AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

AUSTRALIA

CHEMICAL REVIEW PROGRAM

DRAFT REVIEW OF THE MAMMALIAN TOXICOLOGY AND METABOLISM/TOXICOKINETICS OF OMETHOATE

prepared by

Office of Chemical Safety
Office of Health Protection

of the

Department of Health and Ageing

Canberra

May 2007
Revised February 2010
Revised November 2011

TABLE OF CONTENTS

ABBREVIATIONS **4**

EXECUTIVE SUMMARY **6**

TOXICOLOGY HAZARD PROFILE **7**

SUMMARY TOXICOLOGY REPORT **9**

INTRODUCTION 9
METABOLISM AND TOXICOKINETICS 9
ACUTE STUDIES 10
SHORT-TERM REPEAT-DOSE STUDIES 11
CHRONIC STUDIES 11
REPRODUCTION STUDIES 13
DEVELOPMENTAL STUDIES 14
GENOTOXICITY STUDIES 15
NEUROTOXICITY STUDIES 15

HAZARD ASSESSMENT **17**

DISCUSSION 17
IMPURITIES 22

DOSE LEVELS RELEVANT FOR RISK ASSESSMENT **25**

HUMAN EXPOSURE **27**

CONSIDERATION OF PUBLIC HEALTH STANDARDS **27**

APPROVAL STATUS 27
IMPURITY LIMITS 27
ADI/ARFD 28
DRINKING WATER QUALITY GUIDELINES 29
POISONS SCHEDULING 29
FIRST-AID INSTRUCTIONS 29
SAFETY DIRECTIONS 30

RECOMMENDATIONS **34**

MAIN TOXICOLOGY REPORT **38**

1. INTRODUCTION **38**

1.1 HISTORY OF PUBLIC HEALTH CONSIDERATIONS IN AUSTRALIA 38
1.2 INTERNATIONAL TOXICOLOGY ASSESSMENTS 40
1.3 CHEMISTRY – TECHNICAL ACTIVE 40
1.4 PRODUCTS 42

2. METABOLISM AND TOXICOKINETICS **43**

2.1 RATS 43

3. ACUTE STUDIES **46**

3.1 TECHNICAL GRADE ACTIVE CONSTITUENT 46
3.1.1 Median Lethal Dose Studies 46
3.1.2 Skin and Eye Irritation Study 50
3.1.4 Skin Sensitisation Study 51
3.1.5 Potentiation and Antidote Study 52
3.2 FORMULATIONS 52
3.2.1 E 6876 1000 SL 00671/0360 (1000 g/L omethoate) 53
3.2.2 E 6876 500 SL 00671/0360 (500 g/L omethoate) 53
3.2.3 E 6876 5 SL 00671/0360 (50 g/L omethoate) 54

4. SHORT-TERM REPEAT-DOSE STUDIES **55**

4.1. ORAL ADMINISTRATION 55
4.2. DERMAL APPLICATION 56
4.2. INHALATION ADMINISTRATION 57

5. SUBCHRONIC STUDIES 59

5.1. ORAL ADMINISTRATION 59
5.1.1 Rats 59

6. CHRONIC STUDIES 65

6.1 ORAL ADMINISTRATION 65
6.1.1 Mice 65
6.1.2 Rats 71

7. REPRODUCTION STUDIES 77

7.1 RATS 77

8. DEVELOPMENTAL STUDIES 85

8.1 RATS 85
8.2 RABBITS 86

9. GENOTOXICITY STUDIES 89

10. NEUROTOXICITY STUDIES 100

10.1 RATS 100
10.2 HENS 103

REFERENCES 106

APPENDICES 115

APPENDIX I: AUSTRALIAN REGISTERED PRODUCTS CONTAINING OMETHOATE AT THE BEGINNING OF THIS ASSESSMENT 115
APPENDIX II: LIST OF CLINICAL CHEMISTRY, HAEMATOLOGY & URINALYSIS PARAMETERS 116
APPENDIX III: ORGANS FOR WEIGHT DETERMINATION AND HISTOPATHOLOGICAL EXAMINATION 117
APPENDIX IV: REPRODUCTIVE AND DEVELOPMENTAL INDICES 118

ABBREVIATIONS

Time

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

Weight

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

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Dosing

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

Volume

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)
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
ChE	Cholinesterase
CPK	Creatine phosphatase (phosphokinase)
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LH	Luteinising hormone
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NTE	Neurotoxic target esterase
PCV	Packed cell volume (Haematocrit)
PT	Prothrombin time
RBC	Red blood cell/erythrocyte
T_3	Triiodothyroxine
T_4	Thyroxine
TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

Anatomy

CNS	Central nervous system
GIT	Gastro-intestinal tract

Chemistry

DMSO	Dimethyl sulfoxide
GC	Gas chromatography
GLC	Gas liquid chromatography
HPLC	High pressure liquid chromatography
MS	Mass spectrometry
RIA	Radioimmunoassay
TLC	Thin layer chromatography

Terminology

ADI	Acceptable daily intake
ARfD	Acute reference dose
FAISD	First aid instructions and safety directions
GLP	Good laboratory practice
LOEL	Lowest observed effect level
MRL	Maximum residue limit or level
NOEL	No observed effect level
NOAEL	No observed adverse effect level
OP	Organophosphorous pesticide
PPE	Personal protective equipment
TDI	Tolerable daily intake

Organisations & publications

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

EXECUTIVE SUMMARY

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

Omethoate has a long history of use in Australia as a direct and systemic insecticide and acaricide in both commercial and home garden situations. Presently, it is the active constituent in 5 registered products. Omethoate is an OP pesticide, and along with other pesticides of this class, its mode of action is through inhibition of cholinesterase activity. Omethoate and its products are being reviewed because of human health concerns, as well as matters related to residues and trade. Omethoate exists as a pesticide in its own right, but is also a toxic metabolite of dimethoate. Hence, the toxicity of omethoate is also important in the context of omethoate residues arising from the use of dimethoate. The review of omethoate is therefore being conducted in conjunction with the dimethoate review.

This review has concluded that there should be no changes to the approval status of the active constituent. However, based on the potential risks identified in the human health risk assessment of O,O,S-trimethyl phosphorothioate (O,O,S-TMP) (OCS, 2010), a limit of 20 g/kg ai for O,O,S-TMP when present in omethoate technical grade active constituent is recommended.

Due to the high acute oral toxicity of a home garden product containing 50 g/L omethoate, registration of this product is not supported[1]. Amendments have been made to the Safety Directions for the remaining registered products, including one home garden product containing 0.2% omethoate.

The current ADI for omethoate is 0.0003 mg/kg bw, based on a NOEL of 0.025 mg/kg bw/d for inhibition of RBC ChE activity in a 1-year gavage study in dogs, applying a safety factor of 100. This review recommends that the ADI be revised to 0.0004 mg/kg bw, based on a NOEL of 0.04 mg/kg bw/d for inhibition of ChE activity in a 2-year rat dietary study, applying a safety factor of 100. Prior to this review, an ARfD for omethoate had not been set. The ARfD is 0.003 mg/kg bw, based on a NOEL of 0.25 mg/kg bw for inhibition of ChE activity in an acute oral neurotoxicity study in rats, using a safety factor of 100.

Omethoate is in Schedule 7 of the SUSDP with a cut-off to Schedule 6 at 30% or less, or to Schedule 5 in pressurised spray packs containing 0.2% or less of omethoate. The information provided for this review does not raise any concerns regarding scheduling and therefore maintenance of the current scheduling of omethoate is recommended.

[1] Note there are no longer any registered 50 g/L home garden products as of February 2012.

TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption	T_{max}=1 h; Almost complete, with only 2% in faeces.
Distribution	Widely distributed in the tissues, highest levels in the thyroid.
Potential for accumulation	No evidence for accumulation.
Rate and extent of excretion	Rapidly excreted in urine; 88% eliminated after 8 h, 96-97% after 48 h.
Metabolism	Numerous metabolites in urine, ~25-40% present as unchanged omethoate.
Toxicologically significant compounds (animals, plants and environment)	None

Acute toxicity

Rat oral LD_{50} (mg/kg bw)	22
Worst oral LD_{50} in other species	36 in mice
Rat dermal LD_{50} (mg/kg bw)	865; 24 h semi-occlusive
Worst dermal LD_{50} in other species	None tested
Rat inhalation LC_{50} (mg/m^3)	220-425
Worst inhalation LC_{50} in other species	140 in mice
Skin irritation	Non-irritant
Eye irritation	Slight irritant
Skin sensitization	Sensitiser in open epicutaneous test in guinea pigs. Published report of one human case of sensitisation.

Short-term toxicity

Target/critical effect	Inhibition of RBC and brain ChE activity
Lowest relevant oral NOEL (mg/kg bw/d)	No data
Lowest relevant dermal NOEL (mg/kg bw/d)	2.5 (3-week rabbit)
Lowest relevant inhalation NOEC (mg/m^3)	1.0 (3-week rat)

Genotoxicity	Non-genotoxic

Long-term toxicity and carcinogenicity

Target/critical effect	Inhibition of RBC and brain ChE activities
Lowest relevant NOEL (mg/kg bw/d)	0.025 mg/kg bw/d (1-year dog gavage study)

Carcinogenicity	No evidence of oncogenic potential

Reproductive toxicity

Reproduction target/critical effect	Inhibition of RBC and/or brain ChE activity
Lowest relevant reproductive NOEL (mg/kg bw/d)	Parental: 0.04 mg/kg bw/d Offspring: 0.04 mg/kg bw/d
Developmental target/critical effect	Maternal: inhibition of RBC and brain ChE activity; Foetal: malformations (arthrogryposis, epignathus) at the maternal LOEL (clear NOEL)
Lowest relevant developmental NOEL (mg/kg bw/d)	0.2 in rabbits
Delayed neurotoxicity	No effects
Immunotoxicity	No data
Dermal absorption	No data

Summary

ADI: 0.0004 mg/kg bw
[based on inhibition of ChE activity in plasma, RBC and brain]
ARfD: 0.003 mg/kg/bw
[based on inhibition of ChE activity in blood and brain]
Pivotal NOEL for Occupational Health & Safety Assessment
[based on inhibition of ChE activity in plasma, RBC and brain]

NOEL	Study	Safety factor
0.04 mg/kg bw/d	2-year rat dietary study	100
0.25 mg/kg bw/d	Acute neurotoxicity rat gavage	100
2.5 mg/kg bw/d	3-week rabbit dermal	100
1 mg/m^3/d (exposure 6h/d, 5d/week)	3-week rat inhalation	

Health Value in drinking water

Current: none Proposed: 0.001 mg/L

SUMMARY TOXICOLOGY REPORT

Introduction

Omethoate is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under Australia's Chemicals Review Program. Following the data call-in process, two additional data submissions on the toxicology of omethoate were received from industry, and these data, together with all previously submitted data, have been assessed in detail. The detailed report is summarised below.

Omethoate is not registered in the USA, the UK, or in any European country other than Portugal. As of May 2011, omethoate is the active constituent in 11 products registered in Australia. At the beginning of this review, omethoate was the active constituent in five registered products for use as an insecticide and acaricide in both commercial and home garden situations. Four of these products are included in this review. Maximum Residue Limits have been set for omethoate in cereal grains, edible offal (mammalian), eggs, fruits, lupins (dry), meat (mammalian), milks, oilseed, capsicums, edible offal of poultry, poultry meat, tomato and other vegetables. Omethoate is in Schedule 7 of the SUSDP with a cut-off to Schedule 6 at 30% or less, or to Schedule 5 in pressurised spray packs containing 0.2% or less of omethoate. It currently has an ADI of 0.0003 mg/kg bw/d, set in 1989 and based on a NOEL of 0.025 mg/kg bw/d in a 1-year gavage study in dogs. An ARfD has not been set, nor has a National Health and Medical Research Council Health Value for omethoate in drinking water been established. There is an existing upper limit of 5 g/kg for the toxicological impurity O,O,S-trimethyl phosphorothioate.

Metabolism and Toxicokinetics

When radiolabelled omethoate was administered to rats at 0.3 mg/kg bw iv, or orally at 0.3, 1, 5, or 10 mg/kg bw, 96-97% of the radioactivity was eliminated in the urine within 48h, indicating almost total absorption by the oral route. Small amounts of administered radioactivity were accounted for in the faeces, body, GIT and expired air. Radioactivity was widely distributed in body tissues, peaking in the plasma and other tissues at 1 h after oral dosing. Analysis of radioactivity in a limited number of tissues showed the highest concentration in the kidney, but autoradiography showed higher radioactivity in the thyroid relative to other tissues (Weber et al., 1978).

In a study by Ecker and Coelin (1981), rats were dosed orally with radiolabelled omethoate at 5 mg/kg bw. Approximately 88% of the administered radioactivity was found in the urine at 8 h after dosing, with 26-44% present as unchanged omethoate, 13-22% as N-methyl-methyl-sulfinyl-acetamide, 9.5% as O-desmethylated omethoate, and several unidentified metabolites, each of which represented <10% of the radioactivity administered.

In a study using radiolabelled omethoate, single doses were administered to rats by the oral or iv routes at 0.5 or 10 mg/kg bw. A repeat oral dose experiment was also conducted at 0.5 mg/kg bw/d over 15 days, radiolabelled material administered on the final day only. The vast majority of excreted radioactivity (85-96%) was found in the urine, almost all of this appearing in the first 24 h. Most of the remainder was found in the faeces, with ~0.5% or less in the body. Of the organs, radioactivity was most concentrated in the thyroid, where it exceeded plasma levels by a considerable margin at 48 h. Much of the omethoate was not

metabolised, the parent compound representing a greater proportion of recovered radioactivity at the higher dose, and in females relative to males. The main metabolites were N-methyl-2-(methylsulphinyl)acetamide and the O-desmethyl form of omethoate (Hoshino 1990).

Acute Studies

The acute oral toxicity of omethoate is high. In rats, LD_{50} values were in the range of 22-65 mg/kg bw (Flucke 1978, Schrader 1962, Kimmerle 1968, Krötlinger 1989a). The LD_{50} in rats of ~25 mg/kg bw obtained by Flucke (1978) and the (M/F) 22/26 mg/kg bw in Krötlinger (1989a) are consistent with the 29 mg/kg bw reported for fasted rats as part of an antidote study (Bayer 1967). In mice, the oral LD_{50} was 36 mg/kg bw, which was a lower LD_{50} than rats under the same study conditions (Kimmerle 1968). Acute dermal studies in rats gave LD_{50}s from approximately 145 mg/kg bw, up to ~1400 mg/kg bw (Schrader 1962, Kimmerle 1968, Flucke 1978, Krötlinger 1989b). Exposure conditions varied considerably between studies, with a trend showing the LD_{50} decreasing with increasing length of exposure. The dermal study that most closely approximated modern guidelines (Flucke 1978), resulted in an LD_{50} of 865 mg/kg bw in female rats following 24 h exposure under semi-occlusive dressings, with females slightly more sensitive than males. In acute inhalation studies, mice were affected at lower doses than rats (Kimmerle 1968). For 4 h exposures, LC_{50} values in rats were 220-425 mg/m^3, and 140 mg/m^3 in mice.

Overall, deaths occurred in the acute studies within 24 h of dosing, up to day 4 post-dosing, though deaths following iv treatment occurred within 10-15 min of injection. Similar clinical signs (trembling, muscle spasms, red tears, breathing difficulties and behavioural disturbances) were common to exposure by all routes. These were usually rapid in onset (~1 h post-treatment), resolving in 1-12 days in survivors. From the limited number of studies that used animals of both sexes, females appeared more sensitive to the acute effects of omethoate than males by the dermal and inhalation routes.

In a study in rats to determine the effects of acute oral dosing with omethoate on ChE activity, brain ChE activity was the most sensitive to inhibition, followed by plasma, with minimal effects on RBC ChE activity. Inhibition of brain ChE activity at 1.3 mg/kg bw/d was considered treatment-related, with a no-effect level of 0.6 mg/kg bw/d (Flucke 1978).

In rabbits, omethoate was not a skin irritant, but was a slight eye irritant (Pauluhn 1983). It was a skin sensitiser in guinea pigs according to the open epicutaneous test (Flucke 1984).

In a potentiation and antidote study in rats using single oral doses of omethoate, a combination of atropine and PAM (pralidoxime) was considered the best antidote of the combinations tested. The effects of equitoxic mixtures of omethoate and maldison (malathion) administered orally to rats were considered to be additive (Bayer 1967).

Acute studies were performed on rats using several SL (soluble concentrate) formulations containing omethoate (1000, 500 and 50 g/L) and 1-methoxy-2-propyl acetate (MPA) as the sole ingredients. With the exception of the oral studies, females were more sensitive than males to the acute effects of these formulations. The LD_{50} values are therefore given as male/female results. The inhalation LC_{50}s are expressed as mg active/m^3. For the 1000 g/L formulation, the acute oral and dermal LD_{50}s were 30/35 and 584/302 mg/kg bw respectively (Krötlinger 1986a,b), and the inhalation LC_{50}s were 512/224 mg/m^3 (Pauluhn 1986a). In the

case of the 500 g/L formulation, the acute oral and dermal LD_{50}s were 55 (both sexes) and 943/413 mg/kg bw respectively (Krötlinger 1986c, d), and the inhalation LC_{50}s were 774/421 mg/m^3 (Pauluhn 1986b). The oral LD_{50} values for the 50 g/L formulation were 862/872 (Krötlinger 1987). Deaths occurred from one hour after dosing up to day 6. Clinical signs included palmospasms, laboured breathing, apathy, salivation, lacrimation, tremors and piloerection. Reversible weight loss, or reduced weight gain were seen in many of the studies, particularly in females. Macroscopic findings included changes to the appearance of the lungs, spleen, liver and kidneys (mainly pale and patchy), and to the GIT and its contents. The 500 g/L and 50 g/L formulations were not skin irritants in rabbits. The 500 g/L formulation was a moderate eye irritant in rabbits, but a 15% dilution of this mixture was not. The 50 g/L formulation was a moderate to severe eye irritant in rabbits (Pauluhn 1986c, 1987).

Short-Term Repeat-Dose Studies

In a dermal study in rabbits by Flucke and Luckhaus (1979), omethoate was applied to intact or abraded skin at 0, 2.5 or 20 mg/kg bw/d, and left uncovered for 7 h/d on 15 consecutive workdays. At 20 mg/kg bw/d, muscle spasms were observed in the abraded group only, for 2-3 h after the initial 3 treatments, coinciding with the period for which the abraded skin showed an inflammatory reaction. The only other treatment-related finding was inhibition of ChE activity in the brain, plasma and RBC at 20 mg/kg bw. The NOEL was 2.5 mg/kg bw/d, due to clinical signs and inhibition of ChE activity at 20 mg/kg bw/d.

Rats were exposed to 0, 3, 13 or 49 mg/m^3 omethoate by inhalation for 4 h/d for 5 days, avoiding skin contact with the aerosol. One female died at 49 mg/m^3, and clinical signs consistent with ChE inhibition were present at ≥13 mg/m^3. Transient weight loss was likely to have been treatment-related at 49 mg/m^3. Plasma ChE was more sensitive than RBC ChE to inhibition by omethoate. Inhibition of plasma and RBC ChE activity peaked after 3 exposure periods, with full recovery of plasma ChE activity at 3 mg/m^3 by day 8, and partial recovery at the higher doses (Thyssen 1978).

In a 3 week inhalation study, rats were exposed for 6h/d, 5 d/week to omethoate at 0, 0.96, 2.3 or 7.5 mg/m^3, avoiding skin contact with the aerosol. There were no deaths or clinical signs, no effects on bodyweight, and no treatment-related histopathological findings. Plasma, RBC and brain ChE activities were inhibited in both sexes at ≥2.3 mg/m^3, with females less affected than males. Due to these findings, the no effect level was 0.96 mg/m^3 (Thyssen 1979).

Subchronic Studies

In a study supplementary to the chronic study in rats (Schladt, 1995), rats were dosed with omethoate in the drinking water at 0, 100 or 300 ppb, equal to 0/0, 9/11 and 27/32 µg/mg bw/d in males and females respectively, for 32 weeks. None of the parameters measured, including ChE activity in plasma, RBC and brain, was affected by treatment (Schladt, 1994).

In a 13 week study, dogs were treated daily with 0 or 0.0125 mg/kg bw/d omethoate by stomach tube. There were no deaths or clinical signs, or effects on bodyweight, though food consumption was slightly reduced in treated females. All other parameters measured were unaffected, including ChE activity in plasma, RBC and brain (Ruf and Mager 1991).

Chronic Studies

In a 2-year oncogenicity study by Schladt (2001), mice were dosed with omethoate in the drinking water at 0, 0.5, 4 or 32 ppm, equal to 0/0, 0.10/0.11, 0.82/0.80 and 6.41/6.61 mg/kg bw/d in male and female mice respectively. Mortality was not affected by treatment, but tremor was observed in mice at 32 ppm, generally prior to week 8. Weight gain in treated mice exceeded that of controls, and at ≥4 ppm, water consumption was reduced, particularly in the first half of the study. Also at ≥4 ppm, RBC numbers, Hb and MCHC were decreased in males only. Plasma ChE activity was inhibited in both sexes at 32 ppm, as was brain ChE activity at ≥4 ppm. Erythrocyte ChE activity was reduced in both sexes at 32 ppm, and in males at 4 ppm, but results were equivocal for 4 ppm females. Male liver weights were reduced at all doses, but in the absence of any related findings, this was not considered to be toxicologically significant. In the kidneys, there was an increased incidence of calcification (slight), and cortical cysts were present in all treated groups (both sexes) more frequently than in controls. It was considered unlikely that these renal changes were toxicologically significant. The NOEL was 0.5 ppm, equal to 0.1 mg/kg bw/d, based on inhibition of RBC ChE activity in males at 4 ppm and above.

Bomhard et al. (1979) administered omethoate in the diet to rats at 0, 0.3, 1, 3 or 10 ppm, equal to (M/F) 0/0, 12/18, 41/53, 126/166, and 419/542 µg/kg bw/d respectively for 2 years. Fresh batches of feed containing omethoate were prepared twice weekly. Increased mortality in females at 10 ppm was possibly related to treatment. No clinical signs were reported, nor were any treatment-related effects seen in haematology tests. Bilirubin was increased in females at ≥3 ppm, but there were no other findings that indicated altered liver function. Plasma ChE activity was inhibited at 10 ppm in both sexes, and at 3 ppm in males. This occurred mainly early in the study, but persisted for 18 months in 10 ppm males. On the other hand, RBC ChE activity was inhibited at ≥3 ppm throughout the study. Brain ChE activity was also inhibited at these doses at study termination. There were no macroscopic or microscopic findings that were considered treatment-related. The NOEL for this study was 1 ppm, equal to 41 µg/kg bw/d, due to inhibition of plasma, RBC and brain ChE activities at doses of 126 µg/kg bw/d and greater.

A 2-year combined chronic toxicity and carcinogenicity study was performed in rats (Schladt, 1995). Omethoate was administered via the drinking water at 0, 0.5, 4 or 32 ppm, equal to 0/0, 0.04/0.05, 0.30/0.44 and 2.92/3.93 mg/kg bw/d for males and females respectively. Mortality was independent of treatment. Tremor was seen at 32 ppm, mainly in males during the first 7 weeks. Other clinical signs were emaciation and loss of hair at 32 ppm, and eye opacity at ≥4 ppm. Body weight loss occurred in the 32 ppm groups in week one, followed by compensatory weight gain in females, but male bodyweights remained depressed throughout the study. At 32 ppm, water consumption was increased, and there was a slight increase in food consumption. Males at 32 ppm had decreased Hb, Hct and MCV, and increased MCHC and thrombocytes. Plasma ChE activity was inhibited in males at 4 ppm and both sexes at 32 ppm; RBC ChE activity was inhibited at all doses in males and at ≥4 ppm in females; and brain ChE activity was inhibited in both sexes at ≥4 ppm. Adrenal weights were increased in females at ≥4 ppm. Vascularisation of the cornea occurred more frequently at 32 ppm. Treatment-related microscopic changes were limited to the 32 ppm groups and comprised mineralisation of the lens and increased severity of retinal degeneration (males), vacuolation of the lacrimal glands and epididymides (males), and hyperplasia of the mammary glands (females). There was an increased incidence of

follicular cell adenomas of the thyroid in males at ≥4 ppm. A NOEL was not achieved in this study due to inhibition of RBC ChE activity at all doses in males. However, taken in conjunction with the 32-week supplementary study of Schladt (1994), the overall NOEL is 0.3 ppm, equal to 0.03 mg/kg bw/d, based on inhibition of RBC ChE activity in males at the next highest dose of 0.05 mg/kg bw/d.

In a 12-month study by Hoffmann and Schilde (1984), dogs were dosed with 0, 0.025, 0.125 or 0.625 mg/kg bw/d omethoate by stomach intubation. There were no premature deaths, nor any clinical signs, changes in bodyweight, haematology, urinalysis, ophthalmology, organ weights, or macroscopic/microscopic findings that were considered treatment-related. The only change in clinical chemistry was inhibition of ChE activity. Plasma, RBC and brain ChE activities were inhibited in both sexes at 0.625 mg/kg bw/d, with inhibition of RBC and brain ChE activity in 0.125 mg/kg bw/d males also considered likely to be due to treatment. The NOEL was 0.025 mg/kg bw/d due to inhibition of RBC and brain ChE activity in males at 0.125 mg/kg bw/d.

Reproduction Studies

In a one-generation reproduction range-finding study by Dotti et al. (1994), rats were dosed with 0, 10, 30 or 90 ppm omethoate in the drinking water. Due to severe clinical signs at the highest dose, this was reduced to 50 ppm from day 10. Doses were approximately equal to (M/F) 0.8/1.2, 2.6/4.6, and 4.5/9.1 mg/kg bw/d, which represent the bottom-of-the-range values for males throughout the test period, and females in the pre-mating period. Two dams died in the highest dose group, one on day 8 and the other on day 13. Both had dark red discolouration of the lungs, and stomach discolouration or foci. After dose reduction, clinical signs became less severe, with ruffled fur and occasional restlessness the main signs after day 12. Restlessness and occasional tremor were also seen at 30 ppm. Rats lost weight when dosed at 90 ppm, and this corresponded to decreased food consumption. Bodyweight at 50 ppm remained below controls, but bodyweight gain was comparable in control and treated groups. At the lower doses, males gained less weight than controls in the pre-mating period, but weight gain was not affected thereafter. At 50 ppm, pre-coital time was increased, and at ≥30 ppm, fertility and implantations/dam were reduced, and postnatal losses were increased. Gestation time was unaffected. Pup bodyweight was similar in treated and control groups at birth, but there was a dose-related reduction in pup bodyweight across all treated groups from postnatal day 4. Maternal food and water consumption were reduced in all treated groups during lactation. Brain, RBC and plasma ChE activities in dams were inhibited at all doses on PND 21. Pup testes weights were reduced in all treated groups, but other than the rats that died when dosed at 90 ppm, there were no macroscopic findings in pups or parental animals. As part of this study, adult subgroups were dosed for 22 days, at the same doses used in the main study. Findings were similar to the main study, with the additional information that plasma, RBC and brain ChE activities were inhibited at this stage in all treated groups, except for plasma ChE activity in males at 10 ppm. Effects (inhibition of plasma, RBC and brain ChE activities in adults, reduced pup bodyweight and testes weights) were seen at all doses in this study.

In a 2-generation reproduction study in rats (Dotti et al. 1992), omethoate was administered in the drinking water at 0, 0.5, 3 or 18 ppm (approximately equivalent to 0, 0.04, 0.23 and 1.5 mg/kg bw/d respectively). Various parameters were affected at 18 ppm. Food consumption

was reduced in dams during lactation, and water consumption was reduced in both sexes. Body weight was also depressed in both sexes throughout the study. The number of implantations/dam and postnatal loss were increased in both generations, while in the F1, precoital time, the number of non-pregnant females, and post-implantation loss were all increased. Epithelial vacuolation of the epididymides was increased in adult male of both generations. In pups, body weight gain was reduced during lactation, but no external abnormalities were detected. In parental animals, RBC and brain ChE activities were inhibited at ≥3 ppm, while brain ChE activity was inhibited in F2 pups at ≥3 ppm, and in F1 pups at 18 ppm. The NOEL for effects in both parents and offspring was 0.5 ppm, approximately equivalent to 0.04 mg/kg bw/d, due to inhibition of ChE activity at 0.23 mg/kg bw.

In a 3-generation reproduction study (2 litters/generation) by Löser (1981), rats were fed 0, 1, 3 or 10 ppm omethoate in the diet, approximately equivalent to 0, 0.05, 0.15 and 0.5 mg/kg bw/d. In the adults, there were no deaths or clinical signs related to treatment, and bodyweight and mating performance were also unaffected. There were no treatment-related changes in fertility index, litter size, or pup weight at birth. In the F2b, the pup viability index was reduced at 3 and 10 ppm, the lactation index was reduced at 10 ppm, and the mean bodyweight of the pups was reduced during lactation, relative to the controls. As findings in the pups were confined to the F2b, these effects were not considered to be related to treatment. The NOEL for parents and offspring was 10 ppm, equivalent to 0.5 mg/kg bw/d, due to the absence of treatment-related effects at all doses.

Developmental Studies

In a developmental study in rats (Bayer 1975), mated dams were dosed at 0, 0.3, 1, or 3 mg/kg bw/d on gestation days 6 to 15 inclusive. There were no maternal deaths. Effects of treatment were limited to the 3 mg/kg bw/d group. Bodyweight gain was reduced in the dams, with 3/20 experiencing total resorption. There was a slight reduction in foetal bodyweight and placental weight, but no other developmental effects. Both the maternal and foetal NOELs were 1 mg/kg bw/d due to decreased bodyweight in the dams and foetuses, and reduced placental weight at 3 mg/kg bw/d.

Mated female rats were gavaged with omethoate at 0, 0.3, 1, or 3 mg/kg bw/d on gestation days 6 to 15 inclusive. There was one maternal death at 3 mg/kg bw/d on gestation day 11. Also at this dose, tremors were a common clinical sign, and maternal food consumption and bodyweight gain were reduced. There were no gross findings at necropsy. Placental weight was reduced at 3 mg/kg bw/d, but no other developmental effects were observed. The maternal and foetal NOELs were 1 mg/kg bw/d, due to clinical signs and reduced bodyweight in the dams, and reduced placental weights at 3 mg/kg bw/d (Holzum 1990a).

Mated female rabbits were dosed with omethoate by gavage at 0, 0.1, 0.3 or 1 mg/kg bw/d on gestation days 6 to 18 inclusive. Five animals (3 control and 2 treated) were killed *in extremis*, but this was unrelated to exposure to omethoate. Abortions, a resorption, and failure to become pregnant were independent of dose, and considered incidental. Bodyweight gain was unaffected by treatment. The only treatment-related effect observed was inhibition of whole blood ChE activity, which was reduced at 1 mg/kg bw/d. The NOEL for maternal toxicity was therefore 0.3 mg/kg bw/d, with no foetal effects at 1 mg/kg bw/d, the highest dose tested (Tesh et al., 1982).

Omethoate was administered to mated female rabbits by gavage, at 0, 0.2, 1, or 5 mg/kg bw/d. There were no deaths. Clinical signs of tremor, and increased heart rate were seen at 5 mg/kg bw/d, with isolated instances of ataxia. At this dose (5 mg/kg bw), maternal bodyweight gain was reduced during the treatment period. Arthrogryposis of the front extremities occurred in a small number of foetuses at 1 and 5 mg/kg bw/d, without a clear dose response. However, their frequency at both doses exceeded the historical control rate. Epignathus, an abnormality not present in historical control foetuses, was observed in one foetus at 1 mg/kg bw/d. There were no other reproductive or developmental effects. Brain and RBC ChE activities were inhibited in does at 1 and 5 mg/kg bw/d, while plasma ChE activity was inhibited at 5 mg/kg bw/d only. The effects on ChE activity in the blood were seen on gestation days 14 and 19. The maternal and foetal NOELs were 0.2 mg/kg bw/d, due to inhibition of RBC and brain ChE activity in the does, and foetal malformations at 1 mg/kg bw/d (Holzum 1990b).

Genotoxicity Studies

Omethoate was genotoxic in many *in vitro* tests, both in the presence and absence of metabolic activation. Ames tests (*S. typhimurium* strains TA 100 and TA 1535), mitotic crossing over and gene conversion in *Saccharomyces cerevesiae*, and forward mutation and in Chinese hamster ovary (CHO) cells were all positive, and alkaline elution assays were positive in CHO cells. Chromosome aberration and sister chromatid exchange assays using human lymphocytes and CHO cells respectively, also gave positive results, but the omethoate concentrations used in the chromosomal aberration study were cytotoxic. In the absence of metabolic activation, an unscheduled DNA synthesis assay in primary rat hepatocytes and an alkaline elution assay in primary rat testes cells, were both positive. In the presence and absence of metabolic activation, negative results were obtained in reverse mutation tests using *S. typhimurium* strains TA 98 and TA 1537, and two strains of *S. cerevesiae*, as well as in a forward mutation assay in mouse lymphoma cells, and a Pol test in *E. coli*.

With the exception of the mouse spot test, *in vivo* results were negative. The other assays were dominant lethal mutation tests in mice, micronucleus tests (bone marrow) in mice, an alkaline elution assay in rats (testes cells), sister chromatid exchange in Chinese hamsters (bone marrow) and unscheduled DNA synthesis in rats (hepatocytes). In an Ames test using five strains of *S. typhimurium*, an intermediate of omethoate (chloroacetic acid-N-methylamide) produced negative results in the presence and absence of metabolic activation.

Neurotoxicity Studies

In an acute oral neurotoxicity study in rats to determine the time of peak effect for clinical signs and FOB, omethoate was administered by gavage at 0, 5, 10 or 15 mg/kg bw. At 5 mg/kg bw the peak effect occurred in the interval 2-4 h post-treatment, and at 1-4 h for the higher doses. At ≥5 mg/kg bw, ChE activity was inhibited in the RBC of both sexes at 8 and 24 h and in the brain at 24 h. At 8 and 24 h post-treatment, serum ChE activity was inhibited in males at ≥5 mg/kg bw and in females at ≥10 mg/kg bw (Mellert et al. 2002a).

In another acute oral neurotoxicity study, rats were given doses of 0, 0.25, 0.5, 0.75 or 1.5 mg/kg bw omethoate by gavage. Inhibition of RBC ChE activity was observed at ≥0.5 mg/kg bw at 2.5 h post-treatment, but this was reversible by 24 h at all but the top dose. Serum ChE activity was depressed at ≥0.75 mg/kg bw at 2.5 h, but this also did not persist till 24 h. Brain

ChE activity was depressed at 1.5 mg/kg bw at 24 h, the only time it was measured. No effects were seen at 0.25 mg/kg bw (Mellert et al. 2002b).

In a third acute oral neurotoxicity study, rats were dosed with 0. 0.2, 0.25, 0.35 or 5 mg/kg bw omethoate by gavage. Clinical signs and changes in FOB were observed only at 5 mg/kg bw at ~2 h post-treatment. The pupillary reflex was affected, accompanied by a reduction in motor activity and decreases in rearing and grip strength. At 2.5 h after dosing, inhibition of RBC, serum and brain ChE activities were seen, with brain ChE activity also inhibited at 0.35 mg/kg bw. No effects were seen at 0.25 mg/kg bw (Mellert et al. 2003).

In an acute neurotoxicity study, hens pre-treated with atropine were administered a single dose of 92 mg/kg bw omethoate by gastric intubation. There were 4 deaths within 6 days of dosing, but no neurotoxic signs were observed in survivors throughout the 3 week observation period. There were no treatment-related changes in the brain, spinal chord, or sciatic nerve of survivors (Bayer 1972).

Hens were dosed by gavage with single doses of omethoate in the range 20-80 mg/kg bw/d. There were 4 deaths at each of 71 and 80 mg/kg bw/d, in the period 1 to 3 days post-treatment, and survivors at these doses lost weight over the course of the study. Clinical signs seen at all doses were diarrhoea, staggering gait, dry/flaccid comb, salivation (marginal), and respiratory distress, persisting for 1-2 weeks. Moulting was seen in birds treated with 63 mg/kg bw omethoate. Changes to the liver, GIT, spleen and lung were noted at autopsy in animals that died as a result of treatment. There was no evidence of delayed neurotoxic effects in survivors. The acute oral LD_{50} was between 63 and 70 mg/kg bw (Bayer 1990d).

In a study for delayed neurotoxicity in hens, omethoate was administered by gavage at 140 mg/kg bw, as 2 single doses 3 weeks apart. Negative controls received water, and positive controls were dosed with tricresylphosphate. Atropine (20 mg/kg bw) was given subcutaneously 30 min before each omethoate dose, then in combination with PAM (both 50 mg/kg bw) at the same time as omethoate treatment, and at 7, 23, 31, 47 and 55 h post-treatment (25 mg/kg bw each). There were 2 deaths (days 1 and 31) in the omethoate treated group, these animals showing changes to the lungs, spleen, GIT, liver heart and kidneys at necropsy. Clinical signs were typical of acute signs of neurotoxicity, resolving by 8 days or 16 days after the first and second treatments respectively. Omethoate-treated hens lost weight after each treatment, which was followed by partial compensatory weight gain. At microscopic examination of neural tissue, changes were limited to the positive control group. In this study, there were no signs of delayed neurotoxicity in omethoate-treated hens (Bomann and Sykes 1993).

HAZARD ASSESSMENT

Discussion

Reasons for the review

Approval of the active constituent omethoate is being reconsidered on the basis of new toxicological data. Products containing omethoate and all associated labels are being reviewed because of human health concerns, as well as matters of concern related to residues and trade. Due to the similarity in the chemistry of omethoate and dimethoate (omethoate being a metabolite of dimethoate as well as a pesticide in its own right), the review of omethoate is being conducted in conjunction with the review of dimethoate. Since the last consideration of the toxicology database for omethoate in 1993, new data have become available. It is important that this new information be considered as it has the potential to impact on the ADI. As part of the review, the ADI will be reconsidered, an ARfD will be set for the first time, and First Aid Instructions and Safety Directions will be re-evaluated.

Adequacy of the database

New toxicological data submitted for this review comprised a delayed neurotoxicity study in the hen, acute studies (including neurotoxicity) in the rat, short-term, subchronic and chronic oral studies in rats, a chronic oral study in mice, several genotoxicity studies, and a one generation reproduction range-finding study in rats. Though the toxicological endpoints for these studies have been considered previously in some instances, the addition of these new studies provides a more informative database on which to base public health standards for omethoate and its products. However, quality repeat dose studies of 3 months duration or less are under-represented in this database, the studies that are present frequently falling short of modern standards with respect to the purity and stability of the test material administered, the level of reporting, the range of tests performed, and the reliability of the critical ChE assays.

Toxicokinetics and Metabolism

Consistent with its high water solubility, omethoate was rapidly and almost completely absorbed following oral dosing in rats, and was rapidly eliminated. Omethoate is not expected to accumulate in body tissues or organs, even after repeated exposure. Greater than 96% of orally administered radiolabel was collected in the urine of rats within 48 h of dosing, the vast majority of this in the first 12 h. A small amount was excreted in the faeces and in the expired air. Plasma concentrations peaked about one hour after dosing, accompanied by wide distribution in the tissues. Relative to other tissues, the highest concentration of radioactivity was found in the thyroid, with levels in liver, kidney, testes, spleen and lung exceeding plasma levels. However, little radioactivity remained in the tissues after 24 h. About 26-62% of the radioactivity in the urine was unchanged omethoate. These levels were higher in females than in males, indicating a relatively greater capacity for omethoate metabolism in the latter, at least in rats.

The biotransformation of omethoate in mammals has not been studied extensively. In the studies evaluated in this review, the most abundant metabolite identified in the urine of omethoate-treated rats was N-methyl-methyl-sulfinyl-acetamide (16-36%). The only other

metabolite identified was O-desmethylated omethoate at up to 4-9%. Results were similar in two separate studies. In an early published study, rats were treated with a relatively high dose of 50 mg/kg bw ^{32}P-radiolabelled omethoate, and the urine from the survivors was analysed. Cumulative radioactivity in the urine was 16, 19 and 30% of administered radioactivity at 12, 24 and 48 h respectively. Identified metabolites were O,O-dimethyl phosphoric acid (34% of total urinary radioactivity), O,O-dimethyl phosphorothioic acid (9.5%), with two unidentified metabolites comprising 52% and 4.5% of total urinary radioactivity (Dauterman *et al*., 1959). More detailed information is available for the mammalian metabolism of dimethoate, which is oxidised *in vivo* to omethoate. The JMPR (1971) considered that the metabolic route of omethoate would follow that for dimethoate in plants and animals, though the rates of individual reactions may vary.

Acute toxicity of the technical active

For acute exposure to omethoate by the oral, dermal or inhalation routes, there was a very rapid onset of clinical signs that were consistent with inhibition of ChE activity. These symptoms persisted for up to 12 days, with deaths occurring within 1-4 days of dosing. In one study, in which it was possible to directly compare rats and mice, omethoate appeared to be more acutely toxic to mice than rats via the oral route (LD_{50}s ~65 mg/kg bw and 36 mg/kg bw in rats and mice respectively). In other comparable studies in rats, the LD_{50} values were lower than both species in the above study, but this may have been influenced by the chosen vehicle and whether the animals were fed or fasted at dosing. By the inhalation route, the increased sensitivity of mice relative to rats was more pronounced than in the oral dosing experiments. For example, LC_{50} values for 1 h exposures were >1520 mg/m^3 and >250 mg/m^3, and for 4 h exposures, 240 mg/m^3 and 140 mg/m^3 for rats and mice respectively. In contrast, in the 2-year drinking water studies, the NOEL in mice was slightly higher than in rats. In the few cases where acute studies included both male and female rats, females were more sensitive to the acute toxic effects of omethoate than were males, but the differences were small and not expected to be of toxicological significance. For acute exposures, omethoate is considered to be highly toxic by the oral route, and moderately toxic by the dermal or inhalation routes.

Studies in rabbits indicate that omethoate is not likely to be irritating to the skin, but is expected to be a slight eye irritant. Skin irritation was also not apparent in a 3-week dermal toxicity study in rats. Omethoate has been shown to be a skin sensitiser in guinea pigs. Information on human allergic reactions to omethoate is limited to a single case study that reports contact dermatitis in a woman who worked on a daily basis with rose bushes that were sprayed once per week with omethoate, and less often with dimethoate (Haenen *et al*., 1996). During her work, the woman wore leather gloves that rapidly became saturated. Patch tests showed positive reactions to both insecticides at concentrations as low as 0.1% in water. Control tests in 10 subjects were negative. The authors suggested that cross-sensitivity between the two chemicals was probable, but conceded that they could not rule out a common impurity or concomitant sensitivity. It is appropriate for products containing omethoate to be considered potential skin sensitisers in humans.

Stability of omethoate in the feed

Investigators were alerted to the fact that omethoate was unstable in the feed by an early 4-month rat dietary study (Löser and Lorke, 1967), in which symptoms of ChE inhibition became evident on days 2-4 after the rats received their weekly fresh batch of treated food, with the severity of these symptoms decreasing as the week progressed. To overcome this problem, later dietary studies prepared omethoate in the feed on 5 days per week (Löser, 1968) or twice weekly (Bomhard et al., 1979; Krötlinger and Löser, 1982). In the 12-month dog study (Hoffmann and Schilde, 1984), on which the ADI is based, omethoate was administered by stomach tube, working from aqueous concentrates of low pH that were stored for a maximum of 3 days, and diluted immediately prior to dosing. This method of stabilising omethoate was adopted for the most recent chronic rodent studies, in which omethoate was administered via drinking water of low pH (Schladt 1995 and 2001).

Mode of action in mammals

For the studies in which ChE activity was measured, this proved to be the most sensitive endpoint. Of the three compartments in which ChE activity was measured (plasma, RBC and brain), activity in the plasma was the least affected. In most studies, ChE activities in the RBC and brain were generally inhibited at the same dose, though RBC ChE activity was affected more than brain ChE overall. The NOELs/LOELs for ChE activity are summarised in Table 1. The inhibition of brain ChE activity is consistent with the findings in the studies using radiolabelled omethoate, which showed rapid and wide distribution of omethoate to all tissues, including the brain.

In the 2-year study in mice and the 1-year study in dogs, ChE activity in RBC and brain were equally sensitive to the effects of omethoate. In the rat 2-year studies, and rabbit developmental study, similar doses were required to inhibit ChE activity in RBC and brain, but the degree of inhibition was greater in RBC. In 3-week studies where dosing was by the dermal or inhalation route in rabbits and rats respectively, ChE was inhibited in all three compartments at the same dose, though in the inhalation study, inhibition was greatest in brain. Cholinesterase results in the 1-week inhalation study in rats were unusual in that plasma ChE activity was inhibited to a greater extent than RBC ChE activity. However, brain ChE activity was not measured in this study, and plasma and RBC ChE activities were inhibited at all doses.

In the 2-generation reproduction study, ChE activity was less sensitive to omethoate in 21-day-old pups relative to the corresponding measurements in their parents. With the exception of the 2-year drinking water study in rats in which ChE inhibition in the plasma and RBC was slightly greater in males than females, and the 1-year gavage study in dogs in which inhibition of ChE activity in the brain and RBC in males was greater than the corresponding values for females, ChE results were similar for both sexes.

Table 1. Summary of the doses (mg/kg bw, mg/kg bw/d or mg/m^3) at which no inhibition of ChE activity (and the lowest doses at which inhibition of ChE activity) was observed following omethoate administration

Species	Study	Plasma	RBC	Brain
Mouse	2-year drinking water	0.8 (6.5)	**0.1 (0.8)**	**0.1 (0.8)**
Rat	Acute oral	0.6 (1.3)	1.3 (2.6)	**0.6 (1.3)**
Rat	Acute neurotoxicity	0.35 (5)	0.35 (5)	**0.25 (0.35)**

Species	Study	Plasma	RBC	Brain
Rat	1-week inhalation	**(3)**	(3)	ND
Rat	Short-term inhalation (3 weeks)	0.96 (2.3)	0.96 (2.3)	**0.96 (2.3)**
Rat	2-year diet	0.04 (0.13)	**0.04 (0.13)**	0.04 (0.13)
Rat	2-year drinking water	0.04 (0.3)	**0.03 (0.05)**	0.04 (0.3)
Rat	Reproduction (2-generation): parents offspring	NA NA	**0.04 (0.25)** NA	**0.04 (0.25** **0.04 (0.25)**
Rabbit	3-week dermal	2.5 (20)	2.5 (20)	2.5 (20)
Rabbit	Developmental (gavage)	1 (5)	**0.2 (1)**	0.2 (1)
Dog	1-year gavage	0.125 (0.625)	**0.025 (0.125)**	**0.025 (0.125)**

NOTES: The most sensitive endpoint is bolded. If the NOEL is shared between two compartments, where differences in degree of inhibition occur, the compartment with the more severe inhibition is bolded.
ND = no data; NA = details not available.

Carcinogenicity

The only tumours identified as likely to have arisen from treatment were thyroid follicular cell adenomas in rats, for which the incidence in the 2-year drinking water study exceeded the concurrent controls in males at 0.3 and 3 mg/kg bw/d, and was outside the historical control range at 3 mg/kg bw/d. Findings in radiolabelled studies in rats showing higher levels of radioactivity in the thyroid relative to other tissues support the possibility that these neoplasms may have been related to treatment. In the 2-year dietary study in rats, medullary (C-cell) adenomas of the thyroid occurred in females at 0.4 mg/kg bw/d (the highest dose administered) at a higher frequency than in the corresponding control group. This difference was not apparent in males. These are common tumours in Wistar rats, and the incidence in treated females was within the historical control range, which reduces the likelihood that they were treatment-related. The only related finding in mice was one malignant unilateral follicular cell carcinoma of the thyroid, which was observed at the highest dose of 6.5 mg/kg bw/d in the 2-year drinking water study. These tumours have been reported occasionally in NTP studies for male mice of this strain (incidence 1/50 in each of 3/25 studies), so it is possible that the tumour in the omethoate-dosed rat may have arisen independently of treatment. Overall, the thyroid tumours were benign in all but one case, and were present at relatively high doses in studies for which there were clear NOELs. As no other neoplasms were identified as likely to be related to treatment, omethoate is not expected to be carcinogenic in humans.

Omethoate produced positive results in a range of *in vitro* assays to test for gene mutation, DNA damage and repair, and chromosomal effects. There is also a published report that omethoate induced a dose-related increase in sister chromatid exchanges in human lymphocytes *in vitro* (Dolara *et al.*, 1992). Positive results *in vitro* were generally achieved at high omethoate concentrations, and in one study, occurred only at cytotoxic doses. Also, with the exception of the mouse spot test, *in vivo* genotoxicity tests, including tests for gene mutation, chromosome effects and DNA damage and repair, were negative. Based on the balance of evidence indicating that omethoate is not genotoxic *in vivo*, and the lack of evidence for carcinogenicity in the chronic studies in rats and mice, omethoate is not considered to be genotoxic.

A recent published report examined the effects of omethoate and several other OP pesticides on various mammalian cell lines (Isoda *et al.*, 2005). Results showed that low concentrations

of omethoate (0.2 and 0.4 µM) had a proliferative effect on the human breast cancer cell line MCF-7. Omethoate was cytotoxic to these cells at higher concentrations. A mechanism for this effect was not proposed, nor was any *in vivo* evidence presented. Given the difference in results for *in vitro* and *in vivo* genotoxicity tests discussed above, it is considered unlikely that the effects demonstrated in MCF-7 cells are predictive of effects *in vivo*.

Other chronic effects

In the long term studies, there were few other noteworthy effects, all of which occurred at high doses relative to the LOELs for ChE inhibition. Decreases in Hb, Hct, RBC numbers and/or MCV, and increases in MCHC and thrombocytes were seen in male mice and/or rats at relatively high doses (approximately 6 or 3 mg/kg bw/d respectively). In rats at 3 mg/kg bw/d there was a relatively high incidence of various macroscopic and microscopic lesions of the eye, and vacuolation of the lacrimal glands and epididymides in males. At ≥0.8 mg/kg bw/d in mice, there was an increased incidence of atrophy of the ovaries and thymus and findings of fatty bone marrow.

Reproductive/developmental effects

In rats, no foetal abnormalities were observed in two developmental studies for which the maximum dose was 3 mg/kg bw/d. Effects of treatment were limited to decreased placental and/or foetal weights at 3 mg/kg bw/d, coincident with reduced maternal bodyweight and clinical signs. No effects were seen at 1 mg/kg bw/d, but ChE activity was not measured in the rat developmental studies. In addition, no abnormalities were seen in the offspring in reproduction study in rats.

Foetal abnormalities were seen in one developmental study in rabbits. Arthrogryposis of the front extremities occurred at 1 mg/kg bw/d and 5 mg/kg bw/d. This abnormality was present in 5 litters, and exceeded the historical control incidence at both doses. A single instance of epignathus, an abnormality not observed previously in the testing laboratory, was observed in the 1 mg/kg bw/d group. Clinical signs and reduced bodyweight gain were noted in the 5 mg/kg bw/d dams, but the changes detected in the dams at 1 mg/kg bw/d were limited to inhibition of RBC and brain ChE activities. No abnormalities were seen in the other developmental study in rabbits at up to 1 mg/kg bw/d, the maximum dose tested. The possible treatment-relatedness of the foetal abnormalities in the rabbit developmental study cannot be dismissed. However, considering that foetal abnormalities were confined to that study, with no effects in a similar study in the same species, or in developmental and reproduction studies in rats, and that there was a clear NOEL for the developmental abnormalities, they are not of particular concern to human health.

Neurotoxicity

In an investigation of the potential for omethoate to cause delayed neurotoxicity, hens showed acute signs of neurotoxicity which resolved in survivors after approximately 1-2 weeks, but no signs of delayed neurotoxicity were observed. Neuropathy target esterase (NTE) was not measured in this study. In a published study, levels of acetylcholinesterase and NTE were measured in brain autopsy material obtained from a fatal human omethoate poisoning (frozen samples taken 24 h after death) and from hens given omethoate at 4-8 times the LD_{50} without antidote protection (Lotti *et al*., 1981). The NTE activity in the human tissue was within the

normal range, but ChE activity was only about 5% of normal values for human cerebral cortex. *In vitro* experiments using 5 mM omethoate, a concentration at which the authors expected brain ChE activity to be totally inhibited, caused no significant inhibition of NTE in hen or human brain tissue. The IC_{50}s of omethoate were 0.15 mM and 0.14 mM for ChE activity in the hen and human brain tissue respectively. *In vivo* tests in hens also confirmed that brain NTE activity was not inhibited by omethoate at doses that caused >90% inhibition of brain ChE activity in the same birds. It therefore appears unlikely that omethoate has the potential to cause delayed neurotoxicity in humans. The authors criticised a published report of human neuropathy due to omethoate poisoning on the grounds that there was great uncertainty about the agent ingested (Curtes *et al*., 1979).

Impurities

An impurity limit of 5 g/kg currently exists for O,O,S-trimethyl phosphorothioate (OOS-TMP). The Office of Chemical Safety (OCS) has conducted a human health risk assessment (HHRA) of O,O,S-trimethyl phosphorothioate (O,O,S-TMP) when present as an impurity in products containing the active ingredient omethoate (OCS, 2010). O,O,S-TMP was found to be an impurity of toxicological significance based on the hazards identified below.

There are two toxicological concerns regarding the presence of O,O,S-TMP as an impurity in OP pesticides. Firstly, O,O,S-TMP can potentiate the acute toxicity of OP pesticides. Secondly, O,O,S-TMP has intrinsic toxicity which should also be considered. These two concerns are addressed below.

Potentiation of omethoate toxicity by O,O,S-TMP

O,O,S-TMP can potentiate the acute oral toxicity of some OP compounds by inhibiting a "safe" detoxification pathway (carboxylesterase-associated detoxification) and instead driving the formation of the oxon derivatives which are often of greater toxicity than the parent OP compound.

Intrinsic toxicity of O,O,S-TMP

O,O,S-TMP belongs to the organophosphorous class of chemicals, however, available data does not indicate its primary mode of action to be inhibition of AChE in the brain resulting in cholinergic effects. Instead, the available toxicological data indicate that the most sensitive endpoints of toxicity for O,O,S-TMP are immunotoxicity and pneumotoxicity in adults and foetotoxicity. These effects are described briefly below, and the relevant NOELs are included in Table 2.

While a significant pathway for several OPs, omethoate is not detoxified via A-carboxylesterase-catalysed hydrolysis. Omethoate is likely to be detoxified to an extent via non-catalytic stoichiometric binding to B-carboxylesterases, however, this pathway is rapidly saturated by omethoate itself, and since omethoate and O,O,S-TMP are likely to have similar affinity for carboxylesterase enzymes, O,O,S-TMP is unlikely to significantly potentiate the acute toxicity of omethoate when present as an impurity. The impact of the presence of O,O,S-TMP in omethoate products has therefore focused on the inherent toxicity of O,O,S-TMP.

Several studies in mice investigating immunotoxicity induced by O,O,S-TMP have been conducted. O,O,S-TMP has been demonstrated to cause a dose-related and statistically significant effect on humoral and cell-mediated immune responses of mice following single and repeat doses, in addition to associated pathological effects in the spleen and thymus.

Clinical manifestations of delayed pneumotoxicity (decreased respiration and laboured breathing) in addition to pathological effects in the lung (i.e. haemorrhage) were observed in adult rodents in acute oral lethality studies Several studies investigating pneumotoxicity in rats induced by acute oral doses of O,O,S-TMP have demonstrated a dose-related effects in the lungs. Overall the results of the studies indicate that alveolar type I and type II cells are likely involved in the development of delayed pneumotoxicity in rats, with effects initiated early following treatment and increasing in severity with time. Studies also indicate that bronchiolar parameters (bronchiolar epithelium and Clara cells) are affected soon after treatment but appear to recover by day 7. The delayed pneumotoxic effects induced by O,O,S-TMP are similar to those induced by paraquat, although these effects are likely caused via separate modes of action.

Single doses of O,O,S-TMP to rats have been demonstrated to induce adverse pulmonary effects on the foetus. Foetuses from pregnant rats treated with a single doses of 7 mg/kg bw O,O,S-TMP or above on GD19 demonstrated delayed foetal pulmonary development in a dose-related fashion, characterised by biochemical/physiological immaturity observed on GD22, which were not apparent in foetuses from non-treated dams. A separate study demonstrated a dose-related pathological effects in the lungs of foetuses from dams treated with a single dose of 2.5 mg/kg bw O,O,S-TMP (on GD20) and cyanosis at higher doses, not observed in non-treated controls. Neonates from dams treated with single doses of 2.5 mg/kg bw O,O,S-TMP on GD20 demonstrated a dose-related and significant increase in mortality 72 hrs following birth in comparison to non-treated controls. A distribution study demonstrated that single oral doses of 2.5 mg/kg bw O,O,S-TMP or above on GD20 crossed the placental barrier and was present in the foetal lung, measured as the radioactive isotope (Koizumi *et al*, 1988). As such these effects are considered to be a direct result of exposure to O,O,S-TMP across the placenta.

Table 2. Summary of O,O,S-TMP NOELs/LOELs relevant to human health risk assessment

Study (study type)	**NOEL** mg/kg bw/d	**LOEL** mg/kg bw/d	**Toxicological Endpoint**	**Reference**
Acute studies				
Rats (acute developmental toxicity study)	0.5 (dams)	2.5	Decreased food consumption and body-weight gain, and pneumotoxicity	Koizumi *et al*, 1988
Rats (acute haemotoxicity study)	Not determined	10 (single dose)	Adverse effects on blood clotting factors (additional effects not looked for)	Bezencon *et al* 1989 and Keadtisuke *et al*, 1990
Mice (acute immunotoxicity study	Not determined	1.0 (single dose)	Decrease in humoral and cell mediated immune response in the absence of pathological effects in other organs and clinical signs of toxicity	Rogers *et al*, 1986
Repeat-dose studies				
Mice (14-day immunotoxicity study)	0.5*	5 (highest dose tested)	Significant increase in spleen lymphocyte number	Rogers *et al*, 1985b

Study (study type)	**NOEL** mg/kg bw/d	**LOEL** mg/kg bw/d	**Toxicological Endpoint**	**Reference**
Developmental studies				
Rats (acute developmental toxicity study)	0.5 (foetus)*	2.5 (foetus)	Morphological changes in foetal lung, delayed foetal lung development and increased neonatal mortality directly associated with exposure across the placenta	Koizumi *et al*, 1988 and Koizumi *et al*, 1989

*Due to the uncertainties arising as a result of the deficiencies in the study methodology, the use of this NOEL in an OHS risk assessment may require the use of an additional 2-fold safety factor in determining an acceptable MOE.

Establishing an impurity limit for O,O,S-TMP

Impurity limits are generally based on studies conducted using active ingredients containing the impurity at levels comparable to that present in the commercial product, however, in this case, the concentration of O,O,S-TMP in the toxicology studies on omethoate were not specified. Therefore, a maximum impurity limit for O,O,S-TMP present in omethoate active ingredient has been established based on the available toxicological data on the impurity itself.

The risk to workers occupationally exposed to O,O,S-TMP was assessed on the basis of O,O,S-TMP toxicity data and occupational exposure data taken from the draft OCS OHS risk assessment of omethoate submitted to the APVMA in 2006. The greatest potential health risk from exposure to omethoate and O,O,S-TMP arises from mixing, loading and applying products using airblast application methods, and from re-entry into treated fields. The potential exposure in both cases is similar. Based on the estimated exposure to O,O,S-TMP, and the NOEL for occupational risk assessment of 0.5 mg/kg bw, an acceptable margin-of-exposure is achieved if the maximum impurity limit for O,O,S-TMP is 2% (20 g/kg ai). Further details of this calculation can be found in OCS (2010).

The potential risk to the public from dietary exposure to O,O,S-TMP was assessed by comparing the risks following both chronic and acute dietary intake. To address acute dietary intake, the ARfD for omethoate has been compared with the provisional ARfD for O,O,S-TMP. To address chronic dietary intake a qualitative assessment has been conducted, as the database is inadequate to establish a TDI for chronic exposure to O,O,S-TMP. The OCS concluded that there is unlikely to be an increased risk to the public from dietary intake of O,O,S-TMP residues in produce treated with omethoate products.

Conclusion

On the basis of this human health risk assessment, the OCS recommends a maximum impurity limit of 2% (20 g/kg ai) for O,O,S-TMP in omethoate active ingredient.

DOSE LEVELS RELEVANT FOR RISK ASSESSMENT

To determine the lowest NOEL for the establishment of an ADI (health standards for omethoate), a summary of the NOELs determined in those studies deemed adequate for regulatory purposes is shown in Table 3.

Table 3. NOELs for omethoate

Species	Study Type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
Mouse	2-yr drinking water	0.1	0.8	Inhibition of RBC ChE activity (males)	Schladt (2001)
Rat	2-yr dietary	0.04	0.13	Inhibition of plasma, RBC and brain ChE activities	Bomhard et al. (1979)
Rat	2-yr drinking water	0.03	0.05	Inhibition of RBC ChE activity (males)	Schladt (1994, 1995)
Dog	1-yr gavage	0.025	0.125	Inhibition of RBC and brain ChE activity (males)	Hoffmann & Schilde (1984)
Rat	1-generation range-finding reproduction, drinking water	*Parental:* none *Offspring:* none	*Parental:* 0.8 *Offspring:* 0.8	*Parental:* inhibition of RBC and brain ChE activities; *Offspring:* reduced bodyweight and testes weights	Dotti (1994)
Rat	2-generation reproduction, drinking water	*Parental:* 0.04 *Offspring:* 0.04	*Parental:* 0.23 *Offspring:* 0.23	*Parental:* inhibition of RBC and brain ChE activities *Offspring:* inhibition of brain ChE activity	Dotti (1992)
Rat	3-generation reproduction, dietary	*Parental:* 0.5 *Offspring:* 0.5	*Parental:* none *Offspring:* none	No effects at the highest dose tested. *(ChE activity not measured)*	Löser (1981)
Rat	Developmental	*Maternal:* 1 *Foetal:* 1	*Maternal:* 3 *Foetal:* 3	*Maternal:* reduced bodyweight *Foetal:* reduced bodyweight and reduced placental weight	Bayer (1975)
Rat	Developmental	*Maternal:* 1 *Foetal:* 1	*Maternal:* 3 *Foetal:* 3	*Maternal:* clinical signs, reduced bodyweight *Foetal:* decreased placental weight	Bayer (1990b)
Rabbit	Developmental	*Maternal:* 0.3 *Foetal:* 1	*Maternal:* 1 *Foetal:* none	*Maternal:* Inhibition of ChE activity in whole blood *Foetal:* no effects	Tesh (1982)
Rabbit	Developmental	*Maternal:* 0.2 *Foetal:* 0.2	*Maternal:* 1 *Foetal:* 1	*Maternal:* inhibition of RBC and brain ChE activities *Foetal:* malformations	Bayer (1990c)

ADI

The long term studies in mice, rats and dogs listed in the table are the most appropriate for consideration in setting the ADI. All share the same endpoint of inhibition of RBC ChE activity, though in the 2-year rat dietary study, RBC, plasma and brain ChE activities were all inhibited at the same LOEL, as were RBC and brain ChE activities in the 1-year dog study. Of the long term studies, the 1-year gavage study in dogs (Hoffmann and Schilde, 1984) has the lowest NOEL, at 0.025 mg/kg bw/d. The lowest NOEL in a rodent study is from the 2-year study in rats, in which omethoate was administered in the drinking water (Schladt 1994/1995), and at 0.03 mg/kg bw/d, is essentially equivalent to the NOEL from the dog study.

There is a very small margin between the NOEL of 0.03 mg/kg bw/d and the LOEL of 0.05 mg/kg bw/d in the rat 2-year drinking water study, with inhibition of RBC ChE activity seen only in males at the LOEL, and considered to represent a threshold effect. If dose selection is taken into account, the 2-year dietary study in rats (Bomhard et al., 1979), with a slightly higher NOEL of 0.04 mg/kg bw/d, provides the appropriate chronic NOEL for the rat, this dose being less than the LOEL in the corresponding drinking water study. It is reasonable to consider the 2 chronic rat studies in this way, as despite using different vehicles for delivery of the test material, they share the most sensitive endpoint. Accepting that the LOEL in the rat 2-year drinking water study represents a threshold effect, the LOELs for ChE inhibition in the 2-year rat dietary and 1-year dog gavage studies were equivalent (~0.13 mg/kg bw/d). Given the shared endpoint, it is therefore appropriate to select the higher NOEL from these two studies (0.04 mg/kg bw/d in the rat study) as the basis for the ADI. The lowest NOEL in a reproduction study is approximately 0.04 mg/kg bw/d in the 2-generation rat study of Dotti (1992), in which the endpoints were inhibition of RBC and brain ChE activity in the adults, and inhibition of brain ChE activity in the offspring. In a developmental study in rabbits, foetal malformations were observed at the same dose at which maternal RBC and brain ChE activities were inhibited, and with a clear NOEL (see Table). The use of the NOEL from the 2-year rat dietary study is therefore also protective of reproductive and developmental effects. It is proposed that the ADI be revised to 0.0004 mg/kg bw/d, based on a NOEL of 0.04 mg/kg bw/d for ChE inhibition in the 2-year rat dietary study, using a safety factor of 100 for inter- and intra-species variation.

ARfD

In an acute gavage study in female rats (Flucke, 1978), ChE activity was measured in plasma, RBC and brain. Only female rats were used as they had a lower LD_{50} than males, though examination of the results for the LD_{50} study indicates that any sex difference was minimal, at least by the oral route. Cholinesterase activity was clearly inhibited in all 3 compartments at 2.6 mg/kg bw, with marginal inhibition in plasma and brain at 1.3 mg/kg bw. The NOEL was 0.6 mg/kg bw. In the recent acute neurotoxicity study in rats (Mellert et al., 2003), threshold ChE inhibition was observed at 0.35 mg/kg bw. The no-effect level in this study was 0.25 mg/kg bw. Foetal malformations were seen in one of the rabbit developmental studies. As these malformations occurred in the presence of maternal toxicity (inhibition of RBC and brain ChE activities), it is considered that the no-effect levels for acute oral toxicity in rats, based on ChE inhibition, will be protective for developmental endpoints. It is therefore appropriate to set an ARfD of 0.003 mg/kg bw, based on the no-effect level for ChE inhibition in the acute oral neurotoxicity study in rats of 0.25 mg/kg bw/d and incorporating a safety factor of 100 for inter- and intra-species differences.

HUMAN EXPOSURE

In Australia, omethoate is registered for use as a miticide and insecticide, on various commercial food crops and in the home garden.

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There are no objections on toxicological grounds to the ongoing approval of omethoate technical active from the following sources.

Approval holder: Bayer CropScience Pty Ltd
Manufacturing site: Bayer CropScience AG
BCS IOP A.I. Manufacturing
Alte Heerstrasse Building A603
D-41538 DORMAGEN
GERMANY

Approval holder: Bayer CropScience Pty Ltd
Manufacturing site: Sinon Corporation
111 Chung Shan Road
Ta-Tu Hsiang
Taichung Hsien
TAIWAN ROC

Approval holder: TRI-DELTA CHEMICALS PTY LTD
Manufacturing site: TRUSTCHEM CO LTD
89 Hanzhong Rd
NANJING 210029
PEOPLES REPUBLIC OF CHINA
Approval holder: IMTRADE AUSTRALIA PTY LTD
Manufacturing site: Factory No 4
Hangzhou High Tech Industrial Park
ZHEJIANG 310005
PR CHINA

Impurity Limits

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

The current minimum compositional standard for the active constituent omethoate and the maximum level for a toxicologically significant impurity are shown in the following table.

Chemical	**Standard**
Active constituent	Minimum 930 g/kg
O,O,S-trimethyl phosphorothioate	Maximum 5 g/kg

The impurity O,O,S-trimethyl phosphorothioate (O,O,S-TMP), may be present within the omethoate active constituent. A human health risk assessment has been conducted on O,O,S-TMP (OCS. 2010). Based on the potential for human health risks arising from the presence of O,O,S-TMP in technical grade omethoate, an upper limit of 20 g/kg ai for O,O,S-TMP is recommended.

Residue definition

In the existing MRL Standard for Maximum Residue Limits in Food and Animal Feedstuff (APVMA, June 2005), the residue definition of omethoate is defined as 'omethoate'. In its consideration of the residues of omethoate, the JMPR (1971) postulated that the metabolic route of omethoate would follow that observed for dimethoate in plants and animals, though the rates of individual reactions may vary between the two compounds. On this basis, the meeting considered that it was appropriate to determine omethoate residues in terms of the parent omethoate. In 1984, the JMPR considered a radiolabelling study in which the intermediates of omethoate degradation in sugarbeet were identified. This showed "*that residues are converted into natural plant products through a number of well-defined steps*". Omethoate is also included in the residue definition for dimethoate ('sum of dimethoate and omethoate expressed as dimethoate'). This will be considered as part of the review of dimethoate.

ADI/ARfD

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

The current ADI for omethoate of 0.0003 mg/kg bw/d was set in February 1989. It was based on a NOEL of 0.025 mg/kg bw/d for RBC ChE inhibition in a 12-month gavage study in dogs (Hoffmann and Schilde, 1984), using a 100-fold safety factor. This review recommends that the ADI be revised to 0.0004 mg/kg bw/d, based on a NOEL of 0.04 mg/kg bw/d for inhibition of ChE activity in a 2-year rat dietary study, using a 100-fold safety factor.

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

value for omethoate. An ARfD of 0.003 mg/kg bw is established based on a NOEL of 0.25 mg/kg bw for inhibition of ChE activity in an acute oral neurotoxicity study in rats, incorporating a safety factor of 100.

Drinking Water Quality Guidelines

There is no current health guideline value for omethoate in drinking water in Australia. Health Values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The values are derived so as to limit intake *from water alone* to about 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg at a daily water consumption of 2 L over a lifetime. Given that the recommended ADI for omethoate is 0.0004 mg/kg bw/d, the Health Value may be calculated as:

$$\frac{0.0004 \text{ mg/kg bw/d} \times 70 \text{ kg} \times 0.1}{2 \text{ L/d}}$$

$$= 0.001 \text{ mg/L}$$

Hence, the Health Value for omethoate should be set at 0.001 mg/L. This value is included in the current draft of the Australian Drinking Water Guidelines (NHMRC, 2010).

Poisons Scheduling

Omethoate is in S7 except when included in S6 (cut-off to S6 at 30% omethoate), or in pressurised spray packs containing 0.2 % or less of omethoate, when it is included in S5 (SUSMP 1 September 2010). In 1993 the DPSSC reviewed toxicology data in support of an application for re-scheduling formulations containing 5% or less of omethoate to S5. At this time, the cut-off from S7 to S6 was revised from 50% to 30%. The Committee considered the product Folimat 50 Garden Insecticide was not appropriate for home garden use, as its acute oral LD_{50} of 762 mg/kg bw was less than the recommended limit (according to NHMRC Guidelines at that stage) of 1500 mg/kg bw for products destined for the domestic market. The Committee rejected the company's proposal for an S5 entry, except in the case of the 0.2% omethoate aerosol product, and agreed that Folimat 50 Garden Insecticide should not be supported. In response to strong objections from the company regarding the withdrawal of Folimat 50 Garden Insecticide from the home garden market, the Committee re-visited omethoate in 1994. The Committee considered that as one swallow of the formulation could be fatal to a 10 kg toddler, its previous decision was appropriate. The Committee also considered that the child resistant closure on the 100 mL bottle was not adequate to offset the toxicity risk and the tamper-proof seal would not act as a protection after the first use of the container. As none of the new information considered in this review provides any evidence to the contrary, the current scheduling of omethoate is considered appropriate.

First-Aid Instructions

In the edition dated 31 March 2011, the following standard statements for omethoate are specified in the FAISD Handbook – *Handbook of First Aid Instructions, Safety Directions, Warning Statements and General Safety Precautions for Agricultural and Veterinary*

Chemicals (http://www.tga.health.gov.au/docs/pdf/faisd.pdf).

in home garden preparations	a	If poisoning occurs, contact a doctor or Poisons Information Centre. *Phone Australia 131126.*
in 0.2 per cent pressurised spray packs	o	If sprayed on skin, wash thoroughly. If sprayed in mouth, rinse mouth with water.
in other preparations	m	If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131 126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.

The first aid instruction '**a**' is considered appropriate for the product considered acceptable for use in the home garden product (see below), and as this product is packaged in a pressurised container, first aid instruction '**o**' is also appropriate for this product. The Handbook of First Aid Instructions and Safety Directions was amended in 2008, during the course of this review, to replace first aid instructions 'a, h' with '**m**'. This change applies to all organophosphate chemicals, and more details can be found in OCS (2008). The amended instruction should appear on the labels for the omethoate products for commercial use.

Safety Directions

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

Existing Safety Directions

AC 500-800 g/L	
100 101 120 121 130 131 132 133 180 190 181 210 211 212 220 223 373 279 281 290 292 294 296 340 342 350 360 361 362 366	Very dangerous, particularly the concentrate. Product and spray are poisonous if absorbed by skin contact or inhaled or swallowed. Repeated exposure may cause allergic disorders. Repeated minor exposure may have a cumulative poisoning effect. Sensitive workers should use protective clothing. Avoid contact with eyes, skin and clothing. Do not inhale spray mist. Obtain an emergency supply of atropine tablets 0.6 mg. When preparing spray wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and face shield. If product on skin, immediately wash area with soap and water. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, face shield and contaminated clothing.
AC 290 g/L or less	
120 121 130 131 132 133 161 162 180 190 373 210 211 220 223 279	Product and spray are poisonous if absorbed by skin contact or inhaled or swallowed. Will irritate the

281 282 290 292 294 296 (293 298 seed dressing only) 330 332 340 342 340 343 350 360 361 362 366	eyes. Repeated exposure may cause allergic disorders. Repeated minor exposure may have a cumulative poisoning effect. Obtain an emergency supply of atropine tablets 0.6 mg. Avoid contact with eyes and skin. Do not inhale spray mist. When preparing and using the spray wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves and face shield (PVC or rubber apron and impervious footwear, seed dressing only). If clothing becomes contaminated with product, remove clothing immediately. If product on skin, immediately wash area with soap and water. If product in eyes, wash it out immediately with water. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves and face shield and contaminated clothing.

AC 50 g/L or less	
120 129 133 161 162 180 190 373 210 211 220 223 279 281 282 290 292a 294 299 330 332 340 342 340 343 350 360 361 365 366	Product is harmful if swallowed. Will irritate the eyes. Repeated exposure may cause allergic disorders. Repeated minor exposure may have a cumulative poisoning effect. Obtain an emergency supply of atropine tablets 0.6 mg. Avoid contact with eyes and skin. Do not inhale spray mist. When preparing spray and using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), a washable hat, elbow-length PVC gloves and face shield or goggles. If clothing becomes contaminated with product, remove clothing immediately. If product on skin, immediately wash area with soap and water. If product in eyes, wash it out immediately with water. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, face shield and contaminated clothing.
HG AE 2 g/kg or less 350 g pack	
120 129 132 161 162 180 210 211 220 223 351	Product is harmful if inhaled. Will irritate the eyes. Repeated exposure may cause allergic disorders. Avoid contact with eyes and skin. Do not inhale spray mist. Wash hands after use.

The recommended revised safety directions are based on the risks identified in this evaluation and on the OHS risk assessment conducted by the OCS in 2006. A summary of the risks associated with specific omethoate products identified in this evaluation is presented below.

Details of the estimation of the toxicity of the products from the toxicity and concentration of the individual ingredients, are provided in Appendix VIII.

Folimat® 800 Insecticide Spray

This product is available in 5 L and 20 L containers. It is to be applied as a dilute spray at 35-75 mL/100 L, or as a concentrated spray at no greater than 5 times the dilute spraying rate to various crops when pests are first seen, and to be repeated as advised on the label for particular insect pests at the beginning of renewed insect activity, or to coincide with peak hatching. It can also be applied ULV in water using an anti-evaporative oil. The methods of application are by dilute or concentrate spraying equipment, by ground rig or air, and for bananas, by bell injection at 50 mL/5L or as an individual plant treatment at 125 mL/100 L.

This product is expected to have high oral toxicity, and moderate dermal and inhalation toxicity. It is likely to be a moderate eye irritant and a skin sensitiser, but is not expected to cause skin irritation. The current safety directions for AC 500-800 g/L should be amended to take into account the eye irritation potential. As there are data for a 500 SL formulation containing the same excipient as the current product, and for which the toxicity profile is similar to that deduced for Folimat 800 Insecticide Spray, it is appropriate for the entry to cover the concentration range of 500-800 g/L omethoate.

Le-mat 290 SL Insecticide

This product is available in 1 L and 10 L containers, to be applied by boom sprayers or aircraft at 100-300 mL/ha, with a follow-up spray if a second hatching occurs. Actual spray concentrations are not indicated on the label. Estimation of the toxicity of this product from the toxicity of its individual constituents indicates that Le-mat 290 SL Insecticide is expected to have moderate oral and inhalation toxicity, and low dermal toxicity. It is expected to be a moderate eye irritant and a skin sensitiser, but not a skin irritant.

Folimat® Garden Insecticide (AE, 2 g/kg omethoate)

Folimat Garden Insecticide is available as a 0.2% aerosol in a spray can (350 g net). Foliage is to be sprayed from a distance of 30 cm when insects are first seen or if insects re-appear, or in the case of treatment for codling moth, every 14 to 21 days from petal fall until 7 days before harvest.

As this product is for use in the home garden, first aid instructions '**a**' and '**o**' apply. First aid instruction '**a**' is not present on the current label. This should be added. The first sentence of first aid instruction '**o**' is reproduced on the label, but the next sentence on the label states, 'If sprayed in mouth, give milk or water.' This should be amended to 'If sprayed in mouth, rinse mouth with water.'

A 10 kg child would need to ingest approximately 40 mL of a 0.2% omethoate product to reach the dose at which clinical signs were seen in rats after a single oral administration. As little as 6-13 mL would need to be ingested to achieve the dose at which brain ChE activity was inhibited in rats. Considering the presentation of this product, it is unlikely that it will be ingested by a small child. Omethoate is considerably less toxic by dermal exposure, relative to

oral route. The dermal LD_{50} for 24 h exposure was 865 mg/kg bw for female rats, the more sensitive sex, relative to ~25 mg/kg bw by the oral route.

The low concentration of this product, and its presentation in a pressurised spray pack of 350 mL, makes it suitable for application in the home garden. Little human exposure to omethoate is expected from its use according to label instructions. On this basis, it was not considered necessary to perform an operator exposure and risk assessment for this product.

By estimation from the toxicity of its individual ingredients, this product is expected to have low acute oral, dermal and inhalation toxicity. It is likely to be a slight skin irritant and a moderate eye irritant, and possibly a skin sensitiser.

Folimat® 50 Garden Insecticide

This product is packaged in 100 mL containers, for use in the home garden. It is to be diluted in water (60 mL/5L) and sprayed on flowers, ornamental trees and shrubs, vegetables and herbs, eucalyptus, citrus and apples when insects are first seen, and when they re-appear. For codling moth, spraying is recommended every 2-3 weeks from petal fall, until 7 days before picking.

The acute oral toxicity of the product is of concern for use in the home garden situation. A submitted study using a formulation containing 50 g/L omethoate resulted in an oral LD_{50} of 762-862 mg/kg bw, a level well below that considered by the APVMA as appropriate for use in the domestic situation. As the main excipient in Folimat® 50 Garden Insecticide is no longer the same as in the formulation tested, it is appropriate to estimate the acute oral toxicity of the product using an LD_{50} of ~25 mg/kg bw for omethoate. This gives an oral LD_{50} for the product of ~500 mg/kg bw. Therefore, a 10 kg child would need to ingest around 5 mL of the product to achieve a potentially fatal dose. The product is expected to have low acute dermal and inhalation toxicity, to be a moderate eye irritant, a slight skin irritant, and a skin sensitiser. The main excipient in this product also raises concerns, as it has been reported to be a reproductive and developmental toxicant via the dermal route (see Appendix VIII). In 1994 the NDPSC considered that the child-resistant closure was not adequate to offset the toxicity risk, and the tamper-proof seal would not be protective after the first use of the container. Taking all of these considerations into account, it is not appropriate for this product to be registered for home garden use. Home garden products should contain no more than about 17 g/L of omethoate.

RECOMMENDATIONS

1. Approval Status

There is no objection on toxicological grounds to the ongoing approval of omethoate from the existing sponsors and manufacturers. An upper limit of 20 g/kg ai is recommended for the impurity O,O,S-trimethyl phosphorothioate (O,O,S-TMP) based on the potential human health risks identified in the OCS (2010) human health risk assessment of O,O,S-TMP.

2. Acceptable Daily Intake

The current ADI for omethoate is 0.0003 mg/kg bw, derived by applying a 100-fold safety factor to a NOEL for inhibition of RBC cholinesterase activity in a 1-year gavage study in dogs.

This review recommends that the ADI be revised to 0.0004 mg/kg bw, based on a NOEL for inhibition of cholinesterase activity in a 2-year rat dietary study, and incorporating a 100-fold safety factor.

3. Acute Reference Dose

An ARfD of 0.003 mg/kg bw is recommended, derived by applying a 100-fold safety factor to a NOEL of 0.25 mg/kg bw for inhibition of cholinesterase activity in an acute oral neurotoxicity study in rats.

4. Water Quality Guidelines

It is recommended that the NHMRC set the Health Value for omethoate in drinking water at 0.001 mg/L. This value is included in the current draft NHMRC Drinking Water Guidelines (NHMRC, 2010).

5. Poisons Scheduling

The current poisons schedule of omethoate remains appropriate.

6. First Aid Instructions and Safety Directions

NOTE: With the exception of products intended for home garden use, the OHS risk assessment of omethoate conducted by the OCS in 2006 proposed safety directions for registered omethoate products including engineering controls and changes that are necessary to personal protective equipment. These proposed safety directions have been incorporated into the following recommendations to be included in omethoate entry in the FAISD Handbook.

The following FAISD entries for the evaluated omethoate products which are considered acceptable for continued registration are shown below. The current entry for AC 50 g/L or less should be deleted. The current entries for AC 500-800 g/L, AC 290 g/L or less and HG AE 2 g/kg or less 350 g pack should be amended to the following.

AC 800 g/L or less Note: closed mixing/loading and enclosed cab application

HAZARDS

Very dangerous, particularly the concentrate	100, 101
Product and spray are poisonous if absorbed by skin contact or inhaled or swallowed	120, 121, 130, 131, 132, 133
Do not inhale spray mist	220, 223
Will irritate the eyes	161, 162
Repeated minor exposure may have a cumulative poisoning effect	190

PRECAUTIONS

Avoid contact with eyes and skin and clothing	210, 211, 212
Repeated exposure may cause allergic disorders	180

MIXING OR USING

If clothing becomes contaminated with product or wet with spray remove clothing immediately	330, 331, 332
If product on skin, immediately wash area with soap and water	340, 342
If product in eyes, wash it out immediately with water	340, 343
When opening the container and preparing spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), elbow-length chemical resistant gloves, and face shield	279, 280, 281, 290, 292b, 294c, 296

AFTER USE

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, face shield and contaminated clothing	360, 361, 362, 366

In addition to closed mixing and loading a face shield, gloves and cotton overalls have been recommended for workers opening product containers and preparing spray due to the high acute toxicity of omethoate.

AC 500 - 800 g/L Note: hand held application

HAZARDS

Very dangerous, particularly the concentrate	100, 101
Product and spray are poisonous if absorbed by skin contact or inhaled or swallowed	120, 121, 130, 131, 132, 133
Do not inhale spray mist	220, 223
Will irritate the eyes	161, 162
Repeated minor exposure may have a cumulative poisoning effect	190

PRECAUTIONS

Avoid contact with eyes and skin and clothing	210, 211, 212
Repeated exposure may cause allergic disorders	180

MIXING OR USING

If clothing becomes contaminated with product or wet with spray remove clothing immediately	330, 331, 332
If product on skin, immediately wash area with soap and water	340, 342
If product in eyes, wash it out immediately with water	340, 343
When opening the container, preparing spray and using	279, 280, 281, 282, 290,

the prepared spray, protective waterproof clothing, elbow-length chemical resistant gloves, and full facepiece respirator	291, 294c, 301
AFTER USE	
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 respirator (and if rubber wash with detergent and warm water) and contaminated clothing	360, 361, 364, 366

HG AE 2 g/kg or less 350 g pack

HAZARDS	
Will irritate the eyes	161, 162,
May irritate the skin	160, 164
PRECAUTIONS	
Avoid contact with eyes and skin	210, 211
Repeated exposure may cause allergic disorders	180
MIXING OR USING	
If product on skin, immediately wash area with soap and water	340, 342
If product in eyes, wash it out immediately with water	340, 343
AFTER USE	
Wash hands after use	351

The following first aid instructions and warning statement have been recommended to appear on omethoate product labels (all formulations and strengths).

First aid instruction 'h' has been deleted from the Handbook of First Aid Instructions and Safety Directions. The following First Aid Instruction entry now appears for omethoate:

in 0.2 per cent pressurised spray packs	o	If sprayed on skin, wash thoroughly. If sprayed in mouth, rinse mouth with water.
	a	If poisoning occurs, contact a doctor or Poisons Information Centre. Phone Australia 131126, New Zealand 0800 764 766.
in other preparations	m	If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131 126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.

Warning Statement:

"Do not work outside enclosed cabs during application unless wearing gloves, cotton overalls and hat, and a respirator."

7. Product Registration

On the basis of its high acute oral toxicity and the concern that accidental ingestion by a child is likely to lead to irreversible effects, the continued registration of Folimat® 50 Garden Insecticide containing 50 mg/L omethoate is not supported. There are no objections on toxicological grounds to the continued registration of the other omethoate products.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

Omethoate is an organophosphorous compound that as well as existing as a pesticide in its own right, is also a toxicologically important metabolite of dimethoate. Omethoate has both direct and systemic action against a broad range of insect pests in various crops and pastures, and in the home garden. As with other OP pesticides, the mode of action of omethoate is through inhibition of cholinesterase activity, with cholinesterases from invertebrate sources being more sensitive to inhibition than mammalian cholinesterases. As of May 2011, omethoate is the active constituent in eleven products registered in Australia.

1.1 History of Public Health Considerations in Australia

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

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

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

A third committee formerly involved in chemicals management was the NHMRC Standing Committee on Toxicity (SCOT), which was active between 1985 and 1994. SCOT was

responsible for providing specialised advice on complex toxicological matters to all the NHMRC Public Health Committee subordinate committees, including the PACC and NDPSC. In response to referrals from these committees, SCOT undertook evaluation of some drugs, pesticides, food additives, poisons, consumer products, chemicals and other hazardous substances relevant to public health.

Omethoate

Maximum Residue Limits for omethoate have been set in cereal grains, edible offal (mammalian), eggs, fruits, lupins (dry), meat (mammalian), milks, oilseed, capsicums, the edible offal of poultry, poultry meat, tomato, and vegetables other than those mentioned individually. A detailed history of the consideration of omethoate by regulatory committees in Australia is presented in Table 4. Where considerations were limited to MRLs for omethoate, these have been omitted.

Table 4. Consideration of omethoate by regulatory bodies in Australia

Date	Regulatory Activity
July 1968	DPSC: omethoate placed in S7 on the basis of an oral LD50 in the rat of 50 mg/kg bw.
1969	NHMRC: set ADI of 0.000006 mg/kg bw/d, based on a NOEL of 0.125 mg/kg bw/d in a 16-week dietary study in the rat, and applying a safety factor of 2000.
August 1974	DPSC: granted an S6 entry for 50% omethoate or less, as part of an overall review of pesticide scheduling.
August 1982	PACC: In response to a company request, the Committee determined that residues arising from the use of HG omethoate products according to label directions should not cause a hazard to consumers of the fruit and vegetables so treated. This conclusion was based on the Committee's previous review of the toxicology and residue data for omethoate.
November 1988	DPSC: the Committee had no objection to clearance of the TGAC omethoate, but was concerned that such a toxic product, even at 50% (S6) should be available to the home garden market. Scheduling action was postponed until the NHMRC Toxicology Unit submitted a review on S7 poisons with low strengths in S6, and more information about a 0.2% aerosol product in S5 was available.
February 1989	PACC: amended the ADI to 0.0003 mg/kg bw/d based on a NOEL of 0.025 mg/kg bw/d for inhibition of RBC cholinesterase activity and a safety factor of 100. It was noted that a number of studies that had been submitted to the JMPR had not been submitted to the NHMRC. The applicant was to supply the studies.
February 1993	DPSC: Committee rejected a proposal that products containing 5% omethoate or less were appropriate for S5, except in the case of the 0.2% aerosol product. In addition, the cut-off to S6 (which was 50% or less) was reduced to 30% or less because of concerns about omethoate's toxicity and the possibility of severe eye irritation. Furthermore, the Committee recommended that the NRA be advised that a HG product containing 5% omethoate was not appropriate, as it did not meet NHMRC guidelines for pesticide use in the home garden (i.e. the LD_{50} was less than the recommended 1500 mg/kg bw).

Date	Regulatory Activity
April 1994	NDPSC: Consideration of a company request to reconsider the scheduling decisions of the February 1993 meeting for formulations containing omethoate. The Committee noted that the company had made a significant error in their calculations, and concluded that the toxicity of the product was such that little more than one swallow of the formulation could be fatal to a 10 kg toddler. The Committee agreed that the original DPSSC decision not to support the 5% omethoate product for the home garden was appropriate. The Committee also considered the child-resistant closure was not adequate to offset the toxicity risk and the tamper-proof seal would not act as a protection after the first use of the 100 mL container.

ADI

The current ADI for omethoate is 0.0003 mg/kg bw. This ADI was derived from a NOEL of 0.025 mg/kg bw/d for inhibition of RBC cholinesterase activity seen in a 12-month dog study, and using a 100-fold safety factor.

Poisons Scheduling

Omethoate is included in Schedule 7 except when included in Schedule 6 (in preparations containing 30 per cent or less of omethoate except when included in S5) or in Schedule 5 (in pressurised spray packs containing 0.2 per cent or less of omethoate).

Levels in drinking water

Omethoate is not included in the current version of the Australian Drinking Water Guidelines (NHMRC, 2004). However, a health guideline value of 0.001 has been proposed for inclusion in the next publication of the Guideline (NHMRC, 2010).

1.2 International Toxicology Assessments

JMPR

Omethoate was evaluated by the Joint Meeting in 1971, and reviewed in 1975, 1978, 1979, and 1981 (Annex 1, FAO/WHO, 1972a, 1976a, 1979a, 1980a, and 1982a). In 1984 an acceptable daily intake of 0.0003 mg/kg bw/d was set, based on the NOEL of 0.025 mg/kg bw/d for inhibition of erythrocyte cholinesterase activity in a 12-month gavage study in dogs. Prior to this, the ADI for omethoate had temporary status due to the lack of an adequate chronic/carcinogenicity study. Omethoate was also evaluated in 1996 as part of the JMPR evaluation of dimethoate. As the primary manufacturer was no longer producing omethoate, the previous ADI for omethoate was withdrawn.

1.3 Chemistry – Technical Active

Approved common name: Omethoate (ISO)

Alternative names:

Chemical name: (IUPAC) *O,O*-dimethyl-S-methylcarbamoylmethyl phosphorothioate

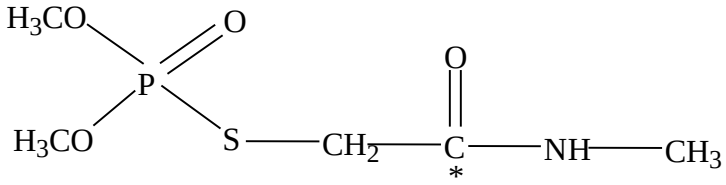
(CAS) *O,O*-dimethyl S-[2-(methylamino)-2-oxoethyl]-phosphorothioate

CAS Registry number: 1113-02-6

Empirical formula: $C_5H_{12}NO_4PS$

Molecular weight: 213.2

Chemical structure:



Isotope label: The position of the radiolabel (^{14}C) is indicated by an asterisk*

Chemical class: Organophosphorous compound

Chemical and physical properties *(technical grade unless otherwise indicated)*

Colour:	Colourless
Odour:	Like mercaptan
Physical state:	Oily liquid
Melting point:	N/A
Density (20^0C):	1.319 g/L
Partition coefficient: (log K_{ow})	Pure active: log P = -0.74
Vapour pressure:	Pure active: 3.3 x 10^{-5} mbar at 20°C
Vapour saturation concentration at 20°C	0.29 mg/m^3 air
Solubility:	
in water:	>200 g/L at 20°C
in organic solvents:	Information not available for technical grade. For pure omethoate, solubility <0.1 g/L in N hexane, 50-100 g/L in toluene, miscible with dichloromethane or 2-propanol.
Stability:	Pure active: stable in air; stable at room temperature; not photolytically degraded in aqueous solution by light of wavelength >290 nm.

Technical active - Declaration of Composition and Batch Analysis

Declarations of composition for technical grade omethoate are shown in Appendix VI.

Impurities of Toxicological Concern

The APVMA Standards for Active Constituents currently stipulate the upper impurity limit for omethoate as:

O,O,S-trimethyl phosphorothioate: 5 g/kg maximum

There are no FAO specifications listed for omethoate.

1.4 Products

As of May 2011, omethoate is the active constituent in eleven products registered in Australia. At the beginning of this assessment, there were 4 registrants for omethoate in Australia and 5 registered products, 4 of which are included in this review. These products are registered for the treatment of a wide range of pests including aphids, thrips, caterpillars, bugs, mealy bugs, mites, whiteflies, leaf eating beetles, citrus leaf miner, codling moth, mirids, jassids, and lucerne flea in flowers, ornamental trees and shrubs, vegetables, herbs, eucalyptus and other native plants, several fruits, various crops and pastures such as cereals, oilseed, legumes, vetch, Faba bean, cotton and lucerne, and as a barrier spray.

2. METABOLISM AND TOXICOKINETICS

2.1 Rats

Weber H, Patzschke K, Wegner LA (1978) [^{14}C]Omethoate (®Folimat active ingredient) biokinetic studies on rats. Pharma Report No. 7669. Lab: not stated. Sponsor: Bayer AG, Isotopenlabor, Institut für Pharmakokinetik, Werk, Elberfeld, Wuppertal. Expt. dates: not stated. Unpublished report date: 29 June 1978. (QA, GLP, Test Guidelines: none).

Materials and Methods

^{14}C-Omethoate (~40 µCi/mg; radiochemical purity >98%) was administered to male rats (Sprague Dawley; average weight 200 g) as single doses at 0.3 mg/kg iv, or 0.3, 1, 5, or 10 mg/kg po in physiological saline. Radioactivity in the urine, faeces, and body (with separate measurement for GIT) was measured at 48 hours post-treatment for all doses. Elimination of radioactivity in the urine was followed at 0.3 and 5 mg/kg bw for 48 h post-treatment. At 5 mg/kg bw, radioactivity was measured in the body (minus the GIT) and a range of tissues at 1, 3, and 8 hours, and 1, 2, 3 and 6 days post-treatment, and in the plasma at various times up to 24 h. Whole body autoradiography was performed, though details of the methods were not reported.

Results

Recovery of administered radioactivity was 84-100%. Almost the entirety of each dose (96-97%) was eliminated in the urine within 48 h of dosing, indicating virtually total absorption. The remaining radioactivity was accounted for in the faeces (1-2%), with minor amounts in the body and GIT. A preliminary study using a dose of 1 mg/kg bw showed that radioactivity in expired air was ~1% of the administered dose. Elimination via the urine was almost complete within 12 h of dosing, at oral doses of 0.3 and 5 mg/kg bw. Peak levels in the plasma and other tissues were reached at 1 hour after oral dosing at 5 mg/kg bw, with little remaining at 24 h. There was not much difference in radioactivity between the tissues, including the brain, but of the organs analysed, the concentration was greatest in the kidney (7 µg equivalents/g), and the lowest in renal fat (~2.7 µg equivalents/g). A supplementary experiment performed as a result of findings in whole body autoradiograms, showed that radioactivity was high in the thyroid gland relative to other tissues. The half-life for elimination in the plasma was ~2 h for the first 6 h post-dosing, and ~6 h from 6 to 24 h. Further elimination was at a much slower rate, with a half-life of approximately 2 days for the period 2-6 days post-treatment.

Whole body autoradiography showed evenly distributed high levels of radioactivity at 5 min after a 0.3 mg/kg bw iv dose. At 8 h after an oral 3 mg/kg bw dose, autoradiographs showed that levels of radioactivity in the thyroid gland, pineal gland, intestines and testes were high relative to other tissues. At 24 h after an oral dose of 5 mg/kg bw, only slight darkening of the autoradiographs was apparent for the majority of tissues, with relatively high levels of radioactivity in the thyroid, pineal gland, eye, olfactory mucosae, seminal vesicles, urinary bladder and testes.

Table 5. Radioactivity in the excreta and body 48 h after dosing (% administered radioactivity)

Dose (mg/kg bw)	Route	Number of rats	Urine	Faeces	Body without GIT	GIT
0.3	iv	4	97.2 ± 1	~1.4	0.41 ± 0.09	0.032 ± 0.004
0.3	oral	5	97.1 ± 3	~1.3	0.49 ± 0.14	0.038 ± 0.002
5	oral	5	97.0 ± 4	1.6 ± 0.3	0.32 ± 0.07	0.027 ± 0.004
10	oral	5	96.0 ± 7	2.1 ± 0.3	0.59 ± 0.3	0.055 ± 0.02

Ecker W, Coelin R (1981) Omethoate: biotransformation of dimethyl S (N-methyl-[^{14}C]carbamoylmethyl)phosphorothioate. Pharma Report No. 10100. PF-Report No. 1574. Lab: PH-E Isotope Lab, Chemistry, Institute of Pharmacokinetics, Wuppertal. Sponsor: Mobay Chemical Corporation, Agricultural Chemicals Division. Unpublished report date: 7 August 1981. (QA, GLP, Test Guidelines: none).

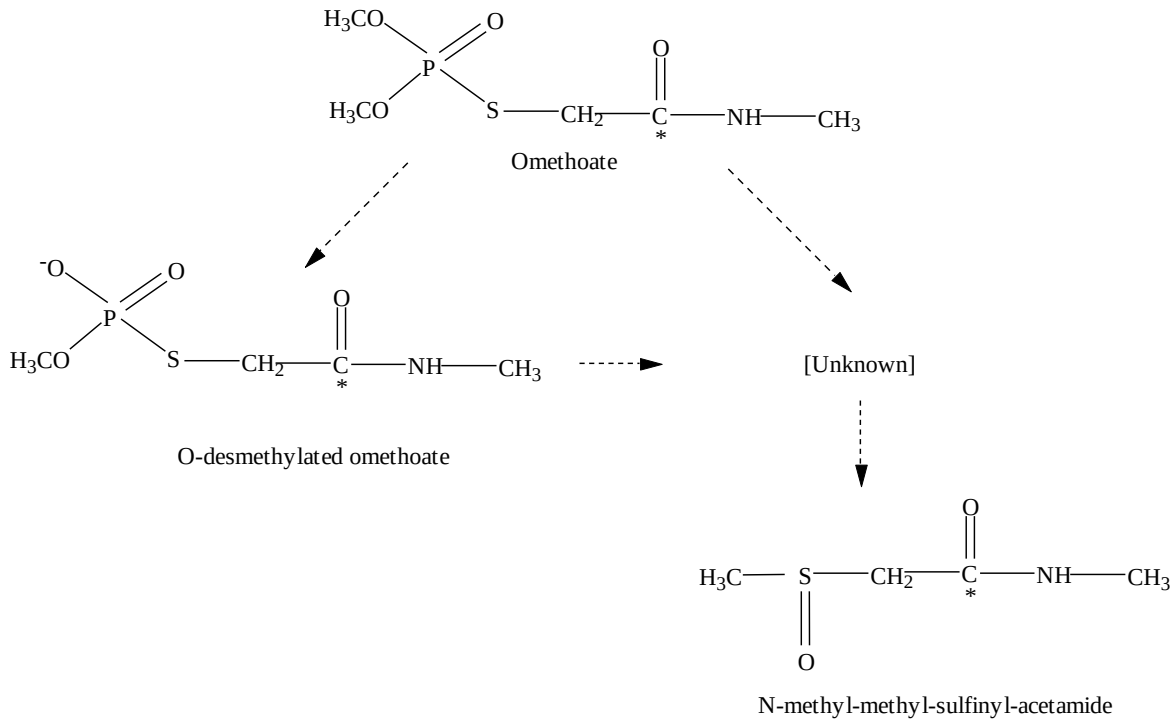
Materials and Methods

Carbonyl-^{14}C-labelled omethoate (part from the sponsor, and part synthesised at the isotope lab., 40 µCi/mg and 97 µCi/mg, and radiochemical purity 99 and 98%, respectively) was administered by gavage at 5 mg/kg bw in an aqueous solution containing 5% Cremophor EL, to male Sprague Dawley rats (SPF breed, Mus-Rattus AG, Munich, ~200 g bw). Urine samples were collected for the interval 0-8 h post-treatment. As no difference was detected in the metabolites present (using TLC), all urine samples were combined. Metabolites were identified using pure standards. For the initial experiment, which used 3 rats, the standards available were the parent compound, and the O-desmethylated and thio-carboxy forms of omethoate. For the second experiment, using the urine collected from one rat, additional metabolites were synthesised (N-methyl-mercaptoacetamide, N-methyl-methylmercapto-acetamide, N-methyl-methylsulfinyl-acetamide, N-methyl-methyl-sulfonyl- acetamide).

Results

Approximately 88% of the administered radioactivity was recovered in the urine within 8 h of dosing. For the 2 experiments respectively, 26% or 44% of the administered radioactivity appeared in the urine as unchanged parent compound, 22% or 13% as N-methyl-methyl-sulfinyl-acetamide, and for the first experiment, 9.5% as the O-desmethylated form of omethoate. Each of the remaining unknown metabolites comprised less than 10% of the administered dose. Based on this limited information, the metabolic pathway shown below was proposed by the study authors.

Metabolic pathway of omethoate



* Indicates position of radiolabel

Hoshino T (1990) [Methylene-^{14}C]omethoate: General metabolism in the rat. Lab. Project ID M 01810019. Bayer AG, Crop Protection Research, Chemical Product Development and Environmental Biology, Institute for Metabolism Research, D-5090 Leverkusen-Bayerwerk, Germany. Sponsor: Bayer AG. Expt dates:18 July 1989 – 8 December 1989. Unpublished report date: 8 August 1990. (QA, GLP: Yes; Guidelines: US EPA 85-1 1982)

Materials and Methods

Single doses of [methylene-^{14}C]omethoate (purity 99.4%, specific activity 97 µCi/mg) were administered to male and female Wistar rats (5/group, from Winkelmann, Versuchstierzucht) at doses of 0.5 or 10 mg/kg bw by the iv or oral route (gavage). A repeat oral dose experiment was also performed in which unlabelled omethoate (99.2% pure) was administered at 0.5 mg/kg bw/d for 14 consecutive days, followed on day 15 by a single oral dose of labelled omethoate, also at 0.5 mg/kg bw. At various times after dosing, radioactivity was measured in the excreta and plasma, and also in the organs and tissues at scheduled necropsy (48 h after administration of the radiolabelled material).

Results and Conclusions

In all cases, the administered radioactivity was rapidly excreted. Total recovery was 89-98%, with 88-98% appearing in the excreta within 48 h of dosing. The vast majority of this was in the urine (85-96% of administered dose), with nearly all (83-95% of administered dose) appearing in the urine in the first 24 h. The AUC was similar for iv and gavage administration, confirming almost total absorption from the GIT. The maximum plasma level after oral administration occurred at 40 min to 1 h post-dosing. Approximately 2-4% of the

administered dose was accounted for in the faeces. Little radioactivity was found in the body at necropsy, with 0.03-0.04% of the administered radioactivity in the GIT and a total of 0.24-0.42% in the rest of the body. In all groups at necropsy, the highest relative tissue concentration of radioactivity was found in the thyroid, representing 0.34-0.59% of the administered dose in the 0.5 mg/kg bw groups, and 0.16-0.19% in the 10 mg/kg bw groups, respectively representing levels 112- to 197-fold and 65- to 75-fold higher concentrations than in the plasma. The liver, kidney, testes, spleen and lung also had high concentrations of radioactivity relative to plasma.

The metabolism of omethoate appeared to be similar in all groups. The parent compound was the main form of radioactivity detected in the urine, representing 26-62% of the administered radioactivity. There were two major metabolites, N-methyl-2-(methylsulphinyl)acetamide (16-36%) and the O-desmethyl metabolite of omethoate (free form 4-9%). Proportionately more radioactivity was detected in the form of omethoate in females, while relative to females, more N-methyl-2-(methylsulphinyl)acetamide was detected in males. A higher percentage of the administered radioactivity was detected as omethoate in the 10 mg/kg bw group relative to the 0.5 mg/kg bw groups. Unidentified metabolites, each amounting to less than 10% of administered radioactivity, were also present. For the small amount of radioactivity present in the faeces, most was present as the O-desmethyl metabolite, the remainder comprising similar amounts of the sulphinyl metabolite and unchanged omethoate.

3. ACUTE STUDIES

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 omethoate is shown in the Tables 7, 8 and 9. In general, the signs of acute omethoate intoxication are consistent with ChE inhibition.

The acute oral toxicity of omethoate was high. Clinical signs such as trembling, muscle spasms, red tears and breathing difficulties appeared within an hour of single oral dose administration, resolving in 1-12 days. Deaths occurred in the interval 1-4 days post-dosing. In the study of Kimmerle (1968) that tested rats and mice under similar conditions, mice were more sensitive than rats to the effects of omethoate poisoning. All but one of the acute oral studies were pre-1980, and except for the study of Flucke (1978) and Krötlinger (1989a), which stated that the rats were fasted before dosing, the animals were fed when treated, or their feeding status was not reported. The relatively low LD_{50}s of the Flucke (1978) and Krötlinger (1989a), studies may be related to the fasted state of these animals. When Schrader (1962) tested a range of species (rats, cats and rabbits) all showed similar susceptibility to the effects of omethoate administered as an acute oral dose, with guinea pigs less so. However, the accuracy of this information is very approximate in cats and rabbits, given the low numbers of doses and animals tested.

Acute dermal studies were conducted under a variety of conditions, with considerable variation in the length of time between application of the test material and its removal (4h – 7 d). The doses at which deaths occurred decreased with increasing length of exposure. Of the studies evaluated, the study by Flucke (1978) most closely matched modern guidelines. This study showed omethoate to have moderate dermal toxicity, with females slightly more sensitive than males. Clinical signs in the acute dermal studies included general infirmity and

behavioural disturbances, trembling, muscle spasms and dyspnoea, persisting in some cases for up to 10 days. Deaths were usually within 24 h of application, though this was extended to up to 6 days in the study of Krotlinger (1989b), in which the dermal toxicity of omethoate was apparently increased considerably by the use of an occluded dressing.

The acute inhalational studies also showed behavioural disturbances (lethargy, failure to groom), and typical cholinergic signs, persisting for 1-10 days. Deaths were within 1-3 d of exposure, the rats becoming heavily sedated prior to death. Mice appeared to be considerably more affected by omethoate than rats. The only study to provide information on particle size indicated MMADs of ~1.4 µm, with 100% of particles <5µm (Pauluhn 1989). Overall, the results of the 4 h exposure studies indicate that omethoate has moderate inhalation toxicity.

Table 6. Acute oral toxicity studies on omethoate.

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Mice [CF1]	M	15	Lutrol	NS	5 - 75 deaths: 3 at 25, 5 at 35, 13 at 50, 15 at 75	36	Kimmerle (1968)
Rat [NS]	M/F	5/sex	water	NS	0.5 - 100 deaths: 1 at 25, 3 at 50, 4 at 75, 5 at 100	>25, <50	Schrader (1962)
Rat [Wistar II]	M	15	Lutrol	NS	5 - 150 deaths: 2 at 35, 4 at 50, 8 at 75, 13 at 100, 15 at 150.	64.6	Kimmerle (1968)
Rat [Wistar]	M/F	15	distilled water	NS	5 - 50 deaths (M/F): 0/2 at 20, 3/4 at 22.5, 6/6 at 25, 10/12 at 30, 13/14 at 35, 15/15 at 50.	27.3 (M) 25.6 (F)	Flucke (1978)
Rat [Wistar strain Bor:WISW (Spf-Cpb)]	M/F	5	Deminer-alised water	96.0% and 94.7%	1.0 – 50 Deaths (M/F): 1/0 at 14, 1M at 20, 4/0 at 25, 1F at 26.5, 4F at 28, 4F at 31.5, 4F at 35.5, 5/5 at 50.	22 (M) 28 (F)	Krötlinger (1989a) GLP
Rabbit	NS	2	water	NS	5 - 100 deaths: 1 at 50, 2 at 100	~50	Schrader (1962)
Cat	NS	2	water	NS	10 - 100 deaths: 1 at 50, 2 at 100	~50	Schrader (1962)
Guinea pig	NS	3	water	NS	10, 25, 50, 100, 250 deaths: 1 at 100, 3 at 250	~100	Schrader (1962)

Abbreviations: NS=not specified;

Table 7. Acute dermal toxicity studies on omethoate.

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD50 (mg/kg bw)	Reference
Rat	NS	3	none	NS	140 - 1400 (4 h; possible oral exposure); 1 death at 1400	~1400	Schrader (1962)
Rat	NS	3	none	NS	140 – 1400 7 d; oral exposure prevented; deaths: 1 at 700, 3 at 1400	~700	Schrader (1962)
Rat [Wistar II]	M	5	none	NS	100 - 1000 (left on skin for 7 d) deaths: 1 at 250, all at 500 and 1000	250-500	Kimmerle (1968)
Rat [Wistar]	M/F	10	none	NS	100 - 1500 24h, with semi-occlusion deaths (M/F): 0/2 at 750, 2M at 850, 5F at 900, 4/9 at 1000, 9M at 1250, 10/10 at 1500	1018 (M) 865 (F)	Flucke (1978)
Rat [Wistar; Bor WISW SPF-Cpb]	M/F	5	Cellulose powder paste	96.0% and 94.7%	10-1000 24h, occluded Deaths (M/F): 1/3 at 160, 4F at 200, 4M at 250, 3/5 at 355, 4M at 500, 5/5 at 1000.	232 (M) ~145 (F)	Krötlinger (1989b) GLP

Abbreviations: NZW=New Zealand White; NS=not specified.

Table 8. Acute inhalation toxicity studies on omethoate.

Species [strain]	Sex	Group Size	Vehicle/ mode	Purity (%)	Concentrations Tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Mouse [CF1]	M	20	Alcohol/ Lutrol 1:1 (no skin contact)	NS	60 – 968 (1 h) deaths: 1 at 60, 3 at 100, 13 at 350, 15 at 670, 20 at 968.	>250 (1 h)	Kimmerle (1968)
					67 – 265 (4 h) deaths: 10 at 102, 13 at 130, 19 at 265	140 (4 h)	
Rat [Wistar II]	M	20	Alcohol/ Lutrol 1:1 (no skin contact)	NS	62 – 1520 (1 h) deaths: 2 at 600, 3 at 750, 4 at 1520.	>1520 (1 h)	Kimmerle (1968)
					75 – 330 (4 h) deaths: 1 at 147 and 183, 11 at 234, 18 at 330	240 (4 h)	
Rat [Wistar II]	M	20	50% formul-ation Alcohol/ Lutrol 1:1 (no skin contact)	NS	1 dose only, no deaths	>1250 (1 h)	Kimmerle (1968)
					28 - 1090 deaths: 3 at 255, 5 at 340, 9 at 430, 19 at 665, 20 at 1090	425 (4 h) (concentration of active)	
Rat [Wistar II]	M/F	10/sex	Ethanol/ Lutrol 1:1/nose only	94.0	56 – 1606 deaths (M/F): 5/2 at 830, 9/10 at 1600	1000 (1 h, M/F)	Thyssen (1978)
					deaths (M/F): 0/1 at 125, 9/8 at 260, 10F at 360, 8M at 680, 18M at 725	300 (4 h, M) 220 (4 h, F)	
Rat [Bor: WISW (SPF-Cpb)]	M/F	5/sex	Poly-ethylene glycol E 400 and ethanol 1:1/ Nose only	97.4%	28.8-508 mg/m^3 Deaths (M/F): 0/0 at ≤88, ½ at 251, 4/5 at 398, 5/5 at 416 and 508.	287 mg/m^3 (4h, M/F)	Pauluhn (1989)

Abbreviations: SD=Sprague-Dawley; NS=not specified.

Flucke W (1978) S 6876, the active ingredient of ®Folimat. Studies on acute toxicity to rats and determination of cholinesterase activity in blood plasma, erythrocytes, and brain. Bayer Report No. 7373. Sponsor: Bayer AG, Institut fuer Toxicologie. Wuppertal-Elberfeld. Unpublished report date: 10 March 1978. (QA, GLP, Guidelines: none)

Fasted Wistar rats (Winkelmann, Borchen; 160-240 g) were dosed by gavage with omethoate dissolved in distilled water, after which they were observed for 7 days. The results of the acute oral and dermal LD_{50} determinations conducted as part of this study are summarised in the above tables (7, 8 and 9). Only the ChE experiment will be discussed here. Female rats (5/dose) were used, as they were shown to be more sensitive than males in the acute oral and dermal toxicity LD_{50} section of this study. Cholinesterase activity was determined by the Ellman method at 2, 5, 24 and 72 h after treatment in plasma, RBC and brain at doses of 0,

0.3, 0.6, 1.3, 2.6, 7.7 or 17.8 mg/kg bw, in two test series as shown in the Tables 10 and 11. At 7.7 and 17.8 mg/kg bw, clinical signs were general behaviour disturbances, muscle tremors, increased secretion, difficult breathing, and tonic and clonic convulsions. Cholinesterase activity was maximally decreased at 2 h and 5 h post-treatment in all 3 compartments. Brain ChE activity was the most sensitive, followed by plasma. Inhibition of plasma ChE activity was marginal at 1.3 mg/kg bw, but the degree of inhibition of brain ChE activity at this dose was considered toxicologically significant. The extent of inhibition of RBC ChE activity was similar across the dose range 2.6-17.8 mg/kg bw, suggesting that this assay lacked sensitivity. The reliability of this assay is also called into question by the large difference in the two control plasma ChE activities. The effects on ChE activity appeared to be reversible, with ChE activity in the RBC normalising by 72 h post-treatment, and in the brain and plasma by day 7. The no-effect level was 0.6 mg/kg bw, due to inhibition of brain ChE activity at 1.3 mg/kg bw.

Table 9. Cholinesterase activity in plasma and RBC (U/mL) at 2 h post-treatment, and % inhibition

Dose (mg/kg bw)	Plasma	RBC
0	1.44	2.26
0.3	1.30	2.05
0.6	1.57	2.06
1.3	1.17 (19%)	1.97 (12%)
0	2.06	2.62
2.6	1.28 (38%)	2.01 (23%)
7.7	0.81 (60%)	1.97 (24%)
17.8	0.53 (74%)	1.91 (27%)

Table 10. Cholinesterase activity in brain (U/mL) and % inhibition over the course of the study

	Time after administration						
Dose (mg/kg bw)	**0**	**2 h**	**5 h**	**24 h**	**72 h**	**7 d**	**14 d**
0	1.60	1.58	1.42	1.31	1.37	1.52	1.53
0.3	1.54	1.52	1.24	1.43	1.40	1.51	1.52
0.6	1.43	1.42	1.19 (16)	1.27	1.38	1.33	1.48
1.3	1.39	1.26 (20)	1.02 (28)	1.28	1.37	1.28 (15)	1.46
0	1.68	- *	1.65	1.46	1.45	1.58	1.28
2.6	1.73	0.53	0.78 (52)	1.34 (8)	1.25 (13)	1.30 (17)	1.27
7.7	1.69	0.38	0.55 (66)	0.94 (35)	1.07 (26)	1.32 (16)	1.23
17.8	1.87	0.32	0.38 (76)	0.84 (42)	0.91 (37)	1.30 (17)	1.37

* No reason was given for the omission of this data point

3.1.2 Skin and Eye Irritation Study

Pauluhn J (1983) S 6876 (The active ingredient of ®Folimat) (Common name: omethoate) Study of the irritant/corrosive effect on the skin and eye (rabbit). Bayer Report No. 11977. Lab: Bayer AG, Institut fuer Toxicologie, Wuppertal-Elberfeld, FRG. Sponsor: Bayer AG. Expt dates: July 1983. Unpublished report date: 2 August 1983. (QA, GLP: no; Guidelines: OECD 404 & 405).

Omethoate (purity 96.9%, batch no. 234 208 022), volume 500 µL, was applied to the clipped skin of 3 rabbits (HC:NZW, from Hacking and Churchill Ltd, Huntington) for 4 h under an occlusive dressing. The scores for erythema/eschar and oedema were all zero. Omethoate was non-irritant to rabbit skin in this test.

A volume of 100 µL was applied to the conjunctival sac of 3 rabbits, the contralateral eye serving as control. The treated eye was washed out with physiological saline after 24 h exposure. Lacrimation (grades 2-3), chemosis (grades 1-2) and redness (grades 1-2) peaked at 1 h after treatment, with chemosis and redness persisting till 24 h. Slight redness persisted in one rabbit till 48h and in another till 72 h. No effects were observed on the cornea or iris. Omethoate was a slight eye irritant in this test.

3.1.4 Skin Sensitisation Study

Flucke W (1984) S 6876 (c.n. omethoate) Study for skin-sensitising effect on guinea pigs in the open epicutaneous test. Study No. T 9016804. Report No. 13084. Lab: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld. Sponsor: Bayer AG. Expt dates: March-April 1984. Unpublished report date: 29 November 1984. (QA: yes; GLP: OECD (1983); Guidelines: no)

Materials and Methods

Omethoate (batch no. 234 208 022, purity 94.6%), formulated in demineralised water with 2% v/v Cremophor EL, was dermally applied to guinea pigs (DHPW strain from Winkelmann, Borchen, bodyweights 275-346 g) to test for skin sensitisation potential according to the open epicutaneous test of Klecak et al. (1977). Four test groups (0.3, 1.0, 3.0 and 10.0% omethoate), and 3 control groups (8 animals/group) were used. Fresh dilutions of the test material were prepared prior to each application. Twenty dermal inductions (5/week over 4 weeks) were performed by applying 0.1 mL of the appropriate mixture to the shorn left flank (8 cm^2), unoccluded. The test concentrations chosen were based on the results of a pilot study in which clinical signs and deaths were seen following 5 dermal applications of 0.1 mL of a 30% omethoate solution. For the challenges, which took place at 4, 6 and 8 weeks after the commencement of the induction period, 0.025 mL of omethoate at each of the concentrations used for induction was applied to each of 4 sites (2 cm^2) on the shorn right flank. The treated areas were examined at 24 h after each induction application and at 24 and 48 h after the challenge applications.

Results and Conclusion

Some skin reactions (redness in places) were observed during the induction period. This occurred in 6 animals in the 3% group, starting halfway through the second induction week, and in 6-8 animals in the 10% group, in which redness was apparent from the 3rd induction application. One animal in the 1% induction group showed a slight skin reaction throughout the 4th week of induction, but as this animal showed no reaction at challenge, this was considered an incidental finding. At challenge, reactions in the 3% and 10% induction groups were the strongest, occurring at all challenge doses, starting from the first challenge application, and were present in most animals at some stage of the challenge period. In each of the 0.3% and 1% induction groups, only 1-2 animals were affected, mostly after the 3rd challenge and at the challenge site at which 10% omethoate was applied, the study author suggesting that the higher dose challenge applications may have had a sensitising effect in these groups. There were no skin reactions in the controls at any stage. Under the conditions of this study, omethoate was a skin sensitiser.

3.1.5 Potentiation and Antidote Study

Bayer AG (1967) Omethoate/antidote effect and potentiation. Sponsor: Bayer AG, Farbenfabriken, Institute for Toxicology. Unpublished report date: 14 February 1967. (QA, GLP, Guidelines: No)

The report stated that the study was performed 'in the usual manner'. No details of route of administration, antidote dose, or timing of antidote administration were provided. Groups of 15 fasted rats were dosed orally with 10-200 mg/kg bw omethoate. Without antidote, the LD_{50} was 29.7 mg/kg bw. In the presence of atropine, PAM (pralidoxime), BH6, atropine + PAM or atropine + BH6, the LD_{50} values were 65.0, 39.3, 44.0, 98.0 and 75.0 respectively. Therefore, the best antidote was a combination of atropine and PAM, resulting in a 3-fold increase in LD_{50}. (Note: the chemical identity of BH6 was not provided in the report).

As part of this study, a potentiation trial was carried out, in which fed male rats were orally administered equitoxic mixtures of omethoate and maldison (malathion), respectively comprising 97.06 % and 2.94% of the mixture. Individual LD_{50} values were 33.6 mg/kg bw omethoate and 1110 mg/kg bw maldison (malathion). The experimental oral LD_{50} for the mixture was 571.6 mg/kg bw, approximately equivalent to the LD_{50} of 525 mg/kg bw calculated by Probit analysis. The combined effects of omethoate and maldison (malathion) in this experiment were therefore considered to be additive.

3.2 Formulations

Rats were exposed to omethoate formulations that contained 1-methoxy-2-propyl acetate (MPA) as the sole excipient, as tabulated in Appendix VII. Deaths occurred from within one hour of dosing, to 6 days post-treatment, the more rapid deaths generally associated with the higher doses. The clinical signs, most of which were common to exposure via the oral, dermal and inhalation routes, included palmospasms, laboured breathing, apathy, salivation, lacrimation, tremors and piloerection. Overall, signs were consistent with cholinergic effects. Treatment-related weight loss and/or reduced weight gain were apparent in the majority of the studies, especially in females, but this was reversible by the conclusion of the observation period (generally 2 weeks). Necropsy findings in animals that died prior to study termination, were lungs patchy, distended, fluid in lung; spleen, liver, kidneys pale, patchy; stomach distended, empty, isolated scattered ulcerous foci, and GIT reddened with contents yellow, slimy and bloody. There were no macroscopic findings in survivors. With the exception of the acute oral studies, these studies consistently showed that females were more sensitive than males to the acute effects of omethoate in these formulations.

3.2.1 E 6876 1000 SL 00671/0360 (1000 g/L omethoate)

Route	Species, strain, sex	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Oral	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex; 10 males at 31.5	1-33.5 (M); 1-45 (F) Male deaths: 1 at 26, 2 at 28, 5 at 31.5, 5 at 33.5 Female deaths: 1 at 25, 1 at 31.5, 3 at 40, 3 at 42.5, 5 at 45 No signs at 1	30 (M) 35 (F)	Krötlinger (1986a)
Dermal	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex	50-2500 (M); 10-2500 (F) Male deaths: 1 at 100, 2 at 250, 1 at 500, 3 at 1000, 5 at 2500 Female deaths: 2 at 50, 2 at 100, 1 at 250, 3 at 500, 3 at 1000, 5 at 2500 No signs in males at 50 and females at 10	584 (M) 302 (F) 24h/occlusive	Krötlinger (1986b)
Inhalation	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex	36-842* Male deaths: 1 at 312, 5 at 842 Female deaths: 5 at 312, 5 at 842 No signs at 36	512* (M) 224* (F) 4h/head-nose only	Pauluhn (1986a)

*concentration refers to the active constituent

3.2.2 E 6876 500 SL 00671/0360 (500 g/L omethoate)

Route	Species, strain, sex	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Oral	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex, but 10 males at 56 mg/kg bw	1-100 (M); 1-71 (F) Male deaths: 2 at 53, 8 at 56, 3 at 63, 4 at 71, 5 at 100 Female deaths: 1 at 50, 3 at 56, 4 at 63, 5 at 71 No signs at 1	55 (M & F)	Krötlinger (1986c)
Dermal	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex, but 10 females at 250 mg/kg bw	50-5000 (M); 10-800 (F) Male deaths: 1 at 500, 2 at 800, 4 at 1250, 4 at 2500, 5 at 5000 Female deaths: 2 at 100, 2 at 250, 2 at 500, 3 at 630, 3 at 710, 5 at 800 No signs at 50 in males, and 10 in females	943 (M) 413 (F) 24h/occlusive	Krötlinger (1986d)
Inhalational	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex	73-978* Male deaths: 1 at 290, 0 at 612, 5 at 978 Female deaths: 5 at 612, 5 at 978 No signs at 73	774* (M) 421* (F) 4h/head-nose only	Pauluhn (1986b)

*concentration refers to the active ingredient

Pauluhn, J (1986c) E 6876 500 SL 00671/0362 (c.n. omethoate) Study for irritant/corrosive potential for skin and eye (rabbit). Study No. T 0021601. Report No. 14342. Lab: Bayer Institute of Toxicology, Wuppertal-Elberfeld. Sponsor: Bayer AG. Study report date: 12 February 1986. Expt dates: December 1985-January 1986. (QA & GLP: no, Guidelines: OECD 404 and 405).

Materials and Methods

The test substance Omethoate (E 6876 500 SL 00671/0362, F1 no. 430B) was administered to female rabbits (HC:NZW from Interfauna UK; 3/group). For the skin irritation test, 500 mg of the test material was applied on a hypoallergenic dressing to a 6 cm^2 area of skin on the shorn flank of each rabbit, under semi-occlusive conditions. Another dressing moistened with water was applied similarly to the opposite shorn flank. After 4 hours, the dressings were removed and the exposed areas of skin washed with water. For the test for ocular irritation, 100 µL of the test material, or the same amount of a 15% aqueous dilution, was placed in the conjunctival sac of one eye, and the eyelids held together for 1 second. The contralateral eye acted as the control. After 24 h, the treated eye was rinsed with physiological saline.

Results and Conclusions

No erythema or oedema was observed for the treated rabbit skin when observed, starting at one hour after removal of the test material. The 500 SL formulation was not a skin irritant in rabbits in this test. In the test for eye irritation, corneal opacity (grade 1) was seen in the 3 rabbits, persisting for 72 h. Conjunctival redness (average score 1.6), swelling (average score 1.3) and lacrimation, were also noted for up to 72 h, intensity decreasing with time. No changes to the iris were observed. There was no evidence of irritation on day 7 post-treatment. The 500 SL formulation was a moderate eye irritant in this test. A 15% dilution of this formulation produced no eye irritation.

3.2.3 E 6876 5 SL 00671/0360 (50 g/L omethoate)

Route	Species, strain, sex	Group Size	Doses Tested (mg/kg bw)	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Oral	Rat, Bor:WISW (SPF-Cpb), M/F	5/sex	10-1600 Male deaths: 1 at 800, 4 at 900, 4 at 1000, 5 at 1600 Female deaths: 1 at 500, 2 at 800, 4 at 1000, 5 at 1600 No signs at 10	862 (M) 762 (F)	Krötlinger (1987)

Pauluhn, J (1987) E 6876 5 SL 00671/0415 A (c.n. omethoate) Study for irritant/corrosion potential for skin and eye (rabbit) to OECD Guideline Nos. 404 and 405. Study No. T 7025659. Report No. 16122. Lab: Bayer Fachbereich Toxikologie, D-5600 Wuppertal 1. Sponsor: Bayer AG. Study report date: 13 October 1987. Expt dates: 23.7.1987-18.8.1987. (QA & GLP: no; Guidelines: OECD 404 and 405).

Materials and Methods

The test material E 6876 5 SL 00671/0415 A, FL NO. 462 A, was administered to rabbits (HC:NZW from Interfauna UK; 2 females and 1 male for the skin irritation test, and 5 males and 1 female for the eye irritation test). The tests were performed as in Pauluhn (1986c), except that the eye irritation study was performed using the undiluted test material only.

Results and Conclusions

In the skin irritation study, no findings of erythema or oedema were reported. The 5 SL formulation was non-irritant to rabbit skin in this test. For the eye irritation test, corneal opacity (grade 1) was present in 4 animals for 48 h, one animal for 72 h, but had not resolved at 21 days in the 6th animal. More than half of the corneal area was affected in most animals, this area decreasing with time. Conjunctival redness was present in all animals (average score 1.3), as was conjunctival swelling (average score 0.4), and dacryorrhoea, all reducing in intensity over time, with no effects on the iris. All conjunctival effects resolved by 7 d, with the exception of the animal with the persistent corneal opacity, in which case slight but barely perceptible conjunctival redness and swelling were present at day 21. The 5% SL formulation was therefore a moderate to severe eye irritant in this study.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1. Oral Administration

Fogleman RW, Levinskas GJ (1963) Report on oxygen analog of dimethoate: twenty-eight day feeding of rats. Report no. 63-12. Lab: American Cyanamid Company, Central Medical Department, Environmental Health Laboratory. Expt dates: commenced 24 March 1963. Unpublished report date: 1 July 1963.

Materials and Methods

Omethoate was fed to groups of weanling rats (25/sex/dose; Nelson strain from Carworth Farms) at 0.2, 0.4, 0.8 or 1.6 ppm for 3, 7, 14 or 28 days for the determination of ChE activity in the plasma, RBC and brain, with an additional group treated at 8 ppm for 25 days to assess the systemic toxicity of the test material. The 8 ppm group were given a complete autopsy and selected organs, including nervous tissue, were examined microscopically. The purity of the test material was described as ‘no impurities seen by thin-layer chromatography’. Omethoate was incorporated into the feed on a weekly basis.

Results and Conclusions

One 0.8 ppm male died due to pneumonia and one 0.2 ppm male was killed accidentally. There were no clinical signs, and no effects on food consumption or weight gain other than what could be attributed to the 8 ppm group being without food on day 20 due to an error in the amount of food prepared at this dose. There were no treatment-related findings at macrosopic or microscopic examination. The values obtained for ChE activity were very variable, especially in the RBC, where, for example, there was a 3-fold difference in control activities at 3, 7 and 14 days. Added to this, the fact that omethoate was shown to be unstable in the feed in a later study (Löser & Lorke 1967), and the lack of information on the purity of

the substance tested, this study is not of value for regulatory purposes.

Löser E (1968a) Bayer 45 432. Subacute toxicological studies on rats. Report no. 634. Lab: Farbenfabriken Bayer AG, Institut fűr Toxicologie, Wuppertal-Elberfeld. Sponsor: Bayer AG. Unpublished report date: 19 February 1968.

The purity of the active ingredient in this study was stated as 82%, with 10% trimethyl thiophosphate as a 'possible impurity'. As the purity level is well below modern requirements, this study is not considered suitable for regulatory purposes.

4.2. Dermal Application

Flucke W and Luckhaus G (1979) S 6876 (Omethoate, the active ingredient of Folimat®) Subacute dermal toxicity study on rabbits. Bayer Report No. 8407. Lab: Bayer AG, Institut für Toxicologie, Wuppertal-Elberfeld. Sponsor: Bayer AG. Expt dates: July 1978. Unpublished report date: 29 May 1979. (QA, GLP, Guidelines: no).

Materials and Methods

Omethoate (batch no. Eg.1/76, purity 94%, dissolved in deionised water) was applied to the clipped skin (area 5 cm x 5 cm) on the backs and flanks of New Zealand white rabbits (2.4-2.9 kg, from Hacking & Churchill Ltd, Huntington, England, 6/sex/dose), at doses of 0, 2.5, 20.0 mg/kg bw/d, and left (uncovered) for 7 h, on 15 consecutive workdays. The skin was superficially abraded with sandpaper in half of the animals in each group, to the extent that erythema and slight oedema resulted. After each exposure period, the area of skin exposed to the test material was washed with soap and water. Animals could not eat or drink during the exposure periods due to the presence of restraining devices. Other procedures were generally to the standard of OECD guidelines.

Results and Conclusion

There were no deaths. After the first 3 treatments, rabbits with abraded skin in the 20 mg/kg bw/d group had slight muscle spasms for 2-3 h. Local irritant effects were apparent after the abrasion procedure, but this was not linked to exposure to the test material. However, the appearance of clinical signs was coincident with the inflammatory reaction precipitated by abrasion, a possible indication that omethoate more readily penetrated the damaged skin. Treatment did not affect bodyweight gain, haematology, urinalysis, or clinical chemistry findings, other than ChE activity. With the exception of plasma ChE activity in females at week 8, plasma, RBC and brain ChE activities were inhibited at all test points in 20 mg/kg bw/d animals with abraded or intact skin. At 2.5 mg/kg bw/d (week 15), RBC ChE activity appeared to be inhibited in males with intact skin. However, as there was no effect in the parallel group of abraded animals, and there was a 30% difference in RBC ChE activities in the male control groups, this was not considered a treatment-related effect. Also at this dose, brain ChE activity appeared to be inhibited in abraded females, but not the corresponding intact group. Given that abraded males at 2.5 mg/kg bw/d had ChE activity 30% higher than the corresponding control, and that both female groups treated at 20 mg/kg bw/d showed similar levels of brain ChE activity, the apparent inhibition of brain ChE activity in females at 2.5 mg/kg bw/d is considered unlikely to represent an effect of treatment. Organ weights were not affected by treatment, and macroscopic and microscopic examination did not reveal any

treatment-related findings. The NOEL was 2.5 mg/kg bw/d, due to clinical signs and inhibition of ChE activity in the plasma, RBC and brain at 20 mg/kg bw/d (Table 12).

Table 11. ChE activity U/mL or U/g (% inhibition relative to control)

			Males			Females		
	Week	**Dose(ppm):**	**0**	**2.5**	**20**	**0**	**2.5**	**20**
Plasma	**8**	Abraded	0.65	0.53 (19%)	0.32 (51%)	0.55	0.59	0.50 9%
		Intact	0.56	0.49 (12%)	0.40 (29%)	0.57	0.61	0.50 (12%)
	15	Abraded	0.74	0.69 (7%)	0.42 (43%)	0.68	0.62 (9%)	0.52 (23%)
		Intact	0.72	0.65 (9%)	0.41 (43%)	0.69	0.70	0.47 (32%)
RBC	**8**	Abraded	1.90	1.92	1.16 (39%)	1.91	1.76 (8%)	1.50 (21%)
		Intact	2.23	1.94 (13%)	1.81 (19%)	2.14	2.04 (4%)	1.55 (27%)
	15	Abraded	1.55	1.59	0.92 (40%)	1.81	1.66 (8%)	1.28 (29%)
		Intact	2.23	1.64 (26%)	1.05 (53%)	1.83	1.69 (7%)	1.09 (40%)
Brain	**15**	Abraded	2.56	3.35	1.52 (40%)	3.27	2.01 (38%)	1.70 (48%)
		Intact	2.60	2.95	1.76 (32%)	3.29	3.00 (8%)	1.59 (51%)

4.2. Inhalation Administration

Thyssen J (1978) S 6876 (The active ingredient of ®Folimat). Acute inhalation toxicity. Bayer Report No. 7888. Lab: Bayer AG. Institut fuer Toxikologie, Wuppertal-Elberfeld. Sponsor: Bayer AG. Expt dates: not stated. 26 October 1978. (QA, GLP, Guidelines: no).

Materials and Methods

As part of this acute study, dynamically nebulised omethoate (purity 94.0%) in ethanol:Lutrol (1:1) was administered by inhalation to rats (Wistar-II from Winkelmann, Borchen; 10/sex/dose) at 0, 3, 13 or 49 mg/m^3 for 4 h/d for 5 days, in a manner that avoided skin contact with the aerosol. Controls were exposed to the vehicle only. Rats were observed for 2 weeks after the exposure period. Bodyweights were determined weekly and ChE activity (RBC and plasma) was assayed before the commencement of treatment, after the 1st, 3rd and 5th exposures, and at 72 h after the final exposure.

Results and Conclusion

One female died at 49 mg/m^3. Behavioural disturbances and signs of ChE inhibition (not specified) were observed at ≥13 mg/m^3. There were no treatment-related findings at macroscopic necropsy. Animals in all groups lost weight during the exposure period, more so at 49 mg/m^3, with compensatory weight gain thereafter. Inhibition of ChE activity in both the plasma and RBC occurred at all doses, peaking after the third exposure, and maintained at this level until after the 5th dose. Inhibition of RBC ChE activity was similar at all doses for both

sexes, suggesting that this assay lacked sensitivity. At 3 days after the 5th dose, plasma ChE activity had recovered fully at 3 mg/m^3, and to a lesser extent at the higher doses, while RBC ChE activity was still inhibited. Effects (inhibition of RBC and plasma ChE activity) were seen at all doses in this study (Table 13).

Table 12. Plasma and RBC ChE activity (U/mL) and maximum ChE inhibition (%) after the 3rd dose

	Males				Females			
Dose mg/m^3	**0**	**3**	**13**	**49**	**0**	**3**	**13**	**49**
Plasma	0.52	0.31 (40%)	0.16 (69%)	0.07 (86%)	1.07	0.61 (43%)	0.28 (73%)	0.07 (93%)
RBC	2.64	2.18 (17%)	1.99 (24%)	1.93 (26%)	2.71	2.07 (23%)	2.08 (23%)	2.00 (26%)

Thyssen J (1979) Folimat active ingredient (S 6876) Subacute inhalational toxicity study on rats. Bayer Report No. 8445. Lab: Bayer AG, Institut für Toxicologie, Wuppertal-Elberfeld. Sponsor: Bayer AG. Expt dates: October 1978 – April 1979. Unpublished report date: 11 June 1979. (QA, GLP, Guidelines: no).

Materials and Methods

Omethoate was administered by the inhalation route to 10 rats/sex/dose (Wistar TNO/W 74, ~180-200 g, from Winkelmann, Borchen) at 0, 0.96, 2.3 or 7.5 mg/m^3, as determined by chemical analysis of chamber air. A total of 15 exposures of 6 h were performed over 3 weeks. Omethoate (purity 92.4%) was diluted with a 1:1 mixture of ethanol and PEG 400, and then aerosolised into inhalation chambers under dynamic flow conditions. Exposure was such that the skin of the rats did not come into contact with the aerosol. Bodyweights were determined weekly for all rats. Overall, other procedures were in accordance with the OECD test guideline for short term inhalation studies, though measurement of the various toxicology parameters was limited to 5 rats/sex/dose (except for organ weights, in which case all rats were assessed), and clotting time was not assessed. Statistical analysis was by the non-parametric ranking test of Wilcoxon.

Results and Conclusion

The measurement of particle size showed that >93% of the particles were within the respirable range (<3.0µm), for all the test concentrations. There were no deaths or clinical signs, nor did exposure to the test material affect bodyweight gain. Mild pulmonary emphysema, chronic pneumonic foci and slightly enlarged bronchial lymph glands were seen in some rats, but incidence was not related to treatment. There were no treatment-related histological findings.

Plasma, RBC and brain ChE activities were clearly inhibited at ≥2.3 mg/m^3 in males and at 7.5 mg/m^3 in females. The extent of inhibition of plasma and RBC ChE activities at these doses at the conclusion of the study was generally similar to that seen at the corresponding dose for weeks 1 and 2, and was slightly greater in males relative to females. In males, plasma ChE activity showed borderline inhibition at 0.96 mg/m^3, with 4/5 rats having levels below the concurrent control range. However, this group also showed low plasma ChE activity relative to the control group prior the commencement of exposure, so the effect at 0.96 mg/m^3 was not considered to be due to treatment. Regarding RBC ChE inhibition in this group, 4/5 results were less than the lowest finding in the control group. However, the extent of

inhibition was variable across the treatment period (13% at week 1, and no inhibition apparent at week 2), so the difference at week 3 is considered a chance event. Inhibition of plasma ChE activity in 2.3 mg/m^3 females was 19% at week 3, but as inhibition reached 26% and 24% for weeks 1 and 2 respectively, this was considered an effect of treatment. In 2.3 mg/m^3 females, RBC ChE activity was inhibited by 20, 29 and 22% at the 3 test points, so this was also considered treatment-related. Brain ChE activity in individual females at 2.3 mg/m^3 were all less than the concurrent controls, as was the case for 0.96 mg/m^3 males. As it has been argued that the changes in plasma and RBC ChE activities at 2.3 mg/m^3 were the result of treatment, and taking into account the finding that brain ChE activity was inhibited to a greater extent than plasma or RBC ChE at 7.5 mg/m^3, inhibition of brain ChE activity in 2.3 mg/m^3 females is considered a treatment-related effect. The clear dose response for inhibition of brain ChE activity in males across the 3 doses is suggestive of an effect at all doses. However, this was also apparent for inhibition of plasma ChE activity in males, and which, with the benefit of pre-test readings, the finding at 0.96 mg/m^3 was dismissed as not biologically relevant. Given the absence of evidence for inhibition of plasma and RBC ChE activity at 0.96 mg/m^3, and the similarity in the extent of inhibition in each of the 3 compartments at the next highest dose, it is considered unlikely that the apparent inhibition of brain ChE activity at 0.96 mg/m^3 is toxicologically significant. Therefore, the no effect level was 0.96 mg/m^3, due to inhibition of plasma, RBC and brain ChE activity at 2.3 mg/m^3 (Table 14)

Table 13. ChE activity at 3 weeks, U/mL or U/g ± SD (% inhibition relative to control)

	Males				Females			
Dose(mg/m^3):	**0**	**0.96**	**2.3**	**7.5**	**0**	**0.96**	**2.3**	**7.5**
Plasma	0.48 ± 0.03	0.39 ± 0.05 (18%)	0.35 ± 0.06 (27%)	0.26 ± 0.04 (45%)	1.68 ± 0.23	1.59 ± 0.33 (5%)	1.35 ± 0.12 (19%)	1.04 ± 0.28 (38%)
RBC	2.93 ± 0.13	2.42 ± 0.32 (17%)	2.04 ± 0.23 (30%)	1.89 ± 0.15 (35%)	3.02 ± 0.17	2.54 ± 0.12 (15%)	2.35 ± 0.30 (22%)	2.02 ± 0.11 (33%)
Brain	1.35 ± 0.17	1.12 ± 0.08 (17%)	0.89 ± 0.10 (34%)	0.57 ± 0.07 (58%)	1.17 ± 0.05	1.12 ± 0.13 (4%)	0.94 ± 0.06 (19%)	0.63 ± 0.08 (47%)

5. SUBCHRONIC STUDIES

5.1. Oral Administration

5.1.1 Rats

Löser E, Lorke B (1967) Bayer 45 432: Subchronic toxicity tests on rats. Lab: Farbenfabriken Bayer AG, Institute for Toxicology, Wuppertal-Elberfeld. Sponsor: Bayer AG. Expt. dates: not stated. Unpublished report date: 7 March 1967. (QA, GLP, Guidelines: none).

Materials and Methods

Omethoate (Bayer 45 432, batch 4/66, purity 88%) was administered in the feed to Wistar rats (SPF, from Winkelman, Paderborn, mean weights M/F, 40.2/40.5 g at study initiation) at 0, 5, 15, 50 or 150 ppm for 4 months. A 50% pre-mix of the test material was prepared in Silkasil S on a weekly basis. Treated groups comprised 15 rats/sex, with 30 rats/sex in the control group. An additional group treated at 2.5 ppm, with its own concurrent control group

of 30 rats/sex, was added later. Bodyweight and food intake were measured weekly. Cholinesterase activity was measured in whole blood at the beginning of the study, and on the 4th day after the rats had been eating from a freshly prepared batch of food, for 5 rats/sex/dose at 4, 8, 12 and 16 weeks. Other blood tests (Hct, Hb, and RBC and WBC counts) and urine tests (glucose, protein, gall-dyestuff, and microscopic sedimentation) were performed towards the end of the study (5/sex/dose). A macroscopic necropsy was performed on all rats, and thyroid, heart, liver, spleen, kidneys, adrenal glands and testes were weighed. Statistical analyses were conducted according to the distribution free grading test of Wilcoxon.

Results

No individual animal data were provided with this study. Deaths were confined to the 150 ppm group (7M/6F). Male rats treated at ≥5 ppm, and females at ≥15 ppm showed symptoms described as 'clinical signs typical of ChE inhibition', including muscle cramps and shivering, persisting throughout the study, except at 15 ppm, at which dose signs were confined to the first 2 months of the study. Typically, the rats presented with these symptoms on days 2-4 after the weekly issue of freshly prepared food, with a reduction in the severity of symptoms in the latter part of each week. Food consumption and bodyweight gain were reduced at 150 ppm. The nominal intake of omethoate was (M/F) 0/0, 4.7/4.1, 10.4/8.3, 32/25, 91/87, 224/199 mg/animal/d. Data were not provided as mg/kg bw/d, but the nominal intake of the test material was approximately equivalent to 0, 0.25, 0.5, 1.5, 5, and 15 mg/kg bw/d. Whole blood ChE activity was reduced to a biologically significant extent in males at ≥5 ppm, and females at ≥50 ppm at all test points. Cholinesterase activity was inhibited in 15 ppm females at week 4 only. No changes were detected in the haematology and urinalysis parameters tested. Absolute liver weights were decreased in both sexes at 150 ppm. Relative liver weight was increased in 150 ppm males, but this was slight (~9%) and probably not of toxicological significance. At 150 ppm, absolute kidney weight was decreased in males, and relative kidney weight was increased in both sexes. The extent of increase in relative kidney weight (~18% relative to control values) was considered sufficient to suggest that this was related to treatment. Other organ weight changes that occurred in the 150 ppm group were considered secondary to lower bodyweight. The results are summarised in Tables 15 and 16.

Table 14. ChE activity in whole blood (% of concurrent control)

Week	Dose(ppm):	0	2.5	5	15	50	150
4	Males	100	87.0	64.2	41.8	17.0	9.2
	Females	100	100	89.1	68.2	28.6	15.5
8	Males	100	98.0	71.6	45.0	20.2	6.1
	Females	100	100	100	96.6	48.4	15.5
12	Males	100	94.6	78.9	52.7	19.1	13.3
	Females	100	100	100	100	50.5	16.7
16	Males	100	91.4	69.5	48.4	26.6	7.1
	Females	100	100	100	100	57.1	15.5

Table 15. Organ weights, absolute (g) and relative (per 100 g bw)

	0 ppm		5 ppm		15 ppm		50 pm		150 ppm	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Liver: absolute	10.58	6.69	9.73	7.09	9.94	7.07	9.99	6.61	6.98*	5.50*
: relative	3.10	3.61	2.88	3.60	2.95	3.68	3.13	3.50	3.39*	3.67
Kidney:absolute	2.11	1.31	2.02	1.42	2.20	1.45	2.02	1.21	1.50*	1.24
: relative	0.62	0.71	0.60	0.72	0.65	0.75	0.63	0.64	0.73	0.83

Note: For simplicity of reporting, results for the 2.5 ppm group are not shown. This group had a separate control group, but no differences in organ weights were apparent between the 2.5 ppm and specific control groups.

Conclusions

In this study, no effects of omethoate were observed at 2.5 ppm for the limited investigations reported. However, from the pattern of appearance of clinical signs, it appears that the test material was unstable in the diet, and therefore the results are not considered true reflections of dosing at the levels nominated. Also, the purity of the test material was well below modern standards. Overall, the standard of this study is not adequate for a NOEL to be set.

Löser E (1968b) Bay 45 432: Subchronic toxicological studies on rats. Report No. 1040. Lab: Farbenfabriken Bayer AG, Institut für Toxicologie, Wuppertal-Elberfeld. Sponsor: Bayer AG. Expt dates: not provided. Unpublished report date 17 October 1968. (QA, GLP, Guidelines: none).

Materials and Methods

Omethoate (BAY 45 432, purity 93.3%) was given to Wistar rats (SPF, from Winkelman, Kreis Paderborn, 15/sex/dose) in the feed for 3 months at 0, 0.5, 1, 2, or 4 ppm. A 50% premix in Silkasil S was prepared on 5 days/week. At study initiation, the rats were 30-34 days old, with average bodyweight 58.4 g (M) or 59 g (F). Rat bodyweights were measured weekly, and food consumption was determined on 5 working days/week. At the end of the study, haematology measurements (Hb, Hct, blood counts [RBC, WBC, differential, thrombocytes, reticulocytes], MCH and MCV), liver function tests (GPT, GOT, ALP, SDH, bilirubin, thymol turbidity), urine examinations (blood, glucose, protein, bile pigment, microscopic examination), kidney function (serum urea and creatinine), and blood glucose were determined for 5 rats/sex/dose. Cholinesterase activity (plasma and RBC) was measured in 5 rats/sex/dose after 1, 4 and 13 weeks of treatment. At the end of the study, all rats were given a macroscopic examination. Brain ChE activity was not assessed. Rats that died prematurely were autopsied. The non-parametric Wilcoxon rank test was used for the statistical evaluation of results.

Results

One male rat died at each of 0.5, 1 and 4 ppm, and one 2 ppm female also died prematurely. The study author attributed the male deaths to hypotonia or atonia of the musculature of the small intestine, a disorder described as typical of SPF rats. The female that died had a whitish tuberosity on the heart muscle. None of these deaths was considered to have resulted from exposure to omethoate. Treatment-related clinical signs were limited to the 4 ppm group, beginning on day 1 and persisting for 3 weeks. They were described by the study authors as ‘typical signs of cholinesterase activity depression’, but no further details were provided. The average omethoate consumption was reported as (M/F) 0/0, 0.9/0.7, 1.7/1.3, 3.4/2.7, 7.1/5.1 mg/animal. Estimating from the ppm levels, this would be approximately equivalent to 0,

0.05, 0.1, 0.2, and 0.4 mg/kg bw/d in ascending order of dose. Food consumption and bodyweight gain were not affected by treatment.

There were trends to increases in GOT, GPT, and total bilirubin in both sexes, and in SDH in females, at times starting from 1 ppm, but as clear dose responses were often lacking, it is uncertain if these changes were due to treatment (Table 17). At ≥2 ppm, ChE activity was inhibited in the RBC in both sexes, increasing with dose. This was considered a treatment-related effect. Cholinesterase activity was decreased in the plasma of females but not males, and this was restricted to the week 1 sampling point. At macroscopic examination there were no findings that could be linked to treatment, and organ weights were not affected.

Table 16. Liver function tests and percent inhibition of ChE activity relative to control

	Dose(ppm):	**0**	**0.5**	**1**	**2**	**4**
GOT (mU)	Males	11.01	9.35	13.14	15.65	15.83
	Females	10.70	11.54	9.94	12.68	15.87
GPT (mU)	Males	8.69	7.68	9.22	13.46	12.45
	Females	8.93	7.05	9.34	12.41	14.30
SDH (mU)	Males	1.3	1.2	1.0	1.1	2.3
	Females	1.4	1.6	2.3	2.4	3.0
Total bilirubin	Males	0.13	0.12	0.14	0.47	0.57
(mg/100 mL)	Females	0.14	0.09	0.16	0.27	0.44
RBC ChE	Males	0	7.7	10.7	46.8	59.1
1 week	Females	0	6.7	15.5	21.2	51.7
RBC ChE	Males	0	10.6	0	29.3	42.8
4 weeks	Females	0	6.0	10.7	21.2	48.0
RBC ChE	Males	0	0	15.8	28.8	36.4
13 weeks	Females	0	0	9.2	26.5	31.2
Plasma ChE	Males	0	8.5	<5.0	10.5	17.3
1 week	Females	0	11.3	18.2	25.7	22.8

Conclusion

As the feed containing omethoate was prepared on 5 days/week, it is expected that the instability problems that were apparent in the previous study (Löser & Lorke, 1967) were largely overcome in the present investigation, though no stability studies were reported. No microscopic investigations were performed, individual animal data were not provided, and only percentage inhibition was reported for ChE activity measurements. Under the conditions of this study, according to the data provided, the highest dose at which no effects were seen was 1 ppm (~0.1 mg/kg bw/d). This study is not considered of sufficient standard for regulatory purposes.

Schladt, L (1994) E 6876 Chronic toxicological study in Wistar rats to determine a no-inhibition level for the cholinesterase activity (32-week administration of test substance in drinking water). Bayer study no. T 2033899. Lab: Bayer AG Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal. Sponsor: Bayer AG, Wuppertal, Germany. Expt dates: 28 May 1990 – 8 January 1991. Unpublished report date: 2 March 1994. (QA: Yes; GLP: not stated; Test guidelines: none)

This 32-week study was designed to determine a NOEL for ChE inhibition, as the results of a chronic study that was underway (Schladt 1995), in which omethoate was administered to

rats at 0, 0.5, 4 or 32 ppm in the drinking water, had not demonstrated a NOEL for inhibition of RBC ChE activity up to the time the present study was undertaken.

Materials and Methods

Omethoate (purity 96.5-96.9%, batch no. 234808038), was dissolved in drinking water acidified to pH 3 with HCl at concentrations of 0, 100 or 300 ppb, and administered *ad libitum* to groups of rats (20/sex/dose; SPF Wistar Bor:WISW(SPF Cpb) from Winkelman breeders, Borchen) as their sole source of drinking water. The rats were 6 weeks old at study initiation and weighed 105-131 g (males) or 91-114 g (females). They were inspected for clinical signs twice daily (once per day on weekends), with weekly detailed examinations. Individual water intakes were determined once per week for 13 weeks, and at 4-weekly intervals thereafter. Interim necropsy was performed at weeks 27/28. The investigators concluded that a NOEL could be determined at that point, so the study was terminated shortly thereafter (32 weeks of treatment). At interim sacrifice, ChE activity was determined in the left half of the brain for 10 rats/sex/group. Plasma and RBC ChE activities were determined for all animals in week 27. Brain, heart, testes, liver, lungs, spleen, kidneys and adrenals were weighed, but only for the interim sacrifice group. All animals were submitted to a gross pathological examination, but microscopic examinations were not performed.

Statistical analyses: The U-test of Mann, Whitney and Wilcoxon was used to compare test and control groups.

Results and Conclusions

The test substance was demonstrated to be stable in the drinking water, and present at the appropriate concentrations. The test compound intake was (M/F) 9.3/10.9 µg/kg bw/d at 100 ppb and 27.1/32.2 µg/kg bw/d at 300 ppb. There were no deaths, and few clinical signs were noted, none of which could be attributed to treatment. The intake of food and water did not differ significantly between groups, and neither did bodyweight gain. Organ weights (absolute and relative) were similar in control and test groups. As shown in the Table, the only statistically significant changes in ChE activity were increases. The mean ChE activities in all groups were similar to historical control data for this laboratory. The associated 2-year rat study (Schladt 1995) showed inhibition of RBC ChE activity at a higher dose than those used in the present study (i.e. 500 ppb).

Cholinesterase activity (kU/L or kU/g)

	Males			Females		
	0 ppb	**100 ppb**	**300 ppb**	**0 ppb**	**100 ppb**	**300 ppb**
Plasma	0.52 ± 0.08	0.52 ± 0.14	0.60 ± 0.09**	2.13 ± 0.57	2.10 ± 0.54	2.20 ± 0.44
RBC	0.76 ± 0.10	0.88 ± 0.18*	0.92 ± 0.21**	0.92 ± 0.25	0.90 ± 0.20	0.93 ± 0.25
Brain	3.54 ± 0.67	3.55 ± 0.54	2.95 ± 0.57	2.28 ± 0.28	2.23 ± 0.25	2.90 ± 0.47**

n=20 for plasma and RBC ChE activities; n=10 for brain ChE activity. *p≤0.05, ** p≤0.01.

5.1.2 Dogs

Hutchison EB, Pope SJ, Schaeffer TR, Varney CH, Woolston SA (1968) Report on oxygen analog of cygon dimethoate: ninety-day feeding to dogs (CL 28,580). Report no. 68-89.

Lab: American Cyanamid Company, Central Medical Department, Environmental Health Laboratory. Sponsor: Bayer AG. Unpublished report date: 12 August 1968.
Omethoate (purity >95%) was administered to purebred beagle dogs (4/sex/group, 6-8 months old) in the diet at 0, 0.4, 0.8 or 1.6 ppm for 14 weeks. Fresh feed batches were prepared at weekly intervals, but no information was provided with respect to the stability of the test material in the feed. In later dog studies, omethoate was administered by stomach tube to overcome instability problems, so it is expected that the present study is flawed in this regard, and therefore is not useful for regulatory purposes. Also, no records were presented of the amount of food consumed, other than a statement that animals ate over 90% of the food offered in most instances. This adds to the uncertainty regarding the amount of the active test substance that the dogs were exposed to in this study. No treatment related effects were seen in this study, including effects on ChE activity.

Ruf J, Mager H (1991) E 6876 Subchronic toxicity study on dogs (Thirteen-week stomach tube dosage test). Study No. T 4 030 768. Report No. 20139. Lab: Bayer AG, Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1. Sponsor: Bayer AG. Expt dates: February 1989 – June 1989. Unpublished report date: 11 April 1991. (QA: yes; GLP: OECD & FIFRA; Guidelines: none stated).

This study was performed as a supplement to a chronic study (T 7 010 303, Hoffmann and Schilde, 1984) to ensure that a NOEL for ChE activity was obtained.

Materials and Methods

Groups of purebred beagle dogs (Bor:Beag strain, Winkelmann Breeders, Borchen; 4/sex/dose; 25-27 weeks old and 6.7-9.4 kg at study initiation) were treated with 0 or 0.0125 mg/kg bw/d omethoate (batch no. 234 808 038, purity 96.6%, adjusted to pH 3-4) by stomach tube for 13 weeks (94 treatments). The vehicle was water. Animals were usually fed 1-3 h prior to treatment. Stability tests showed that the test material was stable in tap water for at least 10 days. Food consumption was determined daily, and bodyweight was measured weekly. Reflex tests, body temperature and pulse rates, ophthalmological examinations, haematology, clinical chemistry and urinalysis were performed 2 weeks before dosing commenced (-2) and in weeks 5 and 12. Laboratory tests were essentially as listed in Appendix III. Cytochrome P450 and N-demethylase activities were determined in liver tissue. Plasma and RBC ChE activities were determined in weeks -2, 1, 2, and 8, and brain ChE activity was measured at termination. All animals were subjected to necropsy, and organs were weighed, but no histology was conducted due to the absence of findings in the chronic study at higher doses. Appropriate t-tests were applied to the ChE data.

Results and Conclusion

No animals died, and clinical signs, reflex testing, and physiological and ophthalmological examinations showed no treatment-related changes. Food intake was slightly reduced in the treated female group, but as this was not reflected in bodyweight gain, it was not considered biologically relevant. All other measurements and tests, including those for ChE activity, gave no indication of treatment-related effects. In this study, there were no observable effects at 0.0125 mg/kg bw/d.

6. CHRONIC STUDIES

6.1 Oral Administration

6.1.1 Mice

Krötlinger F, Löser E (1982) S 6876 (Omethoate, the active ingredient of ®Folimat) Chronic toxicity study on mice (2-year feeding experiment). Bayer Report No. 11161. Lab: Bayer AG, Institut fuer Toxikologie, Wuppertal FRG. Sponsor: Bayer AG. Expt dates: September 1978 - September 1980. Unpublished report date: 14 September 1982. (QA, GLP, Test guidelines: None).

Materials and Methods

Omethoate (batch no. Abf. 75/6, purity 94%) was administered in the feed at 0, 1, 3 or 10 ppm to SPF mice (BOR:CWF1 from Winkelmann, Borchen; 50/sex/group; 30-35 days old; mean initial weight (M/F) 24/21 g). The report states that the stability of omethoate in the feed was established, but no details were provided. Freshly prepared feed was provided twice weekly. Bodyweights were determined weekly for 14 weeks, and every 3 weeks thereafter. Weights of the heart, kidneys, liver, spleen and lungs were recorded for all mice that survived till terminal sacrifice. Major organs and any other organs with gross changes were subjected to histological examination. Haematology and clinical chemistry testing was not performed. Mortality rates were compared using Fisher's Exact Test, and other data were analysed using the Mann-Whitney U test and Wilcoxon.

Results and Conclusion

No inter-group differences in clinical signs were reported. Food intake was similar in all groups. Female bodyweight was similar to controls throughout the treatment period, while male bodyweight in the treated groups overall slightly exceeded corresponding control weights from about 40 weeks onwards. Bodyweight gain was ~11% greater in 10 ppm males than the corresponding control group. Average test compound intake was (M/F) 0/0, 0.21/0.35, 0.69/1.03 and 2.09/3.08 mg/kg bw/d in ascending order of dose. Mortality was not affected by treatment, but exceeded 50% in most groups at study termination (M/F: 47.1/65.3, 62.7/69.4, 53.1/51.0, 58.0/46.0% in ascending order of dose). Spleen weights were less than control values in all male test groups, in the absence of a dose response. This was considered to be the result of high variability in the control group, rather than a toxicological effect (e.g. relative male spleen weights were 450 ± 667, 255 ± 140, 250 ± 125, and 250 ± 151, in ascending order of dose). No other organ weights were affected, nor were there any microscopic changes that were likely to be treatment-related. Neoplastic lesions that occurred only at 10 ppm were 2 malignant leiomyosarcomas of the cutis/subcutis in males, with one malignant polymorphocellular sarcoma of the heart, 2 malignant adenocarcinomas of the mammary gland and one benign tubular adenoma of the ovary in females. These tumours are possibly spontaneous in origin, but in the absence of historical control data, a relationship to treatment cannot be excluded. Due to the limited testing in this study, it is not appropriate to set a NOEL.

Schladt, L (2001) E 6876 Oncogenicity study in B6C3F1 mice (administration in the

drinking water over 24 months; T1032655). Bayer study no. T 1032655. Lab. report no. PH 30972. Lab: Institute of Toxicology, Bayer AG, D-42096 Wuppertal, Friedrich-Ebert-Strasse 217-333, Germany. Histology prepared at Life Science Research, Eye, Suffolk, England. Sponsor: Bayer, Pharmaceutical Business Group, Elberfeld. Expt dates: 22 March 1989-28 March 1991. Unpublished report date: 3 May 2001. (QA and GLP: Yes; Test Guidelines: US EPA 83.2, 1984; OECD 451, 1981).

Materials and Methods

Groups of 50 B6C3F1 mice/sex/dose were dosed with omethoate (purity 96.5-97.4%, batch no. 23480838) in the drinking water (adjusted to pH 3 with HCl) *ad libitum* at doses of 0, 0.5, 4 or 32 ppm for 24 months. Mice were from Winkelmann – Experimental Animal Breeder, Borchen, 5-6 weeks old and 18.1-29.8 g (males) or 17.5-25.7 g (females) at study initiation. Doses were based on a preceding study in which mice were administered 100-200 ppm omethoate in the drinking water for up to 7 weeks, with increased mortality and other signs at 100 ppm and above. In the present study, satellite groups of 10 mice/sex/group were killed after 12 months of treatment. Administration via the drinking water was chosen because omethoate was unstable in the diet. The low pH of the drinking water was necessary to ensure stability of the dissolved omethoate. Haematology, clinical chemistry and organ weights/histology were as listed in Appendices III and IV, except serum electrolytes were not tested; serum enzyme tests were limited to AP, SGPT, and SGOT; the thyroid and ovaries were not weighed, but the lungs were; and histological examination excluded the head, nasal and pharyngeal cavities, ureters, urethra, tattooed ears, oviduct, eyelids, teeth and Zymbal's glands.

Statistical analyses: Test and control groups were compared using the U-test of Mann, Whitney and Wilcoxon. Survival curves were analysed using SAS Routine PROC LIFE-TEST, and subsequently compared separately for each sex using the generalised Wilcoxon test (Breslow test). Fisher's Exact Test was used to compare histopathological findings between control and test groups.

Results and Conclusion

The laboratory reported that there was no evidence that the acidified water affected the parameters tested. The most common clinical sign was tremor, noted only at 32 ppm (15/60 males and 7/60 females). This was first seen in week 2, but after week 8 occurred only in one male for which poor general condition, emaciation and high stepping gait were also recorded. No other signs could be attributed to treatment. Mortality was not affected by exposure to the test substance. The percentage of animals that died in the 2-year groups were, in ascending order of dose, (M/F) 22/40, 28/44, 24/26, 18/34. On a bodyweight basis, water intake in the 4 ppm and 32 ppm was generally less than controls (variable, but roughly 5-15%), the differences achieving statistical significance mainly in the first half of the study. At 0.5 ppm, water consumption was sporadically less than controls, but this was slight and rarely achieved statistical significance. Test compound intake was (M/F) 0/0, 0.10/0.11, 0.82/0.80, 6.48/6.61 mg/kg bw/d in ascending order of dose. Over the study period, mice treated with omethoate gained more weight than controls (M/F 4/14; 14/28; 20/24 % more than control weight gain, in ascending order of dose). However, treated animals consumed less food than controls (M/F: 8/6, 10/16, 17/13 % less than control in ascending order of dose, on a mg/kg bw basis).
Haematology and clinical chemistry

Red blood cell numbers were decreased in males at 4 and 32 ppm, statistically significant at the highest dose. This was accompanied by decreased Hb at study termination, and corresponding increases in MCHC. In males at 4 and 32 ppm, MCH was also increased at 12 months, but this was not sustained till 24 months (not shown). When compared with historical control data, it appears likely that effects on RBC parameters in 32 ppm males were biologically significant. Similar changes were not apparent in females. Decreased thromboplastin time, associated with a trend to increased thrombocyte numbers, was seen in the 4 and 32 ppm female groups at 12 months, but as this was not apparent at 24 months, it is considered unlikely to have been treatment-related. There were no changes in the WBC population that could be attributed to treatment.

Haematology findings ± SD (n=10)

		Males				Females			
Parameter	Month	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
RBC (10^{12}/L)	51/52	10.43 ±0.63	10.07 ±0.91	9.89 ±0.29	9.82* ±0.56	9.88 ±0.52	10.00 ±0.47	10.41* ±0.51	10.16 ±0.43
	103	10.57 ±2.87	9.92 ±1.31	9.10 ±1.07	9.06* ±0.64	9.42 ±0.63	8.90 ±2.03	9.17 ±0.66	9.56 ±0.58
Hb (g/L)	51/52	148 ±5.9	149 ±8.5	150 ±4.8	149 ±6.8	147 ±5.5	152* ±4.1	154* ±4.1	150 ±8.4
	103	150 ±34.1	140 ±8.1	133 ns ±15.5	134* ±7.4	142 ±6.4	135 ±22.8	139 ±7.2	142 ±9.6
Hct (g/L)	51/52	0.485 ±0.025	0.476 ±0.032	0.467 ±0.013	0.463* ±0.026	0.469 ±0.022	0.479 ±0.022	0.497* ±0.023	0.475 ±0.029
	103	0.482 ±0.110	0.450 ±0.041	0.415 ±0.045	0.409** ±0.025	0.441 ±0.027	0.430 ±0.068	0.433 ±0.029	0.444 ±0.028
MCHC (g/L RBC)	51/52	307 ±13.6	312 ±7.2	322** ±5.1	322* ±9.0	314 ±9.0	318 ±11.5	310 ±11.8	315 ±10.7
	103	311 ±15.3	311 ±15.1	319 ±10.6	328* ±11.2	323 ±8.3	313** ±9.5	320 ±7.7	320 ±12.8
Thrombocytes (10^9/L)	51/52	1508 ±181	1416 ±117	1357* ±124	1474 ±230	1164 ±97.5	1145 ±143	1220 ±107	1330** ±134
	103	1698 ±288	1769 ±243	1453 ±350	1561 ±170	961 ±185	1075 ±300	1130* ±139	1006 ±318
Thromboplastin time (s)	51/52	18.9 ±0.98	18.9 ±0.54	19.0 ±0.84	19.0 ±1.28	19.8 ±0.78	19.3 ±0.71	18.7** ±0.60	18.4** ±0.57
	103	19.5 ±1.56	19.7 ±1.42	20.6* ±0.67	20.3 ±1.00	19.9 ±0.63	20.2 ±1.94	19.7 ±0.62	20.0 ±2.77

*p<0.05; **p<0.01

In males at the top two doses, AP, creatinine and urea were decreased in the serum. At 24 months, these apparent differences were largely the result of elevated control means, due to one control animal that was shown at autopsy to have a hepatocellular carcinoma. However, as decreased urea in 32 ppm males was also apparent at 12 months in the absence of elevated control values, the changes in urea were possibly due to treatment.

Females at 32 ppm also showed a statistically significant decrease in urea at 12 months, but this difference was much less pronounced at 24 months, and lacked statistical significance, so it is unclear if this was treatment-related.

Clinical Chemistry ± SD (n=10; % inhibition relative to control is shown for ChE activity)

		Males				Females			
Parameter	**Month**	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
AP (U/L)	**51/52**	101 ±14	101 ±17	104 ±9.7	96 ±22	181 ±39	171 ±43	206 ±47	201 ±37
	103	253 ±307	129 ±79	101 ±19	91* ±23	323 ±179	330 ±186	281 ±103	254 ±92
Creatinine (µmol/L)	**51/52**	26 ±1.9	25 ±2.3	24 ±3.2	24 ±1.9	30 ±4.1	29 ±2.2	29 ±2.6	29 ±3.9
	103	39 ±29	28 ±3.0	26** ±2.5	24** ±2.6	25 ±2.5	27 ±1.8	26 ±1.9	26 ±2.4
Urea (µmol/L)	**51/52**	9.51 ±1.41	9.27 ±1.11	8.46 ±0.85	7.53** ±0.87	9.33 ±1.14	8.54 ±2.17	8.39 ±1.73	7.38** ±1.13
	103	20.69 ±26.1	11.58 ±1.79	10.07* ±1.22	9.99* ±0.82	10.08 ±1.55	9.33 ±1.47	9.69 ±1.33	8.93 ±1.18
Plasma ChE (kU/L)	**52/53**	5.25 ±0.65	4.71 ±0.62 10%	4.57** ±0.30 13%	1.82** ±0.37 65%	6.15 ±0.33	6.21 ±0.27	5.69* ±0.46 8%	2.88** ±0.32 53%
	59	4.85 ±0.48	5.13 ±0.42	4.76 ±0.49 2%	1.48** ±0.31 69%	6.28 ±0.40	5.97* ±0.21 5%	5.39** ±0.55 14%	2.32** ±0.50 63%
	104/105	8.31 ±3.90	6.50 ±2.09 22%	5.04** ±0.50 39%	1.09** ±0.36 77%	-	-	-	-
	105	6.63 ±2.44	6.24 ±1.65 6%	5.22 ±0.96 21%	1.78** ±0.32 73%	7.57 ±0.91	8.23 ±1.82	6.63 ±1.34 12%	3.18** ±0.46 58%
RBC ChE (kU/L)	**52/53**	0.51 ±0.07	0.38** ±0.05 25%	0.22** ±0.03 57%	0.09** ±0.01 82%	0.41 ±0.07	0.31** ±0.03 24%	0.25** ±0.03 39%	0.08** ±0.02 80%
	59	0.52 ±0.08	0.52 ±0.17 0%	0.24** ±0.10 54%	0.06** ±0.01 88%	0.47 ±0.11	0.43 ±0.06 9%	0.28** ±0.05 40%	0.09** ±0.03 81%
	104/105	1.11 ±0.79	0.83 ±0.31 25%	0.56 ±0.28 50%	0.08** ±0.03 93%	0.57 ±0.26	0.79 ±0.63	0.46 ±0.16 19%	0.16** ±0.09 72%
Brain ChE (U/g)	**52/53**	2.21 ±0.41	1.84 ±0.43 17%	1.68** ±0.17 24%	0.71** ±0.06 68%	2.00 ±0.48	2.05 ±0.53	1.55* ±0.21 23%	0.68** ±0.12 66%
	104/105	3.76 ±0.93	3.16 ±0.72 16%	1.76** ±0.58 53%	0.60** ±0.14 84%	2.29 ±0.34	2.17 ±0.19 5%	1.81* ±0.37 21%	0.73** ±0.07 68%

*p<0.05; **p<0.01

Plasma ChE activity was inhibited at 32 ppm in both sexes. At other doses where this parameter differed from controls to a statistically significant extent, the differences were generally too small to be considered biologically significant. In the case of males at weeks 104/105, several control values were extremely high, and even if these were disregarded, 9/10 animals treated at 4 ppm, and all at 0.5 ppm, had plasma ChE activities within the control range, so these were also considered not to be toxicologically significant. Red blood cell ChE activity was strongly inhibited in both sexes at 32 ppm and at 4 ppm in males. In females at 4 ppm, RBC ChE was clearly inhibited at weeks 52/53, and this was confirmed when another group of 10 mice from this dose group was tested in week 59. However, this difference was not apparent at the end of the study, so the finding at this dose in females is equivocal. At 0.5 ppm, RBC ChE activity was inhibited in both sexes at weeks 52/53, but this did not occur in

additional groups tested at week 59, nor at study termination in females. The control male RBC ChE values at termination were highly variable, with 2 extremely high values. The apparent decrease in RBC ChE activity in 0.5 ppm males is therefore not considered to have resulted from treatment. Therefore RBC ChE activity is not considered to have been inhibited at 0.5 ppm. Brain ChE activity was inhibited in males and females at ≥4 ppm.

Organs

In males, absolute and relative liver weights were decreased at all doses. However, results were highly variable, particularly in the control group. Also, as a clear dose response was not present, and there were no histology findings in treated mice that were indicative of liver pathology, these apparent differences were considered unlikely to be of toxicological significance. Decreases in relative testes weights at ≥4 ppm, relative kidney weights in females at ≥4 ppm, and relative heart weights in females at 32 ppm, as well as decreases in relative brain weights in both sexes at ≥4 ppm, were all attributed to higher body weights in these groups relative to the corresponding control groups.

Organ weights at 24 months ± SD (mg or mg/100 g bw)

	Males				Females			
Organ	**0 ppm**	**0.5 ppm**	**4 ppm**	**32 ppm**	**0 ppm**	**0.5 ppm**	**4 ppm**	**32 ppm**
Body weight (g)	40 ±4.6	41 ±4.7	43** ±4.4	42* ±4.3	41 ±9.1	43 ±5.0	44* ±7.1	45* ±7.4
Liver: absolute	2664 ±1194	2235 ±853	2185 ±734	2162 ±588	2081 ±499	2155 ±666	2064 ±428	2208 ±649
: rel to bw	6887 ±3460	5514** ±2415	5207** ±2068	5247** ±1839	5094 ±1357	5055 ±1427	4710 ±1080	5011 ±1624
Testes: absolute	220 ±16	234 ±71	222 ±17	219 ±19	-	-	-	-
: rel to bw	562 ±66	574 ±184	525** ±63	525** ±51	-	-	-	-
Kidney: absolute	741 ±80	749 ±81	774 ±73	786* ±82	549 ±56	536 ±64	527 ±53	535 ±53
: rel to bw	1884 ±166	1826 ±165	1824 ±172	1884 ±187	1369 ±264	1260 ±104	1210** ±187	1211** ±170
Heart : absolute	251 ±24	252 ±32	242 ±31	261 ±47	223 ±37	222 ±31	229 ±33	216 ±40
: rel to bw	644 ±100	617 ±98	572** ±91	629 ±130	560 ±139	524 ±79	525 ±91	488* ±96
Brain: absolute	505 ±21	499 ±29	503 ±27	508 ±25	517 ±23	511 ±23	514 ±21	523 ±26
: rel to bw	1292 ±131	1224 ±132	1194** ±152	1227* ±153	1298 ±264	1214 ±156	1185* ±189	1191 ±183

*p<0.05; **p<0.01;

Calcification of the kidneys occurred more frequently in treated males at all doses, but the degree of change (slight), and the high incidence in the control group, suggested that this finding is unlikely to have toxicological significance. Cortical cysts were observed more often in the kidneys of treated rats of both sexes than in controls, but given the flat dose response, this is also unlikely to be treatment-related. The incidence of atrophied ovaries was increased significantly at ≥4 ppm. At these doses, atrophy of the thymus was also increased, particularly in males, and fatty bone marrow was increased in the femur of females. Due to the technique used, structural losses in the sciatic nerve could not be confirmed as degenerative areas.

The total numbers of tumours (benign or malignant) in the test groups were similar to the number in the corresponding control group. Tumours that occurred at a higher incidence in test groups relative to controls, are tabulated below. The incidences of the listed neoplasms were within their respective historical control ranges, except in the case of Harderian gland adenomas, where the incidence in males at 32 ppm exceeded the highest historical control incidence by one. As these tumours were benign, this small increase is not considered to be toxicologically important.

Tumours that occurred singly at 32 ppm, but not in any other group of that sex, included a benign unilateral adenoma of the parathyroids, a malignant unilateral follicular cell carcinoma of the thyroid and a malignant adenocarcinoma of the rectum in males; benign hepatic haemangiomas in both sexes; a haemangioma of the mesenterial lymph nodes, a bilateral granulosa cell tumour of the ovary, and a cavernous haemangioma of the uterus (all benign) in females; and a malignant keratinised squamous cell carcinoma of the uterus. A malignant adenocarcinoma of the uterus was also present in each treated group, and benign lipomas were also found in 2 females at 32 ppm. Consistent with their single occurrences, and/or their occasional presence in control mice of this strain in NTP studies, all of these tumours are considered likely to have been spontaneous in origin. The NOEL for this study was 0.5 ppm, equal to 0.1 mg/kg bw/d, based on inhibition of RBC and brain ChE activities at doses ≥4 ppm.

Incidence of histopathological findings possibly related to treatment (number/number examined)

	Males				**Females**			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Kidneys								
Calcification	32/48	42/49*	45/48**	48/50***	3/47	1/45	0/49	2/47
Cortical cysts	8/48	15/49	17/48	18/50	1/47	6/45	7/50	8*/49
Ovaries								
Atrophy	-	-	-	-	12/39	18/42	38***/46	31***/44
Thymus								
Atrophy	20/40	21/37	33*/42	33*/43	12/42	5/39	15/43	19/43
Fatty bone marrow increase (femur)	0/48	0/47	0/48	0/50	0/47	3/45	13***/50	12***/49
Lung								
Bronchio-alveolar adenoma [B]	6/48	2/49	2/48	12/50	3/47	0/45	1/50	3/49
Liver								
Hepatocellular adenoma [B]	6/48	5/49	6/48	5/50	2/47	2/45	5/50	6/49
Harderian glands								
Unilateral adenoma [B]	3/48	2/50	3/49	6/50	3/49	2/50	4/50	2/50
Haematopoietic lymphoreticular tissue								
Lymphoma [M]	2/4	2/3	2/4	4/6	9/12	12/13	7/11	10/12

B=benign; M=malignant; *p<0.05; **p<0.01; *** p<0.001.

6.1.2 Rats

Bomhard E, Löser E, Kaliner G (1979) S 6876 chronic toxicity study on rats (2-year feeding experiment). Report No. 8507. Lab: Bayer AG, Institut fur Toxikologie, Wuppertal. Sponsor: Bayer AG. Expt dates: May 1975-May 1977. Unpublished report date: 18 July 1979. (QA, GLP, Test guidelines: none stated)

Materials and Methods

Omethoate (sample no. Eg.1075 Abtg 75/6, technical product no. 2730, purity approximately 94.0%) was administered in the diet to SPF rats (Wistar W.74 strain from Winkelmann, Borchen; 100 controls/sex and 50/sex/dosed group; 30-32 days old; initial average weight M/F 51/50 g) at 0, 0.3, 1, 3 or 10 ppm. Fresh feed was provided once weekly to control rats, and twice weekly to treated rats. Animals were inspected daily for clinical signs. For the first 14 weeks, bodyweights were determined weekly, but fortnightly thereafter. Food consumption was recorded on a weekly basis throughout. Clinical chemistry, haematology and urinalyses (16 h collection period) were performed on 5 rats/sex/dose at 1, 3, 6 and 12 months, and on 10 rats/sex/dose at study termination as listed in Appendix II, except that clinical chemistry tests were limited to AP, SGPT, SGOT, glutamate dehydrogenase, bilirubin, cholesterol, creatinine, glucose and urea. Plasma and RBC ChE activities were assayed in 5 rats/sex/dose at 1, 2, 4, 8, 13, 26, 52, 78 and 105 weeks. Brain ChE activity was determined in 10 rats/sex/dose at study termination. All rats were subjected to gross necropsy. Organs weighed were as listed in Appendix III, though brain was not included, but lungs were. Tissues examined microscopically for all rats in the 0 and 10 ppm groups were essentially as listed in Appendix III, though peripheral nerve, spinal chord and the various glands were not included. Mortality rates were compared using Fisher's Exact Test, and other data were analysed using the Wilcoxon-Mann-Whitney U test.

Results and Conclusion

No treatment-related clinical signs were reported. At the end of the study, percentage mortality was (M/F) 16/18, 34*/22, 26/24, 18/22, 18/30 (*$p \leq 0.05$), in ascending order of dose. It is possible that the increased female deaths at the top dose may have resulted from treatment. Food consumption in all treated groups was 5-10% less than controls, but as these changes were small and a dose response was lacking, this was not considered to be of toxicological significance. There were no appreciable differences in bodyweight between groups. The average test compound intake, calculated using animal bodyweight midway through the study, was (M/F) 0/0, 12/18, 41/53, 126/166, 419/542 µg/kg bw/d in ascending order of dose. These values are close to those predicted by applying a factor 0.05 to convert from ppm to mg/kg bw/d. (Note: the study report gave these figures as mg/kg bw/d, assumed to be a 1000-fold calculation or typographical error).

No effects of treatment were apparent for the haematology parameters measured. Bilirubin was increased to a statistically significant extent at 24 months in females at 3 and 10 ppm, and in males at 3 ppm, the latter lacking a dose response (M/F: 0.17/0.16, 0.18/0.17, 0.18/0.18, 0.20*/0.19*, 0.19/0.19* in ascending order of dose). These changes were not supported by any other findings, so were considered unlikely to have toxicological

significance.

Plasma ChE activity was clearly inhibited at 10 ppm in males for the first 18 months, and in females for 3 months into the study. In 3 ppm males, plasma ChE activity was also inhibited at 3 ppm in weeks 1 and 2, while RBC ChE activity was reduced in both sexes at both 3 and 10 ppm throughout the treatment period, except in 3 ppm males at 26 weeks. Plasma and RBC ChE activities for week 1 are shown in the table, as this, along with the activity seen in week 2, represented the time of maximal inhibition of plasma ChE activity, though inhibition of RBC ChE activity was maintained at a similar level at all test points. Brain ChE activity was reduced in both sexes at ≥3 ppm. At 1 ppm, brain ChE activity was reduced to a statistically significant ($p<0.05$) extent in females, but as 9/10 values were within the range of the concurrent control, this was not considered to represent a biologically significant difference (Table 18).

Table 17. Cholinesterase activity ±SD (µmoles acetylcholine) and percent inhibition relative to the concurrent control

Doses (ppm)	Males			Females		
	Plasma	**RBC**	**Brain**	**Plasma**	**RBC**	**Brain**
0	2.68 ± 0.11	3.62 ± 0.33	1.52 ± 0.14	2.89 ± 0.20	3.81 ± 0.56	1.35 ± 0.17
0.3	2.76 ± 0.20	3.29 ± 0.17	1.31** ± 0.12	3.29 ± 0.51	3.57 ± 0.17	1.25 ± 0.13
1	2.83 ± 0.26	3.49 ± 0.38 (3%)	1.43 ± 0.15 (6%)	2.66 ± 0.28 (8%)	3.28 ± 0.33 (14%)	1.18* ± 0.12 (12%)
3	2.18** ± 0.11 (19%)	2.35** ± 0.23 (35%)	1.09** ± 0.10 (28%)	2.65 ± 0.25 (8%)	2.72** ± 0.22 (28%)	1.11* ± 0.12 (18%)
10	1.79** ± 0.24 (33%)	1.30** ± 0.29 (64%)	0.84** ± 0.18 (45%)	2.18* ± 0.35 (25%)	1.18** ± 0.16 (69%)	0.86 ± 0.09 (36%)

Note: plasma and RBC ChE activities are for week 1, which was the time of maximum inhibition. Brain ChE activity was measured at 24 months.* $p<0.05$ ** $p<0.01$

There were no findings at macroscopic examination, or differences in non-neoplastic findings, that could be attributable to treatment. Neoplastic findings that showed an increased incidence with respect to the corresponding control group are shown in Table 19. The study report states that these findings were within the range of normal variation, and historical control data supports this (Bomhard and Rinke, 1994). The NOEL for this study was 1 ppm, equal to 41 µg/kg bw/d, due to inhibition of plasma, RBC and brain ChE activities at the higher doses.

Table 18. Tumours (incidence at 10 ppm greater than control)

	Males		Females	
Tumour site & type	**0 ppm**	**10 ppm**	**0 ppm**	**10 ppm**
Adrenal cortex – adenoma (B)	2	4	5	6
Brain – glioma (B)	0	1	0	0
Pelvic cavity – adenocarcinoma (M)	0	2	-	-

Ovary – thecal cell tumour (B)	-	-	0	1
Uterus – adenoma (B)	-	-	1	2
Uterus – adenocarcinoma (M)	-	-	4	8
Mammary gland – fibroadenoma (B)	-	-	0	3
Testes – Leydig cell tumour (M)	0	1	-	-
Thyroid – medullary adenoma (B)	6	5	4	10
Mesothelium – mesothelioma (M)	0	0	0	1

M = malignant; B = benign

Schladt L (1995) E6876 (Folimat®) Study for chronic toxicity and carcinogenicity in Wistar rats following two-year administration in drinking water. Bayer study no. T 2030748. Lab: Bayer AG Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal; histopathology performed at the Institute of Experimental Pathology of the Medizinische Hochschule Hannover. Sponsor: Bayer AG, Wuppertal, Germany. Expt dates: 27.2.1989-5.3.1991. Unpublished report date: 21 February 1995. (QA & GLP: Yes; Test guidelines: US EPA 83-5, 1984.)

Materials and Methods

Omethoate (purity 96.5-97.4%), batch no. 234 808 038, was dissolved in drinking water (acidified to pH 3 with HCl) at concentrations of 0, 0.5, 4 or 32 ppm, and administered *ad libitum* to groups of rats (60/sex/dose; SPF Wistar Bor:WISW(SPF Cpb) from Winkelman breeders, Borchen) as their sole source of drinking water for 2 years. Ten rats/sex/dose comprised interim sacrifice groups (12 months). At study initiation, rat weights were 94-145 g (males) and 90-125 g (females). Doses were selected on the basis of a pilot study in which 6 rats/sex/dose were treated at 0, 25, 50 or 100 ppm in the drinking water for 7 weeks. In the pilot study, clinical signs (emaciation, apathy, tremor and tonical spasms), body weight gain, and female deaths were seen at ≥50 ppm, male deaths at ≥100 ppm, and plasma, RBC and brain ChE inhibition at ≥25 ppm. In the present study, animal bodyweights and water intake were recorded weekly for the first 13 weeks, then every 4 weeks from week 16, and food consumption was determined at 4-weekly intervals throughout. Plasma and RBC ChE activities were assayed in weeks 26, 52, 78 and 105 (10 rats/sex/group), and in an additional 10/sex/group in control and 0.5 ppm rats in week 28. Brain ChE activity was determined at 12 and 24 months. Other clinical chemistry, haematology and urinalysis parameters measured were essentially as described in Appendix III (omitting gamma glutamyl transpeptidase, globulin, LDH and CPK). Organs were examined as in Appendix IV, except lungs were weighed and thyroid was not.

Statistical analyses: Results were subjected to the two-tailed U test of Mann & Whitney and Wilcoxon.

Results

Tremor was the most common clinical sign, present mainly at 32 ppm, particularly in males during the first 7 weeks of the study. Otherwise, signs with increased incidence in treated animals were emaciation in both sexes at 32 ppm, loss of hair in 32 ppm females, and eye opacity in males at 32 ppm and females at ≥4 ppm. The number of deaths in the 2-year groups (M/F: 10/15, 10/23, 11/15, 12/22 in ascending order of dose) was elevated relative to

the control in the female 0.5 ppm and 32 ppm groups, but in the absence of any dose response this was not attributed to treatment. Both male and female 32 ppm groups gained little weight relative to the control group in the first week of exposure to the test material. The females then underwent compensatory weight gain, resulting in weights similar to controls from week 36. The weight of the 32 ppm male group, however, remained about 10% below control values for the remainder of the study. Apart from slightly lower body weights observed in the first 8 weeks of the study for the other male test groups, the body weights of other groups were unaffected by treatment. Water intake was elevated at 32 ppm relative to controls in both males (~22%) and females (~16%). Omethoate intake was (M/F) 0/0, 0.04/0.05, 0.30/0.44, 2.92/3.93 mg/kg bw/d in ascending order of dose. Food consumption was slightly increased (8-9%) at 32 ppm, but this was not statistically significant.

Haematology, clinical chemistry and urinalysis

In males at 32 ppm, there were slight but consistent decreases in Hb, Hct and MCV and increases in MCHC and thrombocytes, most of these differences being present at weeks 26/27 and subsequent test points, and therefore possibly treatment-related. In the absence of changes in Hct and Hb in females, the statistically significant increase in MCHC in 32 ppm females is not considered meaningful. Thromboplastin time was decreased in females to a statistically significant extent in all tests prior to 24 months (not shown), but as these changes were generally small (~10% or less) and were not apparent at termination, they were considered unlikely to have toxicological significance.

Haematology at 24 months (n=10)

	Males				**Females**			
Parameter	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Hb (g/L)	155 ±7.2	151 ±10.1	149 ±12.6	142** ±12.8	149 ±10.1	149 ±8.3	147 ±7.4	145 ±10.4
Hct (g/L)	0.491 ±0.022	0.477 ±0.029	0.469 ±0.039	0.438** ±0.036	0.474 ±0.028	0.469 ±0.022	0.459 ±0.019	0.448 ±0.030
MCV (fL)	53 ±1.4	54 ±2.4	52 ±2.3	52* ±4.6	56 ±2.1	55 ±2.5	54 ±2.8	54 ±1.4
MCHC (g/L RBC)	317 ±6.1	317 ±5.5	317 ±5.4	324* ±6.5	315 ±7.9	318 ±5.4	319 ±4.5	324* ±5.2
Thrombocytes (10^9/L)	1088 ±153	1105 ±187	1107 ±170	1277** ±180	982 ±129	826** ±113	953 ±108	1077 ±206

*p<0.05; **p<0.01

Cholesterol and total bilirubin were decreased in 32 ppm males, and creatinine was decreased in treated females, but as these values were similar to historical control levels, and at times lacked dose responses, they were not considered to have toxicological significance. In 32 ppm males, ALT was slightly increased relative to controls, but as there were no concomitant changes in other liver enzymes, and no pathological changes were seen in the liver, this difference is unlikely to be toxicologically significant. Albumin was increased and K decreased in females at ≥4 ppm, but in the absence of similar changes to total protein or other electrolytes, neither of these was expected to signify a toxicological effect. Plasma ChE activity was inhibited in males at ≥4 ppm and in 32 ppm females to an extent considered to be an effect of treatment. Erythrocyte ChE activity was inhibited in all treated male groups and in females ≥4 ppm, while brain ChE activity was inhibited in both sexes at

≥4 ppm. The dose levels at which ChE activity was inhibited in the 3 compartments was consistent across all of the test points.

Clinical Chemistry at 24 months
(n=10; % inhibition relative to control is shown for ChE activity)

	Males				Females			
Parameter	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
ALT (U/L)	43.9 ±6.6	45.9 ±15.2	49.4 ±11.8	52.7* ±12.5	76.4 ±29.9	62.2 ±13.1	71.0 ±20.6	76.3 ±19.4
Cholesterol (mmol/L)	5.13 ±0.88	5.99 ±3.00	4.31 ±1.02	4.48* ±2.20	3.16 ±1.52	2.51 ±0.35	2.95 ±0.61	3.00 ±0.96
Creatinine (µmol/L)	46 ±4.9	60 ±32.7	45 ±4.9	46 ±7.6	59 ±18.1	47* ±4.0	48 ±7.0	45** ±3.6
Total bilirubin (µmol/L)	2.8 ±0.5	2.6 ±0.4	2.4 ±0.4	2.3* ±0.3	2.0 ±0.61	1.9 ±0.28	2.0 ±0.38	2.0 ±0.38
Albumin (g/L)	30.6 ±2.0	28.6 ±4.1	29.8 ±2.5	31.9 ±2.4	36.4 ±3.6	37.9 ±2.5	39.5* ±1.4	39.6* ±3.0
K (mmol/L)	4.7 ±0.2	4.7 ±0.5	4.7 ±0.3	4.6 ±0.3	4.6 ±0.7	4.1* ±0.3	3.8** ±0.3	3.7** ±0.3
Plasma ChE (kU/L)	1.00 ±0.35	0.82 ±0.20 18%	0.79* ±0.35 21%	0.32** ±0.08 68%	1.93 ±0.62	2.05 ±0.53	1.71 ±0.80 11%	0.56** ±0.13 71%
RBC ChE (kU/L)	0.81 ±0.08	0.65** ±0.13 20%	0.21** ±0.05 74%	0.03** ±0.01 96%	0.73 ±0.15	0.63 ±0.12 14%	0.21** ±0.12 71%	0.02** ±0.01 97%
Brain Che (U/g)	2.02 ±0.25	1.91 ±0.28 5%	1.28** ±0.11 37%	0.51** ±0.08 75%	1.86 ±0.27	1.87 ±0.22	0.98** ±0.16 47%	0.34** ±0.09 82%

*p<0.05; **p<0.01

In males, protein in the urine was generally decreased in males at ≥4 ppm. However, this was usually associated with a low urine volume relative to controls, and when expressed as g protein/L urine, there was no clear relationship to treatment. At times protein output in the urine was also decreased in females, but these changes were not consistent throughout the treatment period and usually lacked dose responses, so were not considered to be an effect of treatment.

Organs

At macroscopic examination, lens opacity was identified in (M/F) 2/5, 5/3, 4/9, 9/10 animals in ascending order of dose. Ophthalmological examinations showed an increased incidence of vascularisation of the cornea at 32 ppm (M/F: 1/0, 0/0, 8/3 at 0, 4 and 32 ppm respectively).

Absolute weights of the lung, spleen, liver and kidneys were decreased in 32 ppm males (not shown), but as there was no change in their respective weights relative to body weight, these changes are considered to reflect the lower body weights in this group. An increase in relative heart and brain weight in males at ≥4 ppm was also attributed to bodyweights lower than controls. An effect of treatment cannot be ruled out for the increase in the absolute weight of the adrenals in females at ≥4 ppm and an increase their relative weight at 32 ppm, but as there were no histopathological findings in this tissue, the weight increases in this organ are unlikely to be of toxicological significance.

Body weight and adrenal weights at 24 months (mg or mg/100 g bw)

	Males				Females			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Body weight (g)	476 ±43.0	461 ±51.7	455 ±53.0	433** ±56.3	279 ±23.2	281 ±37.6	279 ±35.1	281 ±31.8
Adrenals: absolute	64 ±14	67 ±19	65 ±18	60 ±15	73 ±17	76 ±19	81* ±17	86** ±15
: rel to bw	13 ±2.8	15 ±4.8	14 ±4.8	14 ±3.9	26 ±6.7	28 ±7.8	29 ±7.5	31** ±6.4

*p<0.05; **p<0.01;

The numbers of animals in which retinal degeneration was observed was similar at all doses. However, severity was clearly increased at 32 ppm in males and was possibly related to treatment. The incidence of mineralisation of the lens was also increased in this group, with keratitis and choroid proliferation more frequent in 32 ppm females than the corresponding control group. Vacuolation of the lacrimal glands and epididymides occurred in all 32 ppm males examined, and as this far exceeded the control incidences, these findings were considered to have resulted from treatment. An increase in hyperplasia of the mammary glands was seen in all female groups, but the relatively high background incidence and the flat dose response across the 0.5 and 4 ppm groups suggested that this may have been related to treatment only at the top dose.

Non-neoplastic lesions at 24 months (incidence/number examined)

	Males				Females			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Eyes Retinal degeneration total	41/50	37/49	36/50	45/49	40/50	36/50	37/44	38/41
slight	5	5	6	1	1	8	2	2
moderate	10	6	3	4	7	4	5	1
severe	26	26	27	40	32	24	37	38
Lens mineralisation	2/50	4/49	2/50	9/40	5/50	3/50	11/50	7/50
Keratitis	1/50	0/49	0/50	1/49	0/50	0/50	0/50	5/50
Choroid proliferation	2/50	1/49	3/50	1/49	1/50	2/50	0/50	6/50
Lacrimal glands Unilateral vacuolation	17/50	16/45	9/47	48/48	28/46	26/50	29/50	32/49
Epididymides Vacuoles	4/50	0/50	10/50	50/50	-	-	-	-
Mammary glands Hyperplasia	1/35	2/25	1/27	1/23	13/47	21/49	23/49	30/47

The incidences of thyroid c-cell tumours and follicular cell adenomas of the thyroid were increased in treated males (respectively 2, 4, 4, 4 and 1, 1, 2, 4 in increasing order of dose). Benign thyroid C-cell tumours are common findings in this strain of rat (historical control range for males 0-19.1%)[2], and considering the flat dose response in this study, the increased incidence in treated males is unlikely to be due to treatment. On the other hand, the historical control incidence of follicular cell adenomas cited in the same source was 0-4.4% for males, so it is likely that the increased incidence of this neoplasm is treatment-related, at least at 32 ppm. Tumours that occurred singly at 32 ppm were a malignant liposarcoma, a carcinoma of the exocrine pancreas, an adenocarcinoma of the salivary

[2] Bomhard E, Rinke M (1994) Frequency of spontaneous tumours in Wistar rats in 2-year studies. Experimental Toxicology and Pathology 46: 17-29.

glands, a malignant fibrous histiocytoma of the skin, and a benign haemangioma of the spleen in males; and a malignant adenocarcinoma of the pituitary gland in females. There were no other neoplasms that were considered likely to have resulted from exposure to omethoate.

Conclusion

A NOEL was not achieved in this study, due to inhibition of RBC ChE activity in males at all doses tested. A supplementary study (Schladt, 1994) found that after 27 weeks of treatment, ChE activity was not inhibited in RBC, plasma or brain at doses up to 0.3 ppm, equal to 0.03 mg/kg bw/d, the highest dose tested. Inhibition of RBC ChE activity in males, the most sensitive endpoint, was maximal in the main study at the week 26 and week 28 test points. Since the supplementary study was performed in the same laboratory under the same conditions as the main study, it is considered acceptable to adopt the highest dose used in the supplementary study, and at which no effects were seen for the most sensitive endpoint, as the overall NOEL. The NOEL for this study is therefore 0.3 ppm, equal to 0.03 mg/kg bw/d, based on inhibition of RBC ChE activity in males at the next highest dose of 0.5 ppm.

7. REPRODUCTION STUDIES

7.1 Rats

Löser E (1981) S-6876 Folimat-Wirkstoff multigeneration reproduction study in rats. Report No. 9731. Lab: Department of Toxicology, Bayer AG, Wuppertal, FRG. Sponsor: Bayer AG. Expt dates: December 1976 – December 1978. Unpublished report date: January 1981. (QA, GLP, Test Guidelines: none).

Note: The information that follows is from an OCS evaluation performed in 1992, and the 1996 JMPR toxicology evaluation of dimethoate.

Materials and Methods

Omethoate (purity 94.0%) was fed to rats (Long Evans FB 30, 10 males and 20 females/group) in the diet at 0, 1, 3, or 10 ppm, approximately equivalent to 0, 0.05, 0.15, 0.5 mg/kg bw/d for 3 successive generations (2 litters per generation). The first mating took place after a pre-mating treatment period of 10 weeks, with each male mated to two females. Offspring of the second litters (F1b and F2b) were selected as parents of the following generation. These animals were maintained on the appropriate dose for their group for at least 100 days post-weaning, prior to their first mating.

At birth, litters were examined for malformations, and were culled to 10 pups/litter on postnatal day 4. If not destined for parenting the following generation, offspring were killed at week 4 and given macroscopic examinations, except in the case of the F3b, for which histopathology examinations were performed on 10 pups/sex/dose at 4 weeks after birth. The survival and growth rates of parents and pups were monitored, and performance indices such as fertility, pup viability, and lactation were calculated.

Results and Conclusions

There were no treatment-related deaths among parents, and no adverse physical or behavioural effects were recorded. Over the 3 generations, parental bodyweight change, mating performance, the fertility index, litter size and pup weight at birth were not affected by treatment. No malformations were observed in the pups, including macroscopic and histopathological examinations of weaned pups of the F3b generation. A number of findings that reached statistical significance ($p<0.05$) were observed in the F2b pups. The viability index was reduced at 3 and 10 ppm, representing 64% and 62.5% of the control value. At 10 ppm, the lactation index was 62% of the control, and the mean bodyweight of F2b pups during lactation was ~20% lower than the control pup bodyweight. As findings in pups were confined to the F2b, they were not considered treatment-related. The NOEL for parents and offspring was 0.5 mg/kg bw/d, the highest dose tested.

Dotti A, Biedermann K, Luetkemeier H (1994) E 6876 (c.n. Omethoate) range finding study to the two-generation reproduction study in the rat. RCC Project 207325. Bayer project T 6029500. Lab: RCC, Research and Consulting Company Ltd, PO Box CH 4452 Itingen, Switzerland. Sponsor: Bayer AG Institut für Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Federal Republic of Germany. Expt dates: 17 April 1989-21 July 1989. Unpublished report date: 12 July 1994. (QA: Yes; GLP: US EPA; Test Guidelines: none stated)

Materials and Methods

In a one-generation reproduction range-finding study, groups of 10 rats/sex/dose (Wistar/HAN rats (kfm:WIST, outbred, SPF quality) from KFM, Kleintierfarm Madoerin AG. CH 4414 Fuellinsdorf, Switzerland, 7-8 weeks old, 176-213 g (males) and 134-165 g (females) at study initiation, were dosed with omethoate (batch no. 234 808 038, purity 96.6-96.9%), dissolved in acidified drinking water (pH 3) at 0, 10, 30, or 90 ppm. The dose levels were as proposed by the sponsor. Subgroups of 5 rats/sex/dose were treated for 22 days for an interim determination of ChE activity. From day 10 of the pre-mating period, the 90 ppm dose was reduced to 50 ppm, as the higher dose resulted in severe clinical signs (ruffled fur, exophthalmia, tremor, ataxia, lateral and dorso-lateral recumbency, stiff gait, sedation, squealing spasm and teeth grinding from day 6, with emaciation evident on day 8). For the parental animals, treatment spanned a pre-mating period of 3 weeks, a mating period, and gestation. Sub-groups of pups (10/dose) were reared for 7 days after weaning on day 21, and during this interval were directly exposed to omethoate in the drinking water at the corresponding parental dose. Parental animals and the remaining pups were sacrificed on the day of weaning. Plasma, RBC and brain ChE activity were determined at this point in 5 dams/dose. The brain, testes, prostate, seminal vesicles and ovaries were weighed for all parental rats as was appropriate for their sex, and 2 pups/sex/litter where possible. Bodyweight and food and water consumption, except during mating, were determined weekly, and on post-natal days 0, 1, 4, 7, and 14. Only bodyweight was recorded for post-natal day 21. Other reproduction parameters were generally as for guideline reproduction studies.

Statistical analyses: Univariate one-way analysis of variance, the Dunnett many-one t-test, a one-way analysis of variance based on Wilcoxon ranks and the Kruskall-Wallis test, and Fisher's Exact test for 2x2 tables were applied to the data as considered appropriate.

Results

At 90 ppm, one dam was found dead on day 8 of the pre-mating period, and another was found dead on day 13 of the same interval, at which point the dose had been reduced to 50 ppm for 3 days. Both animals had dark red discoloured lungs and stomach discoloration or foci in the stomach or forestomach. The clinical signs observed at 90 ppm became less severe when the dose was reduced to 50 ppm, with tremor, ataxia, dorso-lateral recumbency and squealing spasm observed in most animals receiving the reduced dose until day 12 of the pre-mating period, with ruffled fur and occasional restlessness the main clinical signs thereafter. At 30 ppm, restlessness was noted in both sexes during the pre-mating period, and in males for up to 15 days into the post-mating period, with an occasional observation of tremor in females in the pre-mating period, and in one female during lactation.

Males and females dosed at 90 ppm lost weight during the pre-mating period, but when the dose was reduced to 50 ppm, males gained weight at a similar rate to controls, while there was some compensatory weight gain in females during the remainder of this period. These weight changes were associated with decreased food consumption in both sexes of ~50% in week 1 and 17-25% in week 2 of the pre-mating period. Thereafter, food consumption in 50 ppm males was comparable to the corresponding control group, though bodyweight remained below control values. Males at 10 and 30 ppm gained less weight (10 and 30% respectively) than controls during the pre-mating period, but did not differ significantly from controls thereafter. During gestation and lactation, treated dams showed similar body weight gain to controls, though bodyweight at 50 ppm remained ~10% below controls throughout. In females at 30 and 50 ppm there was a slight, but not statistically significant increase (8-18%) in food consumption during gestation, but food consumption in dams during lactation was ~35% and ~15% less than controls at 50 ppm and 30 ppm respectively. During the pre-mating period, water consumption was decreased (34-40%) in both sexes at 90 ppm, and slightly (~14%) in 30 ppm males. Thereafter, water consumption was similar to controls, except in 50 ppm dams during lactation, when it was reduced by 25-50%. The test compound intake is summarised in the accompanying table.

Adult test compound intake (range; mg/kg bw/d)

Group	Interval	10 ppm	30 ppm	90 ppm	50 ppm
Males	Pre- and post-mating periods	0.84-1.72	2.62-4.98	11.93-12.93	4.54
Females	Pre-mating	1.25-1.91	4.61-5.89	13.27-13.31	9.08-9.76
	Gestation	1.16-1.55	3.68-4.90	-	7.03-9.47
	Lactation	1.50-2.51	4.55-8.11	-	3.59-11.23

All animals that survived the pre-mating period mated successfully, though the mean pre-coital time was relatively long for the 90/50 ppm group. Reduced fertility was apparent in the 30 ppm and 90/50 ppm groups, but gestation time was not affected. Of the 3 pregnant dams at 90/50 ppm, one had a single implantation site only, and another lost an entire litter (3 pups), leaving only one dam that successfully reared pups at this dose. At 30 ppm, the 7 pregnant dams all gave birth, but 2 experienced total postnatal loss. All pregnant dams in the control and 10 ppm groups reared pups. Total implantations and implantations per dam were reduced at ≥30 ppm. Sex ratios of the pups appeared to be unaffected by treatment.

Reproduction data

Parameter	0 ppm	10 ppm	30 ppm	90/50 ppm
Mean pre-coital time (days)	2.6	3.7	2.4	6.4

No. females mated	10	10	10	8
No. females pregnant	10	10	7	3
Total litters	10	10	7	2
Total implantations	120	111	59	17
Mean implantations/dam	12.0	11.1	8.4	5.7
Post-implantation loss (no. litters affected)	3 (3)	5 (3)	4 (3)	2 (1)
Live pups at first litter check (% males/females)	117 (44/56)	106 (52/48)	57 (51/49)	15 (53/47)
Postnatal loss days 0-4 (no. litters affected)	0 (0)	1 (1)	3* (2)	7** (2*)
Postnatal loss days 5-21 (no. litters affected)	0 (0)	1 (1)	10** (3)	3** (2*)
Live pups at postnatal day 21 (% males/females)	80 (45/55)	73 (48/52)	35 (51/49)	5 (40/60)

*p≤0.5, **p≤0.01; Note: litters were culled on PND 4 to 4/sex/litter where possible.

Pup bodyweights on postnatal day 1 were similar in all groups, but from day 4, the weights of pups were reduced in the treated groups by 6-10%, 10-19% and 24-40% in increasing order of dose. In the 7-day post-weaning period, pup bodyweight gain was reduced by 13, 32 and 43% of control bodyweight gain, in ascending order of dose. Treated pup weights differed from controls to a statistically significant extent at ≥30 ppm at times during lactation, and in all treated groups in the post-weaning period. During lactation there was a concomitant reduction in maternal food and water consumption in all treated groups.

Red blood cell and brain ChE activities were inhibited in dams at all treatment levels at the time of weaning. Plasma ChE results at 10 ppm were highly variable, but given that inhibition of plasma ChE activity was seen in females in the subgroup after treatment at this dose for 22 days (see below), this was considered to be due to treatment also.

ChE activity in dams on postnatal day 21 (µmol-SH/mL or µmol-SH/g; n=5) and % inhibition relative to corresponding control

	0 ppm	**10 ppm**	**30 ppm**	**90/50 ppm**
Plasma	1.49 ± 0.15	1.16 ± 0.55 22%	1.01 ± 0.22 32%	0.84* ± 0.12 44%
RBC	1.95 ± 0.72	0.49** ± 0.29 75%	0.59** ± 0.24 70%	0.52** ± 0.10 73%
Brain	4.31 ± 0.50	3.02** ± 0.16 30%	2.53** ± 0.18 41%	2.31** ± 0.13 46%

*p≤0.5, **p≤0.01

Macroscopic findings for the adults were limited to those described above for the dams that died in the pre-mating period. There were no macroscopic findings in the pups that could be attributable to treatment. Pup absolute testes weight and testes weight relative to brain weight were reduced in treated groups in a dose-related manner, but no difference was apparent in the corresponding weights expressed relative to bodyweight. As testes weight, along with brain weight, is expected to be preserved with weight change, the changes in testes weight are considered to be treatment-related. Non-statistically significant differences in relative and absolute ovary weight were apparent at the two highest doses, but because of the variability in the control group, and the low numbers in the high dose group, it is not clear if ovary weight was affected by treatment. Relative, but not absolute brain weight was increased in pups of

both sexes at ≥30 ppm, but this was considered secondary to lower body weight in these groups.

Organ weights in pups (number of animals)

	0 ppm	10 ppm	30 ppm	90/50 ppm
Body weight (g) – males	41.8 ± 4.6	38.9 ± 4.4	33.9** ± 3.4	32.4, 32.3*
Body weight (g) – females	40.8 ± 3.6	38.1 ± 4.8	34.9** ± 2.9	26.4, 29.9
Brain – absolute (g) – males	1.41 ± 0.07	1.37 ± 0.06	1.37 ± 0.06	1.37, 1.37
– relative to bw	3.39 ± 0.27	3.55 ± 0.33	4.08 ± 0.33**	4.23 ± 0.01**
Brain – absolute (g) – females	1.37 ± 0.07	1.31 ± 0.11	1.34± 0.04	1.26, 1.32
– relative to bw	3.36 ± 0.23	3.46 ± 0.35	3.87 ± 0.26**	4.57 ± 0.22**
Testes – absolute (g)	0.22 ± 0.04	0.19* ± 0.03	0.18* ± 0.03	0.15, 0.16*
– relative to bw	0.52 ± 0.05	0.49 ± 0.04	0.52 ± 0.05	0.45, 0.50
– relative to brain wt	15.44 ± 2.18	13.87 ± 2.00	12.98* ± 2.25	10.71, 11.78*
Ovaries – absolute (g)	0.016 ± 0.007	0.016 ± 0.005	0.012 ± 0.005	0.008, 0.011
– relative to bw	0.040 ± 0.014	0.041 ± 0.015	0.035 ± 0.014	0.031, 0.036
– relative to brain wt	1.191 ± 0.460	1.201 ± 0.414	0.907 ± 0.355	0.658, 0.825

Note: For males, n=20, 19, 10 and 2; and for females n=20, 20, 10, and 2 in ascending order of dose. The actual scores are provided for the 2 animals in the 90/50 ppm group. *p≤0.5, **p≤0.01

Subgroup results

One male died on day 9 while being dosed at 90 ppm. Dark red discolouration of the lungs, and a reduction in the size of the spleen, prostate and seminal vesicles were seen at necropsy. Clinical signs in subgroup animals were similar to the main group. In both sexes, food consumption at 90/50 ppm was reduced by ~50% during the first week of treatment at 90 ppm, increasing after dose reduction during the second week (28-38% of control), with similar intake to control animals during the final week of treatment. This was associated with an ~75% reduction in bodyweight gain for both sexes at 90/50 ppm over the treatment period, the animals in this group showing a net bodyweight loss during treatment at 90 ppm. Females treated at 10 and 30 ppm showed lower bodyweights than controls, and these differences were statistically significant. However, as these differences were slight (≤7%), they were not considered to be biologically significant. At 90/50 ppm, water consumption was reduced in males (16-44%) till days 15/16 and in females (25-38%) till days 8/9. Intake of the omethoate is summarised in the table.

Subgroup test compound intake (range; mg/kg bw/d)

Group	10 ppm	30 ppm	90 ppm	50 ppm
Males	1.12-1.80	3.62-5.25	11.49-12.43	7.26-7.43
Females	1.42-1.94	3.88-5.04	13.13-14.12	7.71-9.27

*p≤0.5, **p≤0.01

Cholinesterase activities in RBC and brain were inhibited in the parental animals from all treated groups on day 22 of treatment to a similar extent to that found in dams at weaning. Plasma ChE activity was clearly inhibited in females at all doses, but the treatment-relatedness of the inhibition seen in males at ≥30 ppm is doubtful, given the extent of the inhibition, and the absence of a dose response.

ChE activity (µmol-SH/mL or µmol-SH/g; n=5)
and % inhibition relative to corresponding control

			Males				Females	
Dose (ppm)	**0**	**10**	**30**	**90/50**	**0**	**10**	**30**	**90/50**
Plasma	0.57 ±0.04	0.53 ± 0.02 7%	0.45** ± 0.03 20%	0.45** ± 0.02 20%	2.00 ± 0.52	1.43* ± 0.13 28%	0.96** ± 0.05 52%	0.65** ± 0.05 68%
RBC	1.84 ± 0.31	0.67** ± 0.07 64%	0.56** ± 0.09 69%	0.53** 0.08 71%	1.90 ± 0.17	0.80** ± 0.16 58%	0.44** ± 0.08 77%	0.66** ± 0.38 65%
Brain	4.95 ± 0.19	3.78** ± 0.15 24%	2.85** ± 0.14 42%	2.66** ± 0.11 46%	4.65 ± 0.12	3.24** ± 0.09 36%	3.02** ± 0.11 35%	2.79** ± 0.19 40%

Conclusion

Effects were seen at all doses in this study. These included inhibition of plasma, RBC and brain ChE activities in adult animals, as well as reduced bodyweight and reduced testes weights in pups. The LOEL was 10 ppm, approximately equivalent to 0.8 mg/kg bw/d.

Dotti A, Kinder J, Biedermann K, Luetkemeier H, Wright J (1992) E 6876 (c.n. omethoate): Two-generation reproduction study in the rat. Project no. 207336. Study no. T 8029476. Lab: RCC Research and Consulting Company AG, Itingen, Switzerland. Sponsor: Bayer AG. Expt dates: 18 September 1989 – 23 July 1990. Unpublished report date: 22 June 1990. (QA, GLP:Yes; Guidelines: US EPA 83-4, OECD 416).

Materials and Methods

Omethoate (purity ~96.7%, batch no. 234808038) was administered in the drinking water (adjusted to pH 3 with HCl) to rats (Wistar/HAN kfm:WIST, 25/sex/group, M: 124-175 g, F: 89-119 g) at 0, 0.5, 3 or 18 ppm for a 70-day pre-pairing period, and throughout the pairing, gestation and lactation periods for the F1 litters. The F1 generation was weaned at postnatal day 21, whereupon omethoate was administered similarly to 25/sex/group of the F1 for 126 days prior to pairing, and throughout the pairing, gestation and lactation periods for the F2 litters. Omethoate was stable in the drinking water preparations for at least 11 days at room temperature. Determination of ChE activity in plasma, RBC and brain were performed in 10 randomly selected rats/sex/group at necropsy. Measurement of brain ChE activity was also performed for 10 randomly selected F1 and F2 pups at 21 days *post partum*.

Results and Conclusions

There were no deaths in the P generation. In the F1, at 18 ppm one female died 5 days after expected delivery and one male died during blood sampling, and at 3 ppm, one female died on day 2 *post partum*. It was considered unlikely that any of these deaths was related to treatment. Food consumption was not affected by treatment in males, but 18 ppm females of the P generation showed a marginal reduction in food consumption during lactation, with a marked reduction (~20%) in the 18 ppm F1 group for the same period. Water consumption was reduced for both sexes at 18 ppm, most markedly in the F1 females during gestation and lactation. Bodyweight was reduced in 18 ppm males in the P generation, and though bodyweight gain was similar to controls thereafter, and in the F1 generation, absolute

bodyweight remained depressed in this group throughout the experiment. In females, bodyweight gain was retarded slightly throughout treatment of the P generation, but this was more marked in the F1 females during lactation. The amount of omethoate ingested is tabulated below.

Omethoate ingested (mg/kg bw/d)

	Males		**Females**			
			Pre-pairing/gestation		**Lactation**	
Dose (ppm)	**P**	**F1**	**P**	**F1**	**P**	**F1**
0.5	0.04-0.08	0.03-0.12	0.06-0.11	0.06-0.13	0.08-0.12	0.07-0.11
3	0.23-0.57	0.20-0.77	0.36-0.73	0.31-0.80	0.46-0.71	0.39-0.71
18	1.20-3.16	1.06-4.98	2.10-4.38	1.77-5.19	2.41-4.31	2.01-3.44

Reproduction parameters

P generation	**n=25**	**n=24**	**n=25**	**n=22**
No. implantations/dam	12.9 ± 2.1	13.5 ± 1.8	12.0 ± 3.1	11.7* ± 1.7
Postnatal loss (litters affected) (PND 5-21, % living pups PND 4)	0 (0)	0 (0)	2.1 (4)	5.7 (7##)
F1 generation	**n=22**	**n=23**	**n=20**	**n=15**
Mean precoital time (d)	3.6	2.5	2.9	5.6
Fertility index (%)	96	92	88	76
Post-implantation loss (% of implantations)	9.1	7.2	10.1	18.5
Postnatal loss PND 0-4 (litters affected) % living pups	2.5 (5)	3.2 (9)	2.9 (5)	14.5 (9#)
Postnatal loss PND 5-21 (litters affected)	1.1 (2)	1.1 (2)	0 (0)	5.2 (3)
Live pups at first litter check	12.6 ± 2.0	12.1 ± 2.2	11.9 ± 1.8	8.7* ± 3.4
Live pups PND 21	7.9 ± 0.3	7.8 ± 0.4	8.0 ± 0.2	6.1* ± 2.5

PND = postnatal day(s). *Steel test, significant at 5% level. #, ## Fishers Exact Test, significant at 5%, 1% level.

In the P generation, two 16 ppm dams lost all their pups by lactation days 7 or 11, but this was not of particular concern, as two dams in the F1 control group experienced similar losses (lactation days 1 and 2). At 16 ppm, the numbers of implantations/dam were reduced in both the P and F1. In the P generation, implantations/dam were also reduced at 3 ppm, but as this was mainly due to one female with 3 implantations only, and as there was no change at this dose in the F1, this is considered incidental to treatment. Increased postnatal loss occurred at 18 ppm in both generations, with post-implantation loss also increased, but in the F1 only, with a consequent reduction in the number of live pups/dam in the F1. Increases in precoital time and in the number of non-pregnant females was also restricted to the F1 at 18 ppm. Histopathological examination revealed marked epithelial vacuolation of the epididymides in P and F1 males at 18 ppm, but there was no evidence that this had affected reproductive performance. There were no findings in the various other tissues examined, and no teratogenic potential was observed in any group of either generation from external examination of the pups. Bodyweight gain in the pups of both generations was reduced at 18 ppm during lactation, with pup bodyweights reduced by 6–15% relative to controls.

Cholinesterase activity in plasma, RBC and brain (µmol-SH/mL or µmol-SH/g) n=10

			0 ppm	**0.5 ppm**	**3 ppm**	**18 ppm**
Plasma	P	Male	0.65 ± 0.07	0.60 ± 0.03 7%	0.60 ± 0.08 7%	0.49** ± 0.03 24%
		Female	1.13 ± 0.09	1.03 ± 0.09 9%	1.07± 0.24 6%	0.91 ± 0.34 20%
	F1	Male	0.67 ± 0.06	0.75* ± 0.06	0.73 ± 0.08	0.64 ± 0.05

		Female	2.29 ± 0.34	2.29 ± 0.51	2.33 ± 0.56	1.42** ± 0.23 38%
RBC	P	Male	1.73 ± 0.19	1.60 ± 0.18 8%	0.91** ± 0.10 47%	0.31** ± 0.06 82%
		Female	1.72 ± 0.11	1.38** ± 0.10 20%	0.99** ± 0.21 42%	0.25** ± 0.12 85%
	F1	Male	1.84 ± 0.18	1.70 ± 0.21 8%	1.11** ± 0.26 40%	0.34** ± 0.06 82%
		Female	1.48 ± 0.17	1.33* ± 0.10 10%	0.60** ± 0.12 59%	0.35** ± 0.05 76%
Brain	P	Male	4.61 ± 0.25	4.56 ± 0.51	3.80** ± 0.24 18%	2.82** ± 0.16 39%
		Female	4.11 ± 0.32	4.00 ± 0.23 7%	3.26** ± 0.13 21%	2.70** ± 0.18 34%
	F1	Male	7.55 ± 0.68	6.84* ± 0.50 9%	5.32** ± 0.53 30%	3.35** ± 0.60 54%
		Female	6.75 ± 0.35	5.68** ± 0.54 16%	4.72** ± 0.48 30%	3.04** ± 0.16 55%
	F1	Male pups	6.05 ± 0.67	5.92 ± 0.74	6.25 ± 0.52	5.80 ± 0.45
		Female pups	6.33 ± 0.45	6.30 ± 0.57	6.33 ± 0.53	5.56** ± 0.56 12%
	F2	Male pups	7.77 ± 0.41	7.57 ± 0.45	7.60 ± 0.69	6.44** ± 0.74 17%
		Female pups	8.08 ± 0.56	7.67 ± 0.61	6.86** ± 0.95 15%	6.45** ± 0.77 20%

*p≤0.05, **p≤0.01

Plasma ChE activity was reduced in both sexes at 18 ppm, but this was not consistent for the two generations. The difference in female control values suggests poor reproducibility for this assay. Erythrocyte ChE activity was clearly inhibited at 3 and 18 ppm, and was reduced to a statistically significant extent in 0.5 ppm females in both generations. In the P generation, all the RBC ChE findings at 0.5 ppm were less than the lowest concurrent control value, but in the F1, all but one value was within the concurrent control range, with both the control and 0.5 ppm values in the F1 comparable to the 0.5 ppm values in the P generation. Therefore, the greater inhibition in the P generation is a reflection of relatively higher control values. As the extent of inhibition in the P generation was borderline, and this was not a consistent finding across the two generations, the changes in RBC ChE at 0.5 ppm in females are not considered to be biologically significant. For the reduction in brain ChE activity that achieved statistical significance in the F1 at 0.5 ppm, the individual values in the treated groups were within their respective concurrent control ranges, and were therefore not considered to be biologically significant. Inhibition of brain ChE activity was considered to be treatment-related in adults at ≥3 ppm, in the pups of both generations and sexes at 18 ppm, and in the female F2 pups at 3 ppm, (7/10 values lower than the lowest control).

The parental NOEL for this study was 0.5 ppm (~0.04 mg/kg bw/d), due to inhibition of RBC and brain ChE activities at 3 ppm (~0.23 mg/kg bw/d). The NOEL for foetal effects was also 0.5 ppm (~0.04 mg/kg bw/d) due to inhibition of brain ChE activity in the pups at 3 ppm (~0.23 mg/kg bw/d).

8. DEVELOPMENTAL STUDIES

8.1 Rats

Bayer (1975) Evaluation of omethoate for embryotoxic and teratogenic effects in rats following oral administration. Report no. 5235. Lab: Department of Toxicology, Bayer AG, Wuppertal, FRG. Sponsor: Bayer AG. Expt dates: October 1974-January 1975. Unpublished report date: February 1975. ('Conducted in accordance with recommendations of the FDA.')

Note: The information that follows is from an OCS evaluation performed in 1992.

Omethoate was administered to mated female rats (Long Evans FB 30, 20-24/dose) at 0, 0.3, 1 or 3 mg/kg bw/d by gavage from gestation day 6 to 15. The rats were sacrificed on day 20.

There were no deaths. Bodyweight gain in dams at 3 mg/kg bw/d was reduced over the treatment period as a whole (to 54% of control values), and this difference was statistically significant ($p<0.05$). Weight gain in this group was reduced during the gestation period (13% less than controls). At this dose, total resorption occurred in 3/20 dams. This was associated with marked maternal toxicity, demonstrated by the average weight gain of these 3 dams being only 27% of control values during the gestation period, with 2/3 of these dams experiencing net weight loss for the treatment interval. The average foetal bodyweight and placental weights in the 3 mg/kg bw/d group were slightly lower than controls, this difference being statistically significant. The numbers of implantation sites and foetal sex ratios were not affected by treatment. There were no treatment-related increases in the incidence of visceral or skeletal malformations. The maternal and foetal NOELs were both 1 mg/kg bw/d, due to reduced bodyweight gain in the dams, and reduced foetal and placental weights at 3 mg/kg bw/d.

Holzum B (1990a) E 6876 (common name omethoate): Study for embryotoxic effects on rats following oral administration. Study no. T 8030636. Report no. 19222. Lab: Department of Toxicology, Bayer AG, Wuppertal, FRG. Sponsor: Bayer AG. Expt dates: 18 January 1989 – 14 December 1989. Unpublished report date: July 1990. (QA, GLP: yes; Guidelines: US EPA 83-3, OECD 414).

Materials and Methods

Mated female rats (Wistar Bor:WISW SPF Cpb from Winkelmann, Borchen; 25/dose) were dosed by gavage with omethoate (purity 96.6%, batch no. 234808038) in 0.5 Cremophor EL at 0, 0.3, 1, or 3 mg/kg bw/d for gestation days 6 to 15. Dosing solutions were prepared daily. Rats were killed on gestation day 20.

Results and Conclusions

At 3 mg/kg bw/d, one dam died on gestation day 11. Tremor was a prominent clinical sign in 24/25 of the dams at this dose, with isolated cases of exophthalmos, sunken flanks, bristling fur and blood-smeared muzzles. During the treatment period, food intake in the 3 mg/kg bw/d group was ~50% lower than controls (g/animal/day, statistically significant), with bodyweight gain concomitantly reduced to ~2% of control weight gain for this interval. In the 3 mg/kg

bw/d group, overall bodyweight gain (gestation days 0-20), corrected for uterus weight, was ~80% of control. There were no abnormal findings in the dams at gross necropsy.

There were no biologically significant variations in the number of corpora lutea, gestation rate, implantation rate, resorption rate, litter size, foetal sex ratio, or foetal weight following treatment. Placental weight was lower than controls to a statistically significant extent ($p<0.05$) at 3 mg/kg bw/d (0.65±0.06, 0.67±0.07, 0.68±0.12, 0.58*±0.05 g in ascending order of dose), but no differences in the placentas were apparent at macroscopic examination. There were no treatment-related increases in the incidences of malformations or skeletal deviations. The NOEL for maternal and foetal toxicity was 1 mg/kg bw/d due to clinical signs and reduced bodyweight gain in the dams, and reduced placental weight at 3 mg/kg bw/d.

8.2 Rabbits

Tesh JM, Ross FW, Wightman TJ, Wilby OK (1982) S 6876: Effects of oral administration upon pregnancy in the rabbit. 2. Main study. LSR Report No. 82/BAG023/111. Lab: Life Science Research, Stock, Essex, CM4 9PE, England. Sponsor: Bayer AG, Werk Elberfeld Institut fur Toxicologie, Friedrich-Ebert-Strasse 217-319, Wuppertal, Postfach 10 17 09, Germany. Expt dates: 22.11.1981-24.12.1981. Unpublished report date: 13 September 1982. (QA: yes; GLP, Guidelines: None stated).

Materials and Methods

Omethoate (batch no. EG1/81; lot no. 2559) was administered by gavage to New Zealand White rabbits (Morton Rabbits, Stansted, Essex, England; 3.57-4.63 kg at study initiation; 14/dose) at 0, 0.1, 0.3 or 1.0 mg/kg bw/d in distilled water from gestation day 6 to 18. Control animals received vehicle only. Oestrus was synchronised by an iv injection of 25 IU luteinising hormone, followed by a 3 week acclimatisation period, after which the does were artificially inseminated with pooled semen from bucks of established fertility. Animals that aborted were sacrificed on the day of abortion, and subjected to detailed macroscopic examination. Blood samples for assessment of ChE activity were withdrawn on day 6 prior to the first dose, and on gestation day 18. With the exception that food consumption was not recorded, the study was essentially similar to current guidelines.

Statistical analyses: Various tests (t-test or multiple t-test, Mann-Whitney U-test, χ^2-test, Fisher's Exact Probability Test) were used as appropriate to assess inter-group differences.

Results

Three control does and one in each of the 0.1 and 0.3 mg/kg bw/d groups were killed *in extremis*. Necropsy findings showed the deaths were associated with accidental tracheal intubation, respiratory tract infection or GIT disorder, none of which was likely to be due to exposure to the test material. One doe in each of the control and 0.3 mg/kg bw/d groups aborted both showing evidence of upper respiratory tract infection at necropsy. On day 29, resorption of an entire litter was evident in one doe at 0.1 mg/kg bw/d. One doe in each of the control and 0.3 mg/kg bw/d groups, and two at 0.1 mg/kg bw/d, failed to become pregnant. Bodyweight gain during gestation was similar for all groups. At the conclusion of dosing, whole blood ChE activity was inhibited by ~25% in does in the 1 mg/kg bw/d group, both with respect to the pre-dosing activity for that group, and to the concurrent control group.

Effects on ChE activity were not apparent at the lower doses. Due to the relatively low numbers of litters delivered in the control group, control data from a concurrent study were also provided to assist interpretation of data in the present study. There were no maternal or foetal findings that indicated an effect of treatment. The NOEL for maternal toxicity was 0.3 mg/kg bw/d, due to inhibition of whole blood ChE activity at 1.0 mg/kg bw/d (Table 20). The NOEL for foetal effects was 1.0 mg/kg bw/d, due to the absence of treatment-related findings at the maximum dose tested.

Table 19. Whole blood cholinesterase activity (IU/L); % of pre-dosing level and post-dosing control level

Dose (mg/kg bw/d)	0	0.1	0.3	1.0
Number in group	9	10	11	14
Pre-dosing activity	1320 ± 254	1251 ± 176	1306 ± 208	1218 ± 196
Post-dosing activity	1246 ± 277	1295 ± 207	1264 ± 240	914** ± 203
% pre-dosing level	94.4	103.5	96.8	75.0
% control post-dosing level	100.0	103.9	101.4	73.4

**p<0.01

Holzum (1990b) E 6876 (common name: omethoate) Study for embryotoxic effects on rabbits following oral administration. Report no. 19221. Study no. T0032834. Lab: Department of Toxicology, Bayer AG, Wuppertal, FRG. Sponsor: Bayer AG. Expt dates: May 1989–January 1990. Unpublished report date: 4 July 1990. (QA, GLP: yes; Guidelines: US EPA 83-3, OECD 414).

Materials and Methods

Omethoate (purity 96.6%, batch no. 234808038) was administered by gavage to mated female rabbits (Himalayan, 15/dose) at 0, 0.2, 1 or 5 mg/kg bw/d in acidified water containing 0.5% Cremophor EL on gestation days 6 to 18 inclusive. The rabbits were sacrificed on gestation day 29. An additional 5 rabbits/group were treated similarly, and used for the assay of plasma, RBC and brain ChE activities for which blood was withdrawn prior to treatment on gestation days 6, 7, and 14 and on gestation day 19, 24 hours after the last treatment. The stability of dosing solution batches was confirmed for the period of use (5 days).

Results and Conclusions

There were no deaths, no treatment-related effects on food or water consumption, and no abnormal findings at autopsy. At 5 mg/kg bw/d, clinical signs included tremor (all animals at this dose), increased heart rate (13/20), ataxia (2/20) and prostration (1/20). Also at this dose, there was a marked decrease in weight gain during the treatment period (~90%), but this was highly variable. Bodyweight gain for the total gestation period was unaffected by treatment. No significant differences (p>0.05) were observed between control and treated animals with respect to the numbers of corpora lutea and implantation sites, gestation index, foetal sex ratio, mean foetal bodyweights, and placental weights. The average number of foetuses in the 5 mg/kg bw/d group (5.0) was slightly lower than the control (5.6). Also, the mean number of resorptions was increased at 5 mg/kg bw/d (1.0), as well as at 1 mg/kg bw/d (1.1) relative to the control group (0.3). However, as a clear dose response was lacking for the resorption rate, and the numbers of foetuses and resorptions were within the historical control ranges provided, these differences were considered unlikely to have resulted from treatment.

No foetal malformations were seen in the control and 0.2 mg/kg bw/d groups, but at 1 mg/kg bw/d, 4/77 foetuses from a total of 3 litters had malformations, as did 2/70 foetuses from 2 litters at 5 mg/kg bw/d. The rates of malformations in these two groups were higher than the malformation rates in historical controls (representing 10 groups, 1985-1989). The malformations in the omethoate-treated groups comprised arthrogryposis (persistent flexure or contracture of a joint) of the front extremities in 3 (3.9%) of foetuses at 1 mg/kg bw/d and 2 (2.9%) at 5 mg/kg bw/d. There was one case of epignathus (tumour arising from the soft or hard palate) at 1 mg/kg bw/d. The number of instances of arthrogryposis at 1 and 5 mg/kg bw/d exceeded the historical control incidence (1.6% from 10 studies), and epignathus did not occur in any of the historical control groups. Therefore, though clear dose responses were not evident for either of these malformations, a relationship to treatment cannot be dismissed.

Maternal plasma and RBC ChE activities were reduced on gestation days 14 and 19. Plasma ChE activity was inhibited at 5 mg/kg bw/d, while inhibition of RBC and brain ChE activities at 1 and 5 mg/kg bw/d was dose-related (Table 21). The NOEL for maternal and foetal effects was 0.2 mg/kg bw/d. This is based on inhibition of RBC and brain ChE activity in the does, and foetal malformations at ≥1 mg/kg bw/d.

Table 20. Maternal cholinesterase activity in plasma, RBC and brain in kU/L (% inhibition) n=5

Dose (mg/kg bw/d)		0	0.2	1	5
Gestation day 14	Plasma	**0.55 ± 0.04**	**0.64± 0.05****	**0.53 ± 0.04**	**0.31± 0.03 (43%)*****
	RBC	**0.73 ± 0.06**	**0.68 ± 0.10**	**0.53 ± 0.09 (27%)****	**0.19± 0.06 (74%)*****
Gestation day 19	Plasma	**0.47 ± 0.07**	**0.46 ± 0.04**	**0.42 ± 0.05 (10%)**	**0.29± 0.03 (38%)****
	RBC	**0.68 ± 0.08**	**0.64 ± 0.05**	**0.40 ± 0.07 (41%)*****	**0.09± 0.02 (87%)*****
	Brain	**3.77 ± 0.55**	**3.12 ± 0.64 (17%)**	**2.39 ± .35 (36%)****	**1.31± 0.13 (65%)*****

**p≤0.01, ***p≤0.001

9. GENOTOXICITY STUDIES

A summary of findings from submitted *in vitro* genotoxicity studies is tabulated below (Table 22). Evaluations of studies that resulted in positive findings are provided after the table.

Table 22. Summary of *in vitro* Genotoxicity Studies

Assay	Bacterial strain or Cell type	Conc. or Dose	Batch / Purity	Metab. Act.	Result	Reference
Gene Mutation						
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA 1535 TA 1537	20-12,500 µg/plate with or without S9 activation	S 6876 Batch no. 14051979	+, - +, - +, - +, -	-/- +/+ -/+ -/-	Herbold (1980)
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA 1535 TA 1537	775-12,400 µg/plate	unknown	+, - +, - +, - +, -	-/- +/+ +/+ -/-	Herbold (1988a) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 1535 TA 100 TA 1537 TA 98 TA 102	Chloroacetic acid-N-methylamide (an intermediate of Folimat) 16-5000 µg/plate	Batch no. 101 97.9%	+, - +, - +, - +, - +, -	-/- -/- -/- -/- -/-	Herbold (1998) [GLP]
Reverse mutation in *Saccharomyces cerevesiae*	S138 S211α	0.03-66.67 µL/mL with or without S9	S 6876 Batch no. 234 208 022 96.9%	+, - +, -	-/- -/-	Hoorn (1982) [GLP]
Mitotic crossing over and gene conversion in *Saccharomyces cerevesiae*	D7	0.03-66.67 µL/mL with or without S9	S 6876 Batch no. 234 208 022 96.0%	+, -	+/+ (both tests)	Hoorn (1983) [GLP]
Forward mutation in mammalian cells	Mouse lymphoma L5178Y (TK +/-)	500-2000 µg/mL with S9; 500-5000 µg/mL without S9	Batch no. 234-208-022 96.9%	+, -	-/-	Bootman & Rees (1982)
Forward mutation in mammalian cells	CHO cells	500-4000 µg/mL (-S9); 3000-6000 µg/mL (+S9)	97.4%	+, -	-/- (see evaluation below)	Lehn (1989) [GLP]

Summary of *in vitro* Genotoxicity Studies (cont.)

DNA Damage and Repair						
Alkaline elution assay	CHO cells (CHO-WB1 subclone)	50-500 µg/mL with and without activation	07.05.93 B19 95.3%	+,-	+/+	Brendler-Schwaab (1995a) [GLP]
Alkaline elution assay	Primary rat testes cells (Sprague-Dawley)	250-5000 µg/mL without activation	07.05.93 B19 93.5-95.1%	-	+	Brendler-Schwaab (1995b) [GLP]
Pol test (Ames test screening)	E. coli (K12)p 3478 and W 3110	625-10,000 µg/plate	S 6876 234 208 022 96.0%	+,-	-/-	Herbold (1983)
Unscheduled DNA synthesis	Primary rat hepatocytes	0.513 – 5130 µg/mL	97.4%	-	+	Cifone (1989) [GLP]
Chromosomal Effect Assays						
Chromosomal Aberration	Human lymphocytes	10-1000 µg/mL (-S9); 70-7000 µg/mL (+S9)	97.2%	+/-	+/+ *	Bayer (1986)
Sister Chromatid Exchange	CHO cells	Up to 5.0 mg/mL	96%	+/-	+/+	Taalman (1988) [GLP]

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation. * positive results produced at cytotoxic concentrations.

Herbold (1980) S 6876 Salmonella/microsome test for detection of point-mutagenic effects. Report No. 9126. Lab: Bayer Institute of Toxicology. Sponsor: Bayer AG, Institut fur Toxicologie, Wuppertal, Germany. Expt dates: January-February 1980. Unpublished report date 9 May 1980. (QA, GLP, Guidelines: no).

Materials and Methods

Omethoate (S 6876, batch no. 14051979, purity 95.1%) was incubated on plates at concentrations of 0, 20, 100, 500, 2500 and 12,500 µg/plate, with *Salmonella typhimurium* strains TA 1535, TA 1537, TA 100, and TA 98, with and without S9. Positive control substances were endoxan (active ingredient cyclophosphamide, an alkylating agent and known promutagen; 217.5 µg/plate with TA 1535 and TA 100; solvent demineralised water) and trypaflavine (a frameshift promutagen; 200 µg/plate with TA 1537 and TA 98; solvent DMSO). Plates were incubated for 48 h at 37°C)

Results and Conclusion

The test material was not toxic to the bacteria at any of the concentrations used, nor was growth inhibited. There were dose-related increases in the numbers of mutants for strains TA 1535 (-S9) and TA 100 (+/-S9), which were confirmed in repeat experiments, and on this basis were accepted as evidence that omethoate was mutagenic in these strains (Table 23). However, it is noteworthy that the positive findings occurred at relatively high omethoate concentrations (≥3000 µg/plate). An apparent increase in mutants in strain TA 98 (-S9) at 12,500 µg/plate was not reproducible, so was not considered a true effect. The positive

control substances induced appropriate responses. In this study, omethoate was mutagenic at relatively high concentrations in 2 strains of *S. typhimurium*.

Table 23. Revertants/plate

Strain (trial no.) Conc. (µg/plate)	TA 1535 +S9	TA 1535 -S9 (1)	TA 1535 -S9 (2)	TA 100 +S9 (1)	TA 100 +S9 (2)	TA 100 –S9 (1)	TA 100 –S9 (2)	TA 1537 +S9	TA 1537 -S9	TA 98 +S9	TA 98 –S9 (1)	TA 98 –S9 (2)
0	11.0	7.5	8.8	151.5	100.5	143	129.5	8.0	5.0	31.3	8.8	23.5
20	5.8	22.5	5.0	142.0	-	140.5	-	5.5	4.0	36.3	11.8	-
100	2.3	-	8.0	146.0	-	135.5	-	5.0	8.8	30.3	9.0	-
500	6.8	17.0	9.3	180.5	-	120.5	-	7.0	6.3	31.5	10.0	-
2500	14.5	23.5	13.3	219.8	-	132.5	-	9.5	3.5	30.8	15.0	-
3000	-	-	-	-	177.5*	-	238.0*	-	-			19.5
6000	-	-	-	-	313.0*	-	395.5*	-	-			28.8
12000	-	-	-	-	534.8*	-	574.5*	-	-			19.5
12500	11.8	20.0*	50.5*	498.5*	-	289.5*	-	9.8	2.3	31.0	32.8	-

* Scores considered indicative of mutagenicity

Herbold BA (1988a) E 6876 c.n. Omethoate. Salmonella/microsome test to evaluate for point mutagenic effects. Study no. T 8027676. Report no. 17243. Lab: Bayer AG Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333 D-5600 Wuppertal 1, FRG. Sponsor: Bayer AG. Expt. dates: 16 March 1988 – 25 March 1988. Unpublished report date: 18 October 1988. (QA, GLP: yes; Guidelines: none stated).

Omethoate (775-12,500 µg/plate) was tested for induction of gene mutation in *Salmonella* strains TA 98, TA 100, TA 1535 and TA 1537 in the presence and absence of metabolic activation (S9). Positive controls in the absence of S9 were sodium azide (10 µg/plate) and nitrofurantoin (0.2 µg/plate), and 2-aminoanthracene in the presence of S9. Omethoate was not cytotoxic in this test system at concentrations up to 12,500 µg/plate. Positive results were obtained in the TA 100 and TA 1535 strains (+/- S9), but the other two strains were negative. The TA 100 strain was the more sensitive, showing dose-related increases in revertants at ≥1550 µg/plate, with TA 1535 showing increases from ≥2500 µg/plate. The positive controls gave appropriate responses, while their solvent, DMSO, was negative.

Hoorn AJW (1983) S 6876 (c.n. omethoate; a.i. of Folimat) in the induced mitotic crossing over and gene conversion assay in Saccharomyces cerevisiae strain D7. Final report. Bayer study no. T 1008210. Lab: Bayer AG Institut fuer Toxicologie, Postfach 101709, 5600 Wuppertal 1, West Germany. Expt dates: 14.6.1983-24.6.1983. Unpublished report date: July 1983. (QA: yes GLP: yes; Guidelines: none stated).

Materials and Methods

Omethoate (S6876, batch no. 234 208 022, purity 96.0%, diluted in water), at concentrations ranging from 0.03 to 66.67 µL/mL, was incubated with *Saccharomyces cerevesiae* D7, with and without metabolic activation (S9), for 3 h at 30°C, then plated onto minimal media supplemented with adenine and leucine for the mitotic gene conversion test, or onto yeast complete media for the mitotic recombination test. The plates (at least 4/treatment level) were incubated at 30°C for 2-7 days. A preliminary study did not reveal toxicity to the test

organism over the chosen concentration range. The positive controls were ethylmethanesulfonate (EMS) at 1% and sterigmatocystin at 5 µg/mL. The negative control was deionised sterile water.

Results and Conclusion

In the mitotic gene conversion test, the numbers of tryptophan convertants increased at 67 µL/mL (+/- S9), and possibly also at 33 µL/mL in the presence of S9. The total numbers of mitotic recombinants were considered to have increased in a dose-related manner at ≥ 16 µL/mL (–S9) and at ≥ 33 µL/mL (+S9). In the absence of S9, increases in non-reciprocal crossovers were responsible for the increase in recombinants at omethoate concentrations below 66 µL/mL. Survival (%) was similar across all treatment levels, and the positive control produced appropriate responses. The results are summarized in Table 24. Under the conditions of these experiments, omethoate induced gene mutations and mitotic crossing over in yeast cells.

Table 24. Mitotic parameters

	Mitotic gene conversion		**Mitotic recombinants**					
Dose	**Tryptophan convertants**		**Reciprocal crossovers**		**Non-reciprocal crossovers**		**Total crossovers**	
(µL/mL)	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
0	15	11	0	0	7	7	7	7
0.03	8	5	1	2	6	15	7	17
0.33	5	8	1	0	11	11	12	11
3.33	10	10	1	6	12	9	13	15
16.67	15	10	1	1	21	16	22	17
33.33	17	24	5	17	18	18	23	35
66.67	96	105	33	52	66	67	99	119

Lehn H (1989) E 6876 (c.n. omethoate): mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro. Study no. T 0030430. Bayer report no. 17977. Department of Toxicology, Bayer AG, Wuppertal, FRG. Expt dates: 15 November 1988 – 28 December 1988. Unpublished report date: 26 April 1989. (QA, GLP: yes; Guidelines: none stated).

Omethoate (purity 97.4%, batch no. 234808038) was tested *in vitro* for forward mutation at the HGPRT locus in CHO cell cultures, at omethoate concentrations of 500 to 4000 µg/mL without metabolic activation (-S9), and 3000 to 6000 µg/mL (+S9). Preliminary cytotoxicity testing at 100-7500 µg/mL omethoate showed that survival was reduced at ≥3000 µg/mL without activation, and at ≥5000 µg/mL with activation. Positive controls were ethylmethane-sulphonate (0.9 mg/mL) and dimethylbenzanthracene (20 µg/mL) in the presence and absence of metabolic activation, respectively.

Cell survival was reduced to less than 10% at ≥4000 µg/mL (-S9) and ≥4500 µg/mL (+S9). In the absence of S9, the highest dose group was not cloned due to lack of growth during the expression period. Increases in mutation frequency were apparent at 2000 and 4000 µg/mL in the first –S9 trial, though this was not evident at 2000 µg/mL in the repeat experiment. In the presence of S9, all concentrations ≥4000 µg/mL showed increased mutation frequencies above control levels, but dose-responses were lacking and cytotoxicity was high (Table 25). The study authors considered that the results of this study indicated that omethoate was

mutagenic in *S. typhimurium*, but as this occurred at highly cytotoxic doses, and dose responses were generally lacking, it is not considered that the data supports such a conclusion.

Table 25. Mutation frequency

	Mutation frequency							
Omethoate conc. (µg/mL)	**-S9 (Trial 1)**		**-S9 (Trial 2)**		**+S9 (Trial 1)**		**+S9 (Trial 2)**	
0	6.2	8.6	9.6	5.0	6.6	3.0		8.2
500	11.2	9.8	6.5	4.7	-	-	-	-
1000	13.8	5.7	16.1*	7.9	-	-	-	-
2000	30.2*	16.5*	10.3	2.3	-	-	-	-
3000	-	-	-	-	30.0*	5.7	14.7	
4000	83.8*	128.6*	24.3*	13.6*	33.3*	30.5*	26.7*	24.0*
4500	-	-	-	-	44.5*	35.7*	35.3*	
5000	-	-	-	-	53.5*	39.1*	31.3*	29.2*
6000	Not cloned				39.0*	34.7*	44.2*	35.0*
Vehicle control	10.8	4.0	3.1	1.7	4.9	14.9	8.1	12.7
Positive control	417.2*	278.4*	344.5*	284.4*	65.6*	76.2*	54.5*	96.2*

(-) = not tested at this concentration; *p≤0.05.

Bayer (1986) Chromosome aberration in human lymphocyte cultures in vitro. Bayer report no. 15149. Department of Toxicology, Bayer AG, Wuppertal, FRG. Expt dates: February 1986-April 1986. Unpublished report date October 1986. (GLP: yes).

Note: The information that follows is from an OCS evaluation performed in 1992.

Omethoate (97.2% pure) was tested *in vitro* for its potential to induce chromosome aberrations in human lymphocyte cultures. Omethoate concentrations used in the incubations were 10-1000 µg/mL (-S9) or 70-7000 µg/mL (+S9). Mitomycin (0.1µg/mL) and cyclophosphamide (10 µg/mL) were used as positive controls in the absence or presence of metabolic activation, respectively.

The mitotic index was reduced in the presence of omethoate at ≥100 µg/mL (-S9) and at 7000 µg/mL (+S9). Significant increases in clastogenic effects, namely metaphases with aberrations including and excluding gaps, and metaphases with exchanges, occurred at 100 µg/mL (-S9) and at 7000 µg/mL (+S9). That is, omethoate produced clastogenic effects in this assay only at cytotoxic concentrations.

Brendler-Schwaab S (1995a) E6876 Alkaline elution in vitro for the detection of induced DNA single strand breaks in CHO cells. Study No. T 6050308. Report No. 24449. Lab: Institute for Industrial Chemicals, Fachbereich Toxicology, Bayer AG. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. Expt dates: 13.8.1993-13.10.1993. Unpublished report date 7 November 1995. (QA:Yes; GLP: OECD and FIFRA; Test guidelines: None stated).

Materials and Methods

As a result of a preliminary cytotoxicity test performed with omethoate dissolved in PBS (concentration range 50-5000 µg/mL), with or without S9, concentrations of 500, 1000, 2000, 4000 and 5000 µg/mL were chosen for this investigation. For each experiment, CHO cells (CHO-WB1 subclone; 2.5 x 10^6 cells/mL) were incubated at 37°C with vehicle (PBS), a

positive control substance, or omethoate at the above test concentrations, in triplicate, for 45 min (+S9) or 4 h (-S9). The positive controls were ethylmethanesulfonate (EMS; -S9; final conc. 2500 µg/mL); or 2-aminoanthracene dissolved in DMSO (final conc. 30 µg/mL). The S9 was from livers of male Wistar rats induced by Aroclor 1254. The DNA single strand breaks were monitored using the alkaline filter elution technique of Kohn et al. (1981) with minor modifications (Brendler et al., 1992) . Each experiment was repeated once.

Results and Conclusion

As shown in Table 26, the mean % DNA retained on the filter (expressed as % of the total DNA for each incubation), decreased with increasing omethoate concentration. In the presence of S9, and for the first experiment in which S9 was absent, the levels were sufficiently below control values to be considered a genotoxic effect at concentrations of 1000 µg/mL omethoate and higher. In the second -S9 experiment, the decrease in DNA on the filter commenced at a higher concentration, though this was partly due to relatively low levels of DNA bound to the control filters in this instance. The author stated that the frequency of vehicle control single strand breaks were all within the normal range for this assay, though these data were not provided. The positive controls gave appropriate responses. The viability of treated cells relative to vehicle controls was >93% for 3 of the experiments (control viability 81-95%), with moderate cytotoxicity apparent in some incubations for one experiment with S9 (lowest relative viability 72%). Omethoate was considered genotoxic in this assay.

Table 26. Mean % DNA on filters

	-S9		+S9	
Omethoate conc. (µg/mL)	**Experiment 1**	**Experiment 2**	**Experiment 1**	**Experiment 2**
0	100	82.1	93.5	97.6
500	96.8	75.4	86.6	82.3
1000	78.8	68.6	56.9	59.7
2000	25.0	65.9	10.6	47.2
4000	10.1	26.4	1.1	10.6
5000	8.9	12.0	0	1.7
+ve control	3.7	3.5	58.2	60.7

Brendler-Schwaab S (1995b) E6876 Alkaline elution in vitro for the detection of induced DNA single strand breaks in rat primary testes cells. Study No. T 5050307. Report No. 24453. Lab: Institute for Industrial Chemicals, Fachbereich Toxicology, Bayer AG. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. Expt dates: 13.10.1993-23.9.1994. Unpublished report date 8 November 1995. (QA:Yes; GLP: OECD and FIFRA; Test guidelines: None stated).

Materials and Methods

For each experiment, primary rat testes cells from Sprague-Dawley rats (4.1-6.0 x 10^6 cells/mL) were incubated at 37°C with vehicle (PBS), a positive control substance, or omethoate at 250, 500, 1000, 2000, 4000 or 5000 µg/mL in triplicate, for 45 min at 37°C. The positive control was ethylmethanesulfonate (EMS; final conc. 3600 µg/mL). One rat was used

per experiment. Doses were selected following cytotoxicity testing. The alkaline elution procedure was performed essentially as described in Brendler-Schwaab (1995a).

Results and Conclusion

Of 6 experiments performed, 3 were rejected. Two rejections were due to technical problems, and the third resulted from the absence of an appropriate response in the positive control. For all incubations, viability was not affected by exposure to the test material or the positive control substance, with viability relative to control ranging from 91 to 102%. Control viability was 88-92%. The mean % DNA retained on the filter for the 3 successful experiments is shown in the table. An increase in DNA strand breaks occurred in each of the experiments in the presence of omethoate, but there was some variation between experiments with respect to the concentration at which this occurred. In experiments 1 and 3, significant change relative to the corresponding control was present from 1000 and 250 µg/mL respectively, with a dose response generally apparent across the higher doses. In experiment 2, only treatment at 4000 µg/mL was considered to represent a genotoxic effect. The positive control showed appropriate responses in each of the experiments. Omethoate was considered genotoxic in this assay. The results of this trial are summarised in Table 27.

Table 27. Mean % DNA on filters

Omethoate conc. (µg/mL)	Experiment 1	Experiment 2	Experiment 3
0	76.0	72.0	69.9
250	-	82.8	47.5
500	58.8	79.4	37.5
1000	50.1	71.2	35.4
2000	45.0	60.3	27.3
4000	40.4	42.5	20.5
5000	40.8	-	-
+ve control	31.5	19.1	27.1

Cifone MA (1989) Mutagenicity test on E 6876 in the rat primary hepatocyte unscheduled DNA synthesis assay. Bayer report no. R4678. Project no. Lab: Hazelton Laboratories America, Maryland, USA. HLA study no. 10419-0-447. Sponsor: Bayer AG. Expt dates: August 1988-September 1988. Unpublished report date: February 1989. (QA, GLP: yes; Guidelines: none stated).

Omethoate (purity 97.4%, batch no. 234 808 038), ranging in concentration from 0.513 to 5130 µg/mL in 1% DMSO, was tested for its potential to induce unscheduled DNA synthesis in primary cultures of rat hepatocytes. The positive control was 2-acetylamidofluorene (0.1 µg/mL).

Concentrations of omethoate ≥2050 µg/mL were lethal, and cytotoxicity was observed at 1030 µg/mL (62.5% survival) and 513 µg/mL (79.1% survival). At omethoate concentrations of ≤256 µg/mL, survival was >85%. No toxicity was apparent at <103 µg/mL. A concentration-related increase in unscheduled DNA synthesis was observed from 256 to 1030 µg/mL omethoate. The positive control produced a strong response. This study shows that omethoate can induce DNA damage in mammalian cells *in vitro* (Table 28).

Table 28. Unscheduled DNA synthesis results

Omethoate conc (µg/mL)	UDS grains/nucleus	Average % nuclei with >6 grains
25.6	-1.35	2.0
51.3	0.67	6.0
103	0.47	6.7
256	2.03	18.0
513	9.27	76.7
1030	19.05	99.3
Solvent control	0.42	4.7
Positive control	24.15	95.3

Values represent averages from triplicate coverslips.

Taalman RDFM (1988) Clastogenic evaluation of E 6876 in an in vitro cytogenetic assay measuring sister chromatid exchange in Chinese ovary (CHO) cells. Bayer report no. R4469. Study no. T 2027706. Lab: Hazelton Biotechnologies, The Netherlands. Sponsor: Bayer AG. Expt dates: 21 March 1988 – 7 June 1988. Unpublished report date: 7 June 1988. (GLP: yes; Guidelines: none stated).

Omethoate (purity 96%, batch no. 233 790 471) at concentrations of up to 5.0 mg/mL in 1% DMSO, was tested for its potential to cause sister chromatid exchanges (SCEs) in Chinese hamster ovary cells *in vitro*, in the presence and absence of metabolic activation (+/-S9). Positive controls were 5 or 10 ng/mL Mitomycin-C (-S9) and 1.5 or 2.0 µg/mL cyclophosphamide (+S9).

In the absence of metabolic activation, cytotoxicity was apparent at >2.0 mg/mL omethoate, and cell cycle delay occurred at >1 mg/mL. The incubation with 5-bromo-2'-deoxyuridine (BrdU) of cultures showing cell cycle delay was therefore extended by 17.4 hours, in addition to the usual ~27.5 h exposure period. However, in the presence of metabolic activation, neither cell cycle delay nor cytotoxicity was seen at concentrations of omethoate up to 5.0 mg/mL. Dose-related increases in SCE frequency were obtained at 100 µg/mL to 1.0 mg/mL omethoate in the absence of metabolic activation, and at 167 µg/mL to 5.0 mg/mL omethoate in its presence. The untreated and solvent control SCE frequencies were within normal background levels, and the positive controls showed appropriate responses (Table 29). Therefore, omethoate was considered positive for the induction of SCE in this assay, both in the presence and absence of metabolic activation.

Table 29. SCEs/diploid

	SCEs/diploid (mean ± SD)			
Omethoate conc.	**-S9 (trial 1)**	**-S9 (trial 2)**	**+S9 (trial 1)**	**+S9 (trial 2)**
100 µg/mL	13.5* ± 0.56	15.4* ± 0.59	-	-
167 µg/mL	-	-	9.2 ± 0.55	-
250 µg/mL	24.8* ± 0.85	24.1* ± 0.69	-	-
500 µg/mL	39.9* ± 1.2	44.0* ± 0.97	10.8* ± 0.5	-
1 mg/mL	80.6* ± 2.3 #	83.8* ± 1.7	-	-
1.7 mg/mL	-	-	17.3* ± 0.64	-
2 mg/mL	-	-	-	17.9 ± 0.6
3 mg/mL	-	-	-	26.1* ± 0.9
4 mg/mL	-	-	-	33.3* ± 1.0
5 mg/mL	-	-	39.6* ± 1.0	42.7* ± 1.4
Untreated control	7.8 ± 0.41	7.4 ± 0.31	8.4 ± 0.37	7.7 ± 0.4
Solvent control	8.5 ± 0.44	8.3 ± 0.45	9.3 ± 0.51	7.8 ± 0.4
Positive control	27.9* ± 1.0	21.2* ± 0.8	47.6* ± 1.5 (1.5µg/mL) 60.48* ± 1.9 (2 µg/mL)	59.8* 1.4 (1.5 µg/mL)

increased incubation time in BrDu; (-) not assayed at this dose.

A summary of findings from submitted *in vivo* genotoxicity studies is tabulated below (Table 30).

Table 30. Summary of *in vivo* Genotoxicity Studies

Assay	Species (Strain)	Dose	Batch / Purity	Result	Reference
Gene Mutation					
Dominant lethal mutation	Mouse (NMRI)	0, 5 mg/kg bw; gavage, single dose	95.4%	-	Machemer L (1974)
Dominant lethal mutation	Mouse (NMRI SPF Han Wistar)	0, 10, 20 mg/kg bw; gavage, single dose	96.9%	-	Herbold (1991) [GLP]
Spot test	Cross-bred C57B1/6J x T stock	0, 4, 8, 16 mg/kg bw; gavage, single dose	~97%	+	Herbold (1990b) [GLP]
Chromosomal Effect Assays					
Micronucleus (femoral bone marrow)	Mouse (NMRI SPF Han Wistar)	0, 6, 12 mg/kg bw; gavage, 2 doses 24 h apart	97.1%	-	Herbold (1981)
Micronucleus (bone marrow)	Mouse (NMRI SPF Han Wistar)	22.5 mg/kg bw; gavage, single dose	96.0%	-	Herbold (1988b) [GLP]
Alkaline elution assay (testes cells)	Rat (young adult male, Sprague Dawley strain Crl:CD(SD)BR)	0, 8, 15, 30 mg/kg bw, gavage, single dose	07.05.93 B19 93.5-94.4%	-	Brendler-Schwaab (1996) [GLP]
Sister Chromatid Exchange (bone marrow)	Chinese hamsters	0, 5, 10, 20 mg/kg bw, gavage, single dose	96.7%	-	Herbold (1990a) [GLP]
DNA Damage and Repair					
Unscheduled DNA synthesis (hepatocytes)	Rat (Wistar)	0, 3, 10, 30 mg/kg bw, gavage, single dose. (1/3 deaths at 30 mg/kg bw)	96.6%	-	Benford (1989) [GLP]

Results are expressed as +, positive; -, negative; +/-, equivocal.

Herbold BA (1990b) E 6876: Spot test on cross-bred C57B1/6J x T stock mouse fetuses to evaluate for induced somatic changes in the genes of the coat pigment cells. Bayer report no. 19017, Study No. T 2032890. Expt. dates: 14 July 1989 - 24 November 1989. Unpublished report date: 24 April 1990. (QA, GLP: Yes; Guidelines: none stated).

Materials and Methods

Omethoate (purity ~97%, batch no. 234808038) was administered to female mice (C57B1/6JBom from Bomholtgaard Ltd, Denmark; 21-31 g) at 0, 4, 8 or 16 mg/kg bw via stomach tube on the 10th day after mating with adult T stock males (12-24 weeks old). Sufficient females were used to provide 300 F1 animals for evaluation. The vehicle was deionised water, and 1-ethyl-1-nitrosourea (40 mg/kg bw i.p.) was used as the positive control. The F1 animals were examined for coloured spots on the coat once during the period 12 to 16 days after birth, and once again during postnatal days 25 to 35.

Results and Conclusion

Mice treated at 16 mg/kg bw showed overt signs of toxicity (apathy, prone position, shivering, breathing difficulty, white tears, salivation). The numbers of pregnant females bearing litters and the litter sizes were not affected by treatment. The numbers of animals with relevant spots (RS) exceeded controls at all doses, though the dose response was fairly flat (Table 31). The incidence of white mid-ventral spots (WMVS) increased with dose from 8 mg/kg bw, outnumbering the RS at 16 mg/kg bw. It is accepted practice that the WMVS are not regarded as relevant in the measurement of possible genotoxic effects, and their occurrence is attributed to melanocyte death. Doses of omethoate ≥8 mg/kg bw therefore appear to be toxic to melanocytes. The positive control produced an appropriate response. In this test, omethoate was mutagenic at the doses tested.

Table 31. Number and % of F1 mice with/without spots

	Without spots		**With spots**			
			WMVS		**RS**	
Dose (mg/kg bw)	n	%	n	%	n	%
0	339	97.7	6	1.7	2	0.6
4	334	94.6	7	2.0	12**	3.4
8	295	92.2	12	3.8	13**	4.1
16	300	87.5	24**	7.0	19**	5.5
Positive control	284	73.0	22**	5.7	83**	21.3

*p≤0.05, ** p≤0.01 (Chi-square test)

10. NEUROTOXICITY STUDIES

10.1 Rats

Mellert W, Deckardt K, van Ravenzwaay B (2002a) Omethoate. Study for the determination of the peak-effect for clinical signs/FOB in Wistar rats; single administration by gavage and 24 h observation period. Lab. Project no. 11C0709/01063. Lab: Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Sponsor: Dimethoate Task Force (DTF) c/o P. Hopfmann, Wotanstr. 39 D-68305 Mannheim, Germany. Expt dates: 11 March 2002 – 13 March 2002. Unpublished report date: 5 July 2002. (QA: no; GLP: no, as no analytical examinations of the test substance preparations were carried out; test guidelines do not exist for this type of study).

Materials and Methods

Omethoate (96.5% pure; batch no. 676-BSe-74B) was administered as a solution in doubly distilled water prepared immediately prior to dosing, to Wistar rats (CrlGlxBrlHan:W1 from Charles River, Sulzfeld, Germany; 41-45 days old; 5/sex/dose) as a single oral dose of 0, 5, 10 or 15 mg/kg bw, with controls receiving vehicle only. Animals were observed for a range of parameters (clinical signs/FOB) immediately after treatment and at 1, 2, 4, 7 and 24 h post-dosing, in a standard arena outside the home cage. Blood samples were obtained at 8 h after treatment and prior to necropsy (~24 h) for analysis of Hct, and serum and RBC ChE activities. Brain ChE activity was also determined.

Results and Conclusions

There were no deaths. The main signs in the treated groups were tremors, gait impairment, loss of pupillary reflex, irregular respiration, and frequent chewing. No signs were observed in the controls. At 5 mg/kg bw, the time of peak effect was during the interval 2-4 h post-dosing, and at 1-4 h for the 10 and 15 mg/kg bw groups. At 8 and 24 h after treatment, serum ChE activity was inhibited at ≥5 mg/kg bw and ≥10 mg/kg bw in males and females respectively. Erythrocyte ChE activity was inhibited in both sexes at ≥5 mg/kg bw at both time points, as was brain Che activity at 24 h (Table 32).

Table 32. Cholinesterase activity in serum (µkat/L), erythrocytes (µkat/L RBC) and brain ((µkat/g protein) at 8 or 24 hours post-treatment, and percent inhibition relative to the corresponding concurrent control

	Males				Females			
Dose (mg/kg bw):	**0**	**5**	**10**	**15**	**0**	**5**	**10**	**15**
Serum 8 h	11.9 ± 2.2	5.75** ± 0.74 51%	4.90** ± 0.73 58%	4.25** ± 0.77 64%	27.6 ± 8.8	26.4 ± 2.8 4%	13.2** ± 4.9 52%	14.96** ± 4.4 47%
Serum 24 h	10.8 ± 2.0	7.34** ± 0.89 32%	5.62** ± 0.97 47%	5.06** ± 1.03 53%	25.0 ± 7.3	24.6 ± 2.6	12.9** ± 2.7 48%	11.1** ± 3.1 55%
RBC 8 h	27.3 ± 4.2	12.1** ± 1.5 55%	9.64** ± 1.5 64%	7.02** ±0.79 74%	28.4 ± 4.0	11.7** ± 1.9 58%	9.04** ± 1.03 68%	8.71** ± 0.49 69%
RBC 24 h	31.6 ± 4.9	17.6** ±1.2 44%	11.8** ± 2.4 62%	8.9** ±2.0 71%	44.3 ± 2.8	22.7** ± 2.6 48%	16.2** ± 1.6 63%	13.4** ± 1.9 69%

Brain 24 h	1.33 ± 0.7	0.79 ± 0.20 40%	0.59 ± 0.22 55%	0.54* ± 0.20 59%	1.56 ±2.8	0.93 ± 0.38 40%	0.88* ± 0.22 43%	0.69** ± 0.12 55%

Mellert W, Deckardt K, van Ravenzwaay B (2002b) Omethoate. Study for the determination of cholinesterase inhibition in Wistar rats; single administration by gavage and 24 h observation period. Lab. project no. 11C0709/01078. Lab: Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Sponsor: Dimethoate Task Force (DTF) c/o P. Hopfmann, Wotanstr. 39 D-68305 Mannheim, Germany. Expt dates: 10 June 2002 – 11 June 2002. Unpublished report date: 11 October 2002. (QA: no; GLP: no, as no analytical examinations of the test substance preparations were carried out; test guidelines do not exist for this type of study).

Materials and Methods

Omethoate (96.5% pure, batch no. 676-BSe-74B) was administered as a single oral dose to groups of 10 male Wistar rats (CrlGlxBrlHan:W1 from Charles River, Sulzfeld, Germany, 42-44 days old) at 0, 0.25, 0.50, 0.75 or 1.50 mg/kg bw. Controls received the vehicle only (doubly distilled water). Doses were prepared immediately prior to treatment. Blood samples were obtained from non-fasted animals 3 days prior to treatment, 2.5 h after treatment, and prior to necropsy, and analysed for Hct, and serum and RBC ChE activities. Brain ChE activity was determined.

Results and Conclusions

Cholinesterase activity was inhibited in RBC to a biologically significant extent at ≥0.50 mg/kg bw at 2.5 h after treatment, but at 24 h after treatment, inhibition of RBC ChE activity was only present at 1.5 mg/kg bw to an extent considered likely to be treatment-related. Serum ChE activity was inhibited at ≥0.75 mg/kg bw at 2.5 h post-treatment, but not at 24 h. Brain ChE activity was clearly inhibited at 1.50 mg/kg bw, but as the extent of brain ChE activity at 0.50 and 0.75 mg/kg bw was relatively small, and a dose response was lacking, it is unlikely that inhibition of brain ChE activity at the lower doses was related to treatment (Table 33). As the inhibition of RBC ChE activity at 0.25 mg/kg bw was sufficiently slight at 2.5 h post-treatment so as not to be considered biologically significant, 0.25 mg/kg bw is considered the no-effect level for ChE inhibition in this study.

Table 33. Cholinesterase activity in serum (µkat/L), RBC (µkat/L RBC) and brain ((µkat/g protein) at 3 days before treatment, and 2.5 or 24 hours post-treatment, and percent inhibition relative to the corresponding concurrent control

Dose (mg/kg bw)	Serum Day -3	Serum 2.5 h	Serum 24 h	RBC Day -3	RBC 2.5 h	RBC 24 h	Brain 24 h
0	12.1 ± 2.3	12.4 ± 2.3	11.9 ± 1.9	32.3 ± 5.8	32.5 ± 2.7	33.3 ± 3.3	3.20 ± 1.25
0.25	12.1 ± 1.7	11.6 ± 1.3	11.3 ± 1.6	32.6 ± 3.9	28.3** ± 2.4 12%	33.9 ± 3.2	3.23 ± 1.57
0.50	12.1 ± 1.8	10.8 ± 1.6	11.0 ± 1.5	34.7 ± 5.4	23.8** ± 2.6 26%	30.8 ± 3.0	2.8 ± 0.7 13%
0.75	12.5 ± 2.4	10.0* ± 1.9 19%	11.3 ± 2.3	32.9 ± 2.9	20.1** ± 2.7 37%	28.7** ± 3.6 13%	2.85 ± 1.02 10%
1.50	12.4	8.03**	10.8	33.1	14.2**	25.0**	2.21

	± 1.8	± 1.33 35%	± 2.1	± 3.3	± 1.3 56%	± 3.0 24%	± 0.55 30%

*p<0.05 **p<0.01

Mellert W, Deckardt K, Kaufmann W, van Ravenzwaay B (2003) Omethoate – acute oral neurotoxicity study in Wistar rats; single administration by gavage. Lab. project no. 20C0709/01098. Lab: Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Sponsor: Dimethoate Task Force (DTF) c/o P. Hopfmann, Wotanstr. 39 D-68305 Mannheim, Germany. Expt dates: 8 October 2002 – 12 December 2002. Unpublished report date: 4 December 2003. (QA, GLP: yes; Guidelines: OECD 424 and OPPTS 870.6200).

Materials and Methods

Omethoate (batch no. 676-BSe-74B, purity 96.5%) was administered to rats (Wistar CrL\lGlxBrlHan:W1; 25/sex; ~49 days old) by gavage at 0, 0.2, 0.25, 0.35 or 5 mg/kg bw. Controls were given the vehicle only (doubly distilled water). Cholinesterase activity was determined in the serum and RBC of 10 rats/sex prior to administration and in the serum, RBC and brain at 2.5 h post-treatment. Another 10 rats/sex were assessed for functional observation battery (FOB) and motor activity on days -7, 0 (about 2 h post-treatment), 7 and 14 as well as for serum and RBC ChE activity at pre-test and on day 15 post-treatment, at which point brain ChE activity was also assessed. A further 5 rats/sex were also similarly assessed for FOB and motor activity, but were perfused *in situ* and given neuropathological examinations at study termination.

Results and Conclusions

There were no deaths. General clinical signs (apathy, increased respiration, frequent chewing, and tremor) were confined to the 5 mg/kg bw group at about 2 h post-treatment. Differences in the incidence of home cage and open field observations relative to controls were also observed only at this dose at 2 h after dosing. These included increases in the incidence of slight tremors, irregular respiration, urine staining of anogenital fur and impairment of gait in both sexes, increased incidence of soft faeces in males, and frequent chewing, reduced area of exploration and postural differences in females. At 0.35 mg/kg bw, respiration was accelerated in one male, and irregular in another, while one female at this dose showed impairment of gait. It is possible that these symptoms were related to treatment, given the inhibition of brain ChE activity at this dose (see below). Retarded or no adaptation of the pupil to light was recorded in both sexes at 0.25, 0.35 and 5 mg/kg bw/d, also only at 2 h post-treatment. Of the 15 rats/sex/group tested, pupillary reflexes were affected in a total of 3 males and 2 females at 0.25 mg/kg bw, and 2 males and 2 females at 0.35 mg/kg bw, compared to 15 males and 14 females at 5 mg/kg bw. Given the lack of change in ChE activity at 0.25 mg/kg bw, and the absence of a dose response, it is unlikely that this was related to treatment. Also at 2 h post-treatment, 5 mg/kg bw females showed decreases in rearing, grip strength of forelimbs and hindlimbs, while motor activity was reduced in both sexes.

At 2.5 h post-treatment, inhibition of ChE activity was evident in the serum, RBC and brain of both sexes at 5 mg/kg bw. At the same time, brain ChE activity was also inhibited at 0.35 mg/kg bw (Table 34). There was no evidence of biologically significant inhibition of ChE

activity at any other time point. The only microscopic changes reported were axonal degeneration of the peripheral nerves which was present in one rat of each sex at 5 mg/kg bw, but as this also occurred in one of the control females, this is unlikely to be due to treatment. The NOEL for this study is 0.25 mg/kg bw, due to threshold inhibition of ChE activity in blood and brain at 0.35 mg/kg bw.

Table 34. Cholinesterase activity in serum (µkat/L), RBC (µkat/L RBC) and brain (µkat/g protein) at 2.5 hours post-treatment, and percent inhibition relative to the corresponding concurrent control

	Males			Females		
Dose (mg/kg bw)	**Serum**	**RBC**	**Brain**	**Serum**	**RBC**	**Brain**
0	11.7 ± 1.7	31.9 ± 3.4	3.87 ± 0.7	28.7 ± 10.0	33.8 ± 6.1	4.47 ± 0.8
0.20	11.8 ± 1.7	31.5 ± 1.3	3.62 ± 0.5 6%	27.7 ± 5.8 6%	31.4 ± 2.2 7%	3.83 ± 0.8 14%
0.25	11.6 ± 1.7	27.9 ± 2.2 13%	3.57 ± 0.8 8%	29.9 ± 9.0 8%	31.1 ± 4.3 8%	4.03 ± 0.5 10%
0.35	10.7 ± 1.5 9%	26.8* ± 3.2 16%	3.14 ± 0.8 19%	27.2 ± 4.9 19%	29.3 ± 3.4 13%	3.25* ± 0.9 27%
5	5.4** ± 1.1 54%	7.22** ± 1.0 77%	0.75** ± 0.2 81%	16.9 ± 5.6 81%	8.3** ± 1.3 75%	0.89** ± 0.2 80%

*p≤0.05, **p≤0.01

10.2 Hens

Bayer (1972) Acute neurotoxicity in hens. Report no. 3439. Lab: Department of Toxicology, Bayer AG, Wuppertal, FRG. Sponsor: Bayer AG. Unpublished report date: May 1972.

Note: The information that follows is from an OCS evaluation performed in 1992.

In a preliminary acute oral study in white leghorn hens, the LD_{50} for omethoate was 92 mg/kg bw. The lowest dose that led to death was 70 mg/kg bw. Clinical signs of acute ChE inhibition were seen within 2-4 h of omethoate administration (range of doses not specified). The positive control was 350 mg/kg bw tri-ortho-cresyl phosphate (TOCP).

In the main study, after pre-treatment with 50 mg/kg bw atropine by the ip route, 10 hens were dosed with 92 mg/kg bw omethoate by oral intubation. Four omethoate-treated hens died within 6 days of dosing. There were no signs of neurotoxicity in the survivors, which were sacrificed at 3 weeks post-treatment, along with the positive controls treated with TOCP. Macroscopic and histological examinations of the brain, spinal chord and sciatic nerve in omethoate-treated survivors showed no treatment-related changes. Degenerative changes were apparent in the spinal chord or sciatic nerve in hens treated with TOCP.

Bayer (1990) Acute oral toxicity in the hen. Report no. 19318. Lab: Department of Toxicology, Bayer AG, Wuppertal, FRG. Sponsor: Bayer AG. Expt dates: June 1989 – August 1989. Unpublished report date: July 1990. (GLP: yes).

Note: The information that follows is from an OCS evaluation performed in 1992.

Single oral doses of omethoate were administered by gavage to hens (white leghorn, 5/dose) at 5 dose levels in the range 20-80 mg/kg bw/d. They were then observed over a 3-week period. Deaths occurred in 4 hens at each of the two highest doses, 71 and 80 mg/kg bw, between day 1 and day 3 post-treatment. Clinical signs seen at all doses were diarrhoea, staggering gait, dry/flaccid comb, salivation (marginal), and respiratory distress. These signs lasted for 1 to 2 weeks, their intensity increasing with dose. Throughout the study, moulting was observed in all hens treated with 63 mg/kg bw omethoate. Survivors at both 71 and 80 mg/kg bw weighed ~12-40% less at the end of the study than they did on day 1. There was no evidence of delayed neurotoxic signs in survivors. Gross pathological examination of animals that died as a result of treatment revealed signs of lobulation of the liver, pale spleen, mucous membrane of the glandular stomach and duodenum reddened in places, and lung distended and filled with fluid. The acute oral LD_{50} was between 63 and 70 mg/kg bw.

Bomann W, Sykes AK (1993) E 6876 (c.n. omethoate) Study for delayed neurotoxicity following acute oral administration to the hen. Study no. T 7033128. Report no. 21958. Lab/Sponsor: Bayer AG, Wuppertal, Institute of Toxicology Agriculture, Fachbereich Toxikologie, Friedrich-Ebert-Str. 217-333. Expt dates: 19.9.1989-31.10.1989. Unpublished report date: 7 January 1993. (QA: Yes; GLP: OECD and FIFRA; Test guidelines: US EPA 81-7).

Materials and Methods

Omethoate (E 6876 batch no. 234 808 038; 96.7% pure) was dissolved in demineralised water and administered by gavage to 15 non-fasted adult Lohmann Leghorn hens (from Brinkschulte, Senden; 8 months and 17 days old; bodyweight 1.45-1.57 kg) at 140 mg/kg bw with antidote protection. There were 2 treatments, 3 weeks apart. The choice of dose was based on an earlier acute oral toxicity study showing an LD_{50} between 63 and 71 mg/kg bw, with other preliminary studies indicating that 150 mg/kg bw was tolerated in the presence of antidote. Thirty minutes prior to dosing with omethoate, atropine was administered subcutaneously at 20 mg/kg bw. Atropine in combination with pyridine-2-aldoxim 1-methochloride (PAM), each at 50 mg/kg bw, was administered subcutaneously at the time of both omethoate treatments, and at 25 mg/kg bw each, approximately 7, 23, 31, 47 and 55 h post-treatment. As a positive control, tricresylphosphate-MTS 1922 (TOCP, isomeric mix), formulated in demineralised water with 2% v/v Cremophor EL, was administered once by gavage at approximately 400 mg/kg bw to 5 hens. The vehicle control group of 6 hens was gavaged with demineralised water at study initiation and at 3 weeks. Treated and control hens were observed for 21 days after each omethoate treatment.

Results and Conclusion

The positive control hens were killed in a moribund state on day 18. Two hens in the omethoate group died (days 1 and 31). In this group, apathy, staggering gait, diarrhoea, spasms, dry comb, lying on side or prostrated, panting, and ruffled feathers were observed starting on day 1, but had resolved by day 8. After the second omethoate dose, clinical signs were similar to the above, with the addition of flaccid comb, and in one hen, laboured breathing and increased salivation. These signs had resolved 16 days after the second treatment. Normal behaviour was observed in the vehicle control group, while the positive control group showed a progressive deterioration in coordination (from ataxia to paresis) from

day 8, in the absence of acute signs of neurotoxicity. When animals were observed during forced movement, only the positive control group exhibited signs, starting on day 8 and increasing in severity until they were killed as moribund. Hens treated with omethoate lost weight after each treatment, with only partial compensatory weight gain during the ensuing observation periods. The positive control group lost weight steadily, while the negative control group maintained their initial bodyweight. Necropsy findings for the omethoate-treated hens that died prematurely were lung severely distended, containing fluid; spleen pale; crop distended; white slimy film on liver and heart; liver pale; mottled kidneys, somewhat pale and enlarged; ulcerous foci in glandular stomach; mucosa of duodenum reddened in places. Histological examination of the brain, spinal medulla and sciatic nerve showed changes to nerve tissue in the positive control group only. Overall, there were no signs of delayed neurotoxicity in the hens exposed to omethoate up until the end of the study (43 days). The positive control gave appropriate responses.

REFERENCES

[Figures in square brackets are an Australian identification code and indicate the location of the submitted data.]

Bayer AG (1967) Omethoate/antidote effect and potentiation. Sponsor: Bayer AG, Farbenfabriken, Institute for Toxicology. 14.2.1967. Unpublished. [BA; sub: 239, Vol. 2 of 9].

Bayer (1972) Acute neurotoxicity in hens. Report no. 3439. Sponsor: Bayer AG, Wuppertal, FRG. May 1972. Unpublished. [BA; sub 9032].

Bayer (1975) Evaluation of omethoate for embryotoxic and teratogenic effects in rats following oral administration. Report no. 5235. Sponsor: Bayer AG, Wuppertal, FRG. February 1975. Unpublished. [BA; sub 9032].

Bayer (1986) Chromosome aberration in human lymphocyte cultures in vitro. Bayer report no. 15149. Department of Toxicology, Bayer AG, Wuppertal, FRG. October 1986. Unpublished. [BA; sub 9032].

Bayer (1990) Acute oral toxicity in the hen. Report no. 19318. Sponsor: Bayer AG, Wuppertal, FRG. July 1990. Unpublished. [BA; sub 9032].

Benford DJ (1989) Ex vivo hepatocyte UDS study with omethoate. Bayer report no. R4854. Lab: Robens Institute of Health and Safety, University of Surrey, UK. Sponsor: Bayer AG. Expt dates: April 1989-July 1989. October 1989. Unpublished. [BA; sub 9032. CHA; sub: 12564, Vol 3-26 of 39].

Bomann W and Sykes AK (1993) E 6876 (c.n. omethoate) Study for delayed neurotoxicity following acute oral administration to the hen. Study no. T 7033128. Report no. 21958. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 7.1.1993. Unpublished. [BA; sub: 12540, Vol. 10 of 11. CHA; sub: 12564, Vol 3-36 of 39].

Bomhard E, Löser E, Kaliner G (1979) S 6876 chronic toxicity study on rats (2-year feeding experiment). Report No. 8507. Lab: Bayer AG, Institut fur Toxikologie, Wuppertal. Sponsor: Bayer AG. Institut fur Toxikologie, Wuppertal, Germany. 18.7.1979. Unpublished. [BA; sub: 239, Vol. 5 of 9].

Bootman J, Rees R (1982) S 6876: Investigation of mutagenic activity in the TK+/- mouse lymphoma cell mutation system. LSR Report No. 82/BAG027/448. Sponsors Study No. T5006216. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 20.10.1982. Unpublished. [BA; sub: 239, Vol. 7 of 9].

Brendler-Schwaab S (1995a) E6876 Alkaline elution in vitro for the detection of induced DNA single strand breaks in CHO cells. Study No. T 6050308. Report No. 24449. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 7.11.1995. Unpublished. [BA; sub: 12540, Vol. 9 of 11].

Brendler-Schwaab S (1995b) E6876 Alkaline elution in vitro for the detection of induced DNA single strand breaks in rat primary testes cells. Study No. T 5050307. Report No. 24453. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 8.11.1995. Unpublished. [BA; sub: 12540, Vol. 9 of 11].

Brendler-Schwaab S (1996) E6876 Alkaline elution in vivo for the detection of induced DNA-single strand breaks in rats testes. Study No. T 4050306/T 4054069. Report No. 24749. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 2.2.1996. Unpublished. [BA; sub: 12540, Vol. 9 of 11].

Brendler SY, Tompa A, Hutter KF, Preussmann R, Pool-Zobel BL (1992) In vivo and in vitro genotoxicity of several N-nitrosamines in extrahepatic tissues of the rat. Carcinogenesis 13: 2435-2441.

Bomhard E, Rinke M (1994) Frequency of spontaneous tumours in Wistar rats in 2-year studies. Experimental Toxicology and Pathology 46: 17-29.

Bootman J, Rees R (1982) S 6876: Investigation of mutagenic activity in the TK+/- mouse lymphoma cell mutation system. Study no. T 5006216. Sponsor: Bayer AG. 20.10.1982. [CHA; sub: 12564, Vol 3-26 of 39].

Cifone MA (1989) Mutagenicity test on E 6876 in the rat primary hepatocyte unscheduled DNA synthesis assay. Bayer report no. R4678. Sponsor: Bayer AG. February 1989. Unpublished. [BA; sub 9032. CHA; sub 12564 Vol. 3-26 of 39].].

Curtes JP, Develay P, Hubert JP (1979) Late peripheral neuropathy due to an acute voluntary intoxication by organophosphoric compounds. Abstract International Congress of Neurotoxicology, Varese (Italy) 27-30 September.

Dauterman WC, Casida JE, Knaak JB, Kowalcyk T (1959) Animal metabolism of insecticides. Bovine metabolism of organophosphorous insecticides. Metabolism and residues associated with oral administration of dimethoate to rats and three lactating cows. Agricultural and Food Chemistry 7:188-193.

Dolara P, Salvadori M, Capobianco T, Torricelli F (1992) Sister-chromatid exchanges in human lymphocytes induced by dimethoate, omethoate, deltamethrin, benomyl and their mixture. Mutation Research 283: 113-118.

Dotti A, Biedermann K, Luetkemeier H (1994) E 6876 (c.n. Omethoate) range finding study to the two-generation reproduction study in the rat. RCC Project 207325. Bayer project T 6029500. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 12.7.1994. Unpublished. [BA; sub: 12540, Vol. 9 of 11. CHA; sub: 12564, Vol. 3-35 of 39].

Dotti A, Kinder J, Biedermann K, Luetkemeier H, Wright J (1992) E 6876 (c.n. omethoate): Two-generation reproduction study in the rat. PN 207336. Study no. T 8029476. RCC Research and Consulting Company AG, Itingen, Switzerland. Sponsor: Bayer AG.

Ecker W, Coelin R (1981) Omethoate: biotransformation of dimethyl S (N-methyl-

[^{14}C]carbamoylmethyl)phosphorothioate. Pharma Report No. 10100. PF-Report No. 1574. Sponsor: Mobay Chemical Corporation, Agricultural Chemicals Division. 7.8.1981. Unpublished. [BA; sub*:* 239, Vol. 8 of 9].

Flucke W (1978) S 6876, the active ingredient of ®Folimat. Studies on acute toxicity to rats and determination of cholinesterase activity in blood plasma, erythrocytes, and brain. Bayer Report No. 7373. Sponsor: Bayer AG, Institut fuer Toxicologie. Wuppertal-Elberfeld. 10.3.1978. Unpublished. [BA; sub: 239, Vol. 2 of 9; CHA; sub: 12564, Vol 3-25 of 39].

Flucke W (1984) S 6876 (c.n. omethoate) Study for skin-sensitising effect on guinea pigs in the open epicutaneous test. Report No. 13084. Sponsor: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld. 29.11.1984. Unpublished. [BA; sub: 239, Vol. 3 of 9].

Flucke W and Luckhaus G (1979) S 6876 (Omethoate, the active ingredient of Folimat®) Subacute dermal toxicity study on rabbits. Bayer Report No. 8407. Sponsor: Bayer AG. 29.5.1979. Unpublished. [BA; sub: 239, Vol. 3 of 9. CHA; sub: 12564, Vol 3-26 of 39].

Fogleman RW, Levinskas GJ (1963) Report on oxygen analog of dimethoate: twenty-eight day feeding of rats. Report no. 63-12**.** Sponsor: Cheminova. 1.7.1963. Unpublished. [CHA; sub: 12564, Vol 3-25 of 39].

Herbold B (1980) S 6876 Salmonella/microsome test for detection of point-mutagenic effects. Report No. 9126. Sponsor: Bayer AG, Institut fur Toxicologie, Wuppertal, Germany. 9.5.1980. Unpublished. [BA; sub: 239, Vol. 7 of 9].

Haenen C, De Moor A, Dooms-Goossens A (1996) Contact dermatitis caused by the insecticides omethoate and dimethoate. Contact Dermatitis 35: 54-55.

Herbold (1981) S-6876 (Omethoate, Folimat's active ingredient): Micronucleus test on mouse to evaluate S-6876 for mutagenic potential. Report no. 10021. Sponsor: Bayer AG, Institute of Toxicology, Wuppertal, FRG. June 1981. Unpublished. [BA; sub 9032].

Herbold B (1983) S6876 Omethoate. Folimat active ingredient. Pol test on E. coli to evaluate for DNA damage. Report No. 12126. Sponsor: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld. 4.10.1983. Unpublished. [BA; sub: 239, Vol. 7 of 9].

Herbold BA (1988a) E 6876 c.n. Omethoate. Salmonella/microsome test to evaluate for point mutagenic effects. Study no. T 8027676. Report no. 17243. Sponsor: Bayer AG. 18.10.1988. Unpublished. [BA; sub 9032. CHA; sub 12564 Vol. 3-26 of 39].

Herbold (1988b) E 6876 (c.n. omethoate): micronucleus test in the mouse to evaluate for clastogenic effects. Bayer report no. 17231. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, FRG. October 1988. Unpublished. [BA; sub 9032. CHA; sub 12564 Vol. 3-26 of 39].

Herbold BA (1990a) E 6876 sister chromatid exchange in bone marrow of Chinese hamsters in vivo. Bayer report no. 19003. Sponsor: Bayer AG, Department of Toxicology, Bayer AG, Wuppertal, FRG. April 1990. Unpublished. [BA; sub 9032; CHA; sub 12564 Vol. 3-26 of 39].

Herbold BA (1990b) E 6876: Spot test on cross-bred C57B1/6J x T stock mouse fetuses to evaluate for induced somatic changes in the genes of the coat pigment cells. Bayer report no. 19017. Sponsor: Bayer AG, Wuppertal, FRG. April 1990. Unpublished. [BA; sub 9032; CHA; sub 12564 Vol. 3-26 of 39].

Herbold BA (1991) Dominant lethal test on the male mouse to evaluate for mutagenic effects. Report no. 20089. Sponsor: Bayer AG, Wuppertal, FRG. March 1991. Unpublished. [BA; sub 9032. CHA; sub 12564 Vol. 3-26 of 39].

Herbold B (1998) Chloroacetic acid-N-methylmide (Intermediate of Folimat). Special Study. Ames-test screening. Study No. T 4059811. Report No. PH-27744. Sponsor: Bayer AG Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 4.8.1998. Unpublished. [BA; sub: 12540, Vol. 10 of 11].

Hoffmann K, Schilde B (1984) S 6876 (Omethoate) Chronic toxicity to dogs on oral administration (Twelve-month stomach tube study). Report no: 12561. Sponsor: Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, Germany. 26.3.1984. Unpublished. 26 March 1984. [BA; sub: 239, Vol. 6 of 9. CHA; sub: 12564, Vol. 3-26 of 39].

Holzum B (1990a) E 6876 (common name omethoate): Study for embryotoxic effects on rats following oral administration. Study no. T 8030636. Report no. 19222. Sponsor: Bayer AG. 5.7.1990. Unpublished. [BA; sub 9032; CHA; sub: 12564, Vol 3-36 of 39].

Holzum (1990b) E 6876 (common name: omethoate) Study for embryotoxic effects on rabbits following oral administration. Report no. 19221. Study no. T 0032834. Sponsor: Bayer AG. 4.7.1990. Unpublished. [BA; sub 9032; CHA; sub: 12564, Vol 3-36 of 39].

Hoorn AJW (1982) Mutagenicity evaluation of S 6876 – omethoate. Batch no. 234 208 022, content 96.9% in the reverse mutation induction assay with Saccharomyces cerevesiae strains S138 and S211α. Final report. Bayer study no. T 4006215. Sponsor: Bayer AG Institut fuer Toxicologie, Postfach 101709, 5600 Wuppertal 1, West Germany. November 1982. Unpublished. [BA; sub: 239, Vol. 7 of 9].

Hoorn AJW (1983) S 6876 (c.n. omethoate; a.i. of Folimat) in the induced mitotic crossing over and gene conversion assay in Saccharomyces cerevisiae strain D7. Final report. Bayer study no. T 1008210. Sponsor: Bayer AG Institut fuer Toxicologie, Postfach 101709, 5600 Wuppertal 1, West Germany. July 1983. Unpublished. [BA; sub: 239, Vol. 7 of 9].

Hoshino T (1990) [Methylene-^{14}C]omethoate: General metabolism in the rat. Lab. Project ID M 01810019. Bayer AG, Crop Protection Research, Chemical Product Development and Environmental Biology, Institute for Metabolism Research, D-5090 Leverkusen-Bayerwerk, Germany. [BA; sub: 9959; CHA; sub: 12564, Vol 3-25 of 39].

Hutchison EB, Pope SJ, Schaeffer TR, Varney CH, Woolston SA (1968) Report on oxygen analog of cygon dimethoate: ninety-day feeding to dogs (CL 28,580). Report no. 68-89. Sponsor: Bayer AG. 12.8.1968. Unpublished. [CHA; sub: 12564. Vol. 3-25 of 39].

Isoda H, Talorete TPN, Han J, Oka S, Abe Y, Inamori Y (2005) Effects of

organophosphorous pesticides used in China on various mammalian cells. Environmental Sciences 12: 9-19.

Kimmerle G (1968) Bayer 45 432. Toxicological studies. Report No. 582. Sponsor: Bayer AG, Institut für Toxikologie, Wuppertal-Elberfeld. 12.1.1968. Unpublished. [BA; sub: 239, Vol. 2 of 9].

Klecak G, Geleick H, Grey JR (1977) Screening of Fragrance Materials for Allergenicity in the Guinea Pig.-1. Comparison of Four Testing Methods. *Journal of the Society of Cosmetic Chemists.* 28:53-64 (1977).

Kohn KW, Ewing RAG, Erickson LC, Zwelling LA (1981) Measurement of strand breaks and cross-links by alkaline elution. In: Friedburg EC and Hanawalt PC (eds.) DNA repair, a laboratory manual of research procedures. M. Dekker, New York. pp. 379-401.

Krötlinger F (1986a) E 6876 1000 SL 00671/0360 (c.n. Omethoate) Study for acute oral toxicity in rats. Study No. T6021904. Bayer Report No. 14768. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 24.6.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Krötlinger F (1986b) E 6876 1000 SL 00671/0360 (c.n. Omethoate) Study for acute dermal toxicity in rats. Study No. T 7021905. Bayer Report No. 14767. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 24.6.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Krötlinger F (1986c) E 6876 500 SL 00671/0360 (c.n. Omethoate) Study for acute oral toxicity to rats. Study No. T 3021839. Bayer Report No. 14469. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 18.3.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Krötlinger F (1986d) E 6876 500 SL 00671/0360 (c.n. Omethoate) Study for acute dermal toxicity to rats. Study No. 4021858. Bayer Report No. 14769. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 24.6.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Krötlinger F (1987) E 6876 5 SL 00671/0360 (c.n. Omethoate) Study for acute oral toxicity in rats. Study No. T 8025669. Bayer Report No. 14768. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 21.10.1987. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Krötlinger F (1989a) E 6876 [c.n. omethoate] Study for acute oral toxicity in rats. Study No. T 4029689. Bayer Report no. 17566. Bayer AG, Department of Toxicology, Friedrich-Ebert-Str. 217-333 D-5600 Wuppertal 1. 5.1.1989. Unpublished. [CHA; sub: 12564, Vol 3-25 of 39].

Krötlinger F (1989b) E 6876 [c.n. omethoate] Study for acute dermal toxicity in rats. Study No. T 3029688. Bayer Report no. 17665. Bayer AG, Department of Toxicology, Friedrich-Ebert-Str. 217-333 D-5600 Wuppertal 1. 2.2.1989. Unpublished. [CHA; sub: 12564, Vol 3-25 of 39].

Lehn H (1989) E 6876 (c.n. omethoate): mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro. Bayer report no. 17977. Department of Toxicology, Bayer AG, Wuppertal, FRG. April 1989. Unpublished. [BA; sub 9032. CHA; sub 12564 Vol. 3-26 of 39].

Löser E, Lorke B (1967) Bayer 45 432: Subchronic toxicity tests on rats. Sponsor: Bayer AG. 7.3.1967. Unpublished. [BA; sub: 239, Vol. 3 of 9].

Löser E (1968a) Bayer 45 432. Subacute toxicological studies on rats. Report no. 634. Sponsor: Bayer AG. 19.2.1968. Unpublished. [CHA; sub: 12564, Vol. 3-25 of 39].

Löser E (1968b) Bay 45 432: Subchronic toxicological studies on rats. Report No. 1040. Sponsor: Bayer AG. 17.10.1968. Unpublished. [BA; sub: 239, Vol. 3 of 9. CHA; sub: 12564, Vol. 3-25 of 39].

Löser E (1981) S-6876 Folimat-Wirkstoff multigeneration reproduction study in rats. Report No. 9731. Sponsor: Department of Toxicology, Bayer AG, Wuppertal, FRG. January 1981. Unpublished. [BA; sub: 9032].

Lotti M, Ferrara SD, Caroldi S, Sinigaglia F (1981) Enzyme studies with human and hen autopsy tissue suggest omethoate does not cause delayed neuropathy in man. Archives of Toxicology 48:265-270.

Machemer L (1974) Dominant lethal test in the male mouse. Report no. 4794. Sponsor: Department of Toxicology, Bayer AG, Wuppertal, FRG. July 1974. Unpublished. [BA; sub: 9032].

Mellert W, Deckardt K, van Ravenzwaay B (2002a) Omethoate. Study for the determination of the peak-effect for clinical signs/FOB in Wistar rats; single administration by gavage and 24 h observation period. Lab. Project no. 11C0709/01063. Sponsor: Dimethoate Task Force (DTF) c/o P. Hopfmann, Wotanstr. 39 D-68305 Mannheim, Germany. 5.7.2002. Unpublished. [CHA; sub: 12564, vol 3-37 of 39].

Mellert W, Deckardt K, van Ravenzwaay B (2002b) Omethoate. Study for the determination of cholinesterase inhibition in Wistar rats; single administration by gavage and 24 h observation period. Lab. project no. 11C0709/01078. Sponsor: Dimethoate Task Force (DTF) c/o P. Hopfmann, Wotanstr. 39 D-68305 Mannheim, Germany. 11.10.2002. Unpublished. [CHA; sub: 12564, vol 3-37 of 39].

Mellert W, Deckardt K, Kaufmann W, van Ravenzwaay B (2003) Omethoate – acute oral neurotoxicity study in Wistar rats; single administration by gavage. Lab. project no. 20C0709/01098. Sponsor: Dimethoate Task Force (DTF) c/o P. Hopfmann, Wotanstr. 39 D-68305 Mannheim, Germany. 4.12.2003. Unpublished**.** [CHA; sub: 12564, vol 3-38 of 39].

NHMRC (2004). Australian Drinking Water Guidelines 6. National Health and Medical Research Council. Natural Resource Management Ministerial Council. Endorsed 10-11 April 2003. Available online at http://www.nhmrc.gov.au/publications/synopses/eh19syn.htm

NHMRC (2010). Draft Australian Drinking Water Guidelines. National Health and Medical

Research Council. Natural Resource Management Ministerial Council. Draft for public comment. Available online at http://www.nhmrc.gov.au/publications/synopses/eh19syn.htm

Office of Chemical Safety and Environmental Health (2010) *Human Health Risk Assessment of O,O,S-trimethyl phosphorothioate in omethoate.* Australian Government Department of Health and Ageing (DoHA). Canberra.

Office of Chemical Safety (2006) *Draft Occupational and Safety Assessment of Omethoate.* Australian Government Department of Health and Ageing (DoHA). Canberra.

Office of Chemical Safety (2010) A review of emergency first-aid treatment of anticholinesterase pesticide poisoning in Australia. May 2008. Available online at http://www.health.gov.au/internet/main/publishing.nsf/Content/ohp-ocs-anticholinesterase-cnt.htm

Pauluhn J (1983) S 6876 (The active ingredient of ®Folimat) (Common name: omethoate) Study of the irritant/corrosive effect on the skin and eye (rabbit). Bayer Report No. 11977. Sponsor: Bayer AG, Institut fuer Toxicologie, Wuppertal-Elberfeld, FRG. 2.8.1983. Unpublished. [BA; sub: 239, Vol. 2 of 9].

Pauluhn J (1986a) E 6876 1000 SL 00671/0360 (c.n. Omethoate) Study for acute inhalation toxicity. Study No. T 4021920. Bayer Report No. 15199. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 21.10.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Pauluhn J (1986b) E 6876 500 SL 00671/0360 (c.n. Omethoate) Study for acute inhalation toxicity. Study No. T 1021602 & T2021603. Bayer Report No. 15198. Sponsor: Bayer AG, Department of Toxicology, Wuppertal, Germany. 21.10.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Pauluhn J (1986c) E 6876 500 SL 00671/0362 (c.n. omethoate) Study for irritant/corrosive potential for skin and eye (rabbit). Study No. T 0021601. Report No. 14342. Sponsor: Bayer Institute of Toxicology, Wuppertal-Elberfeld. 12.2.1986. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Pauluhn J (1987) E 6876 5 SL 00671/0415 A (c.n. omethoate) Study for irritant/corrosion potential for skin and eye (rabbit) to OECD Guideline Nos. 404 and 405. Study No. T 7025659. Report No. 16122. Sponsor: Bayer Fachbereich Toxikologie, D-5600 Wuppertal 1. 13.10.1987. Unpublished. [BA; sub: 9032, Vol. 2 of 2].

Pauluhn J (1989) E 6876 (c.n. omethoate): acute inhalation toxicity study according to OECD Guideline No. 403. Study No. T 3029642. Report no. 17626. Sponsor: Bayer AG. 19.1.1989. Unpublished. [CHA; sub: 12564, Vol. 3-25 of 39].

Ruf J, Mager H (1991) E 6876 Subchronic toxicity study on dogs (Thirteen-week stomach tube dosage test). Study No. T 4030768. Report No. 20139. Sponsor: Bayer AG, Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, D-5600 Wuppertal 1. 11.4.1991. Unpublished. [BA; sub: 9032, Vol. 2 of 2. CHA; sub: 12564, vol 3-26 of 39].

Schladt L (1994) E 6876 Chronic toxicological study in Wistar rats to determine a no-inhibition level for the cholinesterase activity (32-week administration of test substance in drinking water). Bayer study no. T 2033899. Sponsor: Bayer AG, Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 2.3.1994. Unpublished. [BA; sub: 12540, Vol. 2 of 11. CHA; sub: 12564, vol 3-30 of 39].

Schladt L (1995) E6876 (Folimat®) Study for chronic toxicity and carcinogenicity in Wistar rats following two-year administration in drinking water. Bayer study no. T 2030748. Sponsor: Bayer AG, Institut fűr Toxikologie, Landwirtschaft, Friedrich-Ebert-Strasse 217-333 D-42096 Wuppertal, Germany. 21.2.1995. Unpublished. [BA; sub: 12540, Vol. 6 of 11. CHA; sub: 12564, vol 3-27 of 39].

Schladt, L (2001) E 6876 Oncogenicity study in B6C3F1 mice (administration in the drinking water over 24 months; T1032655). Bayer study no. T 1032655. Lab. Report no. PH 30972. Sponsor: Bayer, Pharmaceutical Business Group, Elberfeld. 3.5.2001. Unpublished. [BA; sub: 12540, Vol. 2 of 11. CHA; sub: 12564, vol 3-31 of 39].

Schrader (1962) Toxicological investigations with the active ingredient. S 6876. Sponsor: Bayer AG. Unpublished. [BA; sub: 239 , Vol. 2 of 9].

Taalman RDFM (1988) Clastogenic evaluation of E 6876 in an in vitro cytogenetic assay measuring sister chromatid exchange in Chinese ovary (CHO) cells. Bayer report no. R4469. Sponsor: Bayer AG. June 1988. Unpublished. [BA; sub 9032. CHA; sub 12564 Vol. 3-26 of 39].

Tesh JM, Ross FW, Wightman TJ, Wilby OK (1982) S 6876: Effects of oral administration upon pregnancy in the rabbit. 2. Main study. LSR Report No. 82/BAG023/111. Sponsor: Bayer AG, Werk Elberfeld Institut fur Toxicologie, Friedrich-Ebert-Strasse 217-319, Wuppertal, Postfach 10 17 09, Germany. 13.9.1982. Unpublished. [BA; sub: 239, Vol. 7 of 9].

Thyssen J (1978) S 6876 (The active ingredient of ®Folimate). Acute inhalation toxicity. Bayer Report No. 7888. Sponsor: Bayer AG. Institut fuer Toxikologie, Wuppertal-Elberfeld. 26.10.1978. Unpublished. [BA; sub: 239, Vol. 2 of 9].

Thyssen J (1979) Folimat active ingredient (S 6876). Subacute inhalational toxicity study on rats. Bayer Report No. 8445. Sponsor: Bayer AG. 11.6.1979. Unpublished. [BA; sub: 239, Vol. 3 of 9].

Weber H, Patzschke K, Wegner LA (1978) [^{14}C]Omethoate (®Folimat active ingredient) biokinetic studies on rats. Pharma Report No. 7669. Sponsor: Bayer AG, Isotopenlabor, Institut für Pharmakokinetik, Werk, Elberfeld, Wuppertal. 29.6.1978. Unpublished. [BA; sub: 239, Vol. 8 of 9].

Sighted but not evaluated

Dr. Fre/Ma. (Translation, 1968) Metabolism of Omethoat. Farbenfabriken Bayer AG. Biological Institute. Leverkusen. Unpublished. Report date: 27.4.1967. [BA; sub: 239, Vol. 8 of 9].

Kimmerle G (1968) Product 5089a. Toxicological studies. Report No. 583. Sponsor: Bayer AG, Institut für Toxikologie, Wuppertal-Elberfeld. 12.1.1968. Unpublished. [BA; sub: 239, Vol. 2 of 9].

Ministry of Agriculture, Fisheries and Food (MAFF), Pesticides Safety Directorate (1993) Evaluation of fully approved or provisionally approved products. Evaluation on: omethoate. November 1993. Project/study no. 83. Department of Environment, Food and Rural Affairs, Pesticides Safety Directorate, York.

APPENDICES

APPENDIX I: Australian Registered Products Containing Omethoate at the beginning of this assessment

APVMA Product Code	Product Description	Product Name
33051	AgChem, aerosol	Folimat Garden Insecticide
33054	AgChem, liquid concentrate	Folimat 50 Garden Insecticide
33055	AgChem, liquid concentrate	Folimat 800 Insecticide Spray
45672	AgChem, aqueous concentrate	Le-Mat 290 SL Insecticide
59300	AgChem, soluble concentrate	ChemAg Omen 290 Insecticide

APPENDIX II: List of clinical Chemistry, Haematology & Urinalysis Parameters

Clinical Chemistry	Haematology	Urinalyses
Albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) cholinesterase activity creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) Phosphorus potassium protein (total) SGPT (serum alanine aminotransferase) SGOT (serum aspartate aminotransferase) Sodium triglycerides urea nitrogen (blood) CPK (creatinine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

APPENDIX III: Organs for weight determination and histopathological examination

Organs Weighed	Tissues Examined		
Adrenals Brain Gonads Heart Kidneys Liver Spleen Thyroid (w/parathyroid)	Adrenals aorta blood smear bone bone marrow brain (3 levels) caecum colon duodenum epididymis eyes eyes (optic nerve) gall bladder Harderian glands head - 3 sections (nasal cavity, para-nasal sinus, tongue, oral cavity, naso-pharynx, inner-ear)	heart ileum jejunum kidneys lacrimal gland liver lungs lymph nodes mammary gland muscle (smooth) muscle (skeletal) nerve (peripheral) oesophagus ovaries pancreas pituitary	prostate rectum salivary gland seminal vesicle skin spinal cord (cervical thoracic, lumbar) spleen sternum stomach testes thymus thyroid (w/parathyroid) trachea urinary bladder uterus vagina Zymbal's gland gross lesions

APPENDIX IV: Reproductive and Developmental Indices

$$\textbf{Male/female mating index } (\%) = \frac{\text{number of males/females with confirmed mating*}}{\text{number of males/females placed with females/males}} \text{x } 100$$

* defined by females with vaginal sperm or that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Male fertility index (\%)} = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females/males}} \text{ x } 100$$

* defined by a female giving birth to a litter or with pups/foetuses in utero

$$\textbf{Female fertility index (\%)} = \frac{\text{number of females pregnant*}}{\text{number of females mated**}} \text{x} 100$$

* defined as the number of females that gave birth to a litter or with pup/foetuses in utero
** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant*}} \text{ x } 100$$

* defined as the number of females that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Live birth index (\%)} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \text{ x } 100$$

$$\textbf{Viability index (\%)} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of liveborn pups on the day of birth}} \text{ x } 100$$

* before standardisation of litters (i.e. before culling)

$$\textbf{Lactation index (\%)} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}} .$$

* after standardisation of litters (i.e. after culling)

$$\textbf{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \text{ x } 100$$

$$\textbf{Conception rate (\%)} = \frac{\text{number of pregnant animals}}{\text{number of fertilised animals}} \text{x } 100$$

$$\textbf{Preimplantation loss (\%)} = \frac{\text{number of corpora lutea – number of implantations}}{\text{number of corpora lutea}} \text{ x } 100$$

$$\textbf{Postimplantation loss (\%)} = \frac{\text{number of implantations – number of live foetuses}}{\text{number of implantation}} \text{ x } 100$$