Feed consumption was measured weekly. Haematology (RBC, WBC, thrombocyte and reticulocyte counts, Hb, Hct, WBC-DC, MCH, MCHC, MCV, thromboplastin time), and clinical chemistry (AP, AST, ALT, creatinine, urea, glucose, cholesterol, bilirubin, total protein) were conducted on 10 rats/sex/dose at months 3, 6, 12 and 24 using blood samples collected from the retro-orbital venous plexus under ether anaesthesia. Glutamate dehydrogenase (GDH) activity was measured only at termination. Blood glucose content and thromboplastin time were measured using blood samples obtained from the caudal vein and the heart (by cardiac puncture) respectively at termination. Urine was analysed for glucose, blood, pH, ketone bodies, bilirubin, urobilinogen and sediments semi-quantitatively and for protein quantitatively. The ChE activity (10 rats/sex/dose) in plasma and RBC was determined on days 1 and 2 and during weeks 1,2, 4, 8, 13, 26, 52, 78, and 105 weeks after initiation of the study, and in brain at termination using a colorimetric method (Ellman *et al*., 1961). The animals found moribund during the experiment and all surviving animals at termination were anaesthetised with ether, sacrificed by exsanguination and necropsied. The weights of the following organs were recorded: thyroid, heart, lungs, liver, spleen, kidneys, adrenals, testes and ovaries. Samples from the following tissues were processed for histopathology: aorta, eyes, intestine, brain, urinary bladder, heart, testes, pituitary, salivary glands, liver, lungs, lymph nodes, stomach, spleen, epididymis, adrenals, kidneys, oesophagus, ovaries, pancreas, prostate gland, seminal vesicles, thyroid, skeletal muscle with femur and sciatic nerve, sternum, trachea and uterus and the tissues that were found to have changes at gross examination. The study authors stated that all processed tissues were subsequently examined histologically, although this was difficult to ascertain from the data presented. A full re-analysis of histopathology on the processed tissues, was performed by Life Science Research in 1987-89 and presented in the addendum.

Findings: Cumulative mortalities among the study groups (including sacrifice of 14 moribund animals) by the completion of the study were: 8 (13%), 8 (13%), 12 (20%) and 6 (10%) in the males and 19 (32%), 16 (26%), 17 (28%) and 14 (23%) in females at control, 67, 200 and 600 ppm respectively. Mortality data presented in the addendum differed slightly from figures given in the main report. The addendum stated that there were 13 deaths among the 200 ppm male group and 17 and 16 deaths in the 67 and 600 ppm females, respectively. Mortalities observed are not considered to be test compound related and statistical significance was not attained.

No differences in general physical appearance or behaviour were seen between the control and treated rats nor were any cholinergic symptoms observed in any of the treated groups. Food consumption in males at 600 ppm was slightly reduced (5%) compared to the concurrent controls during the second year on study. No other inter-group differences in food consumption were observed. Both sexes at 67 ppm gained weight comparable to the parallel controls, as did the 200 ppm female group. However, between weeks 4 and 19, the 200 ppm males showed a consistent deficit in body weight gain compared with controls, leading to a 3 to 8% and statistically significant ($p<0.05$ or 0.01) deficit in bodyweight throughout this period. At 600 ppm, body weights were significantly depressed ($p<0.01$ or 0.05) in animals of both sexes compared to the concurrent controls throughout the study (see Table). At termination, the group mean body weights at 600 ppm were lower in males (approx. 5%) and females (approx. 6%) in comparison to the concurrent controls. The decrease in food consumption in males at 600 ppm was correlated with the reduction in group mean body weight noted in that dose level.

Body weights (g, mean ± SD) of rats fed methiocarb in the diet at selected study weeks

Dose (ppm)	Study Week					
	0	16	27	53	83	105
Males						
0	91± 9	335± 20	370 ± 29	394 ± 36	412 ± 34	405± 47
67	91± 9	331± 25	364 ± 25	394 ± 31	407 ± 35	399±47
200	91± 9	324±24*	360 ± 24	411 ± 25	406 ± 34	404±41
600	91± 9	306±30**	345±3**	384 ± 36	390±40**	384±50*
Females						
0	80 ± 5	189± 19	206 ± 20	212 ± 24	242 ± 25	252 ± 34
67	81 ± 6	189± 15	204 ± 16	211 ± 18	240 ± 26	252 ± 33
200	82 ± 6	189± 15	206 ± 17	223 ± 23	242 ± 28	250 ±34
600	82 ± 6	181±17*	195±17**	213 ± 18	226±22**	234±26**

** Significantly different from the corresponding controls ($p<0.01$).

* Significantly different from the corresponding controls ($p<0.05$).

In haematology, there were several statistically significant but sporadic and biologically insignificant perturbations in various parameters, especially during the first year on study. The only observations that may have been treatment-related were slightly increased reticulocyte counts in females during the first year, accompanied by depressed erythrocyte counts at months 3 and 6 (see Table). Although statistical significance ($p<0.05$ or 0.01) was achieved for either or both of these parameters at all three doses, biological significance is probably restricted to 200 and 600 ppm, because erythrocyte depression in 67 ppm females at 3 months was not repeated at later time points. No inter-group differences were seen at termination.

Reticulocyte and RBC counts in female rats at selected sampling times

Dose (ppm)	Reticulocyte count (%)			RBC count (10^6/µL)		
	3 mo	6 mo	12 mo	3 mo	6 mo	12 mo
0	16	21	16	8.04	8.01	7.51
67	19	22	18	7.73*	7.73	7.75
200	20**	25*	15	7.73*	7.24**	7.88
600	20*	30*	21*	7.94	7.22**	7.75

mo = month

**Significantly different from the corresponding controls ($p<0.01$)

*Significantly different from the corresponding controls ($p<0.05$).

Slight but statistically significant and persistent elevation in ALT activity was noted in females at 600 ppm (at 6, 12 and 24 months) but was probably not treatment-related, being attributable to a high reading in a single animal. Significant reductions ($p<0.01$or 0.05) in serum bilirubin levels were seen in males at 200 ppm at 6 and 12 months and at 600 ppm at 6 and 24 months with a noticeable dose relation at 6 and 24 months. In females, a similar trend in depressed serum bilirubin was only noted at 6 months, attaining statistical significance at 600 ppm ($p<0.05$). These findings are not considered to indicate hepatic dysfunction. Significant reduction (about 58%, $p<0.01$) in GDH activity was observed among 600 ppm males at termination, but this finding is of uncertain biological significance (see Table).

Liver function test results at termination of rats fed methiocarb for 2 years

Dose (ppm)	Study parameter		
	ALT (mU/mL)	Bilirubin (mg/dL)	GDH (mU/mL)

Dose (ppm)	Study parameter		
	ALT (mU/mL)	Bilirubin (mg/dL)	GDH (mU/mL)
Males			
0	43.0	0.20	19.6
67	47.3	0.20	19.8
200	52.4	0.19	25.6
600	41.1	0.17**	8.1*
Females			
0	53.3	0.18	26.7
67	52.9	0.19	33.6
200	44.6	0.19	26.2
600	60.5*	0.20	32.6

** Significantly different from the corresponding controls ($p<0.01$).

* Significantly different from the corresponding controls ($p<0.05$).

Urinalysis and the tests for renal function revealed small (approx. 20%) but statistically significant increases ($p< 0.05$) in plasma urea levels in 600 ppm females at 12 months and termination. Compared with the values at 3 and 6 months, there was a trend towards elevated serum cholesterol content in all male groups at 12 and 24 months, which was more prominent among controls and the 67 ppm group, than at higher doses. Consequently, at 12 months, serum cholesterol showed a dose related decrease compared with controls (significant at both 200 and 600 ppm at $p<0.01$ and 0.05 respectively), and a similar but weaker decreasing trend was also noticeable at termination. However, this finding is not considered indicative of any disease process.

Plasma ChE activity in treated animals at different sampling times are presented in the following Table. Plasma ChE activity in both sexes at 600 ppm was inhibited with either statistical or biological significance (>20% inhibition) and persisted at most of the sampling times. The 20% reduction of plasma ChE activity observed in 200 ppm males at termination was biologically significant. Weak dose-response relationships in plasma ChE activity were observed, but only at a minority of the sampling points.

Plasma ChE activity at different sampling times in rats fed methiocarb

Dose (ppm)	Enzyme activity (group means, u/mL)									
	d 1	wk 1	wk 2	wk 4	wk 8	wk 13	wk 26	wk 52	wk 78	wk 105
Males										
0	0.40	0.53	0.47	0.46	0.41	0.45	0.47	0.50	0.66	0.89
67	0.42	0.53	0.47	0.51	0.45	0.44	0.49	0.47	0.79*	0.73
200	0.42	0.59	0.54	0.51	0.41	0.40	0.41	0.54	0.77	0.71[a]
600	0.34*	0.52	0.47	0.42	0.30**	0.32**	0.38*	0.50	0.56**	0.64*
Females										
0	0.48	0.78	0.87	1.10	1.21	1.26	1.62	1.35	1.78	1.64
67	0.47	0.75	0.92	1.07	1.18	1.29	1.67	1.48	1.81	1.92
200	0.47	0.67	0.87	0.95	1.11	1.30	1.65	1.91*	1.65	1.40
600	0.40*	0.63*	0.68**	0.65**	0.86*	0.99[a]	1.21[a]	1.80	1.60	1.23[a]

d = day, wk = week.

** Significantly different from the corresponding controls ($p<0.01$).

* Significantly different from the corresponding controls ($p<0.05$).

[a]Biologically significant compared to the corresponding controls.

RBC ChE activity (see Table below) tended towards slight depression in treated rats, but a consistent dose response relationship was not observed. In males, statistical significance ($p<0.01$ or 0.05) was achieved at 600 ppm at weeks 8, 13, 78 and 105. The data were consistent with the slight depression in plasma ChE values noted at the same sampling times. Similarly, the 600 ppm female group showed significant ($p<0.05$ or 0.01) RBC ChE depression on day 2 and at weeks 4, 8, 26 and 78. Significant reductions also occurred at 200 ppm on day 2 and at weeks 8, 78 and 105 in males and at weeks 4 and 78 in females ($p<0.01$ or 0.05). At 67 ppm, erythrocyte ChE activity was significantly inhibited ($p< 0.05$) in females at week 78 and in males at week 105. However, it is difficult to attribute this finding to treatment, given that it was limited to a single time point in each sex throughout the study.

Erythrocyte ChE activity at different sampling times in rats fed methiocarb

Dose (ppm)	Enzyme activity (group means, u/mL)									
	d 1	d 2	wk 2	wk 4	wk 8	wk 13	wk 26	wk 52	wk 78	wk 105
Males										
0	2.5	2.5	2.6	2.8	2.6	3.0	2.4	2.9	3.1	3.2
67	2.5	2.5	2.7	2.8	2.6	3.0	2.5	3.0	3.1	3.0*
200	2.6	2.3*	2.7	2.7	2.5*	2.9	2.5	3.1	2.9*	2.9**
600	2.6	2.5	2.6	2.7	2.4*	2.8*	2.5	3.1	2.8**	3.0*
Females										
0	2.4	2.4	2.6	2.8	2.5	2.9	2.4	3.1	3.2	2.9
67	2.4	2.3	2.6	2.8	2.6	2.8	2.5	2.9	3.1*	2.9
200	2.4	2.3	2.6	2.6*	2.6	2.9	2.5	3.0	2.8**	2.8
600	2.5	2.2*	2.5	2.6*	2.7*	3.0	2.6*	2.9	2.8**	2.8

d = Day, wk = Week.

** Significantly different from the corresponding controls ($p<0.01$).

* Significantly different from the corresponding controls ($p<0.05$).

No inhibition in the brain ChE activity was seen (see Table below). However, in females a dose related increase in enzyme activity was noted achieving statistical significance ($p<0.01$) at all dose levels. This finding is of uncertain biological significance.

Brain ChE activity at termination in rats fed methiocarb

Dose (ppm)	Brain ChE activity (u/g)
Males	
0	1.53
67	1.67
200	1.57
600	1.60
Females	
0	1.22
67	1.43**(17%)
200	1.46**(20%)
600	1.52**(25%)

** Significantly different from the corresponding controls ($p<0.01$).

Numbers in parentheses represent percent increase in enzyme activity compared to corresponding controls.

At necropsy, no treatment-related gross tissue abnormalities were observed in animals sacrificed during the experiment. At termination, treatment-related changes in organ weights were confined to the 600 ppm dose level. Relative and/or absolute spleen weights were depressed by 10 - 18% in both sexes ($p<0.01$ in males, $p<0.05$ in females for absolute weight only). In males, relative testes weights were increased by 7% compared with controls ($p<0.05$). Three further findings from 600 ppm females were noted by the study authors, but were not biologically significant. These were: a 3.5% increase in relative kidney weight ($p<0.05$); a 2-fold increase in mean thyroid weights ($p<0.05$ for absolute weight, significance not attained for relative weight, attributable to gross enlargement in a single animal); and approximately 2-fold increases in absolute and relative ovary weights (which failed to achieve statistical significance), attributable to a theca cell tumour in one animal.

In histopathology, about 25% of the animals had evidence of parasitic infection in the bowel, suggesting poor hygiene in the study laboratory and casting doubt on the reliability of the study findings. No treatment-related non-neoplastic or tissue abnormalities were observed. The reported neoplasia were age related neoplastic lesions seen commonly in rats and were not regarded as compound related.

Conclusions: Based on transient depression in body weight in 200 ppm males, elevation in reticulocyte counts in 200 ppm females and plasma ChE inhibition at 200 ppm, the NOEL for this study is established at 67 ppm (3.27 and 4.98 mg/kg bw/day for males and females respectively). Given the uncertainty surrounding the findings of this study, it is considered to be of reduced regulatory value.

6.3 Dogs

6.3.1 Oral

6.3.1.1 2 year dietary study

Doull J, Root M & Meskauskas (1968) Chronic oral toxicity of BAY 37344 to male and female dogs. Project No: not stated. Lab: Toxicology Laboratory, University of Chicago, Chicago IL 60637, USA. Sponsor: Bayer AG, Germany. Study duration: Not stated. Report No: 22115, Report date: February 01, 1968.

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

Study: Methiocarb technical (5% stock concentrate in Dextrose sugar, PF-131), mixed with ground dog food (Rockland Dog Diet, Tecklad Inc., Monmouth, IL, USA), was fed to pure bred Beagle dogs (12-15 weeks old, bw not stated, 2/sex/dose) at 0, 50, 100 and 250 ppm (equivalent to approximately 0, 1.25, 2.5 and 6.25 mg/kg bw/day) for 2 years. The dogs were immunised against distemper and infectious hepatitis and kept under observation for 3 weeks prior to the commencement of the study. The diets were prepared at least biweekly. The test animals were fed twice a day (at 9 am and 4 pm) during the first 8 weeks and once a day (time unspecified) thereafter. They were housed individually in metal metabolic cages and had constant access to water.

Observations: Clinical signs were observed at the time of feeding. The dogs were weighed weekly during the first eight weeks and biweekly thereafter. Food consumption of the animals was not measured regularly. Blood was collected from the saphenous vein at weekly or biweekly intervals for the serum and RBC ChE assay using a manometric method. Brain and liver ChE activities were measured at the termination of the study. The animals were necropsied under ether anaesthesia (phenobarbital) and the following tissues were removed, weighed, sampled and fixed in neutral buffered formalin for histopathology: brain, liver, heart, kidneys, gonads, lungs, thymus, adrenal glands, urinary bladder, mesenteric and

thoracic lymph nodes, thyroid, skeletal muscle, stomach, duodenum and pancreas, ileum, jejunum and colon. A section of the sternum was taken for bone marrow examination.

Findings: There were no mortalities or clinical signs reported, although reporting was limited. The study authors stated that the food consumption among treatment groups was not different from the controls and the inclusion of methiocarb at 250 ppm or less did not alter the acceptability of the diet by the animals (no data provided). Body weight data were presented as line graphs (average weight of males and females vs weeks on diet). No inter-group differences in average body weight were noted approximately up until week 28 (7.5 months) of the study. It was evident that the average body weight at 250 ppm was depressed by about 10% from week 32 to 80; and during the same period it was 10% greater at 100 ppm compared to the controls (no individual data were provided).

Serum ChE activity (as measured by µL of CO_2 produced/10 min/50 mg wet tissue) was inhibited by up to 20% during weeks 15 to 32 of the study at 100 and 250 ppm. The enzyme activity in the control and treated animals was variable between 60-80% of its original value during the last six months of the study (no individual data were provided). This may have been due to ageing. RBC ChE activity was variable in all dose groups but rarely inhibited by more than 20% compared to the controls. Brain and liver ChE activities were not affected by treatment.

At necropsy, adhesion of the kidney capsule was observed in several treated dogs (number unspecified) and two dogs in the control group (sex unspecified) while one female at 100 ppm had a enlarged spleen and one male at 250 ppm displayed thickening of the tricuspid valve. These findings were not considered to be treatment-related. The absolute and relative liver weights of dogs fed 250 ppm diet were slightly higher (6% and 9% respectively) than the controls. No other information on clinical findings or histopathology was provided.

Conclusions: Because of lack of clinical observations, limitations of study data and the low number of dogs/experimental group, this study is not considered appropriate for regulatory purposes.

6.3.1.2 2-Year dietary study

Hoffman K & Schilde B (1980) H321 (Mesurol active ingredient-mercaptodimethur) Chronic toxicity study on dogs (Two year feeding experiment). Project No. H321/006; Mesurol/001. Lab: Pharmacology Research Centre, Bayer AG, Wuppertal-Elberfeld. Sponsor: Bayer AG, Germany. Study duration: April, 1977 to April, 1979. Report No. 9626, Report date: December 4, 1980.

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

Study: H 321 (methiocarb, Batch 234602648, purity 98.4%) was mixed with pulverised dog chow (Altromin H Diet of Altromin GmbH, from week 1 to 37 and Ssniff HH complete Dog Diet of Ssniff Versuchtierdiaten GmbH, from week 38 to termination) and fed to Beagle dogs (F Winkelmann, Borchen, 26-27 weeks old, initial bw 7.1-11.8 kg, 4/sex/dose) at concentrations of either 0, 15 (during the first 15 days), 5 (from week 3 to 104), 60 or 240 ppm for 104 weeks. Based on the food consumption data, actual achieved doses of methiocarb were equivalent to 0.6, 0.2, 2.4 and 9.6 mg/kg bw/day respectively. The dewormed dogs were revaccinated against distemper, infectious hepatitis and leptospirosis during quarantine and were randomly assigned to each treatment group based on their body weight and pre-test plasma ChE activity ranges. The diets were prepared weekly and offered to the animals as a wet mash. In addition to the daily test ration, each animal received about 200 g of Chappi canned chow daily in weeks 16 and 17 and 100 g of Altromin H pellets in

weeks 18 and 37. The initial low dose of 15 ppm was reduced to 5 ppm in week 3 after depression of plasma ChE activity was observed at that dose level. The dogs were housed individually in metabolic cages under conventional laboratory conditions, exercised each morning and were provided with drinking water *ad libitum*. Statistical significances between the control and treatment groups were tested using the Wilcoxon rank test.

Observations: The clinical examinations (corneal, pupillary, patellar, flexor and extensor reflexes, body temperature, pulse rate and ophthalmoscopy), haematology, clinical chemistry and urinalyses tests were performed prior to initiation of feeding and during treatment weeks 14, 16, 27, 40, 53, 66/67, 79, 92 and 104. The dogs were inspected, several times each day for their physical appearance and behaviour. Food and water consumption was measured daily including the time taken to consume the ration. The amount of mash ration not consumed by the animals within about 20 h was weighed before the next feeding. Body weights were measured weekly. The haematology parameters measured were: Hct, Hb, RBC and WBC counts, MCH, MCHC, MCV, thrombocyte, reticulocyte and WBC-DC counts, sedimentation rate and thromboplastin time. The following serum chemistry parameters were measured: glucose, plasma urea, creatinine, total protein, AST, ALT, AP, bilirubin, cholesterol, glutamate dehydrogenase, serum proteins, sodium, potassium, calcium and chloride. Plasma and RBC ChE activities were assessed prior to initiation and at weeks 2, 3, 4, 7, 10, 13, 27, 40, 53, 66, 79, 92 and 104 using jugular vein blood samples taken before feeding and 2 h later. An additional measurement was taken at 24 h after the last feeding at week 104. The plasma and RBC ChE activities were measured using a colorimetric method and a modification of this method was used to assess the brain ChE activity (bulbus olfactorius) in samples collected at necropsy. Urine was tested semi-quantitatively using samples collected in a 6 h period of food withdrawal up until week 40. Starting from treatment week 50 each dog was given a 250 mL of water by oral intubation before being placed in metabolic cages for urine collection. At termination, the dogs were anaesthetised with Evipan, sacrificed by exsanguination and autopsied. The absolute weights of the following organs were recorded: heart, lung, liver, kidney, spleen, testes, ovaries, thyroid, adrenals, prostate gland, brain and pancreas. In addition to the samples collected from the above, the following tissues were sampled and processed for histopathology: pituitary, epididymis, uterus, oesophagus, stomach, intestines, mesenteric glands, thymus, gall bladder, urinary bladder, cerebrum, cerebellum, eye, optic nerve, sciatic nerve, aorta, skeletal muscle.

Findings: One female at 0.2 mg/kg bw/day died during week 98. This animal had the lowest body weight at the commencement of the study. Necropsy revealed mucosal defects in the glandular part of the stomach which resembled perforations, and the death was not considered to be test chemical related. There were no further mortalities.

No difference in general physical appearance was seen between the control and the treated groups. The clinical signs recorded were: occasional mild weakness of the hind limbs accompanied by trembling, lameness in one or both hind limbs and infrequent decreased alertness in 5/8 dogs at 9.6 mg/kg bw/day during the first 14 weeks of the study only. Two dogs (one at 2.4 and one at 9.6 mg/kg bw/day) had epileptic attacks and subsequent clinical examinations revealed that those attacks were not test chemical related. Vomiting was seen occasionally in all groups, the incidence being higher at 9.6 mg/kg bw/day and was considered to be treatment-related. Ophthalmoscopy did not reveal any ocular abnormalities attributable to treatment and the vision of the animals was unaffected.

Food consumption was reduced slightly in 2.4 mg/kg bw/day females (about 5-7%) and in all animals at 9.6 mg/kg bw/day (about 12%) during the second year of the study and this may be treatment-related. The body weights and the nutritional state of the animals were unaffected

by treatment and the animals on test diets gained weight comparable to the controls despite the slight reduction in food consumption occurring at 9.6 mg/kg bw/day.

No significant inter-group differences in haematology or clinical chemistry were seen. Plasma ChE activity expressed as percent of control values is presented in the Table below.

Plasma ChE activity in all animals was depressed at 0.6 mg/kg bw/day and above compared to the controls during week 2 and 3. Therefore, the lowest dose was reduced to 0.2 mg/kg bw/day afterwards. From week 4 onwards the dietary levels of 2.4 and 9.6 mg/kg bw/day induced a dose related and biologically significant depression of the plasma ChE activity in all animals at 2 h post treatment. This inhibition was seen to persist throughout the study. The ChE inhibition at 9.6 mg/kg bw/day pre-treatment was near or above 20% at nearly all sampling times compared to the corresponding controls and was more pronounced in the males than in the females, suggesting slower recovery of the enzyme activity overnight. The RBC ChE activity was variable and inhibition did not reach biological significance at any dose at any of the sampling times. The brain ChE activity was unaffected by treatment.

Urinalysis and necropsy did not reveal any treatment-related abnormalities. No significant differences in absolute or relative organ weights nor histopathological abnormalities attributable to the treatment was seen.

Conclusions: Under the conditions of the study and based on biologically significant plasma ChE inhibition in both sexes and reduced food consumption in females observed at 2.4 mg/kg bw/day, the NOEL was established at 0.2 mg/kg bw/day.

Percent depression of plasma ChE activity in dogs fed methiocarb for 104 weeks (Group means expressed as percent of controls)

Dose (mg/kg bw)	Sampling week																								
	2		4		7		10		13		27		40		53		66		79		92		104		
	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	0	2h	24h
Males																									
0.6	0	28																							
0.2			0	11	0	9	2	10	2	14	3	11	0	7	1	5	1	2	1	3	0	1	0	0	0
2.4*	7	53	4	54	9	66	8	49	8	54	5	54	4	45	2	44	0	44	0	26	0	37	7	31	3
9.6*	2	54	22	77	43	81	19	55	18	77	38	76	16	62	13	72	20	64	34	61	13	48	26	52	23
Females																									
0.6	0	29																							
0.2			0	0	0	0	0	1	0	0	0	0	0	0	0	0	19	9	0	0	0	1	0	0	0
2.4*	7	68	7	63	15	34	3	59	10	58	9	66	0	46	10	52	39	55	10	28	29	35	5	8	3
9.6*	10	80	18	80	2	79	0	78	12	78	12	81	11	78	23	70	56	77	11	68	36	53	23	42	23

*No statistical significance was conducted. Biologically significant (>20%) depression of ChE compared to the concurrent controls.

7. REPRODUCTION STUDIES

7.1 Three-Generation Study

Löser E & Newman AJ (1970) BAY 37 344: Generation studies on rats. Project No. Not stated. Lab: Institute of Toxicology, Farbenfabriken, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: 1968-1969. Report No. 2208. Report date: July 10, 1970 and,

Spicer EFJ (1971) Pathology report of BAY 37 344: Rat breeding - study (Addendum to report No. 2208 of Institute of Toxicology, Farbenfabriken, Bayer, July 10, 1970). Lab: Huntington research Centre, Huntington, England. Sponsor: Bayer AG, Germany. Study duration: July 1970 to February, 1971. Report No. 3838/70/660. Report date: February 16, 1971.

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

Study: Strain FB 30, Elberfeld breed rats (initial bw 45-55 g, approximately 33 days old) were used in the study. The animals were allocated to experimental groups (10 male and 20 females/dose) and methiocarb (source and purity unstated) was administered at 0 (control), 30, 100, and 300 ppm (equivalent to approximately 0, 3, 10, and 30 mg/kg bw/day) in Sniff powder feed for three parental generations of animals and their offspring throughout all phases of this study. A justification for the dose selection was not provided. Fresh diets were prepared weekly. No details were provided for procedures about animal acclimatisation and diet preparation. No data were reported for tests of diet homogeneity, stability or confirmation of the target concentrations of the test compound.

Each generation was mated twice to produce an 'a' and 'b' population for each subsequent generation. Two females were housed together with one male for 19 to 20 days, the males being interchanged during the mating period ensuring that each female was placed together with 3 different males for a period longer than the duration of one oestrous cycle. After matings had taken place (mating confirmation procedure unspecified), the animals were individually housed. The litters that contained more than 10 pups were reduced to 10 after 5 days; all young animals were suckled for up to 4 weeks. The offspring of each of the first matings (F1a, F2a and F3a) and the animals that were used for mating (F0, F1b and F2b) and F3b litters were sacrificed (under ether anaesthesia by exsanguination) after weaning. The pups of each of the second matings (F1b, F2b and F3b) were then weaned, sexed and housed singly until they attained sexual maturity (aged about 100 days) to produce the next generation. Ten rats/sex/dose were selected (from F1b and F2b) for further matings; the mating procedure being similar to the above. The animals were provided with feed and water *ad libitum* and housed under conventional laboratory conditions throughout all phases (including during mating, gestation and suckling) of the study. Statistical significance between the control and treatment groups was tested using the non-parametric rank test of Wilcoxon. The approximate duration (experimental design) of each phase of the study are given in the following Table.

Experimental design

Study phase	Duration (days)
Pretreatment of rats of F0 generation up to first mating	70
Duration of first mating of F0 generation	20
Duration of gestation	21

Lactation of young F1a generation up to sacrifice	28
Waiting period	10
Duration of second mating of F0 generation	20
Lactation of F1b generation and up to sexual maturity and sacrifice of F0 generation	100
Duration of first mating of F1b generation	20
Duration of gestation	21
Lactation of young F2a generation up to sacrifice	28
Waiting period	10
Duration of second mating of F1b generation	20
Duration of gestation	21
Lactation of F2b generation and up to sexual maturity and sacrifice of F1b generation	100
Duration of first mating of F2b generation	20
Duration of gestation	21
Lactation of young F3a generation up to sacrifice	28
Waiting period	10
Duration of second mating of F2b generation	20
Duration of gestation	21
Lactation of young F3b generation up to sacrifice	28

Observations: The animals were weighed weekly. The litter size and the body weights of the young were recorded immediately after birth. The pups were weighed again at 5 days, one week and weekly thereafter. The reproductive parameters that were recorded or indices calculated include: average litter size, gestation rate and lactation index. The pups were subjected to gross examinations for malformations after birth and during the lactation period. The animals which died during the study, were necropsied. The following organs of the F3b generation pups were macroscopically and histologically examined: lungs, heart, liver, spleen, kidneys, adrenals, gonads, thymus and thyroids.

Findings: Graphical representations of group mean body weights (including pre-mating and gestational periods) did not indicate any effect of treatment in F0, F1 and F2 parental generations.

F0 generation: The reproductive parameters and indices of selected generations with respective neonate data including the total pre-cull pup loss (from day 0-5, calculated) are presented in the following table. During lactation, the pups in all generations gained weight at rates comparable to the corresponding controls. A depression in gestation rate of F0 females (by about 11%) associated with a consequent lowering of average litter size (by about 7.6%) was noted at 30 mg/kg bw/day compared to the concurrent controls after the first mating of F0 animals. The study authors stated that the observed gestation rate was within the normal physiological range but no supporting historical control data were provided. The slight increase in average pup weight noted at 30 mg/kg bw/day may have been related to the reduction in average litter size in that group.

No statistically or biologically significant group differences were noted in any of the reproductive parameters or in neonate data at the second mating of F0 generation (data not shown).

Reproductive parameters, indices and neonate data

Parameter	**Dose (mg/kg bw/day)**

	0	3.0	10.0	30.0
After the first mating of F0 generation				
Gestation rate (%)	95 (19/20)	95 (19/20)	95 (19/20)	84 (16/19)
Average number of pups/litter:				
At birth	11.8	12.1	11.6	10.9
Before reduction	10.9	11.8	11.1	10.7
(calculated pre cull pup loss, total)	17	6	9	3
Average pup weight at birth (g)	6.16	6.27	6.06	6.36
Total no. of pups after reduction	164	183	182	141
Lactation performance:				
No. of pups raised up to 4 weeks	163 (99%)	183 (100%)	175 (96%)	137 (97%)
After the first mating of F1b generation				
Gestation rate (%)	95 (19/20)	100 (20/20)	100 (20/20)	90 (18/20)
Average number of pups/litter:				
At birth	12.2	13.7	11.8	10.7
Before reduction	10.9	13.0	10.2	9.8
(calculated pre cull pup loss, total)	25	14	32	16
Average pup weight at birth (g)	6.36	5.98	5.91	6.72
Total no. of pups after reduction	164	197	167	144
Lactation performance:				
No. of pups raised up to 4 weeks	156 (95%)	193 (98%)	151 (90%)	133 (92%)
After the second mating of F1b generation				
Gestation rate (%)	100 (20/20)	95 (19/20)	90 (18/20)	85 (17/20)
Average number of pups/litter:				
At birth	13.8	14.8	11.9	13.0
Before reduction	13.4	14.1	10.1	11.8
(calculated pre cull pup loss, total)	8	13	32	20
Average pup weight at birth (g)	6.01	5.7	5.7	6.26
Total no. of pups after reduction	197	187	146	152
Lactation performance:				
No. of pups raised up to 4 weeks	186 (94%)	184 (98%)	139 (95%)	146 (96%)

Blood, urine and clinical chemistry (AST, ALT, LDH, Bilirubin, SDH, ALP and total serum protein) analyses in representative animals (10/sex/dose) at 10 weeks (before mating) and at 24 weeks (after the second litter of F_1b generation had been reared) did not reveal any biologically significant effects attributable to the treatment in any parameters at either sampling time (Löser, 1969, Report No. 1358).

F1 generation: At the first mating of F1b animals, a slight depression in gestation rate (by about 5%) associated with a lowering of average litter size at birth (by about 12%) and the lactation performance (number of pups raised up to 4 weeks, by about 15%) was noted at 30 mg/kg bw/day compared to the concurrent controls. The slight increase in average pup weight (about 6%) noted at 30 mg/kg bw/day may have been associated with the reduction in average litter size in that group.

Following the second mating of F1b animals a slight dose related decrease in gestation rate was seen. In F2b neonate data, the total number of pups after reduction was depressed by about 26% and 23% at 10 and 30 mg/kg bw/day respectively compared to the parallel

controls. This was associated with a lowering of the lactation performance of F1b animals by about 26% at 10, and by about 22% at 30 mg/kg bw/day compared to the concurrent controls. No statistically or biologically significant inter-group differences were noted.

F2 generation: Average F3a generation pup weight at birth (data not shown) in all treatment groups appeared to be depressed by about 7-10% compared to the parallel controls. No other group differences were seen. No group differences were observed in any of the reproductive parameters after the second mating of the F2b animals.

At necropsy, no macroscopic pathological changes attributable to the treatment were noted in the sacrificed animals (F0, F1b, F2b and F1a, F2a, F3a, F3b). Histopathology did not reveal any inter-group differences nor were any treatment-related malformations observed in any generation at birth or during lactation.

Conclusions: Sporadic changes in some reproductive parameters and neonate data were observed in different generations of animals, but these alterations failed to reveal consistent, statistically or biologically significant treatment-related effects in all generations. Therefore, it is considered that there were no treatment-related effects at the highest dose of 30 mg/kg bw/day. However, due to the age of the study and data limitations (lack of absolute data on maternal body weights, post cull survival, pup sex ratio and ChE) the value of the study findings is reduced.

OECD Guidelines for testing of chemicals No. 416, adopted May 26, 1983 specifies that, in a two-generation reproduction toxicity study, "each test and control group should contain a sufficient number of animals to yield about 20 pregnant females at or near term" for meaningful evaluation of reproductive toxicity.

8. DEVELOPMENTAL STUDIES

8.1 Rats

Lorke D (1971) Mesurol active ingredient (BAY 37 344): Studies on rats for embryotoxicity and teratogenic effects. Report No. 3133. Lab:, Institute of Toxicology, Farbenfabriken, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: not stated. Report date: November 30, 1971.

Includes: Renhof M (1988) BAY 37344 (Mesurol Active Ingredient): Study for embryotoxic effects in rats in oral administration. Addendum to Report No. 3133 of November 11, 1971. Study No: T 8029962. Lab: Bayer AG, Fachbereich Toxikollogie, Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1. Sponsor: Bayer AG, Germany. Report No. 3133 A, Report date: October 21, 1988.

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

Study: Methiocarb (Bay 37 344, batch 11, purity 98.9%, solubility and stability not stated) in a 1% tragacanth suspension was administered once daily by gavage to mated strain FB 30 female rats (2.5-3.5 months old, initial bw 200-250 g, 19-20 animals/dose, source not stated) at 0, 1, 3 and 10 mg/kg bw/day on days 6-15 post-coitum. The control animals received 1 mL of 1% tragacanth suspension per 100 g bw daily during the treatment period. Mating (overnight) was accomplished by natural means using 3-6 months old males of 300-500 g bw (strain, source not stated). The day following the detection of spermatozoa in vaginal smears was considered to be the first day of gestation. It was stated that the dose levels were previously tested for the general tolerance to pregnant rats and possible embryotoxic and teratogenic effects. However, no details of such studies, justification for the doses selected or basis for the dose levels used in the study were provided. The animals were housed

individually in Makrolon cages, provided with pelleted feed (Altromin R) and water *ad libitum* and weighed daily during the experiment. The post-treatment observation period was 5 days. On the 20th day of pregnancy they were anaesthetised using ether and sacrificed (method unspecified).

Observations: All foetuses were recovered after Caesarean delivery and weighed. They were examined thoroughly for external malformations. In addition to observations on foetal malformations the study examined the following developmental parameters: percent fertilisation, resorptions, stunted foetuses (weighing less than 3 g) and weights of the foetuses and placenta (number of corpora lutea and sex of the foetuses were not determined). Of the recovered pups, 30% were examined for visceral abnormalities and the remainder for skeletal malformations following evisceration of the soft tissues with potassium hydroxide and staining the bone tissue with Alizarin Red S. Statistical significance was tested by using the non-parametric Wilcoxon rank test at the 95% probability level ($p<0.05$). Information on clinical observations, food and water intake during the experiment and macroscopic structural abnormalities or pathological changes at sacrifice of dams was not provided.

Findings: The animals did not exhibit any perceptible treatment-related signs of adverse effects. No effects were observed in food consumption (data not provided) and appearance. There were no mortalities or premature abortions recorded during the treatment. A reduction in average weight gain during pregnancy (10%) was noted in the highest dose group (10 mg/kg bw/day) while the average weight gain in 1 and 3 mg/kg bw/day dose groups was comparable to that of control animals during the 20 day experimental period.

No significant differences were observed in percent fertilisation or the number of implantations in treated animals compared to the control group. No significant effects were seen on weights of foetuses or placenta, resorptions, and in foetal bone development in relation to treatment. It was stated that, the nature and the frequency of variation of those parameters observed in this study were within the normal range for this rat strain. However, no historic data were either provided or referred to support this claim. No treatment-related visceral or skeletal malformations were observed.

Conclusions: Under the conditions of the study and based on reduced weight gain at the highest dose, the NOEL for maternal toxicity in rats was 3 mg/kg bw/day. There were no effects on foetal survival, development or growth at the highest dose of 10 mg/kg bw/day.

8.2 Rabbits

8.2.1 Oral

Tesh JM & Ross FW (1981) H 321: Effects of oral administration upon pregnancy in the rabbit. 1. Preliminary study. Study No. not stated. Lab: Life Science Research, Elm Farm Laboratory, Occold, Near Eye, Suffolk, England. Sponsor: Bayer AG, Werk Elberfeld Institute of Toxicology, Friedrich-Ebert-Strasse 217-319, Wuppertal, Postfach 10 17 09, Germany. Study duration: November 19 to December 20, 1980. Report No. 81/BAG012/012. Report date: January 12, 1981.

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

Study: H 321 (methiocarb, a mixture of five batches, purity: unstated) was administered by oral gavage to oestrous synchronised (with luteinising hormone: Pregnyl, Organon: 25 i.u. i.v. injection), artificially inseminated NZW rabbits (Ranch Rabbits, Crawley Down, Sussex, England. about 24 weeks old, 4.07 to 5.13 kg initial bw) at dose levels of either 0, 1, 3 or 10 mg/kg bw/day in distilled water containing 0.5% carboxymethyl cellulose and 0.5% Tween

80 on days 6 through 18 post-insemination. There were 4 rabbits in the control, low and mid dose groups whilst the 10 mg/kg bw/day group consisted of 5 animals. The animals were acclimatised for a minimum of 3 weeks after oestrous synchronisation. The test compound was formulated freshly each day and dosed at a volume dosage of 5 mL/kg bw. The control animals received the vehicle only at the same volume dosage during the treatment period. The volume administered was based on the animal's body weight on that day. Rabbits were housed individually in galvanised steel cages and were provided with food [Beta Rabbit standard Diet, BP Nutrition (UK) Ltd. Witham, Essex, England] and water *ad libitum*. The day of insemination was designated day 0 of gestation.

Observations: All animals were weighed daily and examined thoroughly for visible signs of toxicity including the details of type, severity, time of onset and duration.

On day 29 of gestation, the animals were sacrificed by i.v. overdose of phenobarbitone sodium. After each animal was macroscopically examined for evidence of disease or adverse reaction to treatment, the reproductive tracts including ovaries were dissected out. Any tissues considered abnormal were retained. The following terminal parameters were determined: weight of gravid uterus, numbers of corpora lutea in each ovary, implantation sites, early or late resorption sites, number and the distribution of live and dead foetuses in each uterine horn, weight and sex of individual foetuses, individual placental weight and external abnormalities of individual foetuses, morphological abnormalities of the maternal reproductive tract. The uteri were immersed in a solution of ammonium sulfide to obtain information on the number of implantation sites.

All foetuses were sacrificed by using s.c. injections of phenobarbitone sodium and were observed for external abnormalities. The necks, thoracic and abdominal cavities of foetuses were dissected and the contents were examined for visceral abnormalities. After evisceration, the foetuses were placed in industrial methylated spirit for subsequent examination of skeletal malformations. Data were presented as means with standard deviations (SD).

Findings:
Maternal: There were no mortalities. The animals at 10 mg/kg bw/day showed a loss of body weight (-2.1% by day 12) during the first half of the treatment period compared to the concurrent controls. Thereafter the group mean body weight of this group increased and was comparable to that of the controls at termination. No other inter-group differences were noted. Post-treatment cholinergic responses such as loss of muscular control, muscular tremors and polypnea of about 3 h duration were noted in all animals at 10 mg/kg bw/day commencing from 15 minutes after dosing. The general health condition of the animals at 1 and 3 mg/kg bw/day were comparable to those of the controls. In the control group, there were only two females carrying live young to term. One animal was non-pregnant, and the fourth expelled a dead foetus on day 29 of gestation. At necropsy of this female, all the remaining foetuses were found to be dead. No necropsy findings of the other females were provided nor were food and water consumption determined.

The litter responses as assessed by the numbers of implantations, viable young and the extent of post implantation losses, were unaffected by treatment.

Foetal: Examination of foetuses at terminal necropsy revealed several anomalies such as displacement of the first and the fourth digits medially, agenesis of the median lung lobe, reduction in size of the gall bladder, haemorrhage or constriction of the gall bladder, pale areas on the liver, bilateral hydronephrosis, runts (foetuses of less than 32 g bw) and pale areas on placenta. However, the group incidence of these anomalies did not show any consistent indication of an association with treatment. Further, the study authors stated that

"the majority of these anomalies have previously been found in this strain of rabbits". However, no supporting historic data were provided for evaluation.

Conclusions: From this preliminary investigation, the study authors concluded that dose levels of methiocarb up to 10 mg/kg bw/day would be suitable for use in a main teratology study.

Tesh JM, Ross FW, Secker RC & Wilby OK (1981) H 321: Effects of oral administration upon pregnancy in the rabbit. 2. Main Study. Project No. 81/BAG013/598. Lab: Life Science Research, Stock, Essex CM4 9PE, England. Sponsor: Bayer AG, Werk Eberfeld Institute of Toxicology, Friedrich-Ebert-Strasse 217-319, Wuppertal, Postfach 10 17 09, Germany. Study duration: April 13, 1981 to June 16, 1981. Laboratory Studies completed on August 12, 1981. Report date: December 4, 1981.

Pre GLP, Quality assured study. No test guidelines were cited.

Study: Methiocarb (a mixture of five batches: 234702-602, -607, -609, -610 & -611, purity 97.3%, solubility and stability not stated) was administered by gavage to oestrous synchronised (with i.v. luteinising hormone: Pregnyl, Organon: 25 i.u.) artificially inseminated (with pooled semen) NZW rabbits (Morton Rabbits, Stansted, Essex, England, 18-24 wks old, 3.5-4.7 kg initial bw) at dose levels of either 0, 1, 3 or 10 mg/kg bw/day in distilled water containing 0.5% carboxymethyl cellulose and 0.5% Tween 80 on days 6 through 18 post-insemination. There were 17 rabbits in each treatment group whilst the control group consisted of 19 animals. The animals were acclimatised for a minimum of 3 weeks after oestrous synchronisation. The test substance was prepared freshly each day and dosed at a volume dosage of 5 mL/kg bw. The control animals received the vehicle only at the same volume dosage during the treatment period. The volume administered was based on the animal's body weight on that day.

Rabbits were housed individually in galvanised steel cages and were provided with food [Beta Rabbit standard Diet, BP Nutrition (UK) Ltd. Witham, Essex, England] and water *ad libitum*. Following artificial insemination (time and day not stated) each animal was injected with 25 iu of luteinising hormone (Pregnyl, Organon) i.v. to ensure successful ovulation. The day of insemination was considered to be the day 0 of gestation.

Observations: All animals were weighed daily and examined thoroughly for visible signs of toxicity including the details of type, severity, time of onset and duration and the abortions or premature deliveries during the experiment were recorded. The animals found dead or sacrificed at the point of death were necropsied.

On day 29 of gestation, the animals were sacrificed by i.v. injection of phenobarbitone sodium. After each animal was macroscopically examined for evidence of disease or adverse reaction to treatment, the reproductive tracts including ovaries were dissected out. Any tissues considered abnormal were retained (purpose and preservation medium not stated). The following study parameters were determined: weight of gravid uterus, numbers of corpora lutea in each ovary, implantation sites, resorption sites (early or late), live and dead foetuses in each uterine horn, weight and sex of individual foetus, placental weight and morphological abnormalities of the maternal reproductive tract. The uteri were immersed in a solution of ammonium sulphide to obtain information on the number of implantation sites.

The foetuses were sacrificed by using a s.c. injection of phenobarbitone sodium and were observed for external abnormalities. The necks, thoracic and abdominal cavities of foetuses were dissected and the contents were examined for visceral abnormalities. After evisceration, the foetuses were placed in industrial methylated spirit for subsequent examination of skeletal

malformations using a method modified from the Dawson staining technique. The following foetal skeletal parameters were determined: number of ribs, incomplete ossification of sternebrae, number with heads of long bones un-ossified and the size of the anterior fontanelle. Significance of inter-group differences in study parameters were tested using appropriate statistical procedures which included: t-test or multiple t-test, Mann-Whitney U-test, χ^2 test and Fisher's exact probability test.

Findings: A summary of observations made in methiocarb treated pregnant rabbits from day 6 to sacrifice on day 29 is presented in the following table.

General disposition and mortality of pregnant rabbits treated with methiocarb from day 6 through 29 of gestation

Observation	Dose group (mg/kg bw/day)			
	0	**1**	**3**	**10**
Total number of does inseminated	19	17	17	17
Mortality	6	4	3	3
Not pregnant	2	-	1	1
Premature delivery	-	-	1	-
Abortion	-	-	-	2
Removed from study	-	-	-	1*
Number pregnant with viable young on day 29	11	13	12	10

*Pregnant prior to allocation.

Maternal: Sixteen out of 70 rabbits either died or were sacrificed at the point of death during the experiment. The animals at 10 mg/kg bw/day showed a marked loss of body weight during the first two days of treatment (individual or group mean body weights for these two days were not provided). Consequently, the overall body weight gain of animals in this group was decreased during the remaining test period achieving statistical significance on day 18th of gestation (8% reduction, $p \leq 0.01$) compared to the control group. A slight reduction in weight gain was noticed in the 3 mg/kg bw/day dose group. One animal in this group lost weight (18%) during the experiment causing a reduction in the overall group mean body weight while other animals in the group gained weight comparable to the 1.0 mg/kg bw/day dose group and concurrent controls. Therefore, the insignificant reduction in the weight gain noticed at 3.0 mg/kg bw/day does not appear to be a treatment-related effect. The body weight gain of 1.0 mg/kg bw/day group was comparable to that of the control group during gestation. The group mean body weights showed a dose related downwards trend at termination but statistical significance was not achieved. Weights of the gravid uteri on day 29 appeared comparable among groups and are given in the following table. No data on the weights of other organs, food and water consumption, clinical chemistry or histopathology were provided.

Group mean body weights and gravid uterine weights on day 29

Dose (mg/kg bw/day)	Number of animals	Body wt (kg)	Gravid uterus wt (g)	Corrected body wt (kg)
Control	11	4.66	438	4.22
1.0	13	4.56	473	4.08
3.0	12	4.46	479	3.98
10.0	10	4.44	469	3.97

One doe at 3.0 mg/kg bw/day delivered prematurely on day 28 and two does at 10 mg/kg bw/day aborted on days 22 and 25 of gestation.

Post-treatment cholinergic effects were noted at 10 mg/kg bw/day, where responses such as increased respiratory rate, muscular tremors, salivation/chewing, pupillary constriction, unsteadiness, nervousness and prostration were noted with percent incidences of 70, 28, 13, 12, 68, 9, and 8 respectively. The time of onset of clinical signs ranged from 5 to 64 minutes post dosing and the signs remained for up to 4 h in some cases.

Post-mortem examinations of animals either found dead or sacrificed at the point of death during the experiment revealed evidence of respiratory tract infection and/or gastro-intestinal tract disorder or accidental tracheal intubation. No methiocarb related effect was noted. Reproductive indices including, the number of implantations and viable young, the pre and post- implantation loss and foetal and placental weights were comparable among groups. There appeared to be no compound related effects on any of the test parameters and the values noted were within previously reported background ranges (supporting historic data from 49 studies were provided).

Foetal:

Visceral & morphological: Upon visceral examination of foetuses at necropsy on day 29 (selection or processing methods unspecified), a small number of anomalies were noticed in all groups. The occurrence of pale areas on the foetal liver was increased at 10 mg/kg/day by about 3.5- and 17-fold compared to concurrent and background control data respectively (historic data from 49 studies provided). The study authors stated that the biological significance of this occurrence was "unclear". Although the significance of this finding cannot be determined conclusively without liver histopathology data, its possible association with liver injury cannot be entirely ignored. Data on the number of lobes in the foetal liver and lobular fusion were not provided. Pale areas in the centre of the eyes were noted in control, 1.0 and 3.0 mg/kg bw/day groups but were not observed in foetuses at 10 mg/kg bw/day. With the lack of any incidence at the highest dose level tested, this anomaly was not considered to be treatment-related. All other anomalies observed lay within historic data ranges provided by the study authors. Study data on both of the above mentioned parameters are presented in the table below.

Percent incidence of selected foetal observations at necropsy

Observation[a]	Dose (mg/kg bw/day)				Background[b]	
	0	1.0	3.0	10.0	Mean	Range
Number of foetuses examined	78	106	96	76	4686	-
Pale area in the centre of eyes	1.3	2.8	8.3	0	0.02	0.0-1.1
Pale area on liver	2.6	0.9	1.0	9.2	0.53	0.0-4.8

[a]One foetus may have more than one observation.

[b]Background data from 49 studies.

Skeletal: The results of skeletal examination did not provide evidence for any treatment-related effects.

Conclusions: Under the conditions of the study and based upon the information provided, there was no evidence of teratogenicity. However, maternotoxicity characterised by cholinergic signs and weight loss was evident at 10 mg/kg bw/day. Therefore, a maternotoxicity NOEL can be set at 3 mg/kg bw/day. Based on the discolouration seen in the foetal liver, the embryo/foetotoxicity NOEL can also be set at 3 mg/kg bw/day.

8.2.2 Dermal

Dotti A & Biederman K (1993) Dose range finding embryotoxicity study (including teratogenicity) with H 321 (c. n. Methiocarb) in the rabbit (dermal application). Project no. 297898. Lab: RCC Research and Consulting Company Ltd, PO Box. CH 4452, Itingen, Switzerland. Sponsor: Bayer AG, Institute of Toxicology, Landwirtschaft, Friedrich-Ebert-Strasse 217-333, D-5600, Wuppertal-Elberfeld, Germany. Study duration: May 15, 1991 to June 14, 1991. Report No. R 5929. Report date: April 15, 1993.

The study was neither QA nor conducted according to GLP. No test guidelines were cited.

Study: This dose range-finding test was conducted to assess the effects of H 321 (methiocarb) on embryonic and foetal development in the rabbit to establish suitable dose levels for a subsequent embryotoxicity study. Methiocarb (Batch No: 234002640, purity 99.4 – 99.6%, stability in the vehicle 6 h) was applied dermally to shaved, occluded skin of the backs of mated rabbits (Chinchilla, CHbb:CH, Hybrids, SPF Quality., Dr. Karl Thomae GmbH, Birkendorferstrasse 65, D-W-7960 Biberach/Riss, 13-18 wks old, 3.1-4.2 kg bw, 5/dose) at doses of 0, 250, 500 or 750 mg/kg bw/day in double distilled water with 1% Cremophor vehicle, 6 h/day from days 6 through 18 post coitum. The animals were acclimatised to the test conditions for a minimum of 7 days. Subsequently, they were housed with males until copulation was observed and the day of mating was designated to be the day 0 of gestation. The test formulation was prepared fresh daily before application using a homogeniser and during the application homogeneity was maintained using a magnetic stirrer. The concentration, homogeneity and stability of the test formulation were determined once in samples taken immediately after preparation and 6 h later using a method (HPLC) supplied by the sponsor (results provided).

The test compound was applied evenly on the dorsal surface covering 10% of the total body area at a dose volume of 1.25 mL/kg bw and the site was covered with an occlusive dressing. The dressing was placed in position by wrapping it around the abdomen and using an elastic adhesive bandage. The control animals were treated similarly with the vehicle alone. Six hours after application the dressing was removed and the site was rinsed with lukewarm water. All test animals were housed individually in stainless steel cages equipped with an automatic cleaning system under standard laboratory conditions and pelleted standard rabbit

maintenance diet (Kliba 341, Kliba, Klingentalmuehle AG, CH4303 Kaiseraugst/Switzerland) and tap water was provided *ad libitum* (results of analyses for chemical and bacteriological contaminants were provided).

Observations: The following observations were made: clinical signs (twice daily, types and the time of observation not stated), local skin reactions for erythema, eschar and oedema formation (using Draize scoring system, OECD guidelines for testing chemicals of 1981 and EPA guidelines for primary dermal irritation of 1984), necrosis, scaling, lesions, scabbing shedding of damaged skin and mortalities, food consumption and daily body weight until sacrifice on day 28 of gestation by cervical dislocation. Foetuses were removed by Caesarean section.

At necropsy, gross macroscopic examination was conducted on the uterus, uterine contents, internal organs and position of the foetuses in the uterus and the number of corpora lutea were noted. Further, the weights of the gravid uterus (with live foetuses) and individual foetus, possible haemorrhagic sites of the uterus and external foetal abnormalities were recorded. After dissection, the internal organs of the foetuses were examined and the sex of the foetuses was determined. The degree of ossification of the cranium was assessed by removing the skin. As appropriate the study data were analysed using the following statistical tests: Dunnett many-one t-test, Steel test (many-one rank test) and Fisher's exact test.

Findings:
Maternal: All does in experimental groups were found to carry live foetuses except one animal in the 250 mg/kg bw/day dose group. No mortalities, clinical signs or skin reactions related to treatment were noted. The mean food consumption (presented in the following table) was markedly reduced in all groups during the treatment period compared to the control group.

Food consumption of does post coitum (data are means of 3-5 observations)

Duration (days)	Mean Food Consumption (g/animal/day)			
	Dose (mg/kg bw/day)			
	Control	250	500	750
0-6	208	208	215	199
6-11	210	122**	170	66**
11-15	179	120	155	96**
15-19	175	141	153	110*
19-24	182	185	180	146
24-28	139	146	163	184
Overall mean	182	154	173	133

**Significantly different from control ($p<0.01$).

*Significantly different from control ($p<0.05$).

Reductions in food consumption achieved statistical significance at 250 and 750 mg/kg bw/day from days 6 through 11, and at 750 mg/kg bw/day during days 11 through 15 and again during days 15 through 19. When overall mean values were considered, the does in the highest dose group consumed 27% less food compared to the control animals. The reduction in food consumption however, did not show any dose relationship but is considered to be treatment-related due to its temporal relationship with treatment.

Statistically insignificant moderate loss in body weight was observed at all doses from days 6 through 12 post coitum. This was considered to be test compound related by the study authors

(presented in the following Table). The trend was similar to that observed for food consumption, being most marked at 250 and 750 mg/kg bw/day.

Group mean body weights (g) of does on days 6, 12 and 28 and group mean body weight gain post coitum

Day	Dose (mg/kg bw/day)			
	Control	**250***	**500**	**750**
Group mean body weights				
6	3860	3702	4054	3949
12	3834	3489	3909	3651
28	4101	3939	4214	3941
Group mean body weight gain on day 28 post coitum				
Corrected weight gain (g)	-264	-332	-417	-453
Percent weight gain	-6.8	-8.9	-10.2	-11.2

*One animal excluded from the group being non-pregnant.

Consequently, the mean body weight gain over the treatment period was reduced in all groups. The percent reduction in mean body weight gain in the 750 mg/kg bw/day dose group showed statistical significance from day 7 through 21 and again on day 23 post coitum either at $p<0.01$ or $p<0.05$ level. Macroscopic examination at necropsy on day 28 did not reveal any abnormalities in dams. No test compound related effects were noticed in any of the maternal reproduction parameters studied compared to the control group.

Foetal findings: Dose related reductions in foetal body weight were noted in all dose groups and the data are presented in the following table.

Group mean body weights (g) of live foetuses

Dose (mg/kg bw/day)	Males & Females	Males	Females
Control	35.6 (42)	37.6 (25)	32.1 (17)
250	32.7 (46)	32.7 (25)	32.8 (21)
500	31.3 (61)	30.5 (33)	31.7 (28)
750	28.5 (48)	28.4 (29)	28.9 (19)

Values in parenthesis represent the total number of foetuses in the group.

The study authors stated that the reduction in mean foetal body weights attained statistical significance at 250 (males only) and at 500 mg/kg bw/day on a foetus basis and at 750 mg/kg bw/day on both a foetus and litter basis, but in both cases the level of significance was not stated. Further, when group mean values were considered, a biologically significant reduction in mean group foetal body weights was noticed in both combined males and females by 20% and in males by 24% at 750 mg/kg bw/day. The reductions in the same body weight groups at 500 mg/kg bw/day were 12% and 19% respectively. No test compound related effect on foetal sex ratio was noticed.

One foetus at 500 mg/kg bw/day, and all foetuses of one doe at 750 mg/kg bw/day were of less than 19 g body weight. This observation correlated with the reduced mean foetal body weights that were noted previously. At visceral examination, arthrogryposis was noted in one foetus at 750 mg/kg bw/day, and rudimentary diaphragm and hemidiaphragm were noted in one control foetus and one at 500 mg/kg bw/day respectively. No information on skeletal abnormalities was provided.

Conclusions: Based on the effects seen on maternal food consumption, body weight gain and foetal findings, dose levels of 10, 50, and 250 mg/kg bw/day were chosen for the main embryotoxicity study.

Dotti A & Biedermann K (1992) Embryotoxicity study (including teratogenicity) with H 321 (c. n. Methiocarb) in the rabbit (dermal application). Project no. 297900. Labs: RCC Research and Consulting Company Ltd. and RCC UMWELTCHEMIE AG, PO Box. CH 4452, Itingen, Switzerland. Sponsor: Bayer AG, Institute of Toxicology, Landwirtschaft, Friedrich-Ebert-Strasse 217-333, D-W-5600, Wuppertal-Elberfeld, Germany. Study duration: July 22, 1991 to August 28, 1991. Report No. R 5627. Report date: August 06, 1992.

GLP, quality assured study. Conducted in compliance with US EPA guideline 83-3 of 1989 and OECD guideline TG 414 of 1981.

Study: This study was conducted to assess the effects of H 321 on embryonic and foetal development when applied dermally to mated (1:1) female rabbits. H 321 (methiocarb, batch no: 234002640, purity 99.4 – 99.6%, stability in the vehicle at least 6 h, appearance: white beige powder) was applied dermally to shaved, occluded skin of the backs of rabbits (Chinchilla, CHbb:CH, Hybrids, SPF quality, Dr. Karl Thomae GmbH, Birkendorferstrasse 65, D-W-7960 Biberach/Riss, 19-29 weeks old, 3.1-4.9 kg bw, 16/dose) at 0, 10, 50 or 250 mg/kg bw/day (based on dose range finding study no. 297898) in double distilled water with 1% Cremophor, 6 h/day from days 6 through 18 post coitum. The skin area involved was equivalent to about 10% of the total body surface. The test substance was applied evenly at a dose volume of 1.25 mL/kg bw and the application site was covered with a dressing. The dressing was then wrapped around the abdomen and held in place with an elastic bandage. Individual dose volumes were calculated according to the animal's most recent body weight. The test formulation was prepared fresh daily before application using a homogeniser and during the application the homogeneity was maintained using a magnetic stirrer. The concentration, homogeneity and stability of the test formulation were determined once in samples taken immediately after preparation and 6 h later using a method (HPLC) supplied by the sponsor. The animals were acclimatised to the test conditions for a minimum of 7 days prior to mating and the day of mating was designated as day 0 post coitum.

The control animals were treated similarly with the vehicle alone. Six hours after application the dressing was removed and the site was rinsed with lukewarm water. All animals were housed individually in stainless steel cages equipped with an automatic cleaning system under standard laboratory conditions and offered pelleted standard rabbit maintenance diet (Kliba 341, Kliba, Klingentalmuehle AG, CH4303 Kaiseraugst/Switzerland) and tap water *ad libitum*.

Observations: The following observations were made: clinical signs (twice daily), local skin reactions (prior to application and at the end of exposure period) for erythema, eschar and oedema formation (using Draize scoring system, OECD guidelines for testing chemicals of 1981 and EPA guidelines for primary dermal irritation of 1984), necrosis, scaling, lesions, scabbing shedding of damaged skin and mortalities (twice daily), mean daily food consumption and daily body weight until sacrifice on day 28 of gestation by cervical dislocation. The following reproduction parameters were determined: number of corpora lutea, foetal resorptions, pre-implantation loss, post-implantation loss, live and dead foetuses, runts and foetal sex ratio. Foetuses were removed by Caesarean section.

At necropsy, gross macroscopic examination was conducted on all internal organs with special emphasis on the uterus, uterine contents, and position of the foetuses in the uterus.

Further, the weights of the gravid uteri (with live foetuses) and individual foetuses were recorded (mean foetal weight/group and per litter basis). The foetuses were killed by s.c. injection of pentabarbitone sodium and gross external abnormalities were examined. Subsequently, the foetuses were dissected, internal organs were examined and the sex was determined. The degree of ossification of the cranium was assessed by removing the skin. The heads of 50% of the foetuses were separated from the trunks, fixed in a solution of trichloroacetic acid and formaldehyde, and were then serially sectioned and examined. Following examination, the sections were preserved in a solution of ethyl alcohol and glycerine. The trunks of those foetuses and the remaining 50% of the whole foetuses were placed in a solution of potassium hydroxide for evisceration and subsequently stained with alizarin red S for examination of skeletal abnormalities. As appropriate the study data were analysed using the following statistical tests: univariate one-way ANOVA, Dunnett many-one t-test, Steel test (many-one rank test) and Fisher's exact test.

Findings:

Maternal: One doe at 250 mg/kg bw died on day 16 post coitum, this death was not treatment-related (broken femur).. Macroscopic examination at necropsy did not reveal any abnormalities in this animal. There were no further mortalities or test substance-related clinical signs or skin irritancy in any of the test animals. The mean food consumption was depressed at 250 mg/kg bw during days 6-11, 11-15 and 15-19 post coitum by about 6%, 19.5% and 7.3% respectively in comparison to the controls. The depression in mean food consumption observed during days 11 through 16 was considered to be test compound related by the study authors. When overall mean values were considered, the does at 250 mg/kg bw consumed about 4.5% less food compared to the controls.

About 2-4% body weight loss was noticed in all groups during the first two days of dosing. However, the weight loss was more distinct at 250 mg/kg during days 6 through 22 post coitum (about 1-3%), being significant on days 13 and 16 ($p \leq 0.01$). The depression in body weight was correlated with the reduction in food consumption noted at this dose during the same study period. The mean body weights of the does in other groups were unaffected by treatment and the corrected body weight gain post coitum in all treatment groups were comparable to that of the controls.

Amongst the reproduction data, a two-fold increase in post-implantation loss was observed at 250 mg/kg bw in comparison to the controls. This finding was mainly attributable to one foetal and 5 embryonic resorptions in one doe, and hence was not considered to be test compound related. Further, 1.8-fold increase in pre-implantation loss was seen at 50 mg/kg bw compared to the controls but was not test compound related. No inter-group differences in other reproductive parameters were observed.

Foetal: No methiocarb-related effect on foetal sex ratio was observed. The mean foetal body weights were depressed by about 4% at 250 mg/kg bw compared to the concurrent controls. The incidence of runts was 1/154, 3/175, 1/150 and 3/154 for the control, 10, 50 and 250 mg/kg bw respectively showing no evidence of a dose related effect. Arthrogryposis was observed in two foetuses, one each at 10 and 250 mg/kg bw. Hemidiaphragm was seen in two control foetuses and agenesia of the left kidney and ureter was noted in one foetus at 250 mg/kg bw, all of which were considered to be incidental and not biologically significant. No other visceral abnormalities were observed. However, the data on skeletal anomalies showed an increased incidence of foetuses with incompletely ossified or non-ossified phalangeal nuclei in the limbs. All values recorded were within the historic data range (see Table below). The data on forelimb phalanges did not show any dose-relationship, while the data on foetal hind limb ossification displayed a dose response relationship, although the

magnitude of the response was limited; ie. a 25-fold increase in dose only resulted in an approximately 2-fold increase in incidence to about 16-21%. Overall, the data suggested a possible limited retardation of skeletal development in treated animals compared to the controls, although all reported values lay well within the normal range of historical control data. The finding may correlate with slightly reduced mean foetal body weight at 250 mg/kg bw/day.

The data on the incidence of selected incomplete plus non-ossified phalangeal nuclei in limbs of treated rabbits are given in the following Table.

Selected incidences of incomplete plus non-ossified phalangeal nuclei in limbs (per foetus basis) of methiocarb treated rabbits

Observation	Percent incidence				
	Dose (mg/kg bw/day)				Historical range
	Control	10	50	250	
Number of foetuses examined	154	175	150	154	
Left forelimb					
Metacarpalia 1	37	41	51*	50*	20-100
Digit 1 proximal phalanx	8	21**	29**	26**	0.8-44
Digit 2 medial phalanx	27	45**	48**	40*	0-85
Digit 3 medial phalanx	13	23*	32**	26**	0-90
Right forelimb					
Metacarpalia 1	36	42	51**	51**	0-85
Digit 1 proximal phalanx	8	22**	29**	28**	0-76
Digit 2 medial phalanx	24	40**	44**	37**	0-84
Digit 3 medial phalanx	14	22*	30**	28**	0-87
Left hind limb					
Toe 1 medial phalanx	5	10*	15**	21**	0-82
Toe 2 medial phalanx	3	9*	13**	17**	0-56
Toe 3 medial phalanx	5	10	16**	19**	0-76
Right hind limb					
Toe 1 medial phalanx	5	10	15**	19**	0-76
Toe 2 medial phalanx	3	9*	13**	16**	0-70
Toe 3 medial phalanx	5	10	14**	17**	0-75

*Significantly different from corresponding controls ($p \leq 0.05$, Fisher's exact test).

** Significantly different from corresponding controls ($p \leq 0.01$, Fisher's exact test).

When retarded ossification was examined on a per litter basis, there was a significant ($p \leq 0.05$) effect at 250 mg/kg bw/day (see Table below), which was confined to the hind limbs. The study authors considered this to be related to treatment, as it was correlated with reduced mean foetal body weight in this group.

The incidences of incomplete plus non-ossified phalangeal nuclei in hind limbs (per litter basis) of methiocarb treated rabbits

Observation	Percent incidence				
	Dose (mg/kg bw/day)				Historical range
	Control	10	50	250	
Number of litters examined	14	16	15	14	
Left hind limb					

Toe 2 proximal phalanx	0	13	0	29*	0-25
Toe 2 medial phalanx	21	56	40	64*	0-100
Toe 3 proximal phalanx	0	13	0	29*	0-25
Toe 4 proximal phalanx	0	19	7	29*	0-44
Right hind limb					
Toe 1 proximal phalanx	0	13	0	29*	0-25
Toe 2 proximal phalanx	0	13	0	29*	0-25
Toe 2 medial phalanx	21	56	40	64*	0-100
Toe 3 proximal phalanx	0	13	0	29*	0-25
Toe 4 proximal phalanx	0	19	7	29*	0-100

*Significantly different from corresponding controls ($p \leq 0.05$, Fisher's exact test).

Conclusions: Under the conditions of the study and based on the information provided, there was no evidence of teratogenicity of methiocarb in rabbits. However, based upon reduced food consumption in does at 250 mg/kg bw/day and the weight loss during days 6 through 22 post coitum the maternotoxicity NOEL was established at 50 mg/kg bw/day.

Some statistically significant increases in the incidence of incomplete or non-ossification of phalangeal nuclei seen at 10 and 50 mg/kg bw/day on a per foetus basis was not noticeable when the data were examined on a per litter basis. Hence, the effects seen in phalangeal nuclei at the 2 lower dose levels were considered to be of limited significance. However, the incidence of some statistically significant increases in incompletely ossified hind limb phalangeal nuclei at 250 mg/kg bw/day appear to lie outside the historical data range, suggesting a slight retardation of the ossification process which could be attributed to the test substance.

Because of reduced mean foetal body weight and retarded ossification of hind limb phalanges seen at 250 mg/kg bw, the foetotoxicity NOEL was established at 50 mg/kg bw/day.

9 GENOTOXICITY STUDIES

A summary of submitted and published findings of genotoxicity studies with methiocarb is shown in the Table below.

Results of assays for the genotoxicity of methiocarb

Assay	Bacterial strain or cell type	Concentration/ Dose	Metabolic activation	Results	Reference
Gene mutation					
S. typhimurium Reverse mutation	TA 98 TA 100 TA 1535 TA 1537	4 – 2500 µg/plate DMSO vehicle	+, -	-, -	Herbold (1978)
S. typhimurium Reverse mutation	TA 1535 TA 100 TA 1537 TA 98	20 to 12500 µg/plate	+, -	-, -	Herbold (1986)
CHO-HGPRT Gene mutation	CHO-K1-BH_4	2.5 to 60 µg/mL (with activation) and 1.25 to 30 µg/mL (without activation)	+, -	-, -	Lehn (1989)
Micronucleus formation (marrow cells)	Mouse (NMRI)	5 – 20 mg/kg bw, PO Cremophor EL emulsion		-	Herbold (1979a)

Dominant lethal mutation	Mouse (NMRI)	6 mg/kg bw, PO	-	Herbold (1979b)

Results (+, positive; -, negative) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Assay	Species	Dose	Metabolic activation	Result	Reference
Chromosomal effect assays					
Sister Chromatid Exchange	Chinese Hamster Ovary cells	4.0 – 40.0 µg/mL with activation 2.0 – 20.0 µg/mL without activation	+, -	-, -	Putman (1986)
Chromosome Aberration	Chinese Hamster Ovary cells	9.94 –100 µg/mL and 98.4 – 508 µg/mL with activation, 4.92 –50.8 µg/mL without activation	+, -	+, +	Murli (1990)
Micronucleus formation (marrow cells)	Mouse (NMRI)	5-20 mg/kg bw, po, Cremophor EL emulsion		-	Herbold (1979a)
Other assays					
E.coli polA$^-$ DNA damage	W 3110 (*pol*A$^+$) K 12p, 3478 (*pol*A$^-$)	625 to 5000 µg/plate	+, -	--	Herbold (1983)
Unscheduled DNA Synthesis	Rat primary hepatocytes	0.1 – 100 µg/mL		-	Curren (1988)

9.1 Gene Mutation

Herbold B (1978) H 321 (Active ingredient of Mesurol) Salmonella/Microsome test for determination of point mutations. Study No. H 321/001. Lab: Bayer AG, Institute of Toxicology, Wt.-Elberfeld. Sponsor: Bayer AG, Germany. Study duration: not stated. Report date: December 6, 1978.

Pre GLP, non quality assured study. No test guidelines were cited. Mutagenicity was investigated using the Ames test.

Study: H 321 (methiocarb, purity 98.5%, a composite sample) in DMSO (Dimethylsulfoxide) did not increase the number of histidine revertants in *S. typhimurium* strains TA 1535, TA 1537, TA 100 and TA 98 at 4, 20, 100, 500 or 2500 µg/plate, in the presence or absence of hepatic S9 fraction derived from Aroclor 1254 treated adult male SD rats (200-300 g bw). A reproducible dose related increase in the number of mutations to a level double that of the negative control, obtained with at least one strain, was considered as a positive result. Doses up to and including 500 µg/plate of H 321 did not produce any bacteriotoxic effects. Solvent and positive controls (endoxan at 725 µg/plate and trypaflavin in DMSO at 250 µg/plate) gave the expected results.
Findings and Conclusions: In the Salmonella/microsome assay, no indication of mutagenic effect of methiocarb was seen in the tester strains at doses up to and including 2500 µg/plate.

Herbold B (1986) H 321: c.n. mercaptodimethur Salmonella/Microsome test to evaluate for point mutagenic effect. Study No. T 7019638. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld. Sponsor: Bayer AG, Germany. Study duration: May to June, 1985. Report No. 14205. Report date: January 10, 1986.

Quality assured study conforms to the OECD GLP principles. No test guidelines were cited. Mutagenicity was investigated using the Ames test.

Study: H 321 technical (methiocarb, purity 98.4%, batch 234 402 701) in DMSO did not increase the number of histidine revertants in *S. typhimurium* LT2 mutant strains TA 1535, TA 100, TA 1537 and TA 98 over a concentration range of 20 to 12500 µg/plate, in the absence or presence of S9 fraction from Aroclor 1254 treated unfasted SD rats (6 male rats of 200-300 g bw). Solvent (DMSO) and positive controls sodium azide (10 µg/plate for TA 1535), nitrofurantoin (0.2 µg/plate for TA 100), 4-nitro-o-phenylene diamine (0.5 µg/plate for TA 1537 and TA 98) without S9 fraction and 2-aminoanthracene (3.0 µg/plate) with S9 fraction produced the expected number of revertants. Bacteriotoxic effects were seen at 500 µg/plate and above, both with and without S9 fraction.

Findings and Conclusions: No indication of mutagenic activity of methiocarb in bacterial cells was observed at doses up to and including 250 µg/plate.

Lehn H (1989) H 321: c.n. Methiocarb. Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro. Study No. T9030781. Lab: Institute of Toxicology for Industrial Chemicals, Fachbereich Toxicology, Bayer AG, Friedrich-Ebert-Stra®e, 217-333, D-5600 Wuppertal, FRG. Sponsor: Bayer AG, Germany. Study duration: February 16 to April 19, 1989. Report No. 18280. Report date: August 15, 1989.

Quality assured study conforms to the OECD and FIFRA (40 CFR part 160) GLP principles. No test guidelines were cited. The assay method of Myhr and DiPaolo (1978) was used.

Study: Aroclor 1254 induced S9 fraction derived from SD male rats was used in the study. Statistical significance (increases in mutant frequency) between the treated and control cell cultures was tested using the Poisson heterogeneity test at the $p \leq 0.05$ level. A preliminary cytotoxicity study was conducted over a concentration range of 0.1 to 80 µg/mL (without S9 fraction) and 1.0 to 250 µg/mL (with S9 fraction). H 321 induced concentration related cytotoxic effects (exposure time 5 h) were seen in relative population growth (cumulative growth of the treated cell populations relative to the vehicle control over the expression period and prior to mutant selection) and cloning efficiency (100 x average number of viable colonies per dish/200), under both treatment conditions. The concentration ranges for the main study were chosen on the basis of 0 to 90% reduction in colony forming ability.

Findings: H 321 (methiocarb, purity 99.3%, batch 234702660) in DMSO did not increase the mutant frequency at HGPRT locus in Chinese hamster ovary (CHO) cell cultures (CHO-K1-BH_4, Oak Ridge national Laboratory, Oak Ridge, Tennessee, USA) following treatment over concentration ranges of 1.25 to 30 µg/mL (in the absence of S9 fraction) and 2.5 to 60 µg/mL (in the presence of S9 fraction). No statistically significant increase in mutant frequency (the total number of mutant colonies/number of cells seeded) above that of the negative controls ($1.3 – 2.7 \times 10^{-6}$ mutant frequency) was observed in H 321 treated cultures following 5 h of exposure. Positive controls ethylmethanesulfonate at 0.9 mg/mL (without S9 fraction) and dimethylbenzanthracene at 20 µg/mL (with S9 fraction) gave the expected increase in mutagenic activity (historical data for the negative, vehicle and positive controls were provided).

Conclusions: Methiocarb was considered to be non-mutagenic in the CHO-HGPRT forward mutation assay, both in the absence and presence of metabolic activation.

Herbold B (1979) H 321: Dominant lethal study on male mouse to test for mutagenic effects. Study No. H321/002. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Germany. Study duration: not stated. Report No. 8395. Report date: May 23, 1979.

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

Study: In a dominant lethal assay, 50 male NMRI strain mice (50/group, S. Ivanovas GmbH, Kissleg/Allgau, 31-43 g and 28-33 g initial bw for males and females respectively, 8-12 weeks old, 593 females in the H 321 (methiocarb) group and 598 females in the control group) received a single oral dose of methiocarb (purity 98.5%, a mixed sample from different batches) in 0.5% Cremophor EL emulsion at 6 mg/kg bw in a volume of 10 mg/kg bw. The males in the control group received an equivalent volume of the vehicle. The females were untreated. In a preliminary range finding study, female mice (5/group) received a single oral dose of methiocarb at 10, 12.5, 25 and 50 mg/kg bw in which 10 mg/kg bw was found to be the dose tolerated without inducing any other symptoms besides heavy drowsiness of brief duration (unspecified). Starting from the day of test compound administration, a series of 12 matings were performed by placing virgin females with the treated males (1:1) for 4-days, the procedure which was repeated for each of the subsequent matings with virgin females for a total of 48 days. The females were not inspected for the presence of vaginal plug. All animals were (housed singly after mating) held under conventional laboratory conditions and provided with pelleted ®Altromin feed and water *ad libitum*.

Observations: After an interval of 14 days counted from midway through a mating period, the uterus of each female was examined (method of sacrifice unspecified) for pre-implantation and post-implantation losses. The study parameters determined were fertilisation quota, the total implants, viable implants, dead implants (sum of the deciduomata, the resorptions and dead embryos) and the corpora lutea. Statistical significance between the control and treatment groups was tested using either the 2-way ANOVA, Dunnet test or Kolmogorov-Smirnov non-parametric test where appropriate ($p \leq 0.05$).

Findings: There were three treatment unrelated deaths (2 females and 1 male). Mild drowsiness persisting for up to one hour post treatment was noted in treated males. The appearance and motor activity of the animals were unaffected by treatment. Statistical analysis of the study data did not reveal any inter-group differences in any of the study parameters.

Conclusions: Under the conditions of the study, methiocarb did not produce any mutagenic effect in male mice in a dominant lethal test at 6 mg/kg bw.

9.2 Chromosomal Effects

Putman DL (1986) Sister chromatid exchange assay in Chinese hamster ovary (CHO) cells: Test article Mesurol technical. Study No. T4522.334. Lab: Microbiological Associates, 5221 River Road, Bethesda, MD USA. Sponsor: Mobay Chemical Corporation, 17745 South Metcalf Avenue, Stilwell, Kansas 66085, USA. Study duration: February 25 to September 25, 1986. Report No. 790. Report date: September 25, 1986.

Quality assured study performed in accordance with the US EPA, FDA GLP principles and OECD Guidelines.

Study: Mesurol technical (methiocarb, batch # 0030058, purity not stated) in DMSO was tested in a sister chromatid exchange (SCE) assay using Chinese hamster ovary (CHO) cells (American Type Culture Collection, Rockville, MD, USA) both in the absence and presence of S9 fraction derived from Aroclor 1254 treated adult male SD rats (200-250 g bw). The cells were treated for 26-32 h over a concentration ranges of 2.0 to 20.0 µg/mL without S9 activation system and 4.0 to 40 µg/mL for 2 h with S9 activation (24-30 h recovery period). Preliminary cytotoxicity testing of methiocarb in DMSO was conducted by treating CHO cell cultures for 2 h in the presence and 4 h in the absence of S9 fraction over a concentration range of 0.1 to 1000 µg/mL. Based on cell survival and cell cycle delay seen at 100 µg/mL (35% relative cell growth both in the presence and absence of S9 fraction), the above concentration ranges were selected.

Findings: A significant increase in the mean SCEs/cell (12.98 ± 4.51 at $p \leq 0.05$) compared to the solvent control (11.2 ± 3.92 SCEs/cell) was noted in cells at 2 µg/mL in the absence of S9 fraction compared to the solvent controls. But the small increase noted was not considered as biologically significant since there was no response at any other dose. The positive controls triethylenemelamine (TEM) at 0.025 µg/mL in the non-activated assay and cyclophosphamide (CP) at 2.5 µg/mL in the activated assay gave significantly elevated results of 35.16 ± 11.97 and 25.06 ± 7.00 SCE/cell respectively ($p \leq 0.01$) compared to the negative and solvent controls (11.48 ± 4.60 and 11.20 ± 3.92 SCE/cell respectively).

Conclusions: Under the conditions of the study, methiocarb technical did not cause an increase in the frequency of SCE in CHO cells at any of the dose levels tested either in the presence or absence of exogenous metabolic activation.

Murli H (1990) Mutagenicity test on H 321 in an in vitro cytogenetic assay measuring chromosomal aberration frequencies in Chinese hamster ovary (CHO) cells with a confirmatory assay. Project No. T 6032551. Lab: Hazleton Laboratories America, Inc., 5516 Nicholson Lane, Kensington, MD 20895, USA. Sponsor: Bayer AG, Fachbereich Toxikologie, Postfach 10 17 09, Friedrich-Ebert-Strasse 217-333, 5600 Wuppertal 1, West Germany. Study duration: December 28, 1989 to January 26, 1990. Report No. R 4980. Report date: March 8, 1990.

Quality assured study conducted in compliance with the US EPA FIFRA GLP principles. No test guidelines were cited.

Study: Chinese hamster ovary cells (CHO-WBL, obtained from Dr. S. Wolff, University of California, San Francisco, USA) were maintained in McCoy's 5a culture medium. In preliminary cytotoxicity testing with methiocarb (purity 99.4%, batch 234702660) over a concentration range of 0.0166 to 497 µg/mL, complete cytotoxicity was observed in test cultures treated with 49.7, 166 and 497 µg/mL, severe cell cycle delay at 16.6 µg/mL, with some cell cycle delay persisting at 4.97 µg/mL in the absence of metabolic activation by S9 fraction derived from the livers of Aroclor 1254 treated male SD rats. The CHO cell were incubated with the test substance for 2 h and 20-25 h for activated and non-activated assays respectively.

Findings: The assay with metabolic activation exhibited complete cytotoxicity at 497 µg/mL and severe cell cycle delay at 166 µg/mL. Subsequent incubation trials were conducted with methiocarb over a concentration range of 4.92 to 50.8 µg/mL (without S9 fraction) using 20 h cell harvests, 9.94 to 100 µg/mL using 10 h cell harvests (with S9 fraction) and 98.4 to 508 µg/mL using 20 h cell harvests with metabolic activation. Statistically significant and dose related increases in cells with chromosomal aberrations were noted at and beyond 10.2 µg/mL in the absence of S9 fraction (9.5% cells with aberrations, $p \leq 0.01$) and at and

beyond 98.4 µg/mL in 20 h cell harvests in the presence of S9 fraction (9.0% cells with aberrations, p≤0.01) compared to the solvent (DMSO, 0-0.5% cells with aberrations) controls in the absence of cytotoxicity. The majority of the chromosomal abnormalities were simple aberrations such as chromatid and chromosome breaks and complex three or four armed configurations. Positive controls mitomycin C at 0.04 and 0.08 µg/mL (for non-activation assay) and cyclophosphamide at 12.5, 17.5, 25.0 and 50.0 µg/mL (for activation assay) gave the expected results.

Conclusions: Methiocarb was considered to be positive for inducing chromosomal aberrations in CHO cell cultures at the dose levels tested under both activated and non-activated assay conditions.

Herbold B (1979) H 321 (Active Ingredient of Mesurol) Micronucleus test for mutagenic effect on mice. Study No. H321/003, Lab: Bayer AG, Institute of Toxicology, Wt.-Elberfeld. Sponsor: Bayer AG, Germany. Study duration: February - May, 1979. Report No. 69093. Report date: June 8, 1979.

Pre GLP, non quality assured study. No test guidelines were cited. The micronucleus test of Schmidt (1975) was used.

Study: H 321 (methiocarb, purity 98.5%, composite batch: 06111978) in 0.5% Cremophor EL emulsion was administered to NMRI strain mice (S. Ivanovos GmbH, Kisslegg/Allgau, 22-37 bw, 8-12 weeks old) by oral gavage twice at 5, 10 (5 mice/sex/dose) and 20 (4 mice/sex) mg/kg bw, at an interval of 24 h. Dose selection was based on the results of a preliminary test in which H 321 at 25 mg/kg bw was administered twice at an interval of 24 h and was seen tolerated by the animals (group size, age, bw or sex not stated) with symptoms including lethargy and piloerection. The animals in the positive control group received two i.p. injections of Adriblastin at 5 mL/kg bw at an interval of 24 h. The mice in the negative control group were dosed similarly with 10 mL/kg bw of 0.5% Cremophor emulsion. The animals were housed under standard laboratory conditions and provided with feed and water *ad libitum*.

Observations: Six hours after the second administration, the animals were sacrificed by decapitation and the femoral marrow was prepared. The number of normochromatic RBCs per 1000 polychromatic RBCs (per animal) in femoral marrow smears were counted and the frequency of the cells containing micronuclei was determined. Statistical significance between the control and treatment groups was evaluated using the Wilcoxon non-parametric ranking test at p≤0.05.

Findings: The animals at 20 mg/kg bw exhibited "severe symptoms of damage" (unspecified) and 5/8 (3 males and 1 male) died (time, clinical signs unspecified). Hence the treatment of this group was discontinued and another group was added at the 5mg/kg bw dose level. The samples of the surviving animals at 20 mg/kg bw were processed. No significant increases in the frequency of micronucleated polychromatic RBCs or changes in the ratio of polychromatic to normochromatic cells compared to the negative controls were observed. The negative and positive controls gave the expected results (1.6 and 20.3 micronucleated cells/1000 polychromatic cells respectively).

Conclusions: Methiocarb was not clastogenic at doses up to and including 2 x 20 mg/kg bw in an *in vivo* somatic mutagenicity assay in mice.

9.3 Other Genotoxic Effects

Herbold B (1983) H321 Mercaptodimethur (The active ingredient of Mesurol) Study of DNA damage using the E.coli polA$^-$ test. Study No. T8007939. Lab: Bayer AG, Institute of Toxicology, Wuppertal, Federal Republic of Germany. Sponsor: Bayer AG, Germany. Study duration: July, 1983. Report No. 11928. Report date: July 13, 1983.

Pre GLP, non-quality assured study. No test guidelines were cited. The test procedure described by Rosenkranz and Leifer (1980) was used.

Study: To assess possible direct DNA action resulting in toxicity of bacterial strains having deficient DNA repair mechanisms, a comparison between the survival of normal (W 3110 *pol*A$^+$) and DNA repair enzyme deficient (K 12p, 3478, *pol*A$^-$) *E.coli* strains in the presence of H 321 (methiocarb, purity 98.6%, batch 234 202 611) in DMSO, chloramphenicol (negative control, 30 µg/plate) and methylmethane sulfonate (MMS: positive control, 10 µL/plate) was performed both with and without the S9 fraction derived from livers of Aroclor 1254 treated adult male SD rats. Methiocarb was tested over a concentration range of 625 to 5000 µg/plate. The plates were incubated for 24 h and the mean diameters of zones of inhibition were measured in 4 plates/dose/strain. A reproducible increase of more than 2 mm in the diameter difference of the zone of inhibition was considered as a positive result. No biologically relevant increase in the diameter difference of the zone inhibition was noticed at any of the dose levels both with and without S9 fraction. The solvent, negative and positive control MMS gave the expected results (-2.5 to -5.4 and 14.6 to 13.7 respectively for without and with S9 fraction compared to the solvent control).

Findings and Conclusions: Under the conditions of the study, methiocarb did not cause any DNA damage in the tested bacterial strains at any of the dose levels used.

Curren RD (1988) Unscheduled DNA synthesis in rat primary hepatocytes: Test article Mesurol, Lot No. 86I004. Study No. T5391.380. Lab: Microbiological Associates, Inc., 5221 River Road, Bethesda MD, USA and Microbiological Associates, 9900 Blackwell Road, Rockville, MD, USA. Sponsor: Mobay Corporation, 17745 South Metcalf, Stilwell, KS 66085, USA. Study duration: March 26, 1987 to February 02, 1988. Report No. 1007. Report date: June 01, 1988.

Quality assured study conducted in compliance with the US EPA, FDA GLP principles and OECD Guidelines. The test procedure described by Williams (1977) was used.

Study: Mesurol (methiocarb, purity 98.8%, batch 86I004) was tested in an *in vitro* unscheduled DNA synthesis test in primary hepatocytes freshly isolated from male and female SD rats (Charles River Laboratories Inc.) over a concentration range of 0.1 to 100 µg/mL (preliminary and duplicate tests; 3 cultures/group in each independent test). Test concentrations were chosen on the basis of a preliminary cytotoxicity test (0.3 to 5000 µg/mL; 2 cultures/group) in which 300 µg/mL was identified as the highest usable concentration. The cells were treated for 18-20 h and the net nuclear grains (NNG) in 25 cells/slide in randomly selected areas were counted. An increase in the mean NNG count by at least 5 counts at any dose level was considered significant. The test compound was judged to be positive if it induced a dose related response and at least one dose produced a significant increase in the average NNG compared to the controls. None of the methiocarb doses caused any significant increase in the mean NNG counts. Negative (DMSO) and positive (DMBA at 3 and 10 µg/mL: NNG= 83-100%) controls gave the expected results.
Findings and Conclusions: Methiocarb over a concentration range of 0.1 to 100 µg/mL was negative in an *in vitro* unscheduled DNA synthesis test in primary rat hepatocytes.

10. SPECIAL STUDIES

10.1 Neurotoxicity

Ives M (1965) Demyelination study in chickens. Report No. 16063, Lab: Wedge's Creek Research Farm Inc., Subsidiary of Industrial Bio-Test Laboratories Inc., Biological Evaluations, Neillsville, WI 54456, USA. Report to: Chemagro Corporation, Study duration: not stated, Report date: April 16, 1965.

Pre GLP, non-quality assured study. A US-FDA study protocol proposed to the Chemagro Corporation was followed.

Study: Groups of 8, two year old hens (breed, source, bw not stated) were fed with diets containing either 0, 200, 400 or 800 ppm (equivalent to approximately 25, 50 and 100 mg/kg bw/day respectively) of Bayer 37344 (methiocarb, source, purity not stated) *ad libitum* daily for 30 days in a study aimed to investigate the possible demyelination properties of the chemical. Average body weights of birds at the commencement and after 30 days exposure period were measured.

Four birds from each group were sacrificed after 30 days exposure to the diets and the remainder were fed with stock diet for a 30-day recovery period. No information on animal housing, diet preparation, feeding conditions was provided. Neither food or water intake were measured nor were haematology or clinical chemistry studies conducted. Nerve tissue was preserved and stained (tissue type, preservation medium, staining procedures were not stated) for histopathological studies.

Findings: All birds survived the experimental period. There were no treatment-related effects on body weight after the 30 day treatment period. No histopathological evidence of myelin degeneration or clinical signs of cholinesterase inhibition were found in any of the treated birds. No further clinical observations or methodological information were provided.

Conclusions: Under the conditions of the study, methiocarb when fed at 200, 400 and 800 ppm in diet for 30 days did not produce neurotoxic effects. However, when age and source of the study, lack of detailed methodology, and limitations of the data are considered, it can be concluded that, the findings of this study are of limited regulatory value.

Thyssen J & Schilde B (1978) H 321 (Mesurol active ingredient) Neurotoxicity studies on hens. Report No. 7637. Lab: Institute of Toxicology, Bayer AG, Wuppertal-Elberfeld, Germany. Sponsor: Bayer AG, Wuppertal-Elberfeld, Germany. Study duration: not stated. Report date: June 20, 1978.

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

Study: H321 (methiocarb, a mixed sample from batches: 234702-602, -607, -609, -610, and -611, purity 98.5%) was administered orally (method unspecified) to White Leghorn hens (Brinkschulte, Seden bei Munster, 20 hens, 1-2 kg bw, 15-20 months old) twice at 380 mg/kg bw [equivalent to the LD_{50} (28 days) dose established by the same authors in a previous study] at an interval of 3 weeks. The second treatment was followed by a 3-week observation period. The birds were kept in an air conditioned house and had access to an open pen with natural ground. The hens were acclimatised for 4 weeks prior to commencement of the study and fed with poultry grain diet (Hoveler) and water *ad libitum*. They were treated with i.m. injections of atropine sulphate formulated in physiological saline (50 mg/kg bw) prior to each treatment. The concentrations of emulsified H 321 [in polyethylene glycol 400 (®Lutrol) vehicle] was adjusted for each animal to receive a volume of 0.25 mL of the formulation per 100 g bw. Five hens were treated with tri-orthocresyl-phosphate (TOCP) emulsified in

groundnut oil (DAB 7), administered orally in a single dose of 375 mg/kg bw (0.5 mL/100g bw volume) as positive controls. No negative control group was used in this study.

Observations: Three weeks after the second treatment ten hens out of 16 survivors exhibiting heaviest acute poisoning symptoms, and the birds in the positive control group were anaesthetised with phenobarbital and the hearts were infused with 10% formalin to fix the nerve tissue. The tissues that were collected and processed for histological examination include: brain (cerebrum and cerebellum), spinal marrow (cervical, thoracic and lumbar) and the sciatic nerve. The fixed tissues (in 4% formaldehyde solution, embedded in Paraplast) were stained with hemalum and eosin and examined (5 sections) for histopathological changes.

Findings: Following the first treatment with methiocarb the birds manifested "light behavioural disorders" (unspecified) of brief duration and lethargy on the first day. Two treated hens died after an unspecified period. Similar "symptoms" were noted after the second treatment following which a further 2 hens died after an unspecified period. No ataxia or paralysis were observed in treated hens, compared to positive controls which displayed delayed neurotoxic symptoms such as unsteady gait, ataxia, and lameness starting from day 7 and progressed towards severe paresis during the remaining post-treatment observation period. No other methodological information including food and water intake and body weight data of hens during the experiment was provided.

No gross necropsy findings were provided. In histopathology, 9 out of 10 examined treated hens showed occasional perivascular round cell infiltration (graded from very minimum to minimum) in one or several of the nerve tissues examined. The significance of this finding is difficult to explain as data from untreated hens were not available. Four out of 5 positive controls showed "minimal" degeneration of individual fibres in the sciatic nerve, vacuolar distension of myelin sheaths, Schwann cell proliferation, presence of eosinophilic particles and occasional perivascular round cell infiltration.

Conclusions: Under the conditions of the study, methiocarb when administered twice orally at an interval of 3 weeks at 380 mg/kg bw to White Leghorn hens did not produce any delayed neurotoxic effects. However, the validity of this finding is reduced due lack of negative control data for detailed comparison.

10.2 Immunotoxicity

Casale GP, Vennerstrom JL, Bavari S & Wang T (1993) Inhibition of Interleukin 2 driven proliferation of mouse CTLL2 cells, by selected carbamate and organophosphate insecticides and congeners of carbaryl. Immunopharm Toxicol 15 (2&3) 199-215.

[Although this publication also presents the data on 7 other insecticides, only the results pertaining to methiocarb are included in this evaluation].

Study & Observations: This *in vitro* study was performed to determine the effects of 8 ChE inhibitory insecticides including methiocarb (4 organophosphates and 4 carbamates) and structural analogues of carbaryl on interleukin 2 (IL2) dependent proliferation of a mouse T cell line. Plates containing CTLL2 cells (American Type Culture Collection, Rockville, MD, USA) in a growth medium supplemented with human recombinant IL2 (Cetus corporation, Emeryville, CA, USA) were incubated in the presence of 100 µL of either 0, 0.5, 5.0 or 50 µM methiocarb (purity 98%, Chem Service Inc. West Chester, P, USA. batch not stated) in 0.2 M acetone (purity >97%) for 16 h. IL2 dependent cell proliferation was evaluated by measuring the ^{3}H-thymidine (1 µCi) uptake. Statistical significance of the results of 4 replicate analyses was assessed using one way ANOVA procedure at $p \leq 0.05$.

Findings & Conclusions: Under the conditions of the study, *in vitro* T cell proliferation was inhibited about 80% by 50 µM methiocarb. No inhibition was noticed at other concentration levels. It would appear that the inhibition was brought about in the absence of metabolic activation. Two other carbamates tested, carbaryl and carbofuran produced 97% and 42% inhibition respectively in T cell proliferation at 50 µM in the claimed absence of cytotoxicity. However, no reference to the cytotoxicity was made for methiocarb. The study authors stated that potency to produce acute cholinergic toxicity by the tested chemicals did not predict potency to inhibit T cell proliferation.

11 HUMAN STUDIES

11.1 Dermal irritation

Kimmerle G (1960) Product Dr Wademeyer H 321 (E 37344) Production No. 2410., Toxicological and Industrial Hygiene Laboratory, Bayer AG. Unpublished in company report dated March 25, 1960.

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

Study & Observations: Cotton wool compresses containing H 321 (methiocarb, E 37344, Production No: 2410, dose, source, purity and the dose not specified) in dry form or moistened with oil (type not specified) or water were applied to the forearm of 8 persons (age, sex not stated) for 8 and 24 h respectively. The compresses were held in position with adhesive bandages.

Findings & Conclusions: In some cases symptoms of irritation were noticeable at the site of application after 8 h. Inflammation and swelling were observed at application sites of all the test persons after the 24 h application period. No further methodological information or clinical observations were provided. Based on the information provided, the test compound was an irritant to the human skin. However, it is not possible to comment on the severity of irritation or influence of the vehicle ("oil") on the skin reaction observed, due to lack of information on its identity. The study is therefore of limited regulatory value.

11. 2 Occupational Exposure

Willems PWJM Geursen-Reitsma AM & van Joost T (1997) Allergic dermatitis due to methiocarb (Mesurol). Contact Dermatitis. 36:270.

This case study reports a dermatological effect of methiocarb (98.2%, 0.5% petrolatum, product or constituents not identified) which has not been previously reported. A 35 year old carnation grower developed acute severe hand eczema who continued his work in spite of this condition. Several topical corticosteroids were used without any therapeutic benefit. Patch tests with European Standard Series were positive to potassium dichromate (++) and wool wax alcohols (++). The hand dermatitis failed to improve when contact with the allergens was removed. Additional patch tests were conducted with 11 other pesticide formulations, but yielded negative results. Only a methiocarb based product (Mesurol, 0.5% petrolatum) produced a positive result (++).

Findings of this study are of limited value since, it is not clear whether the allergic reaction was directly related to the active ingredient or other constituents in the product. Information on previous exposure to the chemical/chemical mixtures or length of exposure was also not provided.

Faul J (1993) Mesurol active ingredient-In-company occupational medical experience. Unpublished letter to Dr. Heimann, PF-A/Consulting, Bldg. 6100, Monheim, Germany. Sponsor: Bayer AG, Germany. Report No. not stated. Report date: April 01, 1993.

Study & Observations: About 250 employees (age and bw ranges or sex unspecified) in two Mesurol (methiocarb) manufacturing plants were subjected to yearly medical examinations including assay of whole blood ChE activity for more than 20 years. While one manufacturing plant produced methiocarb, the second plant formulated it, in addition to a multitude of other unspecified crop protection products. The report stated that, whole blood ChE was assayed at regular four week intervals (method unspecified), but the data on these assessments were not provided. The annual medical examination of workers included: examination of the work and health history, measurement of the height and weight, a detailed clinical examination and laboratory tests to determine blood sedimentation rate (BSR), blood count, urinalysis, AST and ALT levels. An X-ray examination of the thoracic organs was conducted at 2-3 year intervals.

Findings & Conclusions: Under the conditions prevailed in the two manufacturing plants, no adverse health effects related to methiocarb were noted in any of the employees, nor were changes in any of the clinical pathology parameters observed.

REFERENCES

[Figures in square brackets are an Australian identification code and indicate the location of the submitted data. BA=Bayer Australia Ltd.]

Andrews P (1998a) H 321 4 GR 00313/1967 (c.n.: Methiocarb) Study for acute oral toxicity in rats. Study No. T 8062613. BA AG, Toxicology, Friedrich-Ebert-Straße, Wuppertal. Unpublished. [BA; sub: 11792, Report No. 27552, Vol. 1]

Andrews P (1998b) H 321 4 GR 00313/1967 (c.n..: Methiocarb) Study for acute dermal toxicity in rats. Study No. T 6062611. Bayer AG, Toxicology, Friedrich-Ebert-Straße, Wuppertal. Report No. 27584. Unpublished. [BA; sub: 11792, Vol. 1]

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APPENDIX I

METHIOCARB TOXICOLOGY DATA SUBMISSION DETAILS

Sponsor/Provider	Submission Number	Data Details
Bayer Australia Ltd	640	15 volumes (40 studies)
	11792	15 volumes (113 studies)
	11823	4 volumes (10 studies)