AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

CHEMICAL REVIEW PROGRAM

HUMAN HEALTH RISK ASSESSMENT

OF

DIMETHOATE

prepared by

Office of Chemical Safetyand Environmental Health
Department of Health and Ageing
Canberra

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PREFACE

Dimethoate is listed as a Priority 1 chemical on the APVMA Priority Chemical Review List due to potential human health concerns related to its current usage.

The Office of Chemical Safety and Environmental Health (OCSEH) has prepared this review of dimethoate for the Australian Pesticides and Veterinary Medicines Authority (APVMA).

EXECUTIVE SUMMARY

Dimethoate has been identified for priority review under APVMA Chemicals Review Program. The review is based on concerns regarding the potential for unacceptable dietary exposure risks resulting from post harvest dipping of fruit and vegetables. Additional toxicology data and data related to occupational exposure were received from industry. These data, together with all previously submitted registration data and relevant published data, have been assessed in detail.

Dimethoate has been registered for use as an insecticide and acaricide in Australia for over 30 years. The current review considered the 8 approved sources of dimethoate active constituent, and 21 registered products as determined at the start of this review. Products containing dimethoate are registered for more than 200 use patterns and to control more than 80 insect pest species, and are used both as a pre-harvest and post-harvest insecticides in orchard and fruit crops.

Dimethoate is a contact and systemic organophosphate pesticide and its major toxicological endpoint in animals and humans is the inhibition of acetylcholinesterase (ChE) activity. Dimethoate is of moderate acute oral toxicity and low to moderate dermal toxicity. It is a slight eye irritant but not a skin irritant. Although dimethoate is not a skin sensitiser, its EC formulation (400 g/L) has showed sensitisation potential. The formulation is also a moderate eye and skin irritant. In repeat dose studies in mice, rats and dogs, dose-related inhibition of plasma, erythrocyte and brain ChE activities was generally the most sensitive manifestation of dimethoate toxicity. Increased liver weight was observed in mice and rats following high-dose chronic exposure. Dimethoate is not considered to be genotoxic or to show any carcinogenic activity. The chemical is not a teratogen but there is evidence for increased pup mortality in developmental neurotoxicity studies.

The current Australian acceptable daily intake (ADI) value for dimethoate of 0.02 mg/kg bw was set in 1988. This ADI was established by applying a 10-fold safety factor to the NOEL of 0.2 mg/kg bw/d for inhibition of ChE activity in whole blood in a 14-57 day human study. This review has identified that this ADI is not appropriate, since the data indicates that the most sensitive toxicological endpoint for repeat-dose exposure to dimethoate is increased mortality and ChE inhibition in pups in two developmental neurotoxicity studies in rats. Hence the revised ADI is 0.001 mg/kg bw, based on a NOEL of 0.1 mg/kg bw/d in these developmental neurotoxicity studies in rats, with a 100-fold safety factor.

This review has also identified the need for an acute reference dose (ARfD) for dimethoate. No ARfD previously been established for dimethoate. It is recommended that an ARfD of 0.02 mg/kg bw be established based on a NOEL of 0.2 mg/kg bw/d for inhibition of ChE activity in whole blood in a 14-57 day human study, with a 10-fold safety factor.

This review also recommends that the NHMRC revises the current (2004) Health Value for dimethoate in drinking water from 0.05 mg/L to a Health Related Guideline Value of 0.004 mg/L, on the basis of a revised ADI of 0.001 mg/kg bw. This value of 0.004 mg/L should also supercede the proposed Health Related Guideline Value of 0.007 mg/L that was published in draft form in October 2009.

The review recommends that dimethoate remain in Schedule 6 of the *Standard for Uniform Scheduling of Drugs and Poisons* (SUSDP), since none of the information evaluated in this review indicates the need for a change to the existing schedule.

The current first aid instruction for dimethoate (namely, 'm'), remains appropriate.

There is no objection on toxicological grounds to the ongoing approval of dimethoate and dimethoate manufacturing concentrate from the existing sponsors and manufacturers. The review recommends, however, that based on toxicological concerns, impurity limits of 2 g/kg for omethoate and 10 g/kg for isodimethoate be established. The existing upper limit for O,O,S-trimethyl phosphorodithioate (5 g/kg) will be reconsidered as part of the occupational health and safety assessment.

The 6 products registered for home garden use will also be considered as part of the occupational health and safety assessment.

RECOMMENDATIONS TO THE APVMA

Approval Status

There is no objection on toxicological grounds to the ongoing approval of dimethoate and dimethoate manufacturing concentrate from the existing sponsors and manufacturers. Occuptaional health and safety concerns will be assessed separately.

The existing upper limit for the impurity O,O,S-trimethyl phosphorodithioate of 5g/kg is to be reviewed in the occupational health and safety assessment. It is recommended that impurity limits of 2 g/kg for omethoate and 10 g/kg for isodimethoate be established on the basis of toxicological concerns, and current practically achievable levels.

Acceptable Daily Intake

The current Australian acceptable daily intake (ADI) value for dimethoate of 0.02 mg/kg bw was set in 1988. This ADI was established by applying a 10-fold safety factor to the NOEL of 0.20 mg/kg bw/d for inhibition of cholinesterase (ChE) activity in whole blood in a 14-57 day repeat-dose human study. The OCS considers this ADI is not appropriate, since the most sensitive toxicological endpoint for repeat-dose exposure to dimethoate is increased mortality and ChE inhibition in pups in developmental neurotoxicity studies in rats. Hence, it is recommended that the ADI is revised to 0.001 mg/kg bw, based on a NOEL of 0.1 mg/kg bw/day in this developmental neurotoxicity studies in rats, and applying a 100-fold safety factor.

Acute Reference Dose

Prior to this review an ARfD had not been established for dimethoate. It is recommended that an ARfD of 0.02 mg/kg bw be established based on a NOEL of 0.2 mg/kg bw/d for inhibition of ChE activity in whole blood in a 14-57 day human study, and applying a 10-fold safety factor.

Water Quality Guidelines

This review also recommends that the NHMRC revises the current (2004) Health Value for dimethoate in drinking water from 0.05 mg/L to a Health Related Guideline Value of 0.004 mg/L, on the basis of a revised ADI of 0.001 mg/kg bw. This value of 0.004 mg/L should also supercede the proposed Health Related Guideline Value of 0.007 mg/L that was published in draft form in October 2009.

Poisons Scheduling

Dimethoate is listed in Schedule 6 of the *Standard for Uniform Scheduling of Drugs and Poisons* (SUSDP). The new information provided for this review supports the current scheduling of dimethoate.

Product Registration

Product registration will be considered in the occupational health and safety assessment.

First Aid Instruction

Amendments to first-aid instructions, precautionary statementsand re-entry statements (see below) should be incorporated onto labels of those dimethoate products supported for ongoing registration according to the results of the occupational heath and safety assessment.

The current standard statements for dimethoate specified in the FAISD Handbook remain appropriate.

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

ACRONYMS AND ABBREVIATIONS

Chemistry and haematology terminology

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)
DMSO	Dimethyl sulfoxide
GC	Gas chromatography
GGT	Gamma-glutamyl transferase
GLC	Gas liquid chromatography
Hb	Haemoglobin
Hct	Haematocrit
HPLC	High pressure liquid chromatography
LDH	Lactate dehydrogenase
LH	Luteinising hormone
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MS	Mass spectrometry
NTE	Neurotoxic target esterase
PCV	Packed cell volume (Haematocrit)
PT	Prothrombin time
RIA	Radioimmunoassay
T_3	Triiodothyroxine
T_4	Thyroxine
TLC	Thin layer chromatography
TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

General terminology

ADI	Acceptable daily intake

AOEL	Acceptable operator exposure level
ARfD	Acute Rreference dose
ChE	Cholinesterase
CRP	Chemical review program
DDVP	2,2-dichlorovinyl dimethyl phosphate (Omethoate)
DFR	Dislodgeable foliar residue
DMP	Dimethylphosphate
EC	Emulsifiable concentrate
GHS	Globally harmonised system for classification and labelling of chemicals
GLP	Good laboratory practice
HG	Home garden
IPM	Integrated pest management
LD	Liquid
LOD	Limit of detection
LOEL	Lowest observed effect level
LOQ	Limit of quantification
MOE	Margin of xposure
MRL	Maximum residue limit
NOEL	No observed effect level
NOAEL	No observed adverse effect level
OHS	Occupational health and safety
OP	Organophosphorus pesticide
PHED	Pesticide handlers' exposure database
POEM	Predictive operator exposure model
PPE	Personal protective Equipment
PVC	Polyvinyl chloride
R	Correlation coefficient
R^2	Regression coefficient
REI	Re-entry interval
RHI	Re-handling Interval
S	Poisons schedule
SD	Safety directions
SO	Solid (formulation type)
SR	Slow release generators (formulation type)
STEL	Short term exposure limit
TWA	Time weighted average
WHP	Withholding Period

Organisations & publications

ACPH	Advisory Committee on Pesticides and Health
AGCS	Advisory Group on Chemical Safety
AHMAC	Australian Health Ministers Advisory Council
APVMA	Australian Pesticides and Veterinary Medicines Authority
CAC	Codex Alimentarius Commission
ECETOC	European Chemical Industry Ecology and Toxicology Centre
FAO	Food and Agriculture Organization 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 Commision
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
NTP	National Toxicology Program
PACC	Pesticide and Agricultural Chemicals Committee
RED	Re-registration Eligibility Document
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
SCOT	NHMRC Standing Committee on Toxicity
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

PART 1 RISK ANALYSIS REPORT

1 BACKGROUND

1.1 Public health consideration of pesticides 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 (FAISD), the human acceptable daily intake (ADI) and the 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 processes under the auspices of the National Health and Medical Research Council (NHMRC). The Food Additives Committee first set "Pesticide Tolerances" in food in 1956. Between 1962 and 1966, this 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, the Pesticide and Agricultural Chemicals Committee (PACC) established Australian MRLs and ADIs for pesticides, 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 National Registration Authority (NRA, renamed the Australian Pesticides and Veterinary Medicines Authority, APVMA) 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 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. Since 2004/2005, the Advisory Group of Chemical Safety (AGCS) has replaced the ACPH.

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 (SUSDP), which recommend 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 (AHMAC), and was re-constituted again in 1999 as a Statutory Committee of the TGA.

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

1.2 History of use of dimethoate

Dimethoate is an organophosphate insecticide. It 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 organophosphorus chemicals, the mode of action of dimethoate is through inhibition of cholinesterase (ChE) activity.

Omethoate, an oxygen analogue metabolite of dimethoate, appears to play a dominant role in toxicity of dimethoate for insects and mammals. Omethoate itself is also used as an active constituent in five products registered in Australia. A separate toxicological review is being prepared for omethoate.

Dimethoate was introduced in 1956 and has been used as an insecticide in Australia for more than 30 years. At the commencement of this review, dimethoate is the active constituent in 21 registered products in Australia. The approvals of the active constituents and the registrations of products are being reconsidered based on concerns related to toxicology, occupational and health and safety, residues and trade.

1.3 Reason for the review of dimethoate

The current review of dimethoate was undertaken under the auspices of the APVMA's Chemical Review Program. Dimethoate was classified as an active constituent of high priority by the Department of Health and Ageing because of concerns over potentially unacceptable dietary exposure risks resulting from post harvest dipping of fruit and vegetables. In addition, assessments performed by the JMPR in 1997 and 2002, and the US EPA in 1999, indicated that there were a number of studies that may impact on the human health risk assessment that had not previously been evaluated by the OCSEH.

The toxicological database for dimethoate is extensive and consists of unpublished reports generated by industry, as well as numerous studies in the published literature. Since the toxicological database for dimethoate was last reviewed in 1988, new information considering a range of toxicological endpoints have become available. In particular, there are now behavioural studies that quantify the extent of functional (task performing) impairment in rats following exposure to dimethoate, as well as reproductive and dermal toxicity studies.

1.4 History of public health considerations of dimethoate

A detailed history of the consideration of dimethoate by regulatory committees in Australia is described below:

Date	Regulatory Activity
November 1974	DPSC & PACC: New entry for dimethoate in Schedule 6.
November 1981	PACC: Agreed that the existing MRL of 2 mg/kg for fruit would cover the use of dimethoate on lychees.
1983	NDPSC: Withdrew registration of dust formulations of dimethoate due to potential dermal exposure risks.

August 1982 (PACC) & February 1983 (NDPSC).	PACC & NDPSC: Received information about US EPA regulatory action on dimethoate: i.e. its potential risks of mutagenicity, reproductive and foetotoxic effects, and its risk of oncogenicity warrants further study; cancellation of registrations for all dust formulations; protective clothing and equipment are mandatory for applications, in particular for aerial application. The committees advised that no dust formulations of dimethoate were registered in Australia, and that labelling proposed by EPA for dust formulations were already in effect in Australia on the emulsifiable concentrate (EC) formulations. Data examined by the EPA had been requested for review.
February 1988	The PACC & NDPSC reviewed toxicology based on further data on mutagenic, reproductive and oncogenic effects of dimethoate (submitted by Boehringer, BASF, A/S Cheminova, Montedison and Schering). It was moderately toxic, and slightly irritating to skin and eyes with non-sensitisation. It showed mutagenic potential in some studies but its carcinogenicity was not evident in the mice and rat studies. A 90-day feeding study in dogs and rats, and an adequate delayed neurotoxicity study were requested. Existing scheduling (S6), FAIs and SDs were considered appropriate. The PACC noted the concern of the South Australian regulatory authorities, which revealed the presence of significant residues in tomatoes from Queensland, and reports that indicated dimethoate was a carcinogen. The report noted that dimethoate was mutagenic in micro-organisms and Drosophila, and positive in sister chromatid exchange (SCE) assay. However, no definitive genotoxicity was demonstrated in other mammalian systems tested. The teratogenic potential of dimethoate was demonstrated in a cat study, but it was difficult to interpret the results of this study due to lack of details on the formulation used, and historical teratology data in this test species. The PACC established an ADI of 0.02 mg/kg bw/d, based on a NOEL of 0.2 mg/kg bw/d for ChE inhibition in a 14-57-day, repeat-dose human study.
December 1988	PACC: Considered an application by Rhone-Poulenc for the establishment of an MRL for dimethoate on lupins, and recommended MRLs for Dimethoate at 0.5 mg/kg lupin – 7D and 1 mg/kg lupin, forage – 7D; and for Omethoate: 0.1 mg/kg lupin – 7D and 0.5 mg/kg lupin, forage – 7D.
February 1990	PACC: Agreed to recommend a reduction in the withholding period for dimethoate in cucurbits from seven days to one day, since trials on both rockmelon and zucchini did not detect dimethoate.
May 1992	DPSSC: Safety directions for a home garden aerosol product "Rogor Aerosol Garden Insecticide" (0.3 g/kg of dimethoate) were accepted with the addition of SD 223 after 219. However, the proposed SDs were not endorsed until the status of the product as a HG product was established (conform with the DPSSC guidelines for household pesticide products).
November 1993	DPSSC: FASDP had reviewed HG/HV products listed in Appendix H for compliance with the NHMRC home-garden protocol. Atropine was not usually appropriate for HG products, and Appendix H safety directions for Dimethoate HG EC 100 g/L would need to be reviewed in the future.

ADI

The current ADI for dimethoate is 0.02 mg/kg bw/d. This ADI was derived from a NOEL of 0.2 mg/kg bw/d in human studies and was established in 1988.

Poisons Scheduling

Dimethoate is currently included in Schedule 6 of the SUSDP. It was first established in 1974 and reviewed in 1988.

MRLs in drinking water

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

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 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg at a daily water consumption of 2 L over a lifetime. At present, the Health Value for dimethoate is 0.05 mg/L.

1.5 International assessments

US EPA

The US EPA has conducted a toxicological evaluation of dimethoate, and the revised and updated Health Effects Division's Chapter for a Re-registration Eligibility Decision Document (RED) was published in 1999. The RED report was updated in July 2006.

According to the review, it appears that the US EPA database includes numerous studies that have never been evaluated by the OCS. These comprise (at least) two rat neurotoxicity studies (acute and subchronic), a five-day rat dermal toxicity study, a one-year dog feeding study and a multi-generation rat reproduction study. These studies were not provided for review.

According to the EPA's evaluation, the dermal absorption of dimethoate was about 7-8% and 9-11% in rats at 1-2 and 5 days after treatment at 10 mg/kg bw, respectively. At 100 mg/kg bw, the absorption was about 1-2% at 1, 2 or 5 days after treatment. Dermal absorption was not measured at 8-10 h after treatment. In this study, the NOAEL for plasma, erythrocyte and brain (cortex) ChE inhibition was also 10 mg/kg bw. Consequently, the review committee recommended the use of the highest dermal absorption value of 11% recorded after 5 days in short- or intermediate term dermal exposure risk assessments. A margin of exposure (MOE) of 300 was included for the intermediate-term dermal risk calculation, since a LOAEL used was based on a 90-day subchronic neurotoxicity study in rats, in which dimethoate was administered via the diet.

The EPA's assessment noted that though several *in vitro* and *in vivo* mutagenicity tests with dimethoate yielded negative results, an unscheduled DNA synthesis assay demonstrated a positive result, together with equivocal findings in a gene mutation assay. In the evaluation of

a one-year dog feeding study, the EPA did not establish a NOAEL for systemic toxicity due to decreased liver weights in females and the presence of a brown, granular pigment in the liver of both sexes at the lowest dose of 0.19 mg/kg bw/d. Statistically significant inhibition of brain ChE was also noted at this dose level, whilst NOAELs for erythrocyte and plasma ChE activities were 0.19 and 0.7 mg/kg bw/d, respectively.

The main regulatory end-points (tolerances) established by the EPA for total residues of dimethoate and its oxygen metabolite, omethoate, were:

- An Acute Reference Dose (acute RfD) of 0.013 mg/kg bw was established by applying a 100-fold uncertainty factor to a $BMDL_{10}$ of 1.3 mg/kg bw/day based on brain ChE inhibition in PND 11 females observed in a comparative ChE inhibition study in rats.

- A chronic RfD of 0.0022 mg/kg bw/d was established by applying a 100-fold uncertainty factor to a BMDL10 of 0.22 mg/kg bw/day based on brain ChE inhibition in females observed in a 2-year chronic feeding study in rats.

- Dimethoate was classified as a Group C (possible) human carcinogen, based on the following observations: equivocal haemolymphoreticular tumours in male B6C3F1 mice at the highest dose of 30 mg/kg bw/d, the compound-related dose-independent weak effect of combined spleen, skin, and lymph tumours in male rats at (0.25 and 5.0 mg/kg bw/d), and positive results seen in some mutagenicity assays.

In 2002, the EPA's Health Effects Division's Hazard Identification Assessment Review Committee re-evaluated the database on the basis of submitted neurotoxicity and dermal absorption studies. The committee established an acute RfD (ARfD) of 0.001 mg/kg/d for females 13-50 years of age, based on a NOAEL of 0.1 mg/kg bw/d established in a developmental neurotoxicity study. The LOAEL was 0.5 mg/kg bw/d based on increased pup death and increases in motor activity during the lactation period.

For the general population an ARfD of 0.005 mg/kg/day was established based on a NOAEL of 0.5 mg/kg bw, applying a 100-fold safety factor. The LOAEL was 3 mg/kg bw for inhibition of brain and erythrocyte ChE activity in adults and pups following single dose exposure.

European Union / United Kingdom

Dimethoate is registered for use in a number of European countries.

The Pesticide Safety Directorate reconsidered dimethoate at their meeting in January 2001. As a result, dimethoate products were suspended until the review is finished.

The UK as rapporteur Member State of EU has recently established a review report on dimethoate (EC: SANCO/10047/2006) which was designated by the EC (EC: SANCO/10047/2006). It is concluded that plant protection products containing dimethoate will fulfil the safety requirements specified, and a list of uses for products containing dimethoate has been recommended.
Following reference values have been finalised:
ADI: 0.001 mg/kg bw/day
ARfD: 0.01 mg/kg bw

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

The FAO/WHO JMPR evaluated dimethoate for toxicological effects in 1963, 1965, 1967, 1984, 1987, 1996 and 2003. The residue chemistry of dimethoate was reviewed by the JMPR in 1998.

In its 1996 evaluation, the JMPR established an ADI of 0.002 mg/kg bw/d for dimethoate by applying a 500-fold safety factor to a NOEL of 1.2 mg/kg bw/d derived from a rat reproduction study. Although a safety factor of 100 would normally be used to calculate an ADI from a study of this nature, the JMPR was concerned about the possibility that reproductive performance may have been affected at the 1.2 mg/kg bw/d dose in this study and therefore used a greater uncertainty factor. The Office of Chemical Safety (OCS) has not reviewed many of the studies, including this pivotal reproduction study considered by the JMPR.

It is noteworthy that the 1996 JMPR review of dimethoate included a toxicology evaluation of omethoate, a metabolic oxygen analogue of dimethoate. Although omethoate was used previously as a pesticide in its own right, based on the information available to the committee, the review indicated that omethoate will no longer be used, since the primary manufacturer no longer produces this compound. However, since the use of dimethoate on agricultural crops could lead to residues of omethoate in treated crops, the JMPR considered that it was important to consider the toxicity of omethoate when evaluating the use of dimethoate. The report noted that omethoate is considerably more toxic than dimethoate (rat oral LD_{50} values being about 310 and 25 mg/kg bw for dimethoate and omethoate, respectively), although the levels of omethoate residues arising from the use of dimethoate on crops are likely to be low. It was recommended that residues of dimethoate and omethoate resulting from the use of dimethoate be expressed as dimethoate and should be assessed in comparison with the ADI for dimethoate. Because the primary manufacturer no longer produces omethoate, and that its review was not supported by toxicology data, the omethoate ADI of 0.0003 mg/kg bw/d, recommended in 1985, was withdrawn at the 1996 JMPR meeting.

The 1996 JMPR review also concluded that there might be a need to re-evaluate the toxicity of dimethoate after the periodic review of the residue and analytical aspects of dimethoate has been completed, and determines whether omethoate is a major residue. The residue chemistry of dimethoate was subsequently reviewed by the JMPR in 1998. Based on the metabolism data in plants and animals, the available analytical methods, and the lack of substantial data on omethoate *per se*, the meeting concluded that the residue for compliance with MRLs should be defined as dimethoate, and that the dietary intake estimates of residues be based on the sum of dimethoate and omethoate, each considered separately.

In 2003, the JMPR established an ARfD of 0.02 mg/kg bw on the basis of an overall NOAEL of 2 mg/kg bw for ChE inhibition in studies in rats, and applying a safety factor of 100. It was considered that this ARfD was supported by the NOAEL of about 0.2 mg/kg bw per day established in studies in human volunteers. Studies identified as useful for further evaluation of the compound were (1) further observations in humans and (2) the two generation reproductive study that was available in abbreviated form at the meeting.

2 HAZARD CHARACTERISATION

2.1 Toxicology hazard profile

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption	Rapid; T_{max}= 0.5 h; almost completely absorbed
Distribution	Well distributed; highest tissue concentrations were found in the liver and kidneys.
Potential for accumulation	No evidence for accumulation.
Rate and extent of excretion	Rapidly excreted in urine; 85-91% within 5 days (52-72% within six h dependent upon dose).
Metabolism	Extensively metabolised to a number of thiophosphate and phosphate esters. Quantitatively minor pathway (in rats) to omethoate.
Toxicologically significant compounds (animals, plants and environment)	Omethoate (See also CRP review of Omethoate) Isodimethoate, O,O,S trimethyl phosphorodithioate

Acute toxicity

Rat oral LD_{50}	150-414 mg/kg bw
Worst oral LD_{50} in other species	60 mg/kg bw (mice)
Rat dermal LD_{50}	>2000 mg/kg bw (no deaths at 2000 mg/kg bw)
Worst dermal LD_{50} in other species	No data
Rat inhalation LC_{50}	1553 mg/m^3 (estimated for technical dimethoate after exposure to an EC formulation)
Worst inhalation LC_{50} in other species	No data
Skin irritation	None
Eye irritation	Slight irritant
Skin sensitisation	Non-sensitiser when dimethoate technical in paraffin oil was applied to guinea pig skin. However, an EC formulation was a sensitizer in guinea pigs using the Buehler method. Two positive patch tests in humans.

Short-term toxicity

Target/critical effect	Cholinesterase inhibition
Lowest relevant oral NOEL	0.43 mg/kg bw/d (4-week dietary dog study) 0.2 mg/kg bw/d (14-57 day human volunteer study)
Lowest relevant dermal NOEL	No data on technical dimethoate
Lowest relevant inhalation NOEC	No data on technical dimethoate

Genotoxicity	Genotoxic *in vitro* in bacteria and mammalian cells. Some equivocal results *in vivo*, but the weight of evidence from guideline studies suggests dimethoate is unlikely to be genotoxic *in vivo*.

Long-term toxicity and carcinogenicity

Target/critical effect	Cholinesterase inhibition

Lowest relevant NOEL	0.04/0.06 mg/kg bw/d in males and female rats respectively (2-year feeding study)
Carcinogenicity	No evidence of carcinogenic potential
Reproductive toxicity	
Reproduction target/critical effect	Decreased pregnancy rate
Lowest relevant reproductive NOEL	0.05 mg/kg bw/d
Developmental target/critical effect	Pup toxicity and mortality (observed in developmental neurotoxicity studies)
Lowest relevant developmental NOEL	0.1 mg/kg bw/d
Delayed neurotoxicity	No data
Immunotoxicity	No data
Dermal absorption	Approximately 5.1% after application at 1 mg/cm^2 for 6 h

Summary

ADI:0.001 mg/kg bw
[Increased pup mortality]

ARfD: 0.02 mg/kg bw
[Inhibition of plasma, erythrocyte and brain ChE activity]

NOEL	Study	Safety factor
0.1 mg/kg/d rat	Developmental neurotoxicity oral study in rat	100
0.2 mg/kg/d Human	Human volunteer study (14-57 days) oral	10

Health Based Guideline Value in drinking water	Current: 0.05 mg/L Proposed: 0.004 mg/L

2.2 Summary of toxicology report

Introduction

Dimethoate is a contact and systemic organophosphorus pesticide (OP) that has been registered for use as an insecticide and acaricide in Australia for over 30 years. It is listed in Schedule 6 of the *Standard for Uniform Scheduling of Drugs and Poisons* (SUSDP). The Australian Acceptable Daily Intake (ADI) value for dimethoate of 0.02 mg/kg bw/d was set in 1988. This ADI was established by applying a 10-fold safety factor to the NOEL of 0.2 mg/kg bw/d for whole blood (ChE) inhibition in a 14 - 57-day repeat-dose human study. No Acute Reference Dose (ARfD) has been set for dimethoate. The current Health Value for dimethoate in Australian drinking water is 0.05 mg/L.

Safety Directions for dimethoate are listed in the *Handbook of First Aid Instructions and Safety Directions*. There are currently eight approved sources of dimethoate active constituent all of which are included in the review. Of the 27 registered products for the control of a range of insects in various agricultural situations, including some home garden use, 21 are included in this review. Six products were registered after the commencement of the review and are therefore not included (in the review), although the APVMA regulatory outcomes of the review will apply to all currently registered products.

Since the toxicological database for dimethoate was last reviewed in 1988, new information considering a range of toxicological endpoints that had previously not been investigated, or that have been assessed in more detail, has become available. In particular, there are behavioural studies that quantify the extent of functional (task performing) impairment in rats following exposure to dimethoate. A review of these studies and all other new data is considered important to determine whether the existing health standards remain appropriate to protect the general public and workers handling products containing dimethoate.

Absorption, distribution, metabolism and excretion

Radiolabeled dimethoate was well absorbed from the gastrointestinal tract following oral administration to rats. Maximum plasma and tissue concentrations were achieved 0.5 hours following dosing and showed a similar distribution in both sexes following a dose of 10 mg/kg bw. In tissues, radioactivity was primarily detected in the liver and kidney, with the lowest levels found in the brain and fat. Excretion of the radiolabel was almost complete by 5 days (89-95% of the administered dose) and occurred primarily in the urine (85-91%), with smaller amounts detected in the faeces (1.2-1.6%) and expired air (2.1-2.2%). Excretion via the urine was rapid; approximately 69-72% and 52-59% of the radiolabel was detected in the urine within six hours of 10 and 100 mg/kg bw doses of dimethoate, respectively. In bile duct cannulated rats, 4-5% of the administered radioactivity was recovered in the bile within 48 hours, independent of low or high doses of dimethoate (Kirkpatrick 1995).

Dimethoate was extensively metabolised in rats, principally by initial cleavage of the C-N bond to yield dimethoate carboxylic acid, and subsequently to a number of thiophosphate and phosphate esters. The quantitatively minor route of elimination involves oxidative metabolism of dimethoate to produce the oxygen analogue, omethoate. The parent compound represented 1-2% of the dose excreted in the urine (Kirkpatrick 1995).

When [^{14}C]-dimethoate was applied dermally as a 1% aqueous solution at a dose of 10 mg/kg bw, approximately 9-11% of the radiolabel was recovered in the urine, faeces and carcass. Approximately 13-17% of the radiolabel was recovered at the treated skin sites at 5 d (Kirkpatrick 1995).

Radiolabeled dimethoate was well absorbed when applied to the skin of rats as a formulation and representative spray dilution. Percentages of radiolabel absorbed varied from 1-42% dependent upon dilution, dose and length of application. The radiolabel was primarily excreted in the urine with smaller amounts detected in the faeces and remaining in the carcass at 5 days. Skin washing fractions contained the large majority of the dose while significant amounts of radiolabel were recovered at the treated skin sites. Proportions of the radioactive metabolites were broadly similar following oral and dermal exposure (Kirkpatrick 1995; Leibold 2001a&b).

An *in vitro* study comparing absorption of dimethoate (from a 400 g/L EC formulation or 1/200 aqueous dilution thereof) through human and rat epidermal membranes, showed that significantly more radiolabel was absorbed through rat skin (Davies 1999).

Metabolites

(Omethoate is 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. Since omethoate is also a pesticide itself, a separate review of omethoate (The OCS, 2006 & 2007) has been conducted in conjunction with the dimethoate review.)

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 tissues. 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

Dimethoate was of moderate toxicity when administered orally to rats. The oral LD_{50} of dimethoate in male and female rats ranged from 150-414 mg/kg bw. Clinical signs in rats prior to death included gait alterations, constricted pupils, salivation, tremors, absent forelimb/hindlimb grasp, laboured and/or shallow respiration and impaired/absent righting reflex. The signs first occurred within 15-90 mins depending on the dose. Hypothermia, lacrimation and staining on various body surfaces appeared later, and all signs persisted (often increasing in severity) through to the time of death. Macroscopic examination revealed dark red lungs in one male and opacity of one eye in the other male at 200 mg/kg bw (Lamb 1993a).

Reported oral LD_{50} values in mice were 60-168 mg/kg bw. Clinical signs observed in mice treated with 20-80 mg/kg bw dimethoate included sedation, dyspnea, hunched posture, ruffled fur, and reddish discharge, while spasms were observed in the 80 mg/kg bw group. In mice treated with 160 and 320 mg/kg bw, additional signs were observed including ventral body position, tremors and coma. Mice that survived recovered within 2 to 6 days. Mortality occurred primarily in the first 5 hours after dosing, but was observed until day 3. No pathological changes were observed in surviving mice on day 14, whereas mice that died were reported to show changes mainly in the lungs, liver, stomach and intestine (Ullman 1985). Oral LD_{50} values in other animals were as follows; hamster 200 mg/kg bw, guinea pig 350-400 mg/kg bw, and rabbit 300 mg/kg bw.

Dermal LD_{50} values in rats ranged from 500 mg/kg bw (for a wettable powder) to greater than 7000 mg/kg bw from a group of studies conducted during 1960s-1070s. In a most recent study (Kynoch 1986) in rats administered 2000 mg/kg bw dimethoate, clinical signs were limited to hunched posture, body tremors and abnormal gait in females on days 8 and 9, and weight loss, which was recorded in 2/5 females on day 8. No macroscopic changes were observed at necropsy. Under the experimental condition, dimethoate technical was of low dermal toxicity.

Dimethoate is not a skin irritant but is a slight eye irritant in rabbits. Dimethoate, in a paraffin oil vehicle, was not a sensitizer in guinea pigs using the closed patch technique (Madison 1984).

Metabolites

A number of metabolites identified in plant metabolism studies or environmental fate studies were administered to rats in single dose studies. The acute oral toxicity of omethoate was high, with LD_{50} values in the range of 22-28 mg/kg bw in rats (Flucke 1978, Krötlinger 1989a). Acute dermal studies in rats gave LD_{50} values from approximately 145 mg/kg bw, up to ~1018 mg/kg bw (Flucke 1978; Krötlinger 1989b). For a 4 h exposure, the inhalation LC_{50} value in rats was 287 mg/m^3 (Pauluhn 1989). Overall, deaths occurred in the acute studies within 24 h of dosing, up to day 4 post-dosing. 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. 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 erythrocyte 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). Omethoate was also evaluated in a separate APVMA CRP report: data contained within that evaluation indicated that in rabbits, omethoate was not a skin irritant, but was a slight eye irritant. It was a skin sensitiser in guinea pigs according to the open epicutaneous test.

Single oral doses of O-desmethyl dimethoate (identified in a water sediment study) at 500, 650, 845, 1099, 1428 and 1856 (1 female/dose) and 2000 mg/kg bw (3/sex) were given to rats by gavage. There were no treatment-related clinical signs or mortality. The oral LD_{50} for O-desmethyl dimethoate was greater than 2000 mg/kg bw (Albrecht 2000).

Dimethoate, omethoate or four potential metabolites of dimethoate (O-Desmethyl omethoate K salt, O-Desmethyl omethoate carboxylic acid K salt, O-Desmethyl N-desmethyl Omethoate

K salt, Des-O-methyl-isodimethoate, dicyclohexylammonium salt) were administered to rats as a single oral dose. Erythrocyte ChE activity was significantly and markedly inhibited following doses of 30 and 5 mg/kg bw dimethoate and omethoate, respectively. Inhibition of erythrocyte ChE activity following exposure to metabolites, administered at doses of 30 mg/kg bw, was less marked than that of dimethoate, but achieved significance and/or greater than 20% compared to pre-treatment values for O-Desmethyl omethoate carboxylic acid K salt and Des-O-methyl-isodimethoate, dicyclohexylammonium salt (Brennan 2001a).

Dimethoate or hydroxy-dimethoate (30 mg/kg bw) was given to groups of male rats as a single oral dose. There were no deaths, no clinical signs and no effects on body weight gain. Dimethoate markedly inhibited erythrocyte ChE activity 2.5 and 25 h after dosing. Inhibition of ChE activity was observed at 2.5 h following dosing with hydroxy-dimethoate, but the effect was reversible at 24 h (Brennan 2002).

Impurities

Iso-dimethoate was identified as an impurity potentially formed at storage. Rats received 25 mg/kg bw (male/female) or 200 mg/kg bw (female) of iso-dimethoate as a single oral dose in distilled water. All rats at 200 mg/kg bw were found dead 30 min or 1 h after dosing. Signs of systemic toxicity noted prior to death were clonic convulsions, prostration, fasciculations, pilo-erection, increased salivation, laboured, gasping and noisy respiration, and body tremors. Haemorrhagic lungs, dark liver, dark kidneys, haemorrhage and sloughing of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small intestine were observed at necropsy. There were no deaths at 25 mg/kg bw. Female rats, but not males, showed hunched posture, lethargy, decreased respiratory rate and laboured respiration from 30 min after dosing and all rats appeared normal by Day 2. The oral LD_{50} for iso-dimethoate was estimated to be in the range of 25-200 mg/kg bw (Dreher 2001a).

Male rats received 30 mg/kg bw of iso-dimethoate as a single oral dose in distilled water. There were no deaths. Slight whole body tremors and “teeth chattering” were noted shortly after dosing. Necropsy on Day 15 revealed no macroscopic abnormalities. Erythrocyte ChE activity was significantly inhibited at 2.5 h and 24 h compared to predose values. The oral LD_{50} for iso-dimethoate was greater than 30 mg/kg bw (Brennan 2001b).

The oral LD_{50} of the impurity O,O-dimethyl S-methoxycarbonylmethyl phosphorodithioate (MPEM) was > 2000 mg/kg bw in rats. Treatment-related clinical signs included piloerection and pinched abdomen in all animals on day 1, while one animal exhibited diarrhoea. Pilo-erection was observed until days 3-4 in males and females, after which all rats displayed normal appearance and behaviour. No gross pathological findings were reported at necropsy (Freytag 1992).

The impurity 0,0,0-trimethyl phosphorothioate had an oral LD_{50} value of 1150 mg/kg bw in mice and an inhalation LC_{50} of 1405 mg/m^3 in rats.

0,0,S-trimethyl phosphorodithioate is an impurity of toxicological significance. Further data on this impurity and an appropriate impurity limit will be addressed in the occupational health and safety assessment.

Formulations

A 400 g/L dimethoate EC product, similar to a formulation registered in the Australian market, had an estimated oral LD_{50} value of between 300 and 500 mg/kg bw in rats. Observed clinical signs were consistent with inhibition of ChE activity (Dreher 2001b). The dermal LD50 of the same formulation was > 2000 mg/kg bw in rats; no clinical signs of toxicity were observed (Dreher 2001c). The formulation was a moderate irritant to the skin and eyes of rabbits (Dreher 2001 d & e). The formulation was a sensitiser when applied to the skin of guinea pigs undiluted or as a 50% w/w solution (Bollen 2001).

Short-term repeat-dose studies

Rats received dimethoate in the diet for 4 weeks at doses of 0, 1, 3 or 12.5 mg/kg bw/day (0/0, 0.83/0.85, 2.48/2.60 and 10.38/11.00 mg/kg bw/d for males/females, respectively). No animals died during the study. There were no treatment related observations for clinical signs, food and water consumption, or body weight. On Day 29 (termination), decreased ChE activity was detected in the serum, erythrocytes and brain of the high dose group, and also in the erythrocytes and brain of the mid dose group. Inhibition of erythrocyte ChE activity was also seen at the high dose on Day 8 (Kaspers *et al*., 2004). The NOEL was 0.8 mg/kg bw/d based on inhibition of ChE activity in erythrocytes and brain, at and above, approximately 2.5 mg/kg bw/d.

Dogs were given dimethoate in the feed at doses of 0, 2, 10, 50, 250 or 1250 ppm (0, 0.09, 0.43, 2.20, 11.12 and 49.81 mg/kg bw/d) for 4 weeks. All dogs at 1250 ppm were killed during Week 3 for humane reasons, and one dog at 250 ppm lost body weight. No treatment-related clinical signs, food consumption or body weight changes were observed at lower doses. Inhibition of plasma ChE activity was observed at ≥ 250 ppm from Day 6, while erythrocyte ChE activity was inhibited at ≥ 50 ppm from Day 13. At termination, brain ChE activity was inhibited approximately 20% and 50% compared to controls at 50 and 250 ppm respectively (Harling *et al*., 1989). The NOEL was 10 ppm (0.43 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 50 ppm (equal to 2.2 mg/kg bw/d) and above.

Rabbits were treated with dimethoate (made into a paste with paraffin oil) at concentrations of 0, 100, 300 and 1000 mg/kg bw/d. The test compound was applied to the shaved dorsal skin for 6 h/day, 5 days/week for 3 weeks, under occlusive conditions. No signs of systemic toxicity were detected in any of the treatment groups up to 1000 mg/kg bw/d. Skin irritation was reported in all groups, including control, but was attributed to the paraffin oil vehicle. No inhibition of cholinesterase was detected in any of the experimental groups, suggesting that dimethoate may not be well absorbed when applied dermally as a suspension in paraffin oil (Madison 1986). A NOEL could not be clearly determined in terms of skin irritation due to interference by paraffin oil.

Metabolites

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 erythrocytes at 20 mg/kg bw/d. The NOEL was 2.5 mg/kg bw/d, due to clinical signs and inhibition of ChE activity at 20 mg/kg bw/d.

Formulations

Dimethoate 4E formulation (43.5% active ingredient, ai) was applied to the semi-occluded skin of rats for 6h/day over a 5 day period at 0 (vehicle control), 5, 10, 20, 40 or 100 mg ai/kg bw/d. There were no deaths during the study period. A significant ($p<0.05$ or $p<0.01$) reduction in erythrocyte and brain (hippocampus, striatum and cortex) ChE activity was observed in males at 100 mg ai/kg bw/d. In females, a dose-related inhibition of erythrocyte ChE activity was observed from 5-100 mg ai/kg bw/d; inhibition at 5 and 10 mg ai/kg bw/d, while not achieving statistical significance, was ≥ 20% and considered treatment-related. Plasma and brain (cortex) ChE activity was also reduced significantly ($p<0.05$ or $p<0.01$) at 20-100 mg ai/kg bw/d in females (Hilaski 1999). The dermal LOEL was 5 mg/kg bw/d based on inhibition of erythrocyte ChE activity in females at all doses.

Dimethoate 400 g/L EC (38% ai) was applied to the semi-occluded skin of rats for 6h/day, 5 days/week, for 4 weeks at doses of 0 (untreated but occluded), 10.5, 21.0, 31.5 or 63.0 mg ai/kg bw/day. There were no deaths, and no clinical signs or local dermal responses. Food consumption and body weight gain were not affected. In a Functional Observation Battery (FOB), treatment was not associated with any behavioural changes which were considered indicative of neurotoxicity. Plasma ChE activity was not affected at any dose level, while inhibition of erythrocyte ChE activity was observed at 63.0 mg ai/kg bw/d on Day 29 in males, but not in females. At termination, a dose-related, statistically significant ($p<0.01$) inhibition (8-17%) of brain ChE activity was observed from 21-63 mg ai/kg bw/d in both sexes. Although related to treatment, effects on brain ChE activity at 21.0 and 31.5 mg ai/kg bw/d were slight (8-10%) and considered of doubtful toxicological significance (Chambers 1999). The dermal NOEL was 31.5 mg ai/kg bw/d, based on inhibition of erythrocyte and brain ChE activity at 63.0 mg ai/kg bw/d.

Dimethoate 400 g/L EC (38% ai) was applied under semi-occlusive conditions to the skin of rats at 0, 5.25, 21, 42, 63 or 105 mg ai/kg bw/day, for 6 h per day, 5 days per week, over a 4 week period. There were no deaths, clinical signs or local dermal responses. Food consumption and body weight gain were not affected. Erythrocyte ChE activity was significantly ($p<0.01$) and dose-dependently (28-61%) decreased in males compared to pre-dose values from 42-105 mg ai/kg bw/d, whereas it was only marginally affected in females (11%; $p<0.05$) at 105 mg ai/kg bw/d. Statistically significant ($p<0.05$ or $p<0.01$) inhibition of brain ChE activity was observed in males (27-43%) and females (10-27%) at 63 and 105 mg ai/kg bw/d (Cheffings 1999). The dermal NOEL was 21 mg/kg bw/d, based on inhibition of erythrocyte ChE activity at 42 mg ai/kg bw/d.

Subchronic studies

Dimethoate was given to rats at dose levels of 0, 0.5, 2, 10, 50, 200 and 800 ppm in their feed for 6 months. Almost all of the 800 ppm animals developed severe toxic effects within a few days of treatment. Signs included fibrillation, weakness, apathy, loss of appetite and reduced weight gain. After withdrawal of test substance from the feed in this group, recovery was apparent within 10-14 days. Animals receiving 200 ppm showed reduced weight gain and slight toxic effects typical of ChE inhibition. These effects abated after several weeks but

behavioural change (aggressiveness) was evident after about 3 months of treatment. No changes in weight gain or clinical signs were seen in the other treatment groups. Marked depression in erythrocyte and brain ChE activity was seen at 50 ppm and above (Edson *et al.,* 1958). The NOEL was 10 ppm (0.5 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 50 ppm (2.5 mg/kg bw/d) and above.

Metabolites

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/kg bw/d in males and females respectively, for 32 weeks. None of the parameters measured, including ChE activity in plasma, erythrocytes and brain, were 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, erythrocytes and brain (Ruf & Mager 1991).

Chronic studies

Dimethoate was administered to mice in the feed at dose levels of 0, 25, 100 or 200 ppm for 18 months. These doses were estimated to be equivalent to approximately 0, 3.6, 13.7 and 31.1 mg/kg bw/d in males and 0, 5.2, 18.2 and 35.6 mg/kg bw/d in females at 0, 25, 100 and 200 ppm respectively. There was no treatment-related change in the survival rate of animals. Dimethoate treatment significantly ($p<0.01$) inhibited plasma and erythrocyte ChE activity at all doses. At 51 weeks of treatment, there were approximate 10%, 50% and 80% reductions in plasma ChE activity and 30%, 70% and 90% reductions in erythrocyte ChE activity at 25, 100 and 200 dimethoate, respectively. A slightly higher incidence of hemolymphoreticular tumours (neoplasm infiltration) was observed in several organs of male mice at 200 mg/kg bw/d and in female rats of all treated groups. However, since lymphoma is a type of common tumours in this species, and the incidences in this study were neither statistically significant nor dose-related, but consistent with the historical control, dimethoate is not considered to be carcinogenic in mice under the experimental condition used. Since dimethoate caused dose-related reductions in erythrocyte ChE levels at all doses tested, a NOEL was not established. The LOEL was 25 ppm or 3.6 mg/kg bw/d (Hellwig *et al*., 1986a).

B6C3F1 hybrid mice were fed dimethoate technical in their diets at levels of 0, 250 or 500 ppm for 80 weeks. As the treated males developed generalized tremors, high dose males were returned to the control diet at 60 weeks and low dose males at 69 weeks. All surviving mice were killed between 93-94 weeks. In the first 12 months of the study, all treated groups except for the low dose females had reduced bodyweight gains. Towards the end of the study period, the physical condition, especially of the high dose males, was generally poor. Dimethoate treatment did not affect mouse survival rates. In males, adrenocortical hyperplasia was observed in all groups with a higher incidence in treated animals, suggesting a treatment related effect. Histopathological examination revealed no increase in tumours associated with treatment (NCI 1977).

Dimethoate was fed to rats at dose levels of 0, 5, 25 or 100 ppm in the diet for 2 years. Additional animals were treated with 0, 1, 5, 25 or 100 ppm dimethoate and used as satellite groups to determine haematological and clinicochemical parameters at interim periods during the study. Doses were equivalent to approximately 0, 0.04, 0.23, 1.2 and 4.8 mg/kg bw/d in

males and 0, 0.06, 0.3, 1.5 and 6.3 mg/kg bw/d in females at 0, 1, 5, 25 and 100 ppm, respectively. Dimethoate-treatment led to an approximate 50% reduction ($p<0.01$) in plasma ChE activity that persisted for the duration of treatment in animals at 100 ppm. Erythrocyte ChE activity was reduced ($p<0.05$ or $p<0.01$) in a dose-related manner for females at 5, 25 and 100 ppm and for males at 25 and 100 ppm. In females, at 5 ppm, the erythrocyte ChE activity was inhibited up to 34% during the first 12 months of treatment; levels were unaffected at this dose after 18 and 24 months of treatment. In males, erythrocyte ChE activity was inhibited (18%, $p<0.01$) at 5 ppm at the 24 month time point only. At necropsy, a dose-related and toxicologically significant decrease in brain ChE activity was seen at 5, 25 and 100 ppm for males ($p<0.05$ or $p<0.01$; 23-62%) and at 25 and 100 ppm ($p<0.01$; 40-52%), for females. Slightly higher incidences of spleen and lymph tumours (hemangioma and/or hemangiosarcoma) occurred in males at 5 ppm and higher doses. Since the tumours were either with a low incidence compared to concurrent and/or historical control, lacking a dose-response relationship, appearing in one sex and one species only, the weight of evidence indicates that dimethoate is unlikely to be a carcinogen in rats. The NOEL, based on inhibition of erythrocyte and brain ChE activity was 1 ppm (0.04/0.06 mg/kg bw/d in males/females) (Hellwig *et al*., 1986b).

Rats (50/sex/group) were fed dimethoate in their diets at doses of 0, 250 or 500 ppm. Males did not tolerate the doses well such that after a 19-week treatment period doses were reduced to 0, 125 or 250 ppm respectively. Females had their doses reduced by half after 43 weeks. At the end of the 80-week period, animals were fed a control diet for a further 34-35 weeks before being necropsied. Reduced body weight gain was seen in treated males and high dose females. Clinical signs were observed during the first weeks at the high dose whereas during the 2nd year all treatment groups exhibited adverse clinical signs. Histopathologic examination revealed no significant increase in tumours associated with dimethoate treatment. However, several non-neoplastic lesions occurred more frequently in treated rats than in control animals. These included interstitial fibrosis of myocardium, focal cytomegaly of adrenal cortex, follicular cell hyperplasia in the thyroid gland and testicular atrophy (NCI 1977). This study was considered unsuitable for establishing a NOEL.

Dogs received 0, 5, 20 or 125 ppm of dimethoate (0.18/0.19, 0.70/0.76 and 4.2/4.3 mg/kg bw/d at 5, 20 or 125 ppm respectively) in the diet for 52 weeks. There were no treatment-related clinical signs. Food consumption and body weight changes were comparable among groups. Inhibition ($p<0.05$ or $p<0.01$) of plasma ChE activity was observed at 125 ppm in males at weeks 13, 26 and 52, and weeks 13 and 26 in females. Erythrocyte ChE activity was significantly ($p<0.05$) decreased at 20 and 125 ppm in both sexes. Brain ChE activity was inhibited 14-18% ($p<0.01$) at 20 ppm and 55-56% at 125 ppm. A minimal (9-10%) statistically significant ($p<0.05$) decrease in brain ChE activity in males and females at 5 ppm was not considered of toxicological relevance. Absolute and relative liver weights were lower in both sexes of the 125 ppm group. Deposit of a brown, granular pigment observed in isolated sinusoidal cells of the liver of treated groups without relationship to dose was considered of doubtful toxicological significance (Burford *et al*., 1990a & b). The NOEL was 5 ppm (0.18/0.19 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 20 ppm (0.70/0.76 mg/kg bw/d).

Metabolites

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 erythrocyte ChE activity in males at 4 ppm and above.

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; erythrocyte 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 treatment related increase in the 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 erythrocyte 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 erythrocyte ChE activity in males at the next highest dose of 0.05 mg/kg bw/d.

In a 12-month study by Hoffmann & 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, erythrocyte and brain ChE activities were inhibited in both sexes at 0.625 mg/kg bw/d, with inhibition of erythrocyte 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 erythrocyte and brain ChE activity in males at 0.125 mg/kg bw/d.

Reproductive studies

In a dose-range finding study, male and female rats received dimethoate in the feed at nominal concentrations of 0, 50, 75 or 100 ppm from 4 weeks prior to mating until all F1 litters had weaned. Selected F1 males and females were retained on the diets to 6 weeks of age. Mating performance was not affected. The implantation rate showed an apparent dose-related reduction in all treated groups that, coupled with an increase in pre-birth losses at 75 and 100 ppm, resulted in a lower litter size at birth. Pup loss from birth to weaning was greater than control in all treated groups, which resulted in a dose-related decrease in litter size by weaning. From Day 4, mean pup weights were also reduced in all treated groups and remained lower through to termination. Plasma, erythrocyte and brain cholinesterase activity were inhibited at all doses in F0 and F1 animals [males 50 ppm plasma ChE activity the only exception where 14% inhibition was observed]. No NOEL was determined for this study. The LOEL for reproductive toxicity was 50 ppm (3.6 mg/kg bw/d) based on dose-related reduction of implantation rate at all doses. The LOEL for maternal toxicity was 50 ppm (equal to 3.6 mg/kg bw/d), based on the inhibition of plasma, erythrocyte and brain ChE activity at and above this dose. The LOEL for paternal toxicity was 50 ppm (equal to 2.7 mg/kg bw/d), based on the inhibition of erythrocyte and brain ChE activity at 50 ppm and above. The LOEL for pup toxicity was 50 ppm (equal to 3.6 mg/kg bw/d) based on decreased pup weight and inhibition of plasma, erythrocyte and brain ChE activity at all doses (Brooker & Stubbs 1991).

In a two-generation reproduction study, dimethoate was administered continuously to rats in the feed at nominal concentrations of 0, 1, 15 and 65 ppm. Treatment with dimethoate did not significantly influence mortality, body weight or food consumption in F0 or F1 parental animals. Body weight gain was slightly lower in high-dose F0 and F1 females during gestation and lactation, however the results did not achieve statistical significance. Water consumption was slightly, but significantly, decreased at some time points in F1 high dose females. Mating performance as assessed by precoital time and duration of pregnancy was unaffected, however the pregnancy rate was lower at 65 ppm and equivocal effects (on pregnancy rate) were seen at 15 ppm. The decreased pregnancy rates were seen at the second mate of the F0 generation at 65 ppm, and at both matings of the F1 generation; results were statistically significant at all doses at the F1 first mate (but within the range of historical controls) and not statistically significant at the second mating (but outside the range of historical controls at 15 and 65 ppm). While results at 15 ppm were not conclusive, on the basis of these data it cannot be ruled out that decreased pregnancy rate at 15 ppm was related to treatment. At 65 ppm, litter size at birth was significantly reduced for F1a and F2b litters and decreased mean pup weight was observed at days 4 and/or 21 (F1a, F1b, F2a). Plasma ChE activity was inhibited ($p<0.01$) in F0 males (28-30%) and females (31-32%) at 65 ppm. Erythrocyte ChE activity was inhibited ($p<0.01$) at ≥ 15 ppm in males (17-67%) and females (36-65%), and brain ChE activity was decreased ($p<0.01$) 18-60% in males and 32-62% in females. In F1 males and females, plasma ChE activity was inhibited 19-33% and 34-41%, respectively, at 65 ppm. Erythrocyte ChE activity was decreased ($p<0.01$) 24-65% in males, and 27-69% in females, at ≥ 15 ppm. Brain ChE activity in F1 males and females was inhibited ($p<0.01$) 28-61% and 30-71%, respectively. A statistically significant (13%) decrease in brain ChE activity was also seen in PND 4 pups at 65 ppm. Minor (11-14%) statistically significant reductions in plasma ChE activity was found at 15 ppm at single time points in F0 and F1 animals. The NOEL for reproductive toxicity was 1 ppm (0.05 mg/kg bw/d) based on decreased pregnancy rate at 15 ppm and 65 ppm. The NOEL for maternal and paternal toxicity was 1 ppm (0.05/0.06 mg/kg bw/d) based on inhibition of erythrocyte and

brain ChE activity at 15 and 65 ppm. The NOEL for pup toxicity was 15 ppm (0.7 mg/kg bw/d) based on decreased body weight at 65 ppm (Brooker *et al*., 1992).

Rats were given daily doses of 0, 0.2, 1.0 and 6.5 mg/kg bw/d dimethoate in the feed in a 2-generation reproduction study. Body weight and food consumption were comparable between control and treated animals with the exception of a significantly decreased body weight gain (40% of the control; $p \leq 0.05$) in F2b dams at 6.5 mg/kg bw/d during lactation. There was a higher rate of F2b pup death at 6.5 mg/kg bw/d, and the lactation index of this group (94%) was out of the range of historical control (95-100%). The increased rate of deaths, considered related to treatment, was attributed to a single litter which were not properly nursed and were cannibalized between days 4 and 6 of lactation. In F0 animals, serum ChE activity was inhibited only at the end of treatment at the high-dose in females. Erythrocyte ChE activity was decreased at 6.5 mg/kg bw/d in F0 males and females prior to mating and at study termination. Brain ChE activity was decreased in F0 males at ≥ 1.0 mg/kg bw/d and in females at 6.5 mg/kg bw/d. In the F1 generation, serum ChE activity was inhibited only in females at 6.5 mg/kg bw/d at the end of treatment. Erythrocyte ChE activity was inhibited in males and females at 6.5 mg/kg bw/d. Brain ChE activity was decreased at ≥ 1.0 mg/kg bw/d in females and 6.5 mg/kg bw/d in males. Reduced prostate weight, associated with diffuse atrophy of the glandular epithelium and reduced secretion, was observed in F1 males at 6.5 mg/kg bw/d. Focal vacuolization of the epididymides was observed in F0 and F1 males at the high dose (Mellert *et al*., 2003b). Under the conditions of this study, the NOEL for reproductive toxicity was 6.5 mg/kg bw/d. The NOEL for maternal toxicity was 0.2 mg/kg bw/d, based on the inhibition of brain ChE activity at 1.0 mg/kg bw/d. The NOEL for paternal toxicity was 0.2 mg/kg bw/d based on the inhibition of erythrocyte and brain ChE activity at 1 mg/kg bw/d or above. The NOEL for pup toxicity was 1.0 mg/kg bw/d based on slightly increased pup deaths during lactation at 6.5 mg/kg bw/d.

Metabolites

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, erythrocyte 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, erythrocyte 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, erythrocyte and brain ChE activities in adults, reduced pup bodyweight and testes weights) were seen at all doses in this study.

In a two-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, erythrocyte 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.

Developmental studies

Dimethoate was administered to rats by gavage at doses of 0, 3, 6, and 18 mg/kg bw/d from GD6 to GD15. Animals were killed on GD20 and litter values determined and foetuses examined for visceral and skeletal abnormalities. Significant signs of maternal toxicity (cholinergic effects) were observed in dams at 18 mg/kg bw/d. These included hypersensitivity, tremors, abnormal gait and reduced weight gain and food intake. At 3 and 6 mg/kg bw/d transient salivation was seen post dosing. There were no instances of total litter loss. The mean incidence of skeletal anomalies was higher in all treated groups than in control, however similar anomalies occurred (at a similar incidence) in historical controls. A single foetus at 6 mg/kg showed retro-oesophageal aortic arch. No malformations were observed at 3 and 18 mg/kg bw/d. Dimethoate was not teratogenic under the conditions of the study (Edwards *et al*., 1984a).

Rabbits received dimethoate technical by gavage at doses of 0, 10, 20 and 40 mg/kg bw/d from day 7 to day 19 of gestation. On day 29, animals were killed, litter values determined and foetuses examined for visceral and skeletal abnormalities. Significant signs of maternal toxicity including muscle tremors, unsteady gait and reduced body weight gain were observed in does at 40 mg/kg bw/d. Reduction in foetal body weight gain was observed at this dose. There were no obvious adverse effects of dimethoate on litter size, upon post implantation losses, on incidence of malformations, visceral and skeletal anomalies or on skeletal variants at any of the treatment levels. Dimethoate was not teratogenic in rabbits under the conditions of the study (Edwards *et al*., 1984b).

Metabolites

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, tremor was 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).

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, 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 erythrocyte 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 erythrocyte and brain ChE activity in does and foetal malformations at 1 mg/kg bw/d (Holzum 1990b).

Formulations

Mated female rats received, by gavage, doses of dimethoate formulation at levels of 0, 3, 6, 12 or 24 mg/kg bw/d on days 6 to 15 of gestation. The formulation used, Cygon 4E, contained 47.3% dimethoate; the non-pesticide ingredients were unknown. The animals were necropsied on day 22. Eight dams of the high dose group showed signs of toxicity including clonic spasms and muscular tremors. No signs of maternal toxicity were observed in the other treated groups. There were no changes in reproduction parameters with dimethoate treatment. A significant increase in the incidence of skeletal anomalies (way ribs, extra ribs) was seen in foetuses of the 12 and 24 mg/kg groups. The NOEL in foetuses was 6 mg/kg bw/d for the formulation or 2.8 mg/kg bw/d of dimethoate (Khera 1979).

Mated female cats received single daily doses of dimethoate formulation (Cygon 4E containing 47.3% dimethoate) in gelatin capsules at levels of 0, 3, 6 or 12 mg/kg bw/d from day 14 to day 22 of gestation. Animals were necropsied on day 43 of gestation. There were no signs of maternal toxicity in any of the treatment groups. In the high dose group polydactyly was observed in 8 of 39 foetuses. This was significantly different from controls. The NOEL was 6 mg/kg bw/d for the formulation or 2.8 mg/kg bw/d of dimethoate (Khera *et al.*, 1979).

Genotoxicity studies

In submitted studies, dimethoate induced an increase in revertants in *Salmonella typhimurium* test strains TA100 (approximately 2-3 fold at 4000-8000 μg/plate) and *Escherichia coli* WP2 uvrA (approximately 2-4 fold at 2500-8000 μg/plate; Engelhart 1993). In the UDS assay, dimethoate exposure *in vitro* induced a reproducible increase in the incorporation of ^{3}HTdR into primary rat hepatocytes compared to the corresponding solvent control (Fautz 1990a & b). In hepatocytes isolated from rats treated with dimethoate *in vivo* at doses of 50, 100 or 200 mg/kg bw, 4 and 12 hours post dosing, UDS induction was not increased compared to controls (Jackh 1991; Engelhardt 1997).

Metabolites

In submitted *in vitro* studies, omethoate was positive in the Ames test (*S. typhimurium* strains TA 100 and TA 1535), and an unscheduled DNA synthesis assay in primary rat hepatocytes (Herbold 1988a; Cifone 1989). Omethoate induced forward mutations at the HGPRT locus in Chinese hamster ovary (CHO) cells, but only at cytotoxic concentrations (Lehn 1989). A sister chromatid exchange using CHO cells also gave positive results in the presence and absence of metabolic activation (Taalman 1988).

With the exception of the mouse spot test (Herbold 1990b), *in vivo* results were negative. The other assays were a dominant lethal mutation test and a micronucleus test (bone marrow) in mice (Herbold 1991; Herbold 1988b), a sister chromatid exchange (bone marrow) in Chinese hamsters (Herbold 1990a) and unscheduled DNA synthesis in rat hepatocytes (Benford 1989).

Neurotoxicity studies

In a range-finding acute study, rats received a single oral dose of 2, 20, 25, 50, 100, 200, 300, 500, 750 or 1000 mg/kg bw of dimethoate. Deaths occurred at 200 mg/kg bw and higher doses, primarily within 24 hours of treatment. The occurrence of clinical signs peaked at approximately 2 h post dosing, and several persisted at 3 h and 8 h. All survivors were clinically normal by Day 4 at 200 and 250 mg/kg bw, and by Day 6 at 300 mg/kg bw. Clinical signs prior to deaths included gait alterations, constricted pupils, salivation, tremors, absent forelimb/hindlimb grasp, laboured and/or shallow respiration and impaired/absent righting reflex. The signs first occurred within 15-90 mins depending on the dose; hypothermia, lacrimation and staining on various body surface appeared later, and all signs persisted (often increasing in severity) through to the time of death. Macroscopic examination revealed dark red lungs in 1 male and opacity of one eye in the other male at 200 mg/kg bw. There were no treatment related effects observed at 2 and 20 mg/kg bw. Dose levels of 2, 20 and 200 mg/kg bw were selected for the acute neurotoxicity study in rats, with an estimated time of peak effect at 2 h post dosing (Lamb 1993a).

In an acute neurotoxicity study, single oral doses of 0, 2, 20 or 200 mg/kg bw of dimethoate were given to groups of rats by gavage. There were no mortalities. Males at 200 mg/kg bw showed a significantly lower body weight gain (38%, $p < 0.01$) than control during the week following treatment, resulting in an overall lower body weight gain for Days 0-14. Clinical signs observed at 200 mg/kg bw, and generally limited to days 1-2 following dosing, included gait alterations (rocking, lurching or swaying in 15/30 rats), tremors (whole body or forelimbs/hindlimbs in 13/30) and constricted pupils (11/30). Body surface staining (the majority of rats), decreased defecation (18/30) and hunched body (in 1 male on Day 2) were also observed in this group. FOB and motor activity evaluations at the estimated time of peak effect (2 hours) revealed a number of alterations at 200 mg/kg bw. Except gait alterations, tremor and constricted pupils, which persisted to Days 1 and/or 2, other responses were transient in nature, and were not apparent on Days 7 and 14. Treatment-related absence of pupil response was also observed at 20 mg/kg bw. There were no treatment related alterations at 2 mg/kg bw (Lamb 1993b). The NOEL for acute neurotoxicity was 2 mg/kg bw/d, based on treatment-related absence of pupil response at 20 mg/kg bw.

In an acute neurotoxicity study, rats were given 0, 1, 2, 3, or 15 mg/kg bw of dimethoate in the diet for 3 h on Day 1. There were no mortalities and no treatment related findings for clinical signs, food consumption or body weight. There were no clearly treatment-related abnormal findings in cageside observations, FOB evaluations or locomotor activity

investigations. On Day 1, males and females at 15 mg/kg bw showed significant reductions in ChE activity in the plasma, erythrocytes, hippocampus, cortex and striatum (up to 50%, 58%, 33%, 36% and 41% reduction respectively for males, and 40%, 65%, 38%, 43% and 47% reduction respectively for females, compared to control). At day 15 at the high dose, statistically significantly lower (15-16%) ChE activity was only detected in hippocampus of male and female rats. Significant inhibition of ChE activity was also noted at 3 mg/kg bw, in erythrocytes from males (29% reduction) and in the cortex of brains from females (11% reduction) on Day 1. At 2 mg/kg bw on Day 1, plasma and erythrocyte ChE activity was decreased greater than 20% (not statistically significant) compared to controls in males (Schaefer 1999a & b). The NOEL for acute neurotoxicity was 1 mg/kg bw, based on inhibition of plasma and erythrocyte ChE activity at 2 mg/kg bw and above.

Rats received 0, 1, 50 or 125 ppm dimethoate in the diet for 91-94 consecutive days. Achieved daily intakes of the test substance were 0, 0.06/0.08, 3.2/3.8 and 8.1/9.9 mg/kg bw/d for males/females respectively. There were no mortalities. Body weight gain was decreased at the high dose. Hairloss and dried tan staining on forelimbs, and/or small faeces were seen in all groups, but were more frequent at 50 and 125 ppm. On occasion, rats at 50 and 125 ppm responded more energetically during tests for the approach response, tail pinch response or touch response than concurrent control. Lower forelimb grip strength was observed in males and females at 125 ppm; reduced rotarod performance was seen in males at 50 and 125 ppm at week 12. Plasma ChE activity was significantly ($p<0.05$ or $p<0.01$) decreased (24-48%) at ≥ 50 ppm in males and also decreased approximately 50% (n.s) compared to controls at 125 ppm in females. Erythrocyte ChE activity was significantly ($p<0.05$ or $p<0.01$) inhibited in males and females at 50 and 125 ppm. A slight, but significant (11%; $p<0.05$) reduction of erythrocyte ChE activity was seen in males at 1 ppm only at week 7. ChE activity was decreased in various brain regions (olfactory, midbrain (with striatum), brainstem, cerebellum and cortex) at the high dose in males and/or females **(**Lamb 1994). The NOEL was 1 ppm (0.06/0.08 mg/kg bw/d in males/females) based on reduction of plasma and erythrocyte ChE activity at 3.2 mg/kg bw/d and above.

Developmental neurotoxicty studies

In a dose-range finding study for developmental neurotoxicity, groups of pregnant female rats received dimethoate by gavage at 0, 0.2, 3 or 6 mg/kg bw/d from GD 6 to PND 10; selected offspring were then dosed directly from PNDs 11-21. Another group of female rats were killed on GD 20 for assessment of litter data and ChE activity in maternal and foetal plasma, erythrocytes and brain. Maternal body weight gain was significantly lower (12% lower from GD 6-20) at 3 and 6 mg/kg bw/d during gestation. There was an increase in post-natal pup mortality at 6 mg/kg bw/d, with a total loss of 2 litters (14 and 12 pups) by PND 2, while 4 out of 14 pups from another dam died by PND 4. Underactivity, hypothermia and lack of feeding (no milk in stomach at necropsy) were seen in these pups soon after birth. The incidence of total pup loss resulted in reductions in live litter size, as well as birth and viability indices of this group, compared with control. Group mean body weight for both male and female offspring at 6 mg/kg bw/d was lower at PND 1 and PND 11. Necropsy revealed no treatment related macroscopic changes in maternal animals or offspring killed on PND 21. Statistical analyses were not conducted on ChE activity data, however plasma, erythrocyte and brain ChE activity was decreased ≥20 % at doses of ≥ 3 mg/kg bw/d in F0 females. In GD 20 male foetuses, ≥20 % reductions in plasma ChE activity were seen following maternal doses of ≥ 0.2 mg/kg bw/d, and erythrocyte and brain ChE activity were inhibited at ≥ 3 mg/kg bw/d. In GD 20 females, erythrocyte ChE activity was inhibited at doses of ≥ 0.2

mg/kg bw/d and erythrocyte and brain ChE activity were inhibited at ≥ 3 mg/kg bw/d. F1 pups directly dosed from PNDs 11-21 showed inhibited plasma, erythrocyte and brain ChE activity at ≥ 3 mg/kg bw/d (Myers 2001a). The results suggest that 6 mg/kg bw/d is unsuitable for use in a developmental neurotoxicity study based on lower pup birth weight and increased early post-natal pup death. A NOEL was not established in this range-finding test, since 0.2 mg/kg bw/d, the lowest dose tested induced decreased plasma ChE activity in male and erythrocyte ChE activity in female F1 foetuses. The LOEL was 0.2 mg/kg bw/d.

In a study to determine the effects of dimethoate on plasma, erythrocyte and brain ChE activity in maternal animals, their pre-term foetuses, in pre-weaning offspring, and in young rats, pregnant female rats were given dimethoate by gavage at doses of 0, 0.1, 0.5 or 3 mg/kg bw/d from GD 6 to GD 20. Another group was similarly treated from GD 6 to PND 10; selected litters of offspring were then dosed directly from PNDs 11-21. The maternal NOEL was 0.5 mg/kg bw/d based on inhibition of plasma, erythrocyte and brain ChE activity at 3.0 mg/kg bw/d. The NOEL for foetotoxicity was 0.5 mg/kg bw/d based on inhibition of plasma, erythrocyte and brain ChE activity at 3.0 mg/kg bw/d. The NOEL for pup toxicity was 0.1 mg/kg bw/d on the basis of inhibition of erythrocyte ChE activity in pups directly dosed with 0.5 mg/kg bw. (Myers 2001b).

In a developmental neurotoxicity study, dimethoate was administered to pregnant female rats by gavage at 0, 0.1, 0.5 or 3 mg/kg bw/d from GD 6 to PND 10, and directly to the offspring from PND 11 to PND 21. Treated dams showed no clinical signs or adverse effects on body weight, food consumption or gestation length. Total pup deaths were 15, 11, 24 and 44 respectively at 0, 0.1, 0.5 and 3 mg/kg bw/d when excluding pups in the totally lost litters (1 and 3 litters at 0.5 and 3 mg/kg bw/d respectively), and 15, 11, 42 and 88 when including those. The affected litters/pups at 0.5 and 3 mg/kg bw/d appeared to be small in size and apparently unfed, exhibited opaque eyes, slow respiration, were cold to touch with little food apparent in the stomach, and/or underactive with the dam paying minimal attention to the litter during lactation. The pup survival rate at 0.1 mg/kg bw/d was not affected. During arena observations on F1 offspring, male and female offspring at 3 mg/kg bw/d tended to be slightly less active than control as shown by consistently, but not significantly lower values for maximum pivoting angle, maximum distance travelled and number of sections entered in the arena on PND 4. Slightly lower values for activity count and/or surface righting reflex were also observed in males and/or females at 3 mg/kg bw/d on PNDs 11 and 21, but not at later stages to PND 60. Motor activity data for pups showed considerable inter- and intra-group variation, and only females at 3 mg/kg bw/d had a significant decrease in high beam score (46, 20, 26, 4.5 at 0, 0.1, 0.5 and 3 mg/kg bw/d respectively) on PND 17 (Myers 2001c; Reiss & Gaylor 2002; Myers 2003). The NOEL for maternal toxicity was not measured but reported as 0.5 mg/kg bw/d based on inhibition of plasma, erythrocyte and brain ChE activity at 3.0 mg/kg bw/d from a previous developmental neurotoxicity study (Myers 2001b). The NOEL for pup toxicity (potential foetotoxicity) was 0.1 mg/kg bw/d based on increased pup mortality at 0.5 and 3.0 mg/kg bw/d. The NOEL for developmental neurotoxicity was 0.5 mg/kg bw/d based on reduced activity and responses during arena observations at 3 mg/kg bw/d. (Myers 2001c)

Metabolites

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 erythrocytes 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 erythrocyte 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 erythrocyte, 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*., 2003a).

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 & Sykes 1993).

Human studies

Twenty subjects ingested 2.5 mg of laboratory grade dimethoate in aqueous solution, corresponding to about 0.04 mg/kg bw/d, for 4 weeks. No signs of toxicity were observed, and there was no significant change in blood cholinesterase activity. The same results were found in two subjects who ingested 9 mg (0.13 mg/kg) and 18 mg (0.26 mg/kg bw) dimethoate respectively, for 21 days. It was also reported that five males ingested single doses of 0.25 mg/kg bw dimethoate, without toxic effect or cholinesterase inhibition (Sanderson & Edson 1964).

Male and female volunteers given dimethoate five days per week, at doses that resulted in average daily intakes of 0.07, 0.20, 0.42, 0.58 and 1.00 mg/kg bw/d for periods of 14 to 57 days, showed no clinical signs or localized gastrointestinal effects. Mean whole blood ChE activity was decreased approximately 24%, 35%, and 21% at 0.42, 0.58 and 1.00 mg/kg bw/d respectively (Edson *et al*., 1967). The NOEL was 0.2 mg/kg bw/d based on whole blood cholinesterase inhibition at higher doses.

2.3 Discussion of dimethoate toxicity

Toxicokinetics/metabolism

Dimethoate was well absorbed from the gastrointestinal tract following oral administration to rats. Maximum plasma and tissue concentrations were achieved 0.5 hours after dosing with 10 mg/kg bw. Excretion of the radiolabel was almost complete by 5 days (89-95% of the administered dose) and occurred primarily in the urine (85-91%). Excretion via the urine was rapid; approximately 69-72% and 52-59% of the radiolabel was detected in the urine within six hours of 10 and 100 mg/kg bw doses of dimethoate, respectively. In tissues, radioactivity was primarily detected in the liver and kidney.

Dimethoate was extensively metabolised in rats, principally by initial cleavage of the C-N bond to yield dimethoate carboxylic acid, and subsequently to a number of thiophosphate and phosphate esters. The quantitatively minor route of elimination involves oxidative metabolism of dimethoate to give its oxygen analogue, omethoate. The parent compound represented 1-2% of the dose excreted in the urine (Kirkpatrick 1995).

Acute toxicity of dimethoate

Dimethoate was of moderate acute oral toxicity with LD_{50} values of 150-414 mg/kg bw in male and female rats. Dimethoate was also moderately toxic following oral administration to mice, hamsters, guinea pigs and rabbits. Reported clinical signs occurred within 15-90 minutes of oral dosing in rats and were consistent with cholinesterase inhibition.

The acute dermal toxicity of technical dimethoate ranged from low [>2000 mg/kg bw; Montedison (1976), Kynoch (1986)] to moderate [700-1150 mg/kg bw; Edson & Noakes (1960); Sanderson & Edson (1964)] in rats. Consistent with these findings, approximately 9-11% of radiolabelled dimethoate was recovered in urine, faeces or the carcass, when applied to the skin of rats in aqueous solution at a dose of 10 mg/kg bw in a dermal absorption study. When the dose was increased to 100 mg/kg bw, the percentage absorbed was decreased to about 1% (Kirkpatrick, 1995). For wettable powders, acute dermal LD50 values of 500 mg/kg bw and 995 mg/kg bw were recorded in rats and guinea pigs, respectively (Sanderson & Edson 1964; West *et al*., 1961). The sponsor stated that no acute inhalation study was conducted with dimethoate due to technical reasons, however in a study with a 400 g/L EC formulation, an LC_{50} of 1553 mg/m^3 was determined when calculated for technical dimethoate.

Dimethoate was not a skin irritant but was a slight eye irritant in rabbits. When administered as a paste in paraffin oil, dimethoate was not a sensitizer to guinea pig skin using the closed patch test technique (Madison *et al*., 1984). In a more recent skin sensitization study, using a dimethoate product similar to that available on the Australian market, a positive result was obtained in guinea pigs using the Buehler test (Bollen 2001). Omethoate, the oxygen analogue metabolite of dimethoate, was also a sensitizer to guinea pig skin in the open epicutaneous test (CRP report on omethoate).

Information on human allergic reactions to dimethoate was limited to two case studies. Contact dermatitis was reported in a woman that worked on a daily basis with rose bushes that were sprayed once per week with omethoate, and less often with dimethoate. 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 purity of the omethoate and dimethoate were not established (Haenen *et al*., 1996). In another report, a 41-year old man presented with erythematous oedematous lesions with a depressed violet centre. The lesions, initially located on the palms and back of the hands as well as the wrist, spread to the rest of the skin. The day before the appearance of lesions the man had been exposed to dimethoate as a result of container breakage. Following resolution of the lesions the man was patch tested with a series of pesticides, and recorded a positive reaction to 1% dimethoate (Schena & Barba 1992).

Metabolites and impurities

Dimethoate is extensively metabolised to a number of thiophosphate and phosphate esters, and its oxygen analogue, omethoate. The acute toxicity of omethoate is higher than that of dimethoate, and has been considered in a separate APVMA Chemical Review Program report. For acute exposures, omethoate is considered to be highly toxic by the oral route, and moderately toxic by the dermal or inhalation routes. Similar to dimethoate, 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.

Several other dimethoate metabolites that are found in plants or the environment were investigated for toxicity in single dose studies. These metabolites were identified as O-desmethyl omethoate, O-desmethyl omethoate carboxylic acid, O-desmethyl N-desmethyl omethoate, O-desmethyl isodimethoate and hydroxy-dimethoate (plants) as well as O-desmethyl dimethoate (water sediment study). In general, these metabolites were of lower acute toxicity than dimethoate, and did not inhibit ChE activity to the same extent as the parent compound.

Iso-dimethoate is a potential impurity formed during storage. The oral LD50 of iso-dimethoate in rats was 25-200 mg/kg bw, and exposed animals showed clinical signs characteristic of cholinesterase inhibition [clonic convulsions, increased salivation, laboured gasping and noisy respiration and body tremors; (Dreher 2001a)]. Consistent with this observation, iso-dimethoate inhibited ChE inhibition to a similar (or slightly greater) extent as dimethoate in rats following single oral doses of 30 mg/kg bw (Brennan 2001b). The impurity 0,0,0-trimethyl phosphorothioate had an oral LD50 value of 1150 mg/kg bw in mice and an inhalation LC50 of 1405 mg/m^3 in rats. 0,0,S-trimethyl phosphorodithioate is an impurity of toxicological significance. Further data on this impurity and an appropriate impurity limit will be addressed in the occupational health and safety assessment.

Cholinesterase inhibition

The primary mode of action of dimethoate is considered to be inhibition of acetylcholinesterase activity as a result of dialkylphosphorylation of the active serine site of a catalytic triad also involving histidine, lysine, glutamine or aspartate. The dialkylphosphorylated enzyme may then undergo reactivation, which may be facilitated by oxime therapy, for example pralidoxime. Alternatively, the dialkylphosphorylated enzyme may also undergo ageing by loss of an alkyl group to form monoalkylphosphoryl-ChE, an irreversibly inhibited form of the enzyme. Most pesticides produce either a dimethoxyphosphorylated enzyme or a diethoxyphosphorylated enzyme. Dimethoate is a dimethoxy OP, and as such the enzyme is more rapidly reactivated, and therefore less likely to

undergo ageing. Furthermore, reactivation is even more rapid if one of the alkyl groups is linked to phosphorous through a sulphur atom, which is the case for dimethoate (Karalliedde *et al*, 2001). Inhibition of acetylcholinesterase causes acetylcholine to accumulate at cholinergic receptor sites and can potentially result in effects similar to excessive stimulation of cholinergic receptors in the central and peripheral nervous systems.

Table 1 summarises the NOELs/LOELs for plasma, erythrocyte and brain ChE activities in laboratory animals following single and repeat dose dimethoate exposure via oral and dermal routes.

In general, inhibition of ChE activity was considered an effect of treatment if the inhibition was greater than 20% compared to control values, and statistically significant, and a dose-response relationship was observed following dimethoate treatment. This level of inhibition was seen consistently across studies, and was the most sensitive toxicological endpoint in the majority of cases.

Table 1: Summary of ChE activity findings in laboratory animals

Study	Plasma ChE		Erythrocyte ChE		Brain ChE		Reference
	NOEL	LOEL	NOEL	LOEL	NOEL	LOEL	
Short term repeated oral dosing *(mg/kg bw/d)*							
Rat (4 w-f)	2.48/2.60 M/F	10.4/11.0 M/F	2.48/0.85 M/F	10.4/2.60 M/F	0.83/0.85 M/F	2.48/2.60 M/F	Kaspers *et al* (2004) [GLP, QA]
Dog (4w-f)	2.20	11.12	0.43	2.20	0.43	2.20	Harling *et al* (1989) [GLP, QA]
Subchronic oral dosing *(mg/kg bw/d)*							
Rat (13 w) neurotoxicity study	0.06/3.8 M/F	3.2/9.9 M/F	0.06/0.08 M/F	3.2/3.8 M/F	3.2/3.8 M/F	8.1/9.9 M/F	Lamb (1994) [GLP, QA]
Rat 6 month	NE	NE	0.5	2.5	0.5	2.5	Edson *et al,* 1958
Chronic oral dosing (*mg/kg bw/d*)							
Rat (2yr)	1.2/1.5 M/F	4.8/6.3 M/F	0.23/0.06 M/F	1.2/0.3 M/F	0.04/0.3 M/F	0.23/1.5 MF	Hellwig *et al* (1986b). [GLP, QA]
Dog (52w)	0.70/0.76 M/F	4.2/4.3 M/F	0.18/0.19 M/F	0.70/0.76 M/F	0.18/0.19	0.70/0.76 M/F	Burford *et al* (1990a&b) [GLP, QA]
Reproduction studies (*mg/kg bw/d*)							
Rat F0	2.7/NE M/F	4.2/3.6	NE	2.7/3.6 M/F	NE	2.7/3.6	Brooker & Stubbs (1991).
F1	NE	7.7/7.2	NE	7.7/7.2	NE	7.7/7.2	
Rat F0 (15 weeks dosing)	0.74/0.83 M/F	3.28/3.75 M/F	0.05/0.06 M/F	0.74/0.83 M/F	0.74/0.06 M/F	3.28/0.83 M/F	Brooker *et al* (1992) [GLP, QA]
F1	0.72/0.86 M/F	3.29/3.99 M/F	0.05/0.06 M/F	0.72/0.86 M/F	0.05/0.06 M/F	0.72/0.86 M/F	
Rat F0 (20 weeks dosing)	6.5/6.5 M/F	NE	1.0/1.0 M/F	6.5/6.5 M/F	0.2/1.0 M/F	1.0/6.5 M/F	Mellert *et al* (2003b) [GLP, QA]

Study	Plasma ChE		Erythrocyte ChE		Brain ChE		Reference
	NOEL	**LOEL**	**NOEL**	**LOEL**	**NOEL**	**LOEL**	
F1	6.5/1.0 M/F	NE/6.5	1.0/1.0 M/F	6.5/6.5 M/F	1.0/0.2 M/F	6.5/1.0 M/F	
Developmental studies (*mg/kg bw/d*)							
Rat F0	0.2 F	3 F	0.2 F	3 F	0.2 F	3 F	Myers (2001a)
F1 (GD 20)	NE/0.2 M/F	0.2/3 M/F	0.2/NE M/F	3/0.2 M/F	0.2/0.2 M/F	3/3 M/F	
F1 (dd PND 11-21))	0.2/0.2 M/F	3.0/3.0 M/F	0.2/0.2 M/F	3.0/3.0 M/F	0.2/0.2 M/F	3.0/3.0 M/F	
Rat F0	0.5 F	3.0 F	0.5 F	3.0 F	0.1 F	0.5 F	Myers (2001b) [GLP, QA]
F1 (GD 20)	0.5	3.0	0.5	3.0	0.5	3.0	
F1 (PND 4)	3.0/3.0 M/F	NE/NE M/F	0.5/3.0 M/F	3.0/NE M/F	3.0/3.0 M/F	NE/NE M/F	
F1 (PND 21) dd	0.5/0.5 M/F	3.0/3.0 M/F	0.5/0.1 M/F	3.0/0.5 M/F	0.5/0.5 M/F	3.0/3.0 M/F	
F1 (sd PND 11)	0.5/3 M/F	3/NE M/F	3/0.5 M/F	NE/3.0 M/F	0.5/0.5 M/F	3.0/3.0 M/F	
F1 (sd adult)	0.5/3.0 M/F	3.0/NE M/F	0.5/0.5 M/F	3.0/3.0 M/F	3.0/3.0 M/F	NE/NE M/F	
F1 (rd adult)	0.5/0.5 M/F	3.0/3.0 M/F	0.5/0.5 M/F	3.0/3.0 M/F	0.5/0.5 M/F	3.0/3.0 M/F	
Neurotoxicity studies (*mg/kg bw*)							
Rat (sd)	1/3 M/F	2/15 M/F	1/3 M/F	2/15 M/F	3/3 M/F	15/15 M/F	Schaefer, (1999a&b) [GLP, QA]
Dermal administration *(mg/kg bw/d)*							
Rat (5 d – 6h)[a]	100/10 M/F	NE/20 M/F	40/NE M/F	100/5 M/F	40/10 M/F	100/20 M/F	Hilaski (1999) [GLP, QA]
Rat (4w-5d-6h)[b]	63/63 M/F	NE	31.5/63 M/F	63/NE M/F	31.5/31.5 M/F	63.0/63.0 M/F	Chambers (1999) [GLP, QA]
Rat (4w-5d-6h)[b]	NM	NM	21/105 M/F	42/NE M/F	42/63 M/F	63/105 M/F	Cheffings, (1999) [GLP, QA]

NM = not measured; NE = not established; w = week; f=feeding; M = males; F = females; QA = quality assured study; GLP = study conducted according to principles of good laboratory practice dd=direct dosing, sd=single dose, rd=repeat dose

[a]Conducted with dimethoate formulations containing unspecified non active constituents.

[b] The dose units are mg (active constituent)/kg bw/day.

Table 1 above demonstrates some inconsistency between studies in relation to which is the most sensitive endpoint of ChE inhibition (i.e. brain, erythrocyte or plasma). For example, brain ChE inhibition was the most sensitive endpoint in males in the 2-year rat study, while for females in this study erythrocyte ChE inhibition was the most sensitive endpoint. Sex differences do not adequately describe all of the disparity, as males in the 13 week study had erythrocyte ChE inhibition as the most sensitive endpoint, with no brain ChE inhibition seen at the relevant dose level.

The Wilcoxon matched pairs signed rank test was used to determine if there is a statistical difference in the distribution of erythrocyte or brain ChE inhibition as the most sensitive endpoint across the animal studies. The conclusion is that there is not enough evidence at the 5% level to conclude that the distribution of erythrocyte and brain ChE inhibition LOELs is different across animal studies. Therefore, it is considered that erythrocyte ChE inhibition will be an adequate surrogate for brain ChE inhibition and will also be protective of plasma ChE inhibition.

ChE inhibition following single dose exposure

In a single dose feeding study, in which dimethoate was administered to 8-9 week old rats in the feed at given 0, 1, 2, 3, or 15 mg/kg bw over a 3 h period, plasma and erythrocyte ChE activity was inhibited greater than 20% ($p>0.05$) in males at 2 mg/kg bw, 2.5-3 hours following removal of the test diet (Schaefer 1999a&b). At 3 mg/kg bw, statistical significance was achieved for erythrocyte ChE inhibition, while ChE activity in different regions of the brain was inhibited only at 15 mg/kg bw. Similar results were observed in preweaned rats and young adult rats when dosed by oral gavage. Single doses of dimethoate given to PND 11 rats resulted in significant inhibition of plasma, erythrocyte and brain ChE activity at 3 mg/kg bw (Myers 2001b). Likewise, in young adult rats in the same study, plasma, erythrocyte and brain ChE activity were clearly inhibited at 3 mg/kg bw. Considering all the single dose studies in the database, plasma, erythrocyte and brain ChE activity was inhibited at single doses of ≥ 2 mg/kg bw dimethoate. No toxicologically significant effects were seen at single doses ≤ 1 mg/kg bw.

ChE inhibition in humans

In a study in humans, dimethoate (purity not specified) was given as a flavoured aqueous solution to male and female volunteers five days per week, at doses that resulted in average daily intakes of 0.07, 0.20, 0.42, 0.58 and 1.00 mg/kg bw/d for periods of 14 to 57 days as indicated in Table 2 (Edson *et al.,* 1967; Table 2). There were no localized gastrointestinal effects and no other noted clinical signs. Inhibition of whole blood ChE activity was seen at ≥ 0.42 mg/kg bw/d (27%), but showed only weak relation to dose. The NOEL was 0.2 mg/kg bw/d. The human study measured plasma and erythrocyte ChE inhibition separately but only reported findings for inhibition of ChE in whole blood (Edson *et al.,* 1967). However, it was reported that erythrocyte ChE activity paralleled that of whole blood ChE activity. It is therefore considered that the NOEL of 0.2 mg/kg bw/day in this study is representative of erythrocyte ChE inhibition.

Table 2: Blood ChE activity in human volunteers

Group	No. subjects	Daily dose (mg)	Duration	Mean Whole Blood ChE[b]		Mean weight (kg)	Daily intake[a]
				Pretest	End		
A	12	5	28	114	110	74	0.067
B	9	15	39	128	124	75	0.200
C	8	30	57	121	92	71	0.423
D	6	45	45	113	74	77	0.584
E	6	60	14	121	96	60	1.00

[a] mg/kg bw/d, [b] defined as 'cholinesterase units' (Δ pH/h x 100).

The OCS recognises that there is some consistency in the NOELs and LOELs for short-term studies across species. Humans, dogs and rats have NOELs within the same order of magnitude. (Table 1). However, when all of the studies for the various species are considered

together, there is a significant lack of consistency in the NOELs and LOELs in relation to duration of treatment. For example, the most sensitive NOEL for cholinesterase inhibition in long-term animal studies (0.04/0.06 mg/kg bw/d) is an order of magnitude lower than the NOEL for ChE inhibition seen in the short-term human study. Similarly, in the 13-week neurotoxicity study **(**Lamb 1994), the NOEL was 0.06 mg/kg bw/day and in F0 rats dosed for 15 weeks (Brooker *et al.,* 1992), the NOELs were 0.05/0.06 (m/f). These data suggest that maximum inhibition of ChE may not be achieved following short-term exposure and that a NOEL derived from a short-term study may not be protective of ChE inhibition following longer-term exposure. The OCS has therefore undertaken a time comparison analysis on the available data (see below).

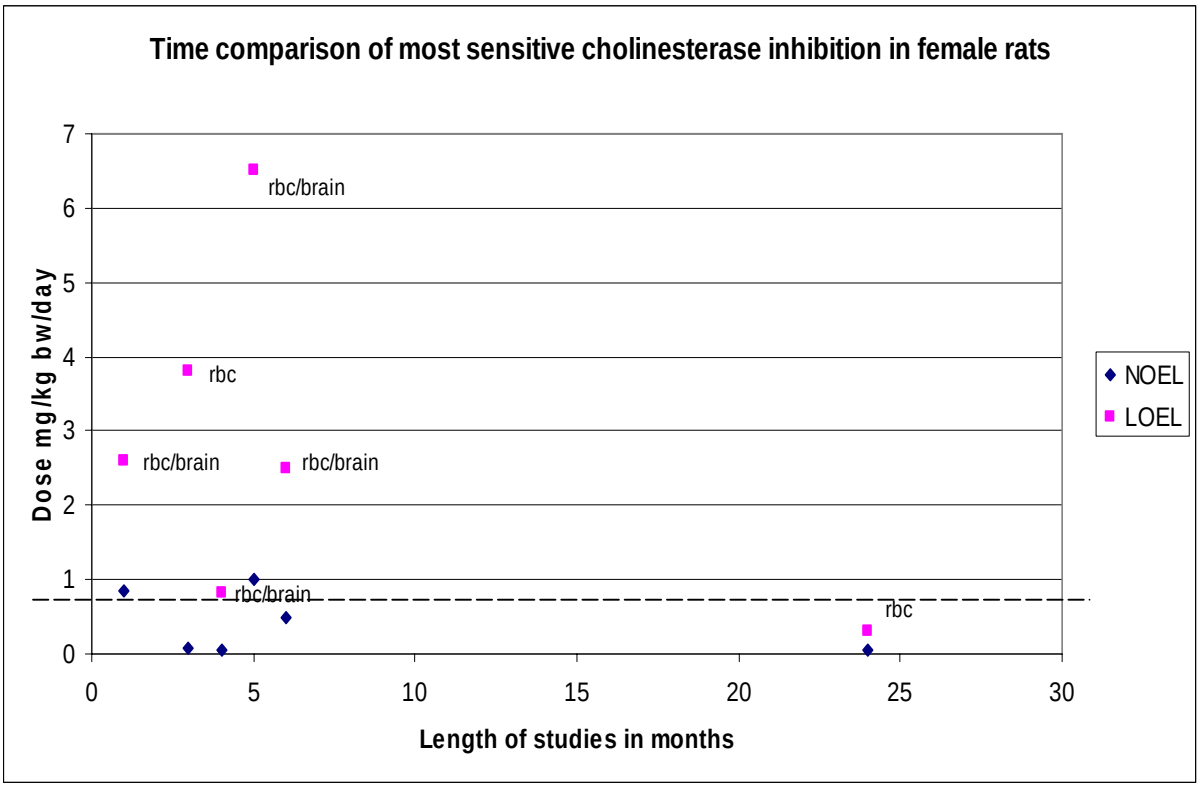
Although the NOELs in the long-term studies are significantly lower than those in the short-term studies (as described above), there is no evidence of an increase in sensitivity at interim time points within the long-term studies. The following paragraphs discuss this in more detail, including interim sampling data from chronic studies together with data from animal studies of different treatment durations.

Analysis of studies accross different durations of treatment demonstrate that some animals may be more sensitive to ChE inhibition following long-term exposure compared to short-term exposure. It is not possible to quantitatively analyse the observed decrease in long-term NOELs and LOELs in comparision to short-term endpoints due to differences in dose spacing across different experiments. However, by graphing NOELs and LOELs from rat studies (Table 3) according to duration of treatment, the data shows that in some cases the LOEL in longer term studies is at or below the NOEL in short-term studies (Figures 1 & 2).

Table 3: Endpoints of ChE inhibition across treatment durations in rat studies

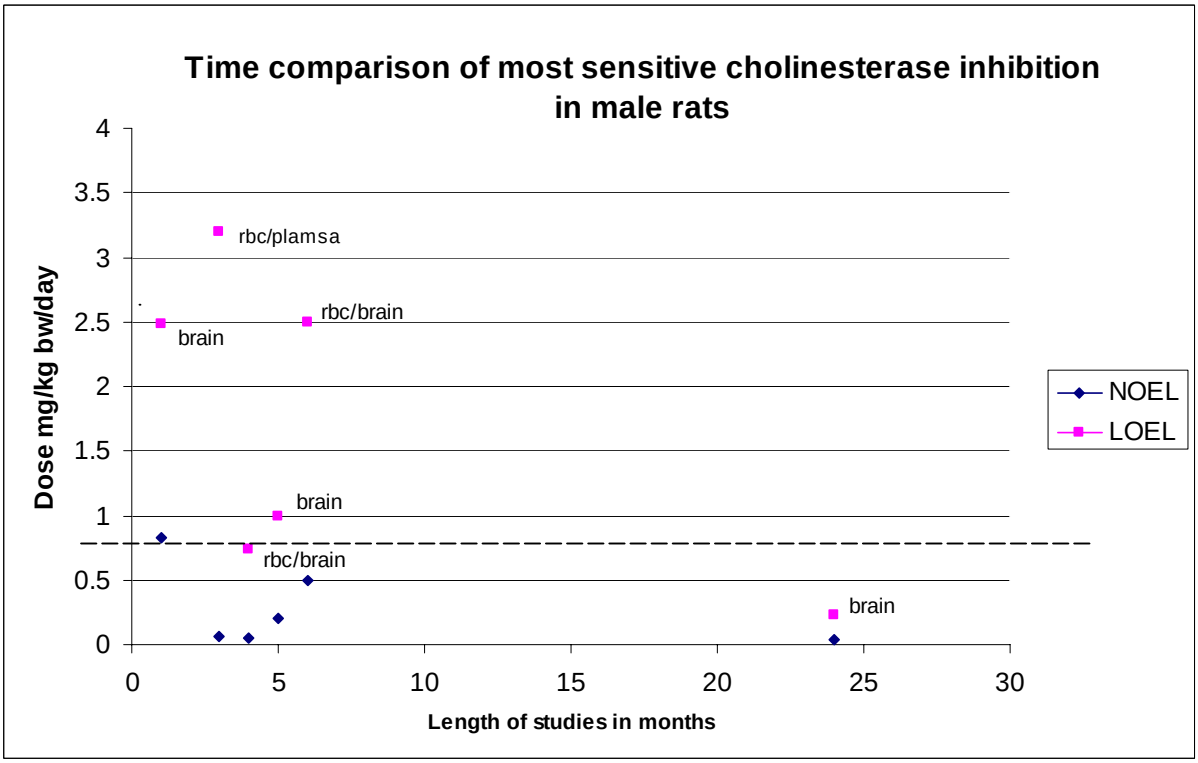
Repeat dose studies (males)	Duration of study (months)	NOEL (mg/kg bw/day)	LOEL (mg/kg bw/day)	Endpoint	Repeat dose studies (females)	Duration of study (months)	NOEL (mg/kg bw/day)	LOEL (mg/kg bw/day)	Endpoint
4 weeks Kaspers *et al* (2004) [GLP, QA]	1	0.83	2.48	Brain	4 weeks Kaspers *et al* (2004) [GLP, QA]	1	0.85	2.6	rbc/brain
13 weeks neurotox Lamb (1994) [GLP, QA]	3	0.06	3.2	rbc/plamsa	13 weeks neurotox Lamb (1994) [GLP, QA]	3	0.08	3.8	rbc
15 weeks repro (F0) Brooker *et al* (1992) [GLP, QA]	4	0.05	0.74	rbc/brain	15 weeks repro (F0) Brooker *et al* (1992) [GLP, QA]	4	0.06	0.83	rbc/brain
20 weeks repro (F0) Mellert *et al* (2003b) [GLP, QA]	5	0.2	1	Brain	20 weeks repro (F0) Mellert *et al* (2003b) [GLP, QA]	5	1	6.5	rbc/brain
6 months Edson *et al,* 1958	6	0.5	2.5	rbc/brain	6 months Edson *et al,* 1958	6	0.5	2.5	rbc/brain
2 year Hellwig *et al* (1986b). [GLP, QA]	24	0.04	0.23	brain	2 year Hellwig *et al* (1986b). [GLP, QA]	24	0.06	0.3	rbc

Figure 1



The endpoints of ChE inhibition are listed next to the LOEL for each study.

Figure 2



The endpoints of ChE inhibition are listed next to the LOEL for each study.

The dotted line in Figures 1 and 2 represents the dose at which the NOEL of 0.83 mg/kg bw/day occurs in the short term study in rats, which is the study most representative of the exposure duration of the human volunteer study. The LOELs for ChE inhibition in F0 rats in the reproductive study (length of study = 4 months) were 0.74 and 0.83 mg/kg bw/day in male and females, respectively, and the LOELs for male and female rats in the 2-year rat study (length of study = 24 months) were 0.04 and 0.06 mg/kg bw/day, respectively. This indicates that in some cases effects are observed in longer term studies at doses where no effects are seen in the short-term study.

In a two-year study in rats, ChE activity was decreased (1) in the plasma at ≥ 4.8 mg/kg bw/d, (2) in erythrocytes at ≥ 0.23 mg/kg bw/d and (3) in brain at ≥ 0.23 mg/kg bw/d. A clear NOEL of 1 ppm in the feed (0.04/0.06 mg/kg bw/d for males brain ChE inhibition and females erythrocyte ChE inhibition, respectively) was observed, (Hellwig *et al*., 1986b). The LOELs for plasma ChE inhibition were 100 ppm in male and female rats at all interim time points from 1 month to 24 months. The LOEL for erythrocyte ChE inhibition was 25 ppm in male rats from 1 month to 24 months and 5 ppm in female rats from 1 month to 12 months. At subsequent interim time points significant ChE inhibition was not observed, indicating a possible adaptive effect in female rats in this dose group.

In a 1-year study, dogs received 0, 5, 20 or 125 ppm of dimethoate (corresponding to intakes of 0.18/0.19, 0.70/0.76 and 4.2/4.3 mg/kg bw/d at 5, 20 or 125 ppm respectively) in the diet for 52 weeks. Inhibition ($p<0.05$ or $p<0.01$) of plasma ChE activity was observed at 125 ppm in males and females from week 13 (22-36%) to week 52. Erythrocyte ChE activity was decreased 22-76 % at 20 and 125 ppm in both sexes ($p<0.05$ or $p<0.01$). Brain ChE activity was inhibited 55-56% ($p<0.01$) at 125 ppm (Burford *et al*., 1990a & b). The NOEL was 5 ppm (0.18/0.19 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 20 ppm (0.70/0.76 mg/kg bw/d). The results from the 52 week dog study indicate consistence in inhibition of plasma and erythrocyte ChE inhibition at interim exposure durations of 13, 26 and 52 weeks. A consistent LOEL of 125 ppm in plasma was reported in both sexes across all interim time points. A LOEL of 20 ppm was reported in both sexes at 13 weeks bot not observed at longer interim durations at this dose, indictating a possible adaptive effect for erythrocyte ChE inhibition.

Protection against ChE inhibition in adults

The short term human volunteer study (Edson *et al.,* 1967) reports a NOEL of 0.2 mg/kg bw/day for inhibition of whole blood ChE (representative of erythrocyte ChE inhibition). Due to conflicting evidence that maximum sensitivity to ChE inhibition is achieved following short term exposure, it is uncertain whether the short-term human study is long enough to establish a NOEL which is protective of longer term exposure. In addition, dosing was carried out for only five days per week in this human study, and therefore it cannot be assured that a kinetic steady state was achieved.

Despite the uncertainty in the available data, the OCS considers that the NOEL of 0.2 mg/kg bw/day derived from this human study can be used to protect against potential ChE inhibition in adult humans, however, two 10-fold safety factors would be required – one for intra-species variation and an additional safety factor to take account of the uncertainty as to whether the maximum sensitivity to ChE inhibition has been achieved. This would result in an acceptable exposure level of 0.002 mg/kg bw/day (ie, an overall 100-fold safety factor), based on ChE inhibition in whole blood.

Although this exposure level is protective of ChE inhibition in adults there is evidence that it may not be protective of sensitive sub-populations based on increased pup mortality and pup ChE inhibition in the absence of maternotoxicity. This is discussed in more detail in the section below on post-natal pup mortality.

Protection against ChE inhibition in offspring

In a developmental neurotoxicity study, ChE inhibition was observed in pups below maternotoxic doses, indicating that pups may be more sensitive to ChE inhibition than adult rats. Significant inhibition of plasma, erythrocyte and brain ChE activity was seen at maternal doses of 3.0 mg/kg bw/d; the NOEL was 0.5 mg/kg bw (Myers 2001b). In the same study, PND 4 male pups showed decreased erythrocyte ChE activity at 3 mg/kg bw/d; plasma ChE activity was slightly (10%) and significantly decreased in females at the same dose. In PND 21 female rats, dosed from PND 11 to PND 21 by gavage, erythrocyte ChE activity was inhibited at 0.5 and 3.0 mg/kg bw/d, the NOEL was 0.1 mg/kg bw/d. Recovery of ChE activity was seen by day 60 (Myers 2001b). The NOEL for effects of dimethoate on ChE activity in offspring was 0.1 mg/kg bw/d.

Other chronic toxicity in repeat-dose and chronic toxicity studies

Dose-related inhibition of plasma, erythrocyte and brain ChE activities was generally the most sensitive manifestation of dimethoate toxicity in short-term, subchronic, and chronic oral studies in mice, rats and dogs. At sufficiently high doses, reduced bodyweight, clinical signs and deaths occurred. Organ toxicity was not generally noted but increased liver weight was observed in mice and rats following high-dose chronic exposure whereas decreased liver weight was reported in a 52-week dog study. In the latter study, deposits of a brown granular pigment were observed at all doses. Slight decreases in ovary weights were also observed in rats and mice following high doses, but these were not associated with histopathological changes. In addition, slight effects were observed at high doses on various clinical chemistry and haematological parameters.

Carcinogenicity

The carcinogenic potential of dimethoate has been investigated in a number of long-term exposure studies in mice and rats. Gibel *et al*. (1973) reported a small, but significant increase in malignant neoplasms in rats receiving dimethoate by gavage at 15 and 30 mg/kg bw/d; the neoplasms consisted mainly of spleen and liver sarcomas. An additional group of rats that received dimethoate (15 mg/kg bw) intramuscularly showed an increased incidence of malignant neoplasms consisting of sarcomas of the spleen, liver, ovary and soft tissues. The significance of these findings was considered unclear given that the increase in incidences was small, the number of test animals was low (20/sex/group) and the doses were given twice weekly by gavage, rather than daily in the diet. An early study by the NCI, while limited in terms of the duration of treatment and selected doses (exceeded the maximum tolerated dose in the early phase of the study), indicated that 75 mg/kg bw/d dimethoate for 60 weeks in mice and 12.5 mg/kg bw/d for 80 weeks in rats was without oncogenic effect (NCI 1977).

A slightly higher incidence of hemolymphoreticular tumours (neoplasm infiltration) was observed in several organs of male mice at 200 mg/kg bw/d and female mice of all treated groups in a 18-month dietary study with B6C3F1 mice (Hellwig et al 1986a). Since

lymphoma is a common type of tumour in this species, and the incidences in this study were neither statistically significant nor dose-related, but consistent with the historical control, dimethoate is not considered to be carcinogenic in mice under the experimental conditions used.

In a 24-month rat dietary study (Hellwig et al 1986b), slightly higher incidences of spleen and lymph tumours (hemangioma and/or hemangiosarcoma) occurred in males at 5 ppm and higher doses. Since the tumours were with a low incidence compared to historical control, lacking a dose-response relationship, appearing in one sex and one species only, the weight of evidence suggests that dimethoate is unlikely to be a carcinogen in rats along with the non-genotoxic nature of the chemical *in vivo*. A NOEL was established at 1 ppm (0.04/0.06 mg/kg bw/d) based on non-neoplastic observations at higher doses.

The only tumours identified as likely to have arisen from omethoate 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.

Genotoxicity

A number of bacterial and mammalian cell assays showed that dimethoate was genotoxic *in vitro*. Dimethoate induced an increase in revertants in test strains *S. typhimurium* TA100 and *E. coli* WP2 uvrA and 5-methyltrytophan resistance mutations in E. coli K12 (Engelhart 1993; Hanna & Dyer 1975; Mohn 1973; Moriya *et al.*, 1983), but did not induce mutations at the HPRT-locus in Chinese hamster ovary cells (Johnson & Allen 1985). Unscheduled DNA synthesis was observed in two studies in isolated rat hepatocytes *in vitro*, and positive results were reported in human lymphocytes *in vitro* using the single cell gel electrophoresis (Comet) assay (Undeger & Basaran 2005; Fautz 1990a; Fautz 1990b). Dimethoate also induced a dose-related increase in sister chromatid exchange in Chinese hamster V79 cells (Chen *et al.,* 1981; 1982).

In the published literature, equivocal or negative results were obtained in bone marrow cytogenetic assays for dimethoate and an EC product containing dimethoate in rats and mice and hamsters (Degraeve & Moutschen 1983; Degraeve *et al*., 1984; Dzwonkowska & Hubner 1986; Nehez *et al.,* 1983 & 1994; Nehez & Desi 1996). Usha Rani *et al*. (1980) reported a positive result for dimethoate of unstated source and purity in a mouse micronucleus assay,

while a dominant lethal test of dimethoate reported >99% pure was negative in mice (Degraeve & Moutschen 1983, Degraeve *et al.,* 1984).

In contrast to results in the published literature, dimethoate was not genotoxic in any guideline compliant *in vivo* study. In hepatocytes isolated from rats treated with dimethoate *in vivo* at doses of 50, 100 or 200 mg/kg bw, 4 and 12 hours post dosing, UDS induction was not increased compared to controls (Jackh 1991; Engelhardt 1997). In addition, dimethoate technical was not clastogenic in the mouse micronucleus assay [(55 mg/kg bw; single or two doses ip; Sorg (1985)] or cytogenetic assay of rat bone marrow [(0, 15, 75 and 150 mg/kg bw ip; San Sebastian (1985)], nor did dimethoate elicit a dominant lethal effect in a dominant lethal assay in mice [(5, 10, 20 mg/kg bw/d for 5 days; Becker (1985)]. Therefore, taken together, while dimethoate was shown to have genotoxic potential in *in vitro* studies in bacteria and mammalian cells, the weight of evidence from guideline compliant studies suggests that the potential genotoxicity of technical dimethoate *in vivo* is considered to be low.

Omethoate produced positive results in a range of *in vitro* assays to test for gene mutation, DNA damage and repair, and chromosomal effects. 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, omethoate is not considered to be genotoxic *in vivo*.

Reproductive toxicity

The dimethoate database contained two 2-generation reproduction studies in rats, one conducted by Brooker *et al.,* (1992) and a more recent study carried out by Mellert *et al*. (2003b). In the earlier study, a NOEL of 0.05 mg/kg bw (1 ppm) dimethoate was established, based on a decreased pregnancy rate compared to control at 15 and 65 ppm (Brooker *et al.,* 1982). Decreased pregnancy rates were seen at the second mate of the F0 generation at the high dose and at both matings of the F1 generation; results were statistically significant at all doses at the F1 first mate (but within the range of historical controls) and not statistically significant at the second mating (but outside the range of historical controls at 15 and 65 ppm). While considered equivocal, it could not be ruled out that the decreased pregnancy rate in this study was attributable to dimethoate treatment. Upon evaluation of this study, JMPR (1996) meeting concluded that the data were clearly indicative of substandard performance at the high dose and showed a possible effect at 15 ppm. Nevertheless, the meeting discounted the effect at 15 ppm when establishing the NOAEL, but applied an extra 5-fold safety factor because of concerns over possible impairment of performance at this dose, and this endpoint served as the basis for establishment of the ADI in the JMPR, 1996 assessment. In a recent publication (Mahadevaswami & Kaliwal, 2004), dimethoate 24 mg/kg bw/day or higher doses given to pregnant albino mice during PDs 7-15 induced a significant decrease in the number of corpora lutea and percent foetal survival, suggesting a toxic effect on the embryo or a hormonal imbalance at the high doses.

The more recent study by Mellert *et al*. (2003b) failed to find evidence of reproductive toxicity at doses of up to 6.5 mg/kg bw/d. However, this study provided evidence of reduced prostate weight, associated with diffuse atrophy of the glandular epithelium and reduced secretion in F1 males at 6.5 mg/kg bw/d. Focal vacuolization of the epididymides was also

observed in F0 and F1 males at the high dose. Under the conditions of this study, the NOEL for reproductive toxicity was 6.5 mg/kg bw/d.

In a very recent publication (Farag et al, 2006) to investigate the effect on male reproduction in mice, dimethoate given by gavage to male mice for 20 days before mating with untreated females, caused decreased sperm production and percent motile sperm at 15 mg/kg bw/day and higher. The NOEL was 7 mg/kg bw/d. A reduction in serum testosterone levels is thought to play an important role in the development of dimethoate-induced infertility, and it was demonstrated that the inhibiton of steroido-genesis by dimethoate was primarily by blocking transcription of the steroidogenic acute regulatory (*StAR*) gene (Walsh et al, 2000).

Post-natal pup mortality

While there was no evidence of reproductive toxicity in the Mellert *et al.* (2003b) experiment, slightly increased pup mortality was seen during lactation at 6.5 mg/kg bw/d. This result was consistent with effects seen in a developmental neurotoxicity study, where Myers (2001c) showed increased pup mortality at 0.5 and 3.0 mg/kg bw/d in rats. Up to weaning, the total pup deaths were 15, 11, 24 and 44 respectively at 0, 0.1, 0.5 and 3 mg/kg bw/d [excluding pups in the totally lost litters (1 and 3 litters at 0.5 and 3 mg/kg bw/d respectively)], and 15, 11, 42 and 88 when all deaths were considered. If only PNDs 1-4 were considered, total pups deaths were 10/359, 8/343, 32/360 and 70/366 corresponding to percentages of 2.7, 2.4, 8.0 and 18.9%, at 0, 0.1, 0.5 and 3.0 mg/kg bw/d respectively. The increased mortality at 0.5 mg/kg bw/d and above was dose-related and statistically significant dependent upon method of analyses. The NOEL for the increased pup loss in Myers (2001c) was 0.1 mg/kg bw/day based on increased pup mortality at 0.5 mg/kg bw/day from offspring dosed GD 6 to PND 21. An internal control for maternal toxicity was not included.

In a dose range finding study by the same author (Myers 2001a), there was also an increase in post-natal pup mortality at 6 mg/kg bw/d, with a total loss of 2 litters (14 and 12 pups) by PND 2, while 4 out of 14 pups from another dam died by PND 4; there were no effects on pup mortality at 3 mg/kg bw/d. It was further noted that in a cross-fostering study in rats carried out to determine whether mortality was a result of dimethoate exposure during gestation or lactation, pup mortality was increased regardless of pre- or postnatal exposure to dimethoate at 3 and 6 mg/kg bw. This study was not submitted to the OCS, but a detailed review by the US EPA was available. In a recently published paper (Reiss & Gaylor 2005), the benchmark dose (5) for pup mortality (defined as the dose for a 5% incidence for pup mortality) for post-natal days 1-4 in rats was 0.64 mg/kg bw/day.

It is not clear whether the increase in postnatal pup mortality is due to foetotoxic effects, due to pup toxicity (exposure post-natal), or due to maternotoxicity affecting lactation. In Myers (2001c) much of the increased pup death at 0.5 mg/kg was due to the total loss of one litter during early lactation, and at the higher dose of 3 mg/kg there was total loss of three litters. Necropsy of the dams for these litters was conducted on the day of litter loss and revealed mammary tissue that was pale and inactive. Additionally, the affected litters/pups at 0.5 and 3 mg/kg bw/day appeared to be small in size and apparently unfed, had little food in their stomachs and were underactive with the dam paying minimal attention to the litter during lactation. However, even if the full-litter losses are not included when considering there is still a dose-related (but not statistically significant) increase in pup mortality at 0.5 mg/kg bw/d. In addition, increased pup mortality was seen in a number of other studies in

the absence of full-litter loss, which suggests that it may be inappropriate to exclude pups from dams that lost their entire litters.

The NOEL for increased pup mortality of 0.1 mg/kg bw/day is clearly a treatment related effect. This dose is also the NOEL for ChE inhibition in pups at a non-maternotoxic dose in Myers (2001b). This is the most sensitive toxicological endpoint for dimethoate. With an incorporated 100 fold safety factor for intra and inter-species variation, this endpoint is protective of ChE inhibition in adult humans (acceptable exposure level for ChE inhibition 0.002 mg/kg bw/day - Edson *et al.,* 1967).

Effects on reproductive performance and pup mortality were also observed for omethoate in one generation and two generation reproductive studies. In these studies, rats were exposed at up to ~4.5 mg/kg bw/d in the drinking water for one generation, and up to ~1.5 mg/kg bw/d for two generations. Reproductive performance was affected at 1.5 mg/kg bw/d and above, evident as increased mean pre-coital time and reduced fertility, with fewer implantations and increased post-implantation loss. At this dose there was also evidence of epididymal vacuolation in parental males. This lesion was also evident in all male rats treated at 3 mg/kg bw/d in the chronic (drinking water) study (Schladt 1995), and also in the 2-generation dimethoate reproductive study (Mellert *et al.,* 2003b), and therefore considered clearly related to treatment.

Developmental toxicity

Studies of the developmental toxicity of dimethoate technical in rats (at 0, 3, 5, or 18 mg/kg bw/d) and rabbits (0, 10, 20 and 40 mg/kg bw/d) revealed no evidence of teratogenicity despite signs of maternotoxicity at high doses (Edson *et al.,* 1984a & b). These results were consistent with the two generation reproduction studies that also showed no evidence that dimethoate was teratogenic. Early published studies, previously evaluated by the OCS, indicated that a dimethoate formulation was teratogenic in cats and caused skeletal variance in rats; the NOELs were 2.8 mg/kg bw/d dimethoate in cats and rats. These studies were however, conducted using a formulation (Cygon 4E) with unknown non-pesticide ingredients and their relation to dimethoate is therefore unclear (Khera 1979; Khera *et al.,* 1979).

Consistent with results for dimethoate, no foetal abnormalities were observed in rats given omethoate in a developmental study for which the maximum dose was 3 mg/kg bw/d. Effects of treatment were limited to decreased placental 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 (Holzum 1990a). Foetal abnormalities were seen in one developmental study with omethoate in rabbits (Holzum B 1990b). 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 erythrocyte and brain ChE activities. The possible treatment-relatedness of the foetal abnormalities in the omethoate rabbit developmental study cannot be dismissed (Holzum 1990b). However, considering that foetal abnormalities were confined to that study only, with no effects in a similar study in the same species (OCS omethoate report), or in

developmental and reproduction studies with dimethoate and omethoate in rats, it is not of particular concern to human health.

Neurotoxicity

Dimethoate and omethoate caused neurotoxicity consistent with cholinesterase inhibition in rats, however there was no evidence for organophosphate-induced delayed neurotoxicity (OPIDN) in rats or in hens (JMPR 1996; Lamb 1993a & b; 1994; Myers 2001a & b & c; 2003; Schaefer 1999a & b). Although complete studies investigating delayed neurotoxicity of dimethoate in hens were not submitted for assessment, previous JMPR summaries were available (JMPR, 1996). Evidence for the development of 'intermediate syndrome' was available from human poisoning incidents following ingestion of large doses of dimethoate (De Bleeker *et al.,* 1992). Despite a number of suicide attempts where large doses of dimethoate were ingested, there was no convincing evidence for development of OPIDN in humans.

2.4 Standards relevant to human health risk assessment

2.4.1 NOELs relevant to public health risk assessment

To determine the lowest NOEL for the establishment of an ADI, a summary of the NOELs determined in those studies deemed adequate for regulatory purposes is shown in **Table 4**.

Table 4: NOELs relevant to establishing an ADI

Species	Study Type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
Short term studies					
Rat	4w dietary	0.83/0.85 M/F	2.48/2.60 M/F	Brain ChE (M) Erythrocyte/brain ChE (F)	Kaspers *et al* (2004) [GLP, QA]
Dog	4w dietary	0.43	2.20	Erythrocyte/brain ChE	Harling et al (1989) [GLP, QA]
Subchronic studies					
Rat	13 w dietary (neurotoxicity)	0.06/0.08 M/F	3.2/3.8 M/F	Plasma/Erythrocyte ChE (M) Erythrocyte (F)	Lamb (1994) [GLP, QA]
Rat	6 month dietary	0.5	2.5	Erythrocyte/brain ChE	Edson *et al* (1958)
Long-term studies					
Rat	2 year dietary	0.04/0.06 M/F	0.23/0.3 M/F	Brain ChE (M) Erythrocyte (F)	Hellwig *et al* (1986b) [GLP, QA]
Dog	1 year dietary	0.18/0.19 M/F	0.70/0.76 M/F	Erythrocyte/brain ChE	Burford *et al* (1990a&b) [GLP, QA]
Reproduction studies					
Rat	2-generation reproduction (approx 15 weeks)	0.05	0.74	Reproductive toxicity: decreased pregnancy rate	Brooker *et al* (1992) [GLP, QA]
		0.06	0.83	Maternal toxicity; erythrocyte/brain ChE	
		0.05	0.74	Paternal toxicity; erythrocyte/brain ChE	
		0.8	3.75	Pup toxicity; decreased body weight, inhibition of brain ChE activity, and delay in the development of startle reflex	
Rat	2-generation reproduction (approx 20 weeks)	6.5	NE	Reproductive toxicity	Mellert *et al* (2003b) [GLP, QA]
		0.2	1.0	Maternal toxicity; brain ChE	
		0.2	1.0	Paternal toxicity; brain ChE	
		1.0	6.5	Pup toxicity	

Species	Study Type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
Developmental studies					
Rat	Developmental ChE investigations	0.5	3.0	Maternal toxicity; plasma/erythrocyte/ brain ChE inhibition	Myers (2001b)* [GLP, QA]
		0.5	3.0	Foetotoxicity (developmental neurotoxicity): plasma/erythrocyte/ brain ChE inhibition	
		0.1	0.5	Pup toxicity: erythrocyte ChE inhitition	
Rat	Developmental neurotoxicity	NE	NE	Maternal toxicity: NE	Myers (2001c) [GLP, QA]
		0.1	0.5	Pup toxicity: increased pup mortality	
		0.5	3.0	Developmental neurotoxicity: reduced activity and responses during arena observations, inhibition of plasma/erythrocyte/ brain ChE	
Human studies					
Human	Repeat dose 14-57 days	0.2	0.42	Whole blood ChE	Edson *et al* (1967)

* This study reported ChE activities, whereas Myers (2001c) reported developmental neurotoxicity data. NE = not established

To determine the lowest NOEL for the establishment of an ARfD, a summary of the NOELs determined in those studies deemed adequate for regulatory purposes is shown in Table 5.

Table 5: NOELs relevant to establishing an ARfD

Species	NOEL (mg/kg bw)	LOEL (mg/kg bw)	Toxicological Endpoint	Reference
Acute studies				
Rats (gavage)	2	20	Pupil response (ChE activity **not** determined)	Lamb (1993b) [GLP, QA]
Rats (dietary*)	1	2	Plasma/erythrocyte ChE	Schaefer (1999a&b) [GLP, QA]
Developmental neurotoxicity studies				
Rats (7-8 weeks) (single dose gavage)	0.5	3.0	Erythrocyte ChE	Myers (2001b) [GLP, QA
Human studies				
Human (as an aqueous solution for 14-57 days)	0.2	0.42	Whole blood ChE	Edson *et al* (1967)

* Administered as a single dose in the diet consumed over a 3-h period.

3 EXPOSURE ESTIMATION

3.1 Public exposure

The majority of dimethoate products are intended for use in the agricultural sector. Residues of dimethoate are expected in food and water.

3.1.1 Residues in food

Assessment of the exposure of the Australian population to residues of agricultural and veterinary chemicals in food crops and target animals is performed by the APVMA, with the support of, and using the procedures and databases provided by Food Standards Australia New Zealand (FSANZ

Recommendation

Due to the proposed change in the Australian ADI for diemthoate, from 0.02 mg/kg bw/day to 0.001 mg/kg bw/day, the OCSEH recommends that a review of the dietary exposure to dimethoate be undertake to ensure that the current MRLs for dimethoate are consistent with the revised ADI.

4 RISK ASSESSMENT

4.1 General public

4.1.1 Dietary risk assessment

Risk assessments from exposure to residues in food and water are performed by the APVMA and FSANZ, comparing the estimated intakes of residues in different population subgroups with health standards established by the Office of Chemical Safety and Environmental Health. Acceptable daily intake (ADI) values are used to consider the dietary intakes of residues on a chronic (lifetime) basis, while the estimated acute intakes (one meal or over one day) of residues in individual commodities are compared with the acute reference dose (ARfD).

The dietary risk assessment for dimethoate will be performed by the APVMA and FSANZ, based on the ADI determined in this review.

4.1.2 Acceptable daily intake (ADI)

In animal studies, the most sensitive toxicological endpoint for repeat-dose exposure to dimethoate is increased pup mortality and ChE inhibition in pups observed in developmental neurotoxicity rat studies, at doses which did not elicit maternotoxicity. The lowest NOEL for this effect is 0.1 mg/kg bw/d in a developmental neurotoxicity study (Myers 2001c). A safety factor of 100 is considered appropriate (10-fold for inter-species extrapolation and 10-fold for intra-species variation). This NOEL is supported by a second neurotoxicity study (Myers 2001b) which demonstrated a NOEL for pup toxicity of 0.1 mg/kg bw/d on the basis of inhibition of erythrocyte ChE activity in pups at 0.5 mg/kg bw/d.

The NOEL derived from a repeat-dose (14-57 days) study in humans was 0.2 mg/kg bw/d after dosing for 39 days based on inhibition of whole blood ChE activity at doses of ≥ 0.42 mg/kg bw/d (Edson *et al*., 1967). This NOEL is considered appropriate for protecting against potential ChE inhibition in adult humans only if a safety factor of 100 is applied (a 10-fold safety factor for intraspecies variation, together with an additional safety factor to take account of the uncertainty as to whether the maximum sensitivity to ChE inhibition has been achieved in a short-term study). This results in an acceptable chronic exposure level of 0.002 mg/kg bw/day (with a 100-fold safety factor) based on the endpoint of ChE inhibition in adult humans. An ADI based on increased pup mortality and pup ChE inhibition in developmental neurotoxicity studies in rats would be protective of potential ChE inhibition in adult humans.

Conclusion

It is recommended that the ADI for dimethoate be revised to 0.001 mg/kg bw based on a NOEL of 0.1 mg/kg bw/d for increased pup mortality and ChE inhibition in pups, observed after repeat-dosing in developmental neurotoxicity studies in rats, and incorporating a 100-fold safety factor.

4.1.3 Acute reference dose (ARfD)

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

There were a number of acute or short-term oral dosing studies conducted in laboratory animals that were identified as relevant for the establishment of an ARfD. In an acute neurotoxicity study, treatment related clinical signs were observed at 20 and 200 mg/kg bw dimethoate; the NOEL was 2 mg/kg bw (Lamb 1993b). In rats, given single doses of dimethoate in the diet for a period of three hours, plasma and erythrocyte ChE activity was inhibited at ≥ 2 mg/kg bw (Schaefer 1999a&b). The NOEL was 1 mg/kg bw dimethoate. Single doses of dimethoate given to rats on PND 11 or to young adult rats resulted in plasma, erythrocyte and/or brain ChE inhibition at 3 mg/kg bw, with a NOEL of 0.5 mg/kg bw (Myers 2001b). A slight increase in pup mortality was seen at 0.5 mg/kg bw/d dimethoate and a clear increase was seen at 3.0 mg/kg bw/d (Myers 2001c); a NOEL was observed at 0.1 mg/kg bw/d. However, these effects are not considered to be representative of a single day exposure as offspring were dosed from GD 6 – PND 10 and then directly from PND 11- PND 21. In a human volunteer study, no clinical signs were observed following repeated doses of dimethoate at up to 1 mg/kg bw/d for 14-57 days; however a clear NOEL was observed for whole blood ChE inhibition at 0.2 mg/kg bw. The LOEL was 0.42 mg/kg bw/d (Edson *et al*., 1967). This NOEL will be protective of a single dose effect in humans and is considerd appropriate for setting the ARfD.

Conclusion

It is recommended that an ARfD of 0.02 mg/kg bw be set based on a NOEL of 0.2 mg/kg bw/day for inhibition of ChE in whole blood based on a 14-57 day human volunteer study, and incorporating a 10-fold safety factor for intra-species variation.

4.1.4 Impurity limits in the technical grade active

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

The current minimum compositional standard for the active constituent dimethoate and the maximum level for a toxicologically significant impurity are as follows:

Chemical	**Standard**
Active constituent	Minimum 950 g/kg
O,O,S-trimethyl phosphorodithioate	Maximum 5 g/kg

O,O,S-trimethyl phosphorodithioate

O,O,S-trimethyl phosphorodithioate is an impurity of toxicological significance. Further data on this impurity and an appropriate impurity limit will be addressed in the occupational health and safety assessment.

Omethoate

Omethoate, the oxygen analogue of dimethoate, may be formed during manufacture or during storage of EC products. The acute oral toxicity of omethoate was high with LD_{50} values in the range of 22-28 mg/kg bw in rats (Flucke 1978, Krötlinger 1989a). Acute dermal studies in rats gave LD_{50} values from approximately 145 mg/kg bw, up to ~1018 mg/kg bw (Flucke 1978, Krötlinger 1989b). For a 4 h exposure, the inhalation LC_{50} value in rats was 287 mg/m^3 (Pauluhn 1989). Overall, deaths occurred in the acute studies within 24 h of dosing, up to day 4 post-dosing. 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. 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 erythrocyte 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). Omethoate was also evaluated in a separate report by the OCS, and data contained within that evaluation indicated that in rabbits, omethoate was not a skin irritant, but was a slight eye irritant. It was a skin sensitiser in guinea pigs according to the open epicutaneous test. Considering the toxicity of omethoate which is considerably higher than dimethoate, the impurity limit of omethoate should be set at the lowest practicable level. Declarations of Composition for dimethoate from all of the approved sources show omethoate concentrations of less than 2 g/kg. In accordance with this observation, the maximum impurity limit for omethoate (CAS No. 1113-02-6, CAS name O,O-dimethyl S-[2-(methylamino)-2-oxoethyl]phosphorothioate) in dimethoate technical material stipulated in the May 2005 FAO specifications was 2 g/kg.

Conclusion

The impurity limit for omethoate in dimethoate technical should be established at 2 g/kg.

Isodimethoate

Isodimethoate was identified as an impurity potentially formed at storage. The oral LD_{50} for isodimethoate in rats was estimated to be in the range of 25-200 mg/kg bw (Dreher, 2001). In consideration of the slightly higher toxicity of isodimethoate compared to dimethoate, the impurity limit of isodimethoate should be set at the lowest practicable level. Declaration of Compositions for technical dimethoate indicates that the impurity limit established on a practicable basis would be 1% or 10 g/kg for isodimethoate. Although this value is slightly higher than 3 g/kg established in the May 2005 FAO specifications, its present in the technical-grade material should be no particular concern since health standards are established on the basis of toxicology studies conducted using the mixture containing isodimethoate approximately at this level.

Conclusion

The impurity limit for isodimethoate should be established at 10 g/kg.

4.1.5 Residue definition

In the existing MRL Standard for Maximum Residue Limits in Food and Animal Feedstuff (APVMA, October 2006), the residue definition of dimethoate is defined as 'Sum of dimethoate and omethoate, expressed as dimethoate.' As it appears that the toxicology studies of dimethoate and omethoate have adequately covered the potential toxicity of dimethoate metabolites that may be present as residues, from a toxicological perspective, the current residue definition is considered appropriate.

Conclusion

The current residue definition for dimethoate is considered appropriate.

5 RISK MANAGEMENT

5.1 General public

5.1.1 First-aid instructions

In the edition current to May 2007, the following standard statements for dimethoate are specified in the FAISD Handbook – *Handbook of First Aid Instructions, Safety Directions, Warning Statements and General Safety Precautions for Agricultural and Veterinary Chemicals*.

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

5.1.2 Water quality guidelines

Health Values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or mis-use 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 ADI for dimethoate is 0.001 mg/kg bw/d, the Health Value may be calculated as:

$$\frac{0.001 \text{ mg/kg bw/d} \times 70 \text{ kg} \times 0.1}{2 \text{ L/d}}$$

$$= 0.0035 \text{ mg/L}$$

The current Health Value for dimethoate of 0.05 mg/L is not supported. It is recommended that the amended Health Value for dimethoate be established at 0.004 mg/L. This value should also supercede the health-based value of 0.007 mg/L proposed in the 2009 NHMRC Draft Water Quality Guidelines

5.1.3 Products for homegarden use

Registration of homegarden products will be considered as part of the occupational health and safety assessment.

PART 2 MAIN TOXICOLOGY REPORT

1 CHEMISTRY

1.1 Technical Active

Approved Common Name: Dimethoate

Alternative names: El 12880, L395, BAS 152, OMS 94, OMS 111, ENT 24 650, chemathoate, cygon, fosfamid, cekuthoate, daphene, devignon, dimet, dimethogon, trimetion

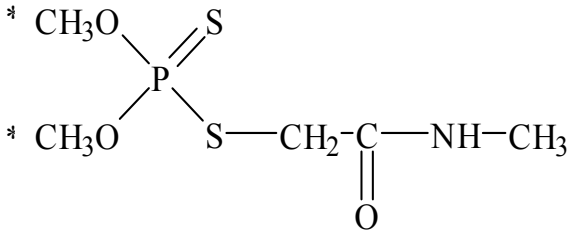
Chemical name: O,O-dimethyl-S-(N-methylcarbamoylmethyl) phosphorodithioate,
2-dimethoxyphosphinothioylthio-N-methylacetamide (IUPAC)
Phosphorodithioic acid O,O-dimethyl S-[2-(methylamino)-2-oxoethyl] ester

CAS Registry number: 60-51-5

Empirical formula: $C_5H_{12}NO_3PS_2$

Molecular weight: 229.3

Chemical structure:



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

Chemical class: Organophosphorus

Structural analogues: Omethoate

1.2 Chemical and physical properties

Colour:	White
Odour:	Mercaptanic odour
Physical state:	Solid
Melting point:	50.0-51.5^{o}C
Density (20^{0}C):	1.28
Partition coefficient: (log K_{ow})	0.704
Vapour pressure:	2.46 x 10^{-4} Pa at 25^{o}C
Aqueous solubility:	23.3 g/L pH 5 23.8 g/L pH 7 25.0 g/L pH 9
in water:	25 g/L at 21^{o}C
in organic solvents:	Readily soluble in most
Stability:	Information not available; does not dissociate in water

1.4 Impurities of toxicological concern

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

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

The May 2005 FAO specifications for dimethoate technical material requires that the dimethoate content shall be declared not less than 950 g/kg and, where determined, the mean content shall not be lower than the declared minimum. Maximum limits for relevant impurities are as follows:

Omethoate (CAS No. 1113-02-6, CAS name O,O-dimethyl S-[2-(methylamino)-2-oxoethyl]phosphorothioate). Maximum 2 g/kg

Isodimethoate (CAS No. 3344-11-4, CAS name phosphorodithioic acid, O,S-dimethyl S-[2-(methylamino)-2-oxoethyl] ester). Maximum 3 g/kg

Water. Maximum 2g/kg

2 METABOLISM AND TOXICOKINETICS

2.1 Technical grade active constituent

Kirkpatrick D (1995). ^{14}C-Dimethoate: the biokinetics and metabolism in the rat. Huntingdon Life Sciences Ltd. Study period: January 1993 – December 1995. Guidelines: US EPA F 85-1. GLP/QA: yes. DTF Doc No: '651-001' Ref: 3-1/Vol 3-2.

Methods [^{14}C]-Dimethoate (Batch: MR-DTF17-3, NPE-DTF17-5; purity: >98%) was labelled at the two O-methyl carbon atoms with a specific activity of 14.55 μCi/mg, and mixed with non-radiolabelled dimethoate (batch: 00315-00, 20522-00; purity: 99.5%, 99.1%) before dosing.

The [^{14}C]-dimethoate was administered to male and female rats (5/sex/group; approx 7-10 weeks of age; 195-224 g; Charles River UK Ltd, Margate, Kent) at 10 mg/kg bw (a single radioactive dose or 14 non-radiolabelled doses + 1 radioactive dose) or 100 mg/kg bw. Doses were given either (1) orally (in solution with water), (2) intravenously (in solution with isotonic saline) or (3) dermally (as a suspension in 1% aqueous sodium carboxymethylcellulose solution on approximately 10 cm^2 of clipped area at the back for 6 h followed by a wash). Following oral and intravenous administration, animals were kept in glass metabolism cages and samples were collected after dosing at 0-6, 6-12, 12-24 h and at 24 h intervals up to 5 days for urine, at 24 h intervals up to 5 days for faeces, and at 0-6, 6-24 h and at 24 h intervals up to 3 days for expired air. Five days after dosing, a blood sample was taken from the heart by cardiac puncture immediately prior to sacrifice. Tissues (stomach and remaining gastrointestinal tract, adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, spleen, testes, thyroid, uterus, muscle, fat, bone, bone marrow, skin, and residual carcass) were taken for analysis.

Following dermal application, rats were transferred to restraining cages and urine and faeces were collected at 6-24 h, and then at 24 h intervals to sacrifice at 120 h (5 days). At sacrifice, tissues (as above), the treated skin area, dressing and covers, and cage washes were also taken for analysis. In a separate group of bile duct cannulated rats (3/sex/group) orally dosed with 10 or 100 mg/kg bw of [^{14}C]-dimethoate, urine and faeces were collected at 0-24 and 24-48 hrs, and bile was sampled at 0-3, 3-6, 3-12, 12-24 and 24-48 hrs after dosing. At 48 hrs, the rats were killed and the GIT including contents, liver and carcass were taken for analysis. Another group of rats were used for a plasma radioactivity kinetic study (3/sex/dose per time point) after single oral doses of 10 or 100 mg/kg bw [^{14}C]-dimethoate. Blood samples were withdrawn pre-dose, 0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, 120, 144h and 168 h post dosing. Similarly, groups of 3 rats/sex/dose per time point were used for a plasma parent compound kinetic study following an oral dose of 10 or 100 mg/kg bw [^{14}C]-dimethoate. In this experiment, blood samples were taken by cardiac puncture prior to sacrifice and at 0.5, 2, 6 and 24 h post dosing. Quantitative analysis of tissue distribution (tissues as described above) was carried out in rats (3/sex/dose per time point) 0.5, 2 and 48 h following an oral dose of 10 or 100 mg/kg bw [^{14}C]-dimethoate. Whole body autoradiography was carried out on pairs of rats (1/sex) killed 0.5, 2, 6, 48 and 120 h after dosing.

Samples were radioassayed by liquid scintillation counting (LSC) with or without pre-treatment. High performance liquid chromatography (HPLC), thin-layer chromatography

(TLC) and mass spectrometry (MS), as well as autoradiography, alone or in combination, were also used for separation, quantitation and quantification of samples.

Results In a pre-test dose range finding study, body tremors were reported in rats after a single oral dose of 100 mg/kg bw/d, but no signs of toxicity were observed following dosing of other groups.

The radiolabel was well absorbed from the gastrointestinal tract after oral administration of [^{14}C]-dimethoate to male and female rats. Single low or high oral doses, or multiple low oral doses, resulted in similar patterns of excretion of radioactivity in both sexes, with 89-95% of the dose excreted within five days (Table 1). In all cases, radioactivity was mainly excreted in the urine (85-91%) with small amounts detected in expired air (2.1-2.5%) and in the faeces (1.2-1.6%). Urinary excretion was rapid, with 69-72% of the low dose and 52-59% of the high dose detected within 6 h. A similar excretion pattern was seen following iv administration of 10 mg/kg bw [^{14}C]-dimethoate.

Table 1: Mean excretion & retention of radioactivity (% of the dose) within 5 days

Dose (mg/kg bw)	10 (single, iv)		10 (single, po)		10 (multiple, po)		100 (single, po)	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Urine	88.9	89.4	91.3	85.4	90.6	88.9	90.8	90.8
Expired air	1.76	1.62	2.10	2.17	2.07	2.28	2.44	2.53
Faeces	1.15	1.35	1.15	1.56	1.30	1.22	1.45	1.45
Total excreted	**91.8**	**92.4**	**94.6**	**89.1**	**94.0**	**92.4**	**94.7**	**94.8**
Total carcass	0.81	0.96	0.67	1.45	1.04	1.72	1.14	1.88
Total recovery	**92.6**	**93.3**	**95.3**	**90.6**	**95.0**	**94.1**	**95.9**	**96.6**

Table 2 shows the mean excretion and retention of radiolabel following dermal administration as a percentage of the applied dose. For dermal dose levels of 10 and 100 mg/kg bw, absorption was approximately 10% and 1% respectively (approximately 1 mg/kg bw). Absorbed radioactivity was excreted mainly in the urine (80-96%).

Table 2: Mean excretion & retention of radioactivity (% of the dose) after dermal administration

Dose (mg/kg bw)	10		100	
	Male	female	Male	Female
Urine	8.27	9.26	1.13	1.32
Faeces	0.21	0.58	0.05	0.13
Carcass	0.76	0.76	0.11	0.22
Total absorbed	**9.19**	**10.6**	**1.18**	**1.64**
Skin wash	62.5	62.1	84.1	83.7
Dressing extracts	3.42	3.16	1.35	3.64
Treated skin	17.3	13.3	3.65	2.18
Total recovery	**92.5**	**89.1**	**90.2**	**91.1**

In rats with cannulated bile ducts, biliary excretion within 48 h represented 4-5% of the low or high dose and most of the remaining dose (82-87%) was excreted in the urine, suggesting bile was a minor route of excretion (Table 3).

Table 3: Mean excretion & retention of radioactivity (% of the dose) after in bile duct cannulated rats

Dose (mg/kg bw)	10		100	
	Male	Female	Male	Female
Bile	4.05	3.68	4.07	4.52
Urine (with cage wash)	82.8	81.6	82.6	87.0
Faeces	1.40	3.31	2.39	2.69
Total recovery	91.3	90.8	91.8	96.3

The maximum plasma radioactivity concentration was reached at 0.5 h after dosing. Plasma kinetics of radioactivity were similar between sexes at 10 mg/kg bw, whereas the values of C_{max}, T_{max} and area under the curve (AUC) were higher in females than males at 100 mg/kg bw. For a 10-fold increase in dose level, the mean AUC increased by a factor of 8 and 14 for males and females, respectively (Table 4). After an oral dose of 100 mg/kg bw, concentrations of dimethoate (the parent compound) fell rapidly from 6-7 mg/L 0.5 h after administration, to 1-2 mg/L after 2 h and 6 h, and below detectable levels (0.051 mg/L) 24 h following dosing.

Table 4: Pharmacokinetic parameters of plasma radioactivity

	C_{max} (mg equiv/L)[b]		Tmax (h)		AUC (mg equiv.h/L)		Terminal half-life[a] (h)	
Dose (mg/kg bw)	Male	female	Male	Female	Male	female	male	Female
10	8.62	7.68	0.5	0.5	49.4	48.9	42.0	59.3
100	50.7	93.2	0.25	0.5	417.0	686.6	36.1	46.4

[a] Terminal half-lives were calculated using the 12 h to 168 hr concentrations [b] mg dimethoate equivalents/L were calculated by multiplying the mean proportion of the administered dose in the sample by the mg of dimethoate administered to the rats.

The distribution of radioactivity in tissues after an oral dose of 10 mg/kg bw was similar in both sexes (Table 5). Peak concentrations in almost all tissues occurred in rats sacrificed 0.5 h after dosing, with highest concentrations (maximum in the range of 9 to 25 mg dimethoate equivalents/kg tissue) found in the liver and kidneys, the lowest (approximately 1 mg dimethoate equivalents/kg tissue) in the brain and fat, and 1-5.5 mg dimethoate equivalents/kg in other tissues. Concentrations of radioactivity in tissues declined with time at similar rates in all cases, and was less than 1 mg dimethoate equivalents/kg bw in all tissues at 48 h. Tissue concentrations after multiple doses of 10 mg/kg bw were slightly higher than those after a single dose. A ten-fold higher dose (100 mg/kg bw) resulted in peak concentrations in tissues 5-18 times higher, as well as higher ratios in adrenals and fat in male rats.

Table 5: Tissue distribution of dimethoate in rats after an oral dose of 10 mg/kg bw dimethoate

Tissue	Timepoint					
	0.5 h		2 h		48 h	
	Male	female	male	female	male	Female
Adrenal	2.22	4.23	1.20	1.83	0.17	0.24
Bone	1.12	1.39	0.64	0.98	0.08	0.13
Bone marrow	1.61	3.04	0.86	1.31	0.19	0.26
Brain	0.65	1.36	0.39	0.69	0.04	0.05
Fat	0.99	1.10	1.16	0.59	0.07	0.06
Heart	2.60	3.47	1.26	1.75	0.10	0.12
Intestines and contents	6.20	6.71	4.73	6.70	0.18	0.81
Kidneys	20.0	24.6	7.22	7.91	0.30	0.35
Liver	8.57	11.7	6.11	7.53	0.53	0.63
Lungs	3.28	5.47	1.73	2.58	0.16	0.20
Muscle	1.19	1.89	0.60	0.88	0.07	0.08
Ovaries		3.77		1.64		0.17
Pancreas	2.94	4.33	1.83	2.08	0.28	0.45
Skin	2.24	3.56	1.02	1.53	0.14	0.29
Spleen	1.76	2.93	0.93	1.35	0.17	0.18
Stomach and contents	146	178	83.0	82.0	0.08	0.25
Testes	1.72	-	1.14	-	0.08	-
Thyroid	1.99	3.31	0.94	1.64	0.15	0.27
Uterus	-	3.52	-	1.73	-	0.17
Whole blood	4.70	6.08	1.63	2.08	0.10	0.13
Plasma	6.09	7.81	2.20	2.65	0.08	0.12

Units are expressed as mg/kg dimethoate equivalents: calculated by multiplying the mean proportion of the administered dose in the sample by the mg of dimethoate administered to the rats.

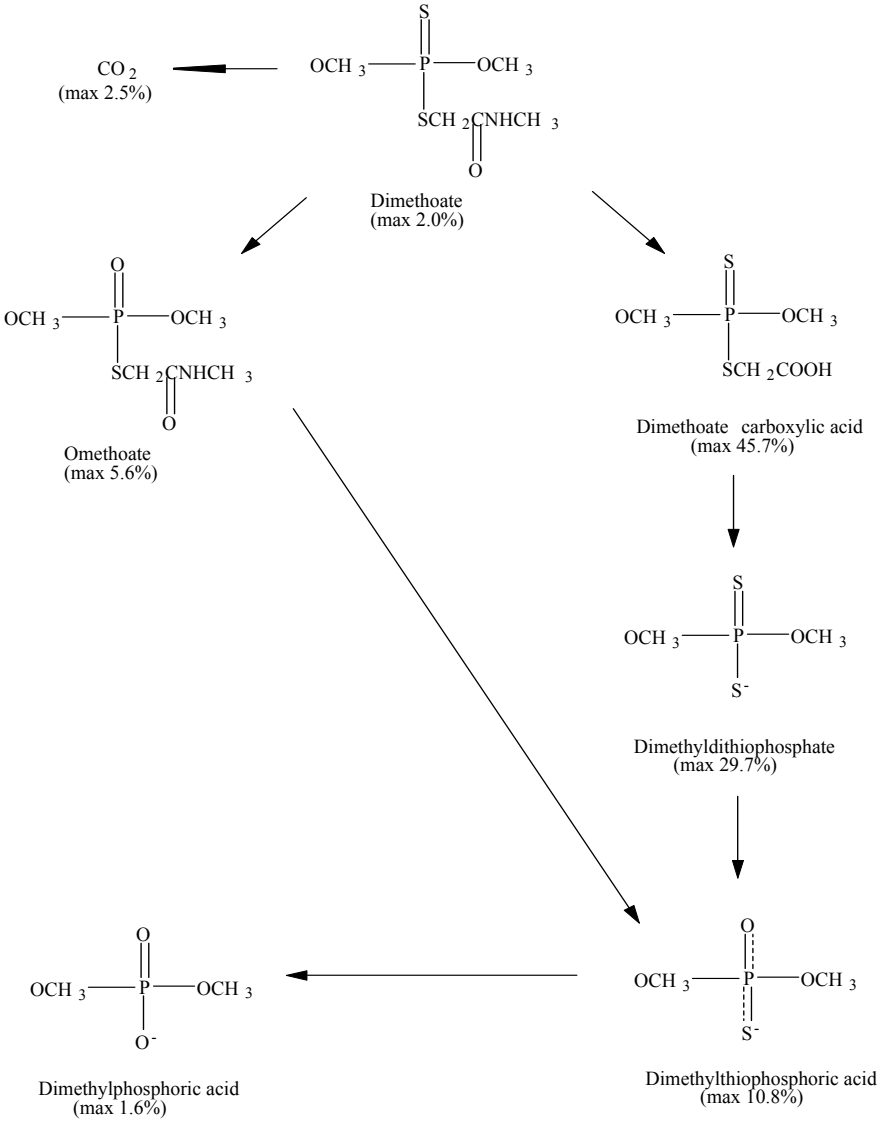
Rates of elimination were similar in all tissues at each dose level. At 120 h, mean tissue concentrations of radioactivity were about or below 0.1 mg dimethoate equivalents/kg bw (with exception of slightly higher levels in kidneys, liver and pancreas) after the single oral or iv dose of 10 mg/kg bw. Mean tissue concentrations were slightly higher after multiple doses of 10 mg/kg bw/d, and below 3 mg dimethoate equivalents/kg bw after the single dose of 100 mg/kg bw. In many cases, levels were below the limit of accurate determination following a single dermal dose of 10 or 100 mg/kg bw.

Whole-body autoradiography after a single oral dose at 10 mg/kg bw indicated that the highest concentrations were present in the contents of the GIT, liver and kidneys, with some accumulation of radioactivity in the Harderian gland, the intra- and exorbital lachrymal glands and the preputial gland, even at 48 and 120 h after dosing. The CNS contained the lowest concentrations of radioactivity.

Chromatographic and spectroscopic techniques identified a number of metabolites of dimethoate in urine, which accounted for 70-80% of an oral or iv dose. Dimethoate was extensively metabolised, mainly by initial cleavage of the C-N bond to yield dimethoate carboxylic acid and subsequently a number of thiophosphate and phosphate esters, but also by oxidation to its oxon analogue, omethoate. In all cases, after oral and iv dosing, dimethyldithiophosphate (22-30% dose) and dimethoate carboxylic acid (29-46%), represented most of the excreted radioactive dose, followed by dimethylthiophosphoric acid (4-11%) and omethoate (1-6%), as well as some unidentified metabolites. The proportions of the radioactive components in dermally dosed rats were broadly similar to those detected

following oral dosing. Unchanged dimethoate in urine accounted for 1-2% of the dose. At least 13 radioactive components were revealed in bile, with no qualitative or significant quantitative differences apparent between dose levels and between sexes. Main components include dimethoate carboxylic acid (1-2%), dimethyldithiophosphate (0.2-0.4%), dimethoate (0.1-0.2%), and at least 9 polar components (each < 0.8%) some of which might be present in the urine.

The following is proposed biotransformation pathway of dimethoate in rats.



2.2 Formulations (400 g/L EC formulation)

Leibold E (2001a). Study on the dermal penetration of ^{14}C-dimethoate in rats. BASF Aktiengesellschaft. Study period: September 2000 – February 2001. Guidelines: OECD (1996); US EPA OPPTS 870.7600 (1998). GLP/QA: yes. DTF Doc No: '654-002' Ref: 3-2/Vol 3-3.

Leibold E (2001b). Study on the dermal penetration of ^{14}C-dimethoate in rats: Amendment No 1 to the report. Study period: September 2000 – February 2001. Guidelines: OECD (1996); US EPA OPPTS 870.7600 (1998). GLP/QA: yes. BASF Aktiengesellschaft. DTF Doc No: '654-002' Ref: 3-3/Vol 3-3.

Methods [^{14}C]-dimethoate (formulation concentrate or 1/10 and 1/200 aqueous dilutions) was instilled on to the skin of rats under semi-occlusive conditions at concentrations of 0.02, 0.4 or 4.0 mg/cm^2. These doses corresponded to 0.2, 4.0 or 40 mg/rat, or approximately 0.67, 13.3 or 133 mg/kg bw, respectively. The test material was applied for 1, 10 or 24 h, following which the animals were killed, with or without a post exposure observation period, as shown in Table 6.

Table 6: Exposure times & times of sacrifice

Dose	**0.02 mg/cm^2 (0.2 mg/rat, 0.67 mg/kg bw)**			
Exposure time (h)	1	10	24	24
Time of sacrifice (h)	1	10	24	72
Dose	**0.4 mg/cm^2 (4.0 mg/rat, 13.3 mg/kg bw)**			
Exposure time (h)	1	10	24	24
Time of sacrifice (h)	1	10	24	72
Dose	**4.0 mg/cm^2 (40 mg/rat, 133 mg/kg bw)**			
Exposure time (h)	1	10	24	24
Time of sacrifice (h)	1	10	24	72

Dosing formulations were prepared by mixing Dimethoate 400 g/L EC (Batch: 70917-00, 38% active constituent; See Appndix IX for formulation details) with [^{14}C]-dimethoate (labelled at O-methyl carbon atoms; Batch: MEF24/8/TW; Specific activity: 42.56 mCi/mmol). The rats (4/dose/timepoint, obtained from Charles River Laboratories, Sulzfeld, Germany) were about 14 weeks old, and weighed 266-328 g at the commencement of dosing. During the experiments, rats were housed in glass metabolism cages and supplied with feed (Kliba lab diet) and tap water *ad libitum*. After each exposure period, the protective cover was removed and the exposure site was washed with a mild soap. For animals with a post-exposure observation period, a new gauze and bandage was applied and another skin wash performed prior to sacrifice. Urine and faeces were collected up to 72 h. At the end of the various collection periods, rats were killed and all specimens (excreta, blood, carcass, skin wash, treated and non-treated skin areas) were assayed for remaining radioactivity by LSC.

Results There was no mortality and no treatment-related clinical signs were reported.
Total recovery of radioactivity from all groups ranged from 94% to 106% of the dose. Radiolabel (as a percentage of administered dose) recovered from the skin wash fractions was approximately 60-70% at 4.0 mg/cm^2, 60-90% at 0.4 mg/cm^2 and 40-80% at 0.02 mg/cm^2. Percentages of administered radioactivity recovered at the application site were about 3-8%, 3-6% and 9-13% at 4.0 mg/cm^2, 0.4 mg/cm^2 and at 0.02 mg/cm^2, respectively. The total

amounts absorbed (radioactivity detected in excreta, cage wash, blood and carcass) in different dose groups at different time points are shown below (Table 7).

Table 7: Percentage & total amount of radioactivity absorbed

Exposure time (h)	Sacrifice time (h)	0.02 mg/cm^2 (0.2 mg/rat, 0.67 mg/kg)		0.4 mg/cm^2 (4.0 mg/rat, 13.3 mg/kg)		4.0 mg/cm^2 (40 mg/rat, 133 mg/kg)	
		% absorbed	mg/rat	% absorbed	mg/rat	% absorbed	mg/rat
1	1	5.68	0.014	5.68	0.228	1.03	0.435
10	10	24.40	0.055	24.98 (20.23*)	0.952	5.78	2.238
24	24	38.06	0.089	25.33	0.973	12.06	4.602
24	72	41.81	0.095	31.69	1.238	13.16	5.143

*n=5 when including 2 rats from the pretest

Davies DJ (1999). Dimethoate: In vitro absorption from a 400 g/L EC Formulation through human and rat epidermis. Central Toxicology Laboratory. Study period: July – December 1999. Guidelines: OECD (1996); EEC/91/414. GLP/QA: yes. DTF Doc No: '469-001' Ref: 3-4/Vol 3-3.
&
Heylings JR (2000). Statement regarding SCC project no: 104-065 CTL contract CO9027 – JV1591. Dimethoate: In vitro absorption from a 400 g/L EC Formulation through human and rat epidermis. Central Toxicology Laboratory. DTF Doc No: '481-036' Ref: 3-5/Vol 3-4.

Methods The *in vitro* absorption of dimethoate was measured by applying [^{14}C]-dimethoate to human and rat epidermal membranes at a rate of 10 µL/cm^2 unoccluded for 8 or 24 h. The dosing formulations were prepared by mixing [^{14}C]-dimethoate (Source: ISOTOPCHIM, France; Batch: 291-BSe-75B; Radiochemical purity: 96%; Specific activity: 2.072 GBq/mMol) with a nominal 400 g/L EC formulation (Source: Cheminova, Denmark, Batch: 70917-00; 38% active constituent) as the concentrate and as a 1:200 v/v spray strength aqueous dilution (2 g/L).

Human whole skin samples of extraneous tissue were obtained from female subjects (age not specified; abdomen) post mortem, and rat skin was taken from the dorsal and flank region of Wistar male rats (Charles River UK Ltd, aged 28 ± 2 days). Samples of epidermis with an exposed area of 2.54 cm^2 were mounted in receptor chambers of glass diffusion cells. The receptor chambers contained saline with 5% w/w bovine serum albumin and were maintained at 32 ± 1^{o}C in a water bath. Study phase I was designed to measure the absorption of dimethoate over an 8 h time period. Following washing at 8 h, the distribution of dimethoate within human stratum corneum/epidermis was assessed using a tape stripping technique, followed by a mass balance procedure. Phase II was designed to incorporate washing at 8 h with the measurement of the absorption of dimethoate through isolated human and rat epidermis over a 24 h time period. This was followed by a tape stripping technique and a mass balance procedure at 24 h. Samples collected were analysed by LSC in combination with repurification and purity determination by HPLC and TLC.

Results Total mean recovery of radioactivity was 119% of the applied dose for humans and 103% for rats. Mild skin washing at 8 h removed the majority of the dose (133% and 114% of dose for the concentrate and dilution) from the surface of human skin, but lesser amounts (59% and 16%) from rat skin; the latter was probably due to a large proportion adhering to the hair of rat skin.

As shown in Table 8, small amounts of dimethoate were absorbed through human epidermis over a 24 h exposure that involved a washing procedure at 8 h, i.e. 0.09% and 3.05% of the applied dose for the concentrate and spray dilution respectively were detected from the receptor fluits. In addition, 0.51% and 2.06% of the dose of concentrate and spray dilution respectively were shown to be still associated with the human skin 24 h after application. Since the chemical remaining in the skin at the end of study may be absorbed continuously, this portion should be included in the total amount of skin absorption unless it can be demonstrated that absorption can be determined from fluid values alone (OECD Guideline 428).

Table 8: Mean absorption rates & dose absorbed

	Absorption rate (μg/cm²/h ± SEM)			**Dose absorbed (μg/cm²)**			**Percentage (%) of dose absorbed**	
	Time (h)	**Human**	**Rat**	**Time (h)**	**Human**	**Rat**	**Human**	**Rat**
Concentrate formulation, 24h exposure, (human n=6) (rat n=8)				6	*3.56	77.7	*0.09	1.96
	0-8	*<0.44	12.7±2.66	8	*3.56	96.7	*0.09	2.43
	8-24	-	11.0+1.45	10	3.70	161	0.09	4.06
	0-24	*<0.15	12.9+1.76	24 (- in skin)	3.60	**293**	**0.09**	**7.37**
				24 (+ in skin)			**0.60**	**9.03**
				LOQ	3.56	3.01	0.09	0.08
1:200 aqueous dilution, 24 h exposure, n=8				6	0.27	7.14	1.28	33.8
	0-8	0.05+0.01	0.94+0.16	8	0.34	8.04	1.61	38.1
	8-24	0.02+<0.01	0.08+0.01	10	0.42	9.23	2.00	43.7
	0-24	0.03+<0.01	0.35+0.06	24 (- in skin)	0.64	**10.0**	**3.05**	**47.6**
				24 (+ in skin)			**5.11**	**75.6**
				LOQ	0.01	0.01	0.04	0.03

LOQ: Limit of quantitation.
*The LOQ values have been used as positive values in the calculation of the mean where values were <LOQ.

Absorption was considerably faster through rat epidermis than through human epidermis for all applications of the concentrate and dilution. Small amounts (0.60% of the concentrate dose and 5.1% of the dilution dose) were absorbed through human epidermis over a 24 h exposure that involved a washing at 8 h. Larger amounts (9.0% and 75.6%) were absorbed at a higher rate by rat skin. The mean factors of difference between human and rats were approximately 15 for both the concentrate and spray dilution.

This study suggests that dermal absorption of dimethoate from an EC formulation concentrate or its 1:200 spray dilution through human epidermis is slow and at a low rate (5.1%), and the vast majority which may contact human skin will be removed during normal washing procedures.

2.3 Metabolites

Hoshino T (1990). [Methylene-^{14}C]omethoate: General metabolism in the rat. Bayer AG, Crop Protection Research, Chemical Product Development and Environmental Biology, Institute for Metabolism Research, D-5090 Leverkusen-Bayerwerk Study duration: July – December 1989. Guidelines: US EPA 85-1 1982. DTF Doc No: '512-001' GLP/QA: yes Ref 3-46/Vol 3-25.

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 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

Median Lethal Dose

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

Oral studies

[Note: the information in the table is from a 1988 OCS evaluation report (Submission No. 1345)]

Species [strain]	Sex	Batch no / Purity (%)	LD50 (mg/kg bw)	Reference
Rat	-	Technical	307	BASF (22-9-66)
Rat (SD)	-	Technical	150-325	BASF (4-7-77)
Rat (Wistar)	-	Technical	300-335	BASF (4-7-77)
Rat (Wistar)	M/F	Technical	391	Montedison (1976)
Rat (SD)	M/F	Technical	M/F: 358/414	A/S Cheminova: HRC No: 851338D/ -CHV33/AC (1986)
Rat	M	Technical	152	Boyd & Taylor (1971)
Rat	M	Technical	247	Edson & Noakes (1960)
Rat	M/F	Technical	185-245	West *et al* (1961)
Rat	M/F	Pure Laboratory grade Technical	M/F: 500-600/570-680 280-350/300-356 180-325/240-336	Sanderson & Edson (1964)
Rat	M	Pure	300	Battelle-Institute, No101-11 (1961)
Rat		Pure	200-300	Ben-Dyke *et al* (1970)
Mouse	M/F	Technical, purity 97.3%	160	Ullman (1985)
Mouse		Technical	80.2	BASF (27-9-66)
Mouse (KFM-NMR 1)	M/F	Technical	M/F: 168/152	A/S Cheminova: RCC No: 038981 (1985)
Mouse	-	Pure	140	Hewitt *et al* (1958)
Mouse	F	Pure Technical	60 60	Sanderson & Edson (1964)
Hamster	M	Laboratory grade	200	Sanderson & Edson (1964)
Guinea-pig	M/F	Pure Laboratory grade Technical	550 600 350-400	Sanderson & Edson (1964)
Rabbit	M/F	Pure Laboratory grade Technical	500 450 300	Sanderson & Edson (1964)
Hen	-	Pure	50	Sanderson & Edson (1964)
Dog	-	Pure	> 100	Hewitt *et al* (1958)

M = males F = females; In Sanderson and Edson, (1964) pure dimethoate was described as an odourless grade obtained by repeated recrystallization from anhydrous ether; laboratory grade dimethoate was defined as a white crystalline solid with a marked thiol odour; technical dimethoate was defined as about 93% pure and varied from off white crystals to a grey semi-crystalline material.

In mice, clinical signs at 20-80 mg/kg bw dimethoate included sedation, dyspnea, hunched posture, ruffled fur, and reddish discharge, while spasms were observed in the 80 mg/kg bw

group. In mice treated with 160 and 320 mg/kg bw, additional signs were observed including ventral body position, tremors and coma. Mice that survived recovered within 2 to 6 days. Mortality occurred primarily in the first 5 hours after dosing, but was observed until day 3. No pathological changes were observed in surviving mice on day 14, whereas mice that died were reported to show changes mainly in the lungs, liver, stomach and intestine (Ullman, 1985).
The oral LD_{50} of dimethoate in male and female rats ranged from 150-414 mg/kg bw. Clinical signs in rats prior to death included piloerection, hunched posture, gait alterations (rocking, lurching or swaying or prostration), constricted pupils, salivation, tremors (whole body and/or forelimbs/hindlimbs), absent forelimb/hindlimb grasp, laboured and/or shallow respiration and impaired/absent righting reflex. The signs first occurred within 15-90 mins depending on the dose, Hypothermia (body cool to touch), lacrimation and staining on various body surfaces appeared later, and all signs persisted (often increasing in severity) through to the time of death. Macroscopic examination revealed dark red lungs in one male and opacity of one eye in the other male at 200 mg/kg bw (Lamb, 1993a).

Dermal studies

[Note: the information in the table is from 1988 OCS evaluation reports (Submission Nos. 150, 415, 437, 439)]

Species [strain]	Sex	Purity (%)	LD50 (mg/kg bw)	Reference
Rat (Wistar)	M/F	Technical	> 7,000 (24 h)	Montedison (1976)
Rat (SD)	M/F	Technical	> 2,000	Kynoch (1986)
Rat	M	Technical	1120	Edson & Noakes (1960)
Rat	M	Technical	> 800	West *et al* (1961)
Rat		Technical	700-1150 (24 h)	Sanderson & Edson (1964)
Rat		Technical	500[b] (24 h)	Sanderson & Edson (1964)
Guinea-pig		Technical	96[a]	West *et al* (1961)
Guinea-pig		Technical	995[b]	West *et al* (1961)

SD=Sprague Dawley
[a] 46% liquid concentrate in methyl cellosolve, [b]wettable powder

Dimethoate technical (83.3% w/v in distilled water) was instilled onto the skin of Sprague-Dawley rats at 2000 mg/kg bw for 24 hours under semi-occlusive conditions. Application sites were washed after 24 hours and the animals were observed for 14 days for mortality, clinical signs, dermal irritation, and body weight. A necropsy was performed at study termination. There was no mortality. No treatment related signs were seen in male rats. Hunched posture, body tremors and abnormal gait was observed in females on days 8 and 9 and resolved by day 13. Weight loss was recorded in 2/5 females on day 8. No macroscopic changes were observed at necropsy (Kynoch, 1986). Under these experimental conditions, dimethoate technical was of low dermal toxicity.

Inhalational studies

[Note: the information in the Table is from a 1988 OCS evaluation report (Submission No. 1345)]

Species [strain]	Sex	Group Size	Vehicle/ mode	Purity (%)	Concentrations Tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Rat				Technical		1.55 (b) g/m^3(4h)	Merck No 4/108/73 (1973)

Abbreviations: SD=Sprague-Dawley
(b) LC_{50} value based on calculated aerosol concentration only.

The sponsor stated that no acute inhalation study was conducted with dimethoate technical because it is not possible to mill the technical test substance to less than 10 microns or to sieve the powder (Vol 3-39, page 5-27; Detailed summaries of Toxicological and Metabolism studies as submitted to the EU). The LC_{50} 1553 mg/m^3 for technical dimethoate was attained with a 400 g/L EC formulation.

Skin Irritation

Liggett, MP & Parcell BI (1985a). Irritant effects on rabbit skin of Chemathoate (Dimethoate) technical. Huntingdon Research Centre, Great Britain. Report No: 851223 D/CHV 35/SE. Guidelines: U.S. EPA/FIFRA, Subdivision 81-5 (1982); GLP/QA: yes
[Note: This study has been evaluated previously by OCSEH (Submission Nos. 150 and 415)]

Six (3 male/3 female) NZW rabbits (Froxfield rabbits UK, 1.9 to 2.5 kg; 9-11 weeks of age) received applications of 0.5 g dimethoate (Source/batch number not indicated: purity 96-98%) in distilled water (0.5 mL) to their intact, shaved, dorsal skin under semi-occlusive dressing for 4 h. After 4 h, the dressing was removed and the application site was washed with water. Skin was assessed for signs of irritation at 4 h, 24 h, 48 h and 72 h.

Very slight transient oedema and erythema was observed in 3/6 rabbits after 4 hours (Table 9). No signs of irritation were observed at later time points. Under the conditions of this study dimethoate technical was not an irritant to the skin of rabbits.

Table9: Skin irritation scores following application of 0.5 g dimethoate to rabbit skin for 4 h

Animal no	Erythema						Oedema					
	733	746	789	790	791	792	733	746	789	790	791	792
4 h	0	0	0	1	1	1	0	0	0	1	1	1
24 h	0	0	0	0	0	0	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0	0	0	0	0

Eye Irritation

Liggett MP & Parcell BI (1985b). Irritant effects on the rabbit eye of Chemathoate (Dimethoate) technical. Huntingdon Research Centre, Great Britain. Report No: 851218 D/CHV 36/SE. Guidelines: U.S. EPA/FIFRA, Subdivision 81-4 (1982); GLP/QA: yes

[Note: This study has been evaluated previously by OCSEH (Submission Nos. 150 and 415)]
83 mg of dimethoate (Source/batch number not indicated; purity 96-98%) was instilled into the conjunctival sac of one eye of each of 6 NZW rabbits (Froxfield rabbits, UK; 2.0 to 2.4 kg; 9-11 weeks of age). The unwashed eyes were observed for signs of irritation 1 h after instillation of the test substance and at 1, 2, 3, 4 and 7 days.

Corneal effects (Grades 1-2) were observed in 4/6 rabbits from 24 h to 96 h and were resolved at 7 days (Table 10). Effects on the iris (Grade 1) were confined to 2/6 rabbits at 24 hours and persisted at 96 hours in one rabbit. Conjunctival redness (Grades 1-2) was observed in all rabbits at 24 h and persisted in two rabbits until 96 h. Chemosis (Grade 1) was seen in 4/6 rabbits at 1 h and had resolved in all rabbits at 48 h. Under the conditions of this study dimethoate technical was a slight irritant to the eyes of rabbits.

Table 10: Eye irritation scores following administration of 83 mg dimethoate technical

Animal no.	Cornea						Iris						Conjunctivae						Chemosis					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 h	0	0	D	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1	1
24 h	2	1	2	0	2	0	0	1	1	0	0	0	1	1	2	1	1	1	0	0	1	0	0	0
48 h	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0
72 h	1	1	2	0	1	0	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0
96 h	1	1	2	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
7 days	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

D = dulling of cornea

Skin Sensitisation

Madison, W.A. (1984). Dermal sensitisation study with technical dimethoate CL 12,880 in guinea pigs. Hazleton Laboratories, Wisonsin, USA. Guidelines: U.S. EPA/FIFRA, Subdivision 81-6 (1982), equivalent to OECD 406; GLP/QA: yes
[Note: This study has been evaluated previously by OCSEH (Submission Nos. 150 and 415 and 1345)]

Dimethoate technical (0.2 g mixed to paste with paraffin oil) was applied on an adhesive pad to the clipped surface of ten male Hartley albino guinea pigs for 6 h using the closed patch technique. Ten male animals served as controls. A total of 3 applications were carried out (1/week for 3 weeks). Two weeks following the last application a challenge dose of 0.2 g was applied. Erythema and oedema were assessed at 24 and 48 h following each application. DNCB (2,4-dinitrochlorobenzene) was used as a positive control. During induction, 0.4 mL of 0.3% w/v DNCB in 80% v/v ethanol in deionised water was administered. Challenge was carried out using 0.4 mL of 0.1% w/v DNCB in acetone.

No dermal irritation was observed during the induction or challenge phase. All animals appeared normal throughout the study with the exception of slight body weight loss of 2-21 g by in some animals in the last 2 days of the study. Dimethoate was not a skin sensitizer in guinea-pigs when tested under the conditions of this study using the closed patch technique.

Reviewers comment: This study deviated from current guideline standards in the number of animals used (ie the test group consisted of only 10 animals not 20). In addition, the rationale for the use of paraffin oil as a vehicle is unclear and may affect the validity of the study conclusions. It was noted that in a three-week repeat dose study in rabbits at doses of up to 1000 mg/kg bw, no inhibition of plasma or erythrocyte ChE activity was detected in any of the experimental groups, suggesting that dimethoate may not be well absorbed when applied dermally as a suspension in paraffin oil.

3.2 Metabolites

Median Lethal Dose

A summary of findings from submitted acute median lethal dose studies with the oxygen analogue of dimethoate, omethoate, is shown in Table 11 (Note: A separate CRP review of omethoate has also been conducted). An oral LD_{50} study on O-desmethyl dimethoate, submitted as part of the data call in process is also summarised below.

Table 11: Acute LD_{50} for omethoate

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral							
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
Dermal							
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
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)
Inhalation							
Rat [Bor: WISW (SPF-Cpb)]	M/F	5/sex	PG 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)

PG = polyethylene glycol

Omethoate is of high acute oral toxicity in rats, and of moderate toxicity by dermal and inhalational routes.

Albrecht A (2000). Acute oral toxicity up-and down method with O-desmethyl dimethoate (free acid). Bioservice Scientific Laboratories GmbH. Study duration: October-November 2000. Guidelines: OECD 425. GLP/QA: yes. DTF Doc No: '421-019' Ref: 3-23/Vol 3-9.

Methods A single oral dose of O-desmethyl dimethoate (Source: DTF Dimethoate Task Force, Batch: 656-OSJ-10P; Purity: 94.7%) was given to Wistar rats (HsdBrl:WH) by gavage. Dosing started at 500 mg/kg bw, and increased with a factor of 1.3 to 650, 845, 1099, 1428 and 1856 mg/kg bw, respectively (all 1 female/dose). A final dose of 2000 mg/kg bw was used for 3 animals/sex. Each increase was based on the absence of mortality at the previous dose.

Results There was no mortality or treatment-related clinical signs. No treatment-related effects were seen at necropsy. The LD_{50} for O-desmethyl dimethoate was greater than 2000 mg/kg bw.

Cholinesterase inhibition

A number of studies were submitted to characterise the ability of dimethoate metabolites to inhibit ChE activity following single dose oral exposure. These studies are summarised as follows.

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 AG, Institut fuer Toxicologie. Wuppertal-Elberfeld. Study duration: March 1978. Guidelines: None GLP/QA: None. DTF Doc No: '522-001' Ref: 3-47/Vol 3-25.

Methods Fasted Wistar rats (Winkelmann, Borchen; 160-240 g) were dosed by gavage with omethoate dissolved in distilled water, after which they were observed for seven days. Only the ChE experiment is described below; acute toxicity data are tabulated above. 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, erythrocyte 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 Table below.

Results 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 (Table 12), but the degree of inhibition of brain ChE activity at this dose (Table 13) was considered toxicologically significant. The extent of inhibition of erythrocyte 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 erythrocyte normalising by 72 h post-treatment, and in the brain and plasma by day 7. The NOEL was 0.6 mg/kg bw, due to inhibition of brain ChE activity at 1.3 mg/kg bw.

Table 12: Cholinesterase activity in plasma & erythrocyte at 2 h post-treatment

Dose (mg/kg bw)	Plasma (U/mL)	Erythrocyte (U/mL)
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)

Numbers in parentheses represent % inhibition *cf*. controls

Table 13: Cholinesterase activity in brain over the course of the study

Dose (mg/kg bw)	Time after administration*						
	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

*Units are expressed as U/mL and numbers in parentheses represent % inhibition *cf*. controls. **No reason given for omission of this data point.

Brennan C (2001a). Dimethoate, omethoate, 4 metabolites: comparison of toxicity and cholinesterase inhibition potential following a single oral gavage administration to male CD rats. Huntingdon Life Sciences Ltd. DTF Doc No: '463-006' Ref: 3-24/Vol 3-9.

Methods Dimethoate (Source: Cheminova Agro A/S; Batch No. 291-BSe-75B; Purity 99.5%), omethoate (Source: Cheminova Agro A/S; Batch No. 512-BSe-37A; Purity 96.3%) and 4 potential metabolites of dimethoate (as a solution in distilled deionised water) were given to groups of 5 male Crl:CD (SD) IGS BR rats (Charles River UK Ltd, approximately 8 weeks of age and weighing 252 to 310 grams) as single oral doses as shown in the Table below. The metabolites were identified as O-desmethyl omethoate K salt (Source: Cheminova Agro A/S; Batch No. 324-OSJ-6A; Purity 98.6%), O-Desmethyl omethoate carboxylic acid K salt (Source: Cheminova Agro A/S; Batch No. 352-OSJ-56B; Purity 89.5%), O-Desmethyl N-desmethyl Omethoate K salt (Source: Cheminova Agro A/S; Batch No. 352-OSJ-17A; Purity 96.4%) and Des-O-methyl-isodimethoate, dicyclohexylammonium salt (Source: Cheminova Agro A/S; Batch No. 302-OSJ-10B; Purity 98.2%)

Animals were observed for 14 days after dosing. Body weight was recorded on the day of treatment and twice weekly thereafter. Detailed observations of the rats were made in association with dosing and then on a weekly basis. Blood samples were obtained pre-treatment and at 2.5 h and 24 h following dosing to test erythrocyte ChE activity. On day 15, all rats were killed and examined macroscopically. Statistical analyses were conducted using a three way anova; times were then compared separately using the pooled variance error.

Results There were no deaths, no clinical signs and no effects on body weight gain. Inhibition of erythrocyte ChE activity at 2.5 and 24 h post dosing is shown below. Significant inhibition of erythrocyte ChE activity was observed 2.5 h following dosing with dimethoate

and omethoate; partially reversibility was seen at 24 h (Table 14). The metabolites tested showed a lower potential to inhibit ChE than dimethoate and omethoate.

Table 14: Percentage inhibition of erythrocyte ChE activity

Chemical	Dose (mg/kg bw)	Purity	% Inhibition	
			2.5 h	24 h
Dimethoate	30	99.5	53***	40**
Omethoate	5	96.3	74***	34***
O-Desmethyl omethoate K salt	30	98.6	19	16
O-Desmethyl omethoate carboxylic acid K salt	30	89.5	25*	21
O-Desmethyl N-desmethyl Omethoate K salt	30	96.4	+2	9
Des-O-methyl-isodimethoate, dicyclohexylammonium salt	30	98.2	28*	20

*p<0.05; p<0.01 and p<0.001 compared to predose values.

No treatment-related findings were observed at macroscopic examination on day 15.

Brennan C (2002). Dimethoate and hydroxy dimethoate: comparison of toxicity and cholinesterase inhibition potential following a single oral gavage administration to male CD rats. Huntingdon Life Sciences Ltd. DTF Doc No: '463-007' Ref: 3-25/Vol 3-9.

Methods Single oral doses of 30 mg/kg bw of dimethoate (Source: Cheminova Agro A/S; Batch No. 20522-00; Purity: 99.1%) or hydroxy-dimethoate (Source: Cheminova Agro A/S; Batch No. 621-BSe-7A; Purity: 96.9%) in distilled deionised water were given to groups of 5 male Crl:CD (SD) IGS BR rats (Charles River UK Ltd, approximately 8 weeks of age and weighing 263 to 293 grams) by gavage. Clinical signs and body weights were monitored prior to treatment, on the day dosing, and at sacrifice. Blood samples were obtained pre-treatment and 2.5 h and 24 h after dosing to measure erythrocyte ChE activity. Upon completion of the 24 h blood sampling, all rats were killed and examined macroscopically. Brain ChE activity was also examined, but without control for comparison. Statistical analyses were conducted using a three way anova; times were then compared separately using the pooled variance error. Brain ChE data was analysed using a t test.

Results There were no deaths or treatment-related clinical signs. Mean body weight in both groups was less (8.2-8.5 g) than pre-dose values. Dimethoate markedly inhibited erythrocyte ChE activity 2.5 and 25 h after dosing (Table 15). Inhibition of erythrocyte ChE activity was observed at 2.5 h following dosing with hydroxy-dimethoate, but the effect was reversible at 24 h.

Table 15: Erythrocyte & brain ChE activity

Chemical	Dose (mg/kg bw)	% Inhibition of erythrocyte ChE		Brain ChE (U/kg)
		2.5 h	24 h	
Dimethoate	30	71***	46**	11518
Hydroxy-dimethoate	30	33*	2	14882

*p<0.05; p<0.01 and p<0.001 compared to the predose value.

3.3 Impurities

Median Lethal Dose

Acute oral LD_{50} studies on impurities of dimethoate are summarised in Table 16.

Table 16: Acute oral LD_{50} studies on impurities of dimethoate

Chemical	Species [strain]	Route	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
0,0,0-trimethyl phosphorothioate[a]	Mouse	Oral	1150	Toia *et al* (1980)
	Rat	inhalation	1405 mg/m$^{3@}$/4h	#
phosphorodithioic acid, O,S-dimethyl S-[2-(methylamino)-2-oxoethyl] ester (iso-dimethoate)	Rat	Oral	25-200	Dreher (2001a)
O,O-dimethyl S-methoxycarbonylmethyl phosphorodithioate	Rat	Oral	>2000	Freytag (1992)

[a] Note: These data have been evaluated previously by OCSEH (Submission No.1345)
@ as LCLo: the lowest concentration in air which has been reported to cause death.
# Registry of toxic effects of chemical substances (1978). US Department of Health, Education and Welfare, National Institute for Occupational Safety and Health p947.

Dreher DM (2001a). Iso-dimethoate: acute oral toxicity in the rat: acute toxic class method. Safepharm Laboratories Limited. Study duration: 2001. Guidelines: OECD 423. GLP/QA: yes. DTF Doc No: '463-004' Ref: 3-36/Vol 3-21.

Methods A group of 3 fasted female Sprague-Dawley rats (CD Crl: CD (SD) IGS BR, ~ 8 weeks of age) received 200 mg/kg bw of iso-dimethoate (Batch: 621-BSe-40A; Purity: 95.6%) as a single oral dose in distilled water. Based on the results for the 200 mg/kg bw group, a further group of 3 rats/sex was treated at a dose level of 25 mg/kg bw of iso-dimethoate.

Results All rats at 200 mg/kg bw were found dead 30 min or 1 h after dosing. Signs of systemic toxicity noted prior to death were clonic convulsions, prostration, fasciculations, pilo-erection, increased salivation, laboured, gasping and noisy respiration, and body tremors. Haemorrhagic lungs, dark liver, dark kidneys, haemorrhage and sloughing of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small intestine were observed at necropsy.

No deaths occurred in rats at 25 mg/kg bw. Female rats, but not males, showed hunched posture, lethargy, decreased respiratory rate and laboured respiration from 30 min after dosing, and all rats appeared normal by Day 2. Body weight gains were as expected over the study period. No abnormalities were noted at necropsy. The LD_{50} for iso-dimethoate was estimated to be in the range of 25-200 mg/kg bw.

Freytag B (1992). MPEM: Assessment of acute oral toxicity in rats. Scantox Germany. Study duration: October, 1992. Guidelines: OECD 401. GLP/QA: yes. Ref: 3-80/Vol 3-40.

Fasted male and female Wistar rats (5/sex; Mollegaard Breeding Centre Ltd, Lille Skensved; 6-7 weeks of age; 134–164 g) were given single doses of O,O-dimethyl S-methoxycarbonylmethyl phosphorodithioate (Cheminova; Batch No. 20825) in peanut oil (dose volume 10 mL/kg bw) by oral gavage at 2000 mg/kg bw. Rats were observed at 1 h, 3 h and 6 h, and daily thereafter for a period of 14 days. Body weight was recorded on days 0, 7 and 14. At day 14, all rats were subjected to gross necropsy.
There were no deaths during the observation period. Group mean body weights indicated weight gain throughout the study. Treatment-related clinical signs included piloerection and pinched abdomen in all animals on day 1, while one animal exhibited diarrhoea. Piloerection was observed until days 3-4 in males and females, after which all rats displayed normal appearance and behaviour. No gross pathological findings were reported at necropsy. The oral LD_{50} of O,O-dimethyl S-methoxycarbonylmethyl phosphorodithioate (MPEM) was > 2000 mg/kg bw.

Cholinesterase inhibition

The following study was submitted to characterise the ability of iso-dimethoate to inhibit ChE activity following single dose oral exposure, in comparison to dimethoate and omethoate.

Brennan C (2001b). Iso-dimethoate: toxicity and cholinesterase inhibition potential following a single oral gavage administration to male CD rats. Huntingdon Life Sciences Ltd. Study duration: November – December 2000. Guidelines: OECD 423. GLP/QA: yes. DTF Doc No: '463-005' Ref: 3-37/Vol 3-21.

Methods A group of 5 fasted male Sprague-Dawley rats (CD Crl: CD (SD) IGS BR strain, ~ 8 weeks of age) received 30 mg/kg bw of iso-dimethoate (Batch: 621-BSe-40A; Purity: 95.6%) as a single oral dose in distilled water. Blood samples were obtained prior to dosing (0 h) and 2.5 h and 24 h after dosing for determination of erythrocyte ChE activities.

Results There were no deaths. Slight whole body tremors and "teeth chattering" were noted in 2 rats 1 h after dosing, and disappeared 2 h later. Slight and temporary weight loss was seen in one rat on Day 4 (for the period Days 0-3) of the study, and total body weight gain over the 14 day period remained slightly lower for this animal. Necropsy on Day 15 revealed no macroscopic abnormalities. The LD50 for iso-dimethoate is greater than 30 mg/kg bw.
As shown in Table 17, data from this study on iso-dimethoate (30 mg/kg bw) and a study run concurrently investigating the effect of a single oral dose of dimethoate (30 mg/kg bw) and omethoate (5 mg/kg bw), revealed significantly inhibited erythrocyte ChE activity relative to pre-dose values at both time points.

Table 17: Percent inhibition of erythrocyte ChE activity

Test article	2.5 h		24 h	
	% inhibition	P value	% inhibition	P value
Iso-dimethoate 30 mg/kg bw	68***	0.0001	68***	0.0001
Dimethoate 30 mg/kg bw	53***	0.0001	40**	0.0021

Test article	2.5 h		24 h	
	% inhibition	P value	% inhibition	P value
Iso-dimethoate 30 mg/kg bw	68***	0.0001	68***	0.0001
Omethoate 5 mg/kg bw	74***	< 0.0001	34***	0.0009

3.4 Formulations

Median Lethal Dose

Oral studies

Dreher DM (2001b). Dimethoate 400 g/L EC, stabilized. Acute oral toxicity in the rat – Acute toxic class method. Safepharm Laboratories Limited. Study Duration March – April, 2001. Guidelines: OECD 423. GLP/QA: yes. Ref: 3-85/Vol 3-41.

Methods Danadim insecticide (dimethoate 400 g/L EC, Source: Cheminova, Batch No. 3619-02-00209) was administered to fasted male and female Sprague-Dawley CD (Crl: CD® (SD) IGS BR) strain rats by oral gavage at doses of 200 and 2000 mg/kg bw. The rats weighed at least 200 g and were approximately 8 weeks of age at study initiation. The test article was administered as a freshly prepared emulsion in distilled water at 200 mg/kg bw (dose volume 10 mL/kg bw) to three male and female rats, or undiluted at 2000 mg/kg bw (dose volume 1.90 mL/kg bw) to three female rats only. Animals were observed for mortality and clinical signs 0.5, 1, 2, and 4 hours after dosing and then once daily up to day 14. Body weight was recorded prior to dosing and weekly thereafter. A gross necropsy was performed on all animals.

Results All rats treated at 2000 mg/kg bw died within four hours of dosing; there were no deaths at 200 mg/kg bw. At 200 mg/kg bw all animals gained weight during the observation period (49-97 g). Animals treated at 200 mg/kg bw exhibited clinical signs including splayed gait, chromodacryorrhea and occasional tremors. Hunched posture, lethargy, ataxia, decreased respiratory rate, laboured respiration and increased salivation were observed at both 200 and 2000 mg/kg bw. Clinical signs had resolved by day 2 in rats treated at 200 mg/kg bw. High-dose animals also exhibited ptosis, noisy respiration, exophthalmos and prostration. Necropsy revealed haemorrhagic lungs, dark liver and kidneys and haemorrhage and sloughing of gastric mucosa at 2000 mg/kg bw. No treatment related abnormalities were detected in animals treated at 200 mg/kg bw. The oral LD_{50} of Danadim insecticide was estimated to be approximately 300–500 mg/kg bw in rats.

Dreher DM (1998a). CHA 3620-Fresh : Acute oral toxicity test in the rat. Safepharm Laboratories Limited. Study duration: September – October, 1998. Guidelines: none. GLP/QA: yes. Ref: 3-83/Vol 3-40.

Methods A dimethoate 400 g/L EC (Cheminova, Danadim insecticide, Batch No: 4031400was prepared as a solution in arachis oil BP and administered to three male Sprague-Dawley rats (Crl:CD®BR strain supplied by Charles river (UK) Ltd, Margate, Kent, UK; weighing 251-264 g; 8-12 weeks of age) by oral gavage as a single dose of 300 mg/kg bw. The dose volume was 10 mL/kg bw. Rats were observed for mortality and clinical signs at 0.5, 1, 2, and 4 h, then at daily intervals until day 14. Body weight was recorded on days 0, 7 and 14. Gross pathological examination was conducted at the end of the observation period.

Results No deaths were observed during the study period. Body weight gain was observed in all animals. Clinical signs observed up to three days following dosing, included hunched posture, ataxia, decreased respiratory rate, occasional body tremors, fasciculations and lethargy. There were no abnormalities at necropsy.

Dreher DM (1998b). CHA 3620-stored: Acute oral toxicity test in the rat. Safepharm Laboratories Limited. Study duration: September – October, 1998. Guidelines: none. GLP/QA: yes. Ref: 3-84/Vol 3-40.

Methods A dimethoate 400 g/L EC (Cheminova, Danadim insecticide, Batch No: 4031400was stored for 2 years; the content of dimethoate at this time was analysed as 35.2% w/w. The test material was then prepared as a solution in arachis oil BP and administered to three male Sprague-Dawley rats (Crl:CD®BR strain supplied by Charles river (UK) Ltd, Margate, Kent, UK; weighing 239-264 g; 8-12 weeks of age) by oral gavage as a single dose of 300 mg/kg bw. The dose volume was 10 mL/kg bw. Rats were observed for mortality and clinical signs at 0.5, 1, 2, and 4 h, then at daily intervals until day 14. Body weight was recorded on days 0, 7 and 14. Gross pathological examination was conducted at the end of the observation period.

Results No deaths were observed during the study period. Body weight gain was observed in all animals. Clinical signs observed up to 5 days following dosing, included hunched posture, lethargy ataxia, occasional body tremors, decreased respiratory rate, laboured respiration, fasciculations and red/brown staining around the eyes, chromodacryorrhea, diarrhoea, exophthalmos, increased salivation and tiptoe gait. There were no abnormalities at necropsy.

Dermal studies

Dreher DM (2001c). Dimethoate 400 g/L EC, stabilized. Acute Dermal Toxicity (Limit Test) in the rat. Safepharm Laboratories Limited. Study Duration March – April, 2001. Guidelines: OECD 402. GLP/QA: yes. Ref: 3-86/Vol 3-41.

Methods Danadim insecticide (dimethoate 400 g/L EC, Source: Cheminova, Batch No. 3619-02-00209) was applied to the skin of Sprague-Dawley rats [CD (Crl: CD® (SD) IGS BR) (5/sex; weighing at least 200 g; aged approximately 8 weeks)] at a dose of 2000 mg/kg bw. The back and flanks of each animal were clipped free of hair prior to treatment and the test article was applied in a dose volume of 1.90 mL/kg bw to approximately 10% of the body total surface area using a syringe. Surgical gauze was applied, and the area semi-occluded for a 24 h exposure period using a self-adhesive bandage. Animals were observed for mortality and clinical signs 0.5, 1, 2, and 4 hours after dosing and then once daily up to day 14. Body weight was recorded prior to dosing and weekly thereafter. The test sites were examined and scored for signs of irritation following removal of the dressings and subsequently once daily for 14 days. A gross necropsy was performed on all animals.

Results There was no mortality and no clinical signs were observed. All animals gained weight during the study period (males 77-113 g; females 28-32 g). No abnormalities were seen at necropsy. There was no evidence of dermal irritation. The acute dermal LD_{50} was estimated to be >2000 mg/kg bw.

Skin Irritation

Dreher DM (2001d). Dimethoate 400 g/L EC, stabilized. Acute dermal irritation in the rabbit. Safepharm Laboratories Limited. Study Duration March – April, 2001. Guidelines: OECD 404. GLP/QA: yes. Ref: 3-87/Vol 3-41.

Methods Undiluted Danadim insecticide (dimethoate 400 g/L EC, Source: Cheminova, Batch No. 3619-02-00209was applied to the clipped skin on the dorsal flank of New Zealand White rabbits [Supplied by David Percival Ltd, Cheshire, UK; weighing 2.0 to 3.5 kg; aged approximately 12 to 16 weeks)]. The test article was initially instilled onto three test sites of one rabbit and held in place under a cotton gauze patch secured by surgical adhesive tape. The patches were removed 3 minutes, 1 h or 4 h after application. An additional two rabbits were treated at a single site and the test material was allowed to remain in contact with the skin for a period of 4 h. The test sites were observed for signs of irritation at 1, 24, 48 and 72 h, and at days 7 and 14.

Results No mortality or clinical signs were reported. As shown in Table 18, well-defined erythema was seen in all animals at 1 h and persisted in one animal at 72 h. Slight oedema was observed in two animals, and at 72 h very slight oedema persisted in two rabbits. Crust formation was observed at two treated skin sites at 7 days.

Table 18: Individual skin irritation scores following a 4 h exposure period

Animal no.	Erythema			Oedema		
	6 male	9 male	115 female	6 male	9 male	115 female
1 h	2	2	2	1	2	1
24 h	2	2	1	2	2	0
48 h	2 Le	2	1	1	2	0
72 h	1 Le	2 Le	0	1	1	0
7 days	0 Cf	0 Cf	0	0	0	0
14 days	0	0	0	0	0	0

Le = loss of elasticity, Cf = crust formation

Scores for skin irritation in the single rabbit exposed for 1 h and 3 minutes to Danadim insecticide are shown in Table 19. After three-minute exposure to Danadim, very slight erythema was observed at 24 h; no other signs of irritation were observed. Well-defined erythema was observed until 48 h and very slight erythema was seen at 72 h following the 1 h exposure time. Slight oedema was seen at 24 h and 48 h, persisting as very slight oedema at 72 hours. There were no effects at seven days (except crust formation) or 14 days.

Table 19: Skin irritation scores in one rabbit following 1 h & 3 minute exposure to Danadim insecticide

Animal no.	Erythema		Oedema	
	1 h exposure	3 minute exposure	1 h exposure	3 minute exposure
1 h	2	0	1	0
24 h	2	1	2	0
48 h	2	0	2	0
72 h	1 Le	0	1	0
7 days	0 Cf	0	0	0
14 days	0	0	0	0

Le = loss of elasticity, Cf = crust formation

Under the conditions of this study, Danadim insecticide was a moderate irritant to the skin of rabbits.

Eye Irritation

Dreher DM (2001e). Dimethoate 400 g/L EC, stabilized. Acute eye irritation in the rabbit. Safepharm Laboratories Limited. Study Duration March – April, 2001. Guidelines: OECD 405. GLP/QA: yes. Ref: 3-88/Vol 3-41.

Methods Undiluted Danadim insecticide (dimethoate 400 g/L EC, Source: Cheminova, Batch No. 3619-02-00209was instilled into the conjunctival sac of the right eye of one New Zealand White rabbit [Supplied by David Percival Ltd, Cheshire, UK; weighing 2.0 to 3.5 kg; aged approximately 12 to 16 weeks)]. Another two rabbits received the same treatment with the addition of one drop of local anaesthetic one to two minutes prior to treatment. Eyes were examined at 1, 24, 48 and 72 h, and a further observation was made at 7 days.

Results Individual scores for eye irritation for the three rabbits are shown in Table 20. Dulling of the normal lustre of the cornea was observed in two animals at 1 h and scattered or diffuse areas of corneal opacity were seen in these animals at the 24 and 48 h time points. Inflammation of the iris was reported in all treated eyes at 1 h, and persisted until 48 h in one animal. Conjunctival redness (Draize score =2) was seen in two animals until 48 h, and persisted until 72 hours (Draize score = 1). Obvious swelling of the conjunctivae was seen in all animals at 1 h, and at 48 h in one animal. Discharge (score = 3) was seen in all animals at 1 h. No treatment-related effects were seen at seven days.

Table 20: Eye irritation scores in rabbits following instillation of a 400 g/L dimethoate EC

Animal no.	116					15					19				
Time	1 h	24 h	48 h	72 h	7 d	1 h	24 h	48 h	72 h	7 d	1 h	24 h	48 h	72 h	7 d
Cornea	d	1	1	0	0	0	0	0	0	0	d	1	1	0	0
Iris	1	1	0	0	0	1	0	0	0	0	1	1	1	0	0
Conjunctivae															
Redness	2	2Pt	2Pt	1	0	2	1	1	1	0	2	2	2	1	0
Chemosis	2	2	1	1	0	2	1	1	0	0	2	2	2	1	0
Discharge	3	2	1	0	0	3	1	1	0	0	3	2	2	1	0

d = dulling of the cornea, Pt – petechial haemorrhage scattered over the nicitating membrane

Under the conditions of this study, Danadim insecticide was a moderate irritant to the eyes of rabbits.

Skin Sensitisation

Bollen LS (2001). Test for delayed contact hypersensitivity using the Buehler Test. Scantox. Study Duration March – May, 2001. Guidelines: OECD 406. GLP/QA: yes. Ref: 3-89/Vol 3-41.

Methods During the induction phase, 0.3 mL of the undiluted test compound (dimethoate 400 g/L EC, stabilized, Source: Cheminova, Batch No: 3619-02-00209-LV) was applied to the left flank region of 20 female Dunkin Hartley guinea pigs (M&B, Ejby, DK-4623 Lille Skensved, Denmark; weighing between 258 and 357 g; approximately 5-6 weeks of age) for 6 h using a Hill Top chamber. These applications were carried out on days 1, 3, 5, 8, 10, 12, 15, 17 and 19. Skin irritation was assessed daily; due to irritation in treated animals after the third application, the test concentration was lowered to 50% (presumably in water). A control group, containing 10 animals was similarly treated with sterilized water. On day 30, all animals were challenged with about 0.3 mL of the undiluted test compound in a Hill Top

chamber at both the anterior and posterior part of the right flank for 6 h. Rechallenge was performed on the entire test group and control group two weeks after the first challenge using three Hill Top chambers containing the undiluted test substance, a 50% w/w dilution of the test substance, and vehicle.

Results During the induction phase, skin irritation, described as no to well-defined erythema with scale and small crust formations, was seen following the 3rd dose in the treated group. After the concentration was lowered to 50% (w/w), no to well defined erythema was observed and some of the animals showed crust formations.

At challenge, discrete or patchy erythema (Score=1) was observed in one control animal at 24 h, following treatment with either distilled water or the test substance. There were no signs of skin irritation in control animals at 48 h.

Skin irritation scores for treated (induced) guinea pigs following challenge with the vehicle and undiluted test compound are shown in Table 21. Discrete or patchy erythema was observed in 9/20 animals at 24 h, while moderate and confluent erythema was observed in one animal. At 48 h, 9/20 animals showed discrete or patchy erythema, and three animals showed moderate and confluent erythema. No signs of irritation were observed at the right posterior site where distilled water was applied.

Table 21: Skin irritation scores of guinea pigs challenged with undiluted test substance (right anterior) & distilled water (right posterior)

Animal No.	24 h		48 h	
	Right anterior	Right posterior	Right anterior	Right posterior
221	1	0	0	0
222	0	0	0	0
223	1	0	1	0
224	1	0	2	0
225	0	0	1	0
226	1	0	2	0
227	1	0	2	0
228	0	0	0	0
229	0	0	1	0
230	0	0	1	0
231	0	0	0	0
232	1	0	1	0
233	1	0	1	0
234	0	0	0	0
235	0	0	0	0
236	2	0	1	0
237	1	0	1	0
238	1	0	1	0
239	0	0	0	0
240	0	0	0	0

Skin irritation scores at re-challenge are shown in Table 22. Discrete or patchy erythema was observed infrequently in control animals, as well as in an extra naïve control group (not shown). In induced animals, rechallenge with the undiluted test compound resulted in discrete or patchy erythema in 5/20 animals at 24 h. At 48 hours, discrete or patchy erythema was seen in 5/20 animals, confluent erythema was observed in 1/20 animals, and intense erythema and swelling was seen in 3/20 animals. At the left middle site (re-challenged with a 50% dilution of the test compound), discrete or patchy erythema in 7/20 animals at 24 h. At 48 h, confluent

erythema was observed in 3/20 animals and intense erythema and swelling was seen in 3/20 animals. At the left posterior site (re-challenged with distilled water) 3/20 animals showed discrete or patchy erythema at 24 h, there was no sign of skin irritation at 48 h.

Table 22: Skin irritation at re-challenge in guinea pigs exposed to the test compound or distilled water*

Animal no.	24 h			48 h		
	Left anterior	Left middle	Left posterior	Left anterior	Left middle	Left posterior
221	0	1	0	3	3	0
222	0	0	0	0	0	0
223	0	0	0	0	0	0
224	0	0	0	1	1	0
225	0	0	0	0	0	0
226	0	0	0	0	0	0
227	0	0	0	1	1	0
228	0	0	1	1	2	0
229	1	1	0	1	2	0
230	0	0	0	0	0	0
231	1	1	0	2	2	0
232	1	1	0	3	3	0
233	0	1	0	1	1	0
234	1	1	0	0	0	0
235	0	0	0	0	0	0
236	1	1	1	3	3	0
237	0	0	0	1	1	0
238	0	0	0	0	0	0
239	0	0	1	0	0	0
240	0	0	0	0	0	0

*Left anterior – undiluted test article; Left middle – 50% w/w test article; Left posterior – distilled water

In total, 8/20 (40%) animals in the test group showed skin irritation scores ≥2 at challenge or rechallenge with the undiluted, or a 50% w/w dilution, of the test article. Under the conditions of this study, Danadim insecticide was a sensitizer to the skin of guinea pigs.

4 SHORT-TERM REPEAT-DOSE STUDIES

4.1 Technical grade active constituent

Oral

Rats

Kaspers U, Kaufmann W, Deckardt K, van Ravenzwaay B (2004). Dimethoate – range finding study in Wistar rats administration via the diet over 4 weeks (Volume I-III). Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen/Rhein, Germany. Study period: January-February 2002. GLP/QA: yes. DTF Doc No: '432-009' Ref: 3-45/Vol 3-24.

Methods Wistar rats (5/sex/dose) received 0, 1, 3 or 12.5 mg/kg bw/d of dimethoate (Source: not specified; Batch: 20522-00in the diet for 4 weeks. The calculated mean daily intakes of the test substance were 0.83/0.85, 2.48/2.60 and 10.38/11.00 mg/kg bw/d for males/females in the 1, 3 and 12.5 mg/kg bw/d dose groups, respectively. The stability of the test substance in the diet was verified. The rats (CrlGlxBrlHan:WI, supplied by Charles River Germany GmbH) were 5 weeks old and mean group weights ranged from 190-197 g for males and 139-149 g for females on day 0. Clinical signs and mortality were observed daily, while food and water consumption, and body weight, were measured weekly. Haematological and clinical chemistry parameters were examined at termination. Serum and erythrocyte ChE activities were measured on Days -7, 2, 8 and 29, and brain ChE at termination (Day 29). All rats were assessed for organ weights (adrenal, brain, epididymides, heart, kidneys, liver, spleen, testes or ovaries, thymus and uterus), and gross pathology, while histopathology was not performed. Statistical analyses were conducted using the non-parametric Kruskal-Wallis one way analysis of variance. Pairwise comparison was conducted using the Wilcoxon test.

Results No animal died during the study. There were no treatment related observations for clinical signs, food and water consumption, or body weight. Haematological parameters and clinical chemistry were within normal ranges.

On Day 29 (termination), decreased activities of ChE were detected in the serum, erythrocytes and brain of the high-dose group, and also in the erythrocytes and brain of the mid-dose group (Table 23). Although results for brain ChE activity at 3 mg/kg bw/d did not achieve statistical significance, inhibition was ≥20% compared to control and dose-related and was considered biologically significant. Inhibition of erythrocyte ChE activity was also seen at the high-dose on Day 8.

Table 23: ChE activity in plasma, erythrocytes & brain

Dose (mg/kg bw/d) @	Male*				Female*			
	0	1 (0.83/0.85)	3 (2.48/2.60)	12.5 (10.4/11.0)	0	1 (0.83/0.85)	3 (2.48/2.60)	12.5 (10.4/11.0)
Serum ChE, µkat/L								
Pre-test	13.9	13.5	12.8	15.1	25.2	24.5	28.1	26.5
Day 8	12.4	12.6	11.3 (9)	11.7 (6)	33.7	28.7 (15)	39	27.5 (18)
Day 29	11.0	11.5	10.1 (8)	8.5** (23)	46.5	40.1 (14)	47.7	28.6** (38)
Erythrocyte ChE, µkat/L								
Pre-test	32.3	34.2	32.8	33.9	35.9	39.0	35.2	39.7
Day 8	34.2	33.1	34.4	24.9 (27)	32.3	36.2	31.4	21.2** (34)
Day 29	38.7	35.1 (9)	33.9 (12)	11.8** (70)	35.9	36.8	26.7** (26)	10.9** (70)
Brain ChE, µkat/g protein								
Day 29	2.24	2.27	1.78 (20)	0.78** (65)	2.18	3.11**	1.53 (30)	0.65** (70)

@ Numbers in parentheses under doses represent calculated mean daily intakes of the test substance.
* Numbers in parentheses represent % inhibition *cf.* control values.
** $p<0.01$ by Kruskal-Wallis test and Wilcoxon-test, two-sided; n=5.

Absolute and relative weights of the ovaries were significantly ($p<0.05$) increased at 1 (absolute; 35%, relative 30%) and 12.5 mg/kg bw/d (absolute 25%, relative 27%), but not at 3 mg/kg bw/d; these changes were dose-independent and therefore not considered treatement-related.

The NOEL was 0.83/0.85 mg/kg bw/d based on inhibition of erythrocyte and brain ChE activity at higher doses.

Dogs

Harling RJ, Burford P, McLean TA, Buist DP, Crook D (1989). Dimethoate dietary toxicity study in Beagle dogs (Final report – repeated daily dosage for 4 Weeks). Huntingdon Research Center. Study duration: August – October 1988. GLP/QA: yes. DTF Doc No: '432-003' Ref: 3-6/Vol 3-4.

Methods Pure-bred Beagle dogs (2/sex/dose) received 0, 2, 10, 50, 250 or 1250 ppm of dimethoate in the daily diet for 4 weeks. The dogs were supplied by Interfauna UK Ltd, Wyton, Huntingdon, 24-29 weeks old, and weighed 7.7-10.1 kg at the beginning of the study. Dietary mixtures with the test substance (Source: Predotti Chinici SpA; Purity: 96.44%; Batch no: 611A) were prepared and replaced weekly. The concentration, homogeneity and stability of the test substance were analysed. Achieved intakes of the test substance were 0.09, 0.43, 2.20, 11.12 and 49.81 mg/kg bw/d for 2, 10, 50, 250 or 1250 ppm respectively. Dogs were observed daily for clinical signs and mortalities. Food consumption was measured daily and body weight twice per week. Blood was collected from all dogs twice daily (at 4 and 24 h after feeding) before dosing and on dosing Days 6, 13, 20 and 27 for analysis of plasma ChE and erythrocyte ChE activity. At termination, organ weights were measured and gross and histopathology was performed on organs. A sample of brain was frozen for analysis of brain ChE. No statistical analyses were performed.

Results All dogs at 1250 ppm were killed during Week 3 for humane reasons. Vomiting, reduced food consumption, body weight loss (up to - 2.3 kg) and/or notably dark/black faeces were observed prior to sacrifice. One dog at 250 ppm lost body weight (up to -1.2 kg), which led to a nil mean body weight gain of the group. No treatment-related clinical signs, food consumption or body weight changes were observed in other groups.

As shown in the Table 24, greater than or equal to 20% inhibition of plasma ChE activity was observed at ≥ 250 ppm from Day 6, while erythrocyte ChE activity was inhibited at ≥ 50 ppm from Day 13. At termination, brain ChE activity was inhibited approximately 20% and 50% compared to control at 50 and 250 ppm, respectively.

Table 24: ChE activity in plasma, erythrocytes & brain[a]

Dose (ppm)	0	2	10	50	250	1250
Plasma ChE [(µmol/mL/min (% inhibition from pre-dose)]						
Pre-test	1.52	1.64	1.59	1.62	1.48	1.70
Day 6	1.40	1.53	1.53	1.42	0.98 (30)	0.90 (35)
Day 13	1.54	1.48 (10)	1.50 (6)	1.39 (14)	0.96 (35)	0.84 (51)
Day 20	1.33	1.5	1.52	1.47	1.0 (25)	-
Day 27	1.26 (16)	1.64	1.62	1.44 (12)	0.94 (37)	-
Erythrocyte ChE [(µmol/mL/min (% inhibition from pre-dose)]						
Pre-test	1.88	2.32	2.26	2.05	2.14	1.91
Day 6	1.78	2.23	2.14	1.84	1.23 (31)	0.56 (68)
Day 13	1.72 (9)	2.30 (3)	2.07 (9)	1.64 (21)	0.85 (61)	0.51 (71)
Day 20	1.81	2.17	2.02	1.45 (20)	0.67 (63)	^
Day 27	1.66 (12)	2.08 (10)	1.86 (18)	1.25 (39)	0.39 (81)	^
Brain ChE [(µmol/mL/min (% inhibition from control)]						
Terminal at day 28	3.86	4.30	4.21	3.14 (19)	1.81 (53)	1.15 (n=1)

[a] Mean values from males and females from samplings either in weeks 1 or 2 pre-test or 4h and 24h after feeding during the dosing period (n=4). *Numbers in parentheses represent % inhibition *cf*. pre-dose or control values. ^All dogs at 1250 ppm were killed during week 3 for humane reasons.

At termination, mean thymus weight was increased (118-181% of control) at doses of 2 - 250 ppm, however there was no clear relationship with dose. The increase at 250 ppm (181%) was largely accounted for by one animal with a thymus weight over twice the mean, therefore the relationship to treatment is unclear. Macroscopic examination revealed a firm swelling in the cortex of the right kidney of 1 female dog at 250 ppm. Kidney weight for this animal, and kidney mean weights for other treated groups did not differ from control; therefore it was considered that this effect could not be clearly attributed to treatment. Examination of the kidney of animals that were killed at the high-dose, revealed a treatment-related 'dark red' or 'dark' discoloration of the medulla of both kidneys in 2/4 animals.

The NOEL was 10 ppm (0.43 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 50 ppm (equal to 2.2 mg/kg bw/d) and above.

Dermal

Rabbits

Madison WA (1986). 21-Day dermal study with dimethoate in rabbits. Hazleton Laboratories Guidelines: not indicated. GLP: yes. No: 6123-125.
[Note: This study has been evaluated previously by OCSEH (Submission Nos. 150 and 415 and 1345)]

Method New Zealand White rabbits (6/sex/group) were treated with dimethoate (made into a paste with paraffin oil) at concentrations of 0, 100, 300 and 1000 mg/kg bw. Dimethoate was applied to the shaved dorsal skin for 6 h/day, 5 days/week for 3 weeks, using the closed patch technique. Half of the animals in each group had their dorsal surface abraded prior to application of the test compound, the skin of the remaining animals was intact. Two control groups were used in this study; an untreated control group and a vehicle control group. All animals were examined daily for dermal irritation, signs of intoxication and mortality. Animals were necropsied at the end of the treatment period.

Results Dermal irritation seen in the vehicle control group consisted of slight to severe erythema and oedema and slight to moderate desquamation and fissuring and slight atonia. The levels of dermal irritation seen in this group were very similar, in type and severity, to that seen in treated groups. The highest levels of dermal irritation occurred in the 100 mg/kg bw animals. The degree of dermal irritation was similar on abraded and intact skin.

Macroscopic changes consisting of red areas, mild thickening and crusting of the treated skin of vehicle control and test group animals was accompanied by mild chronic or chronic/active dermatitis and acanthosis. These changes were not dose-related and appear to be associated, in part, with the vehicle, paraffin. No inhibition of cholinesterase was detected in any of the experimental groups suggesting that dimethoate may not be absorbed when applied dermally as a suspension in paraffin oil. No signs of systemic toxicity were detected in any of the treatment groups up to 1000 mg/kg bw/d. A NOEL could not be clearly determined in terms of skin irritation due to interference by paraffin oil.

4.2 Metabolites

Oral

Rats

Fogleman RW & Levinskas GJ (1963). Report on oxygen analog of dimethoate: twenty-eight day feeding of rats. American Cyanamid Company, Central Medical Department, Environmental Health Laboratory. Study duration: March 1963. Guidelines: None GLP/QA: None. DTF Doc No: '532-003' Ref: 3-52/Vol 3-25.

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, erythrocytes 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 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 macroscopic or microscopic examination. The values obtained for ChE activity were very variable, especially in the erythrocytes, 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, and the lack of information on the purity of the substance tested, this study is not of value for regulatory purposes.

Dermal

Rats

Löser E (1968a). Bayer 45 432. Subacute toxicological studies on rats. Farbenfabriken Bayer AG, Institut fűr Toxicologie, Wuppertal-Elberfeld. Sponsor: Bayer AG. Report date: 19 February 1968. Guidelines: None GLP/QA: None DTF Doc No: '532-001' Ref: 3-53/Vol 3-25

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.

Rabbits

Flucke W & Luckhaus G (1979). S 6876 (Omethoate, the active ingredient of Folimat®) Subacute dermal toxicity study on rabbits. Bayer AG, Institut für Toxicologie, Wuppertal-Elberfeld. Study duration: July 1978. Guidelines: None stated QA/GLP: No. DTF Doc No: '532-002' Ref: 3-58/Vol 3-26.

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 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, erythrocyte 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), erythrocyte 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 erythrocyte 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 (Table 25). 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, erythrocyte and brain at 20 mg/kg bw/d.

Table 25: ChE activity

Tissue	Week	Dose (ppm):	Males			Females		
			0	2.5	20	0	2.5	20
Plasma (U/mL)	**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)
Erythrocyte (U/mL)	**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 (U/g)	**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)

Numbers in parentheses represent % inhibition *cf.* control values.

4.3 Formulations

Dermal

Rats

Hilaski R (1999). A 5-day dermal toxicity study of dimethoate 4E (neat formulation) in rats. MPI Research, Inc. Study period: November – December 1998. Guidelines: FIFRA. GLP/QA: yes. DTF Doc No: '431-001' Ref: 3-11/Vol 3-6.

Methods Dimethoate 4E formulation (Micro-Flo Co., Sparks, Georgia; Lot No: 8-307-47; 43.5% active constituent) was administered at 0 (vehicle control), 5, 10, 20, 40 or 100 mg (active constituent)/kg bw/d to the clipped skin area (~10 cm^2) of the dorsum between the limb griddles of Sprague-Dawley rats (16/sex/dose, from Charles River Laboratory, Portage, Michigan). Male rats were aged approximately 6.5 weeks and in the weight range of 151-199 g, and female rats were approximately 8 weeks of age and weighed 173-204 g. The test compound was applied under semi-occlusion (with a collar), 6 h per day for a total of 5 days. Clinical signs and food consumption were observed daily. An abbreviated FOB (including home cage observations on posture, clonic and tonic movements, and palpebral closure; observations during handling on ease of removal, handling reactivity, lacrimation, salivation, palpebral closure, piloerection, exophthalmos; open field observations on gait, clonic and tonic movements, defecation, urination, arousal, mobility, vocalization, stereotypy, bizarre behaviour and respiration) was performed on 8 rats/sex/dose on Days 3 and 5, prior to sacrifice. Body weights were measured pretest, and on Days 3 and 5. On Days 3 and 5, blood samples from 8 rats/sex/dose were collected, macroscopic evaluations were conducted, and brains were collected. The rats were examined for external abnormalities, and abnormalities in abdominal, thoracic and cranial cavities. The brain was weighed and brain weight ratios were calculated. ChE inhibition was evaluated in the plasma, erythrocytes, and 3 brain regions (hippocampus, striatum and cortex) on Days 3 and 5. Statistical analyses were made by pairwise comparison using Dunnett's test or Welch's t-test, or the Chi-Square test for homogeneity (FOB observations).

Results There were no mortalities and body weights were not affected by treatment. Tremors, exophthalmos, excessive lacrimation, pale eyes, and shallow breathing were observed in 2 females at 100 mg/kg bw/d. Collars of these animals were reported to have come off during the exposure period, suggesting that the test material may have been ingested. Higher incidences of lacrimation (1/16 vs 6/16) and pale eyes (0/16 vs 3/16) were observed in high-dose females compared to controls. Home-cage palpebral closure (drooping half-way or completely shut) was seen at higher incidences in treated groups than control however there was no clear dose-response relationship. An increased incidence of dermal desquamation was observed in males at doses of 5-40 mg (active constituent)/kg bw/day, but not at the high-dose (1/16, 2/16, 3/16, 4/16, 3/16, 1/16 at 0, 5, 10, 20, 40 or 100 mg (active constituent)/kg bw/d respectively).

As shown in Table 26, a significant ($p<0.05$ or $p<0.01$) reduction in erythrocyte and brain (hippocampus, striatum and cortex) ChE activity was observed in males at 100 mg (active constituent)/kg bw/d. In females, a dose-related inhibition of erythrocyte ChE activity was observed from 5-100 mg (active constituent)/kg bw/d; inhibition at 5 and 10 mg (active constituent)/kg bw/d, while not achieving statistical significance, was ≥ 20% and considered

treatment related. Plasma and brain (cortex) ChE activity was also reduced significantly ($p<0.05$ or $p<0.01$) at 20-100 mg (active constituent)/kg bw/d in females.

Table 26: ChE activity in plasma, erythrocytes & brain tissues

Dose (mg/kg bw/d)	Male						Female					
	0	**5**	**10**	**20**	**40**	**100**	**0**	**5**	**10**	**20**	**40**	**100**
Plasma ChE (mM/L/min)												
Day 3	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.5 (17)	0.5 (17)	0.5 (17)	0.3 ** (50)	0.4 ** (33)
Day 5	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.5 (17)	0.5 (17)	0.4 * (33)	0.4 * (33)	0.3 ** (50)
Erythrocyte ChE (mM/L/min)												
Day 3	1.8	1.9 (0)	1.7 (6)	1.7 (6)	1.7 (6)	1.2* (33)	2.0	1.5 (25)	1.4 (30)	1.3 * (35)	0.7 ** (65)	0.6 ** (75)
Day 5	1.8	1.7 (6)	1.5 (17)	1.5 (17)	2.0 (0)	0.9 ** (50)	2.0	1.6 (20)	1.4 (30)	1.3 * (35)	1.0 ** (50)	0.5 ** (75)
Brain ChE (mM/g/min)												
Hippocampus Day 3	0.81	0.75 (7)	0.75 (7)	0.77 (5)	0.78 (4)	0.70 (14)	0.87	0.80 (8)	0.74 (15)	0.71 (18)	0.54 ** (38)	0.45 ** (48)
Hippocampus Day 5	0.81	0.90 (0)	0.80 (1)	0.82 (0)	0.74 (9)	0.56 ** (31)	0.87	0.81 (7)	0.81 (7)	0.72 (17)	0.69 (21)	0.45 ** (48)
Striatum Day 3	1.04	0.97 (7)	1.06 (0)	1.04 (0)	1.04 (0)	0.80 * (23)	1.06	0.93 (12)	0.90 (15)	0.87 (18)	0.64 ** (40)	0.57 ** (46)
Striatum Day 5	1.04	1.06 (0)	0.95 (9)	0.95 (9)	0.98 (6)	0.81 ** (22)	1.06	1.05 (1)	1.02 (4)	0.84 (21)	0.86 (19)	0.59 ** (44)
Cortex Day 3	1.62	1.51 (7)	1.50 (7)	1.52 (6)	1.41 (13)	1.29 ** (20)	1.60	1.46 (9)	1.44 (10)	1.33 (17)	0.94 ** (41)	0.82 ** (49)
Cortex Day 5	1.62	1.51 (7)	1.57 (3)	1.55 (4)	1.51 (7)	1.13** (30)	1.60	1.56 (3)	1.48 (8)	1.26 ** (21)	1.27 ** (21)	0.78 ** (51)

*Numbers in parentheses represent % inhibition *cf.* control values. **$p<0.01$, compared with control. n=8. Control is a pooled value of two tests from Days 3 & 5. Four females whose collars came off had decreased ChE values & were excluded from statistical evaluation.

There were no treatment-related macroscopic findings. A statistically significant ($p \leq 0.05$) decrease (21%) in mean absolute striatum weight was seen in males at 100 mg/kg bw/d killed on Day 3, but not on Day 5, nor in females of either day. This finding did not clearly indicate a treatment-induced effect

No NOEL was established; the LOEL was 5 mg/kg bw/d based on inhibition of erythrocyte ChE activity in females at all doses.

Chambers PR (1999). Dimethoate 400 g/L EC: Toxicity study by dermal administration to Han Wistar rats for 4 weeks. Huntingdon Life Sciences Ltd. Study period: May - June 1999. Guidelines: OECD 410 (1998); OPPTS-870.3200. GLP/QA: yes. DTF Doc No: '432-005' Ref: 3-9/Vol 3-5.

Methods Dimethoate 400 g/L EC (Source: Dimethoate Task Force; Lot No: 70917-00; 38% active constituent) was applied at 0 (untreated), 10.5, 21.0, 31.5 or 63.0 mg (active constituent)/kg bw/d to the clipped skin area of the dorsum between the limb griddles of Han Wistar rats (10/sex/dose). The test compound was applied under semi-occlusion for 6 h per day, 5 days per week for 4 weeks. The rats (from Harlan UK Limited, Shaw's Farm, Blackthorn, UK) were 9 weeks old, and weighed 214-262 g for males, and 13 weeks old and 189-222 g for females, at the beginning of treatment. Clinical signs including skin reaction at the application site were observed daily. Neurobehavioural screening including FOB and motor activity assessments were performed pre-test and during Week 4. Pupillary response was measured daily pre-test, once weekly during treatment and at the end of test. Food consumption and body weight were measured weekly. Ophthalmic examinations were performed pre-test and in Week 4. Blood was collected from all rats before dosing, and on dosing days 7 and 29 for analysis of plasma ChE and erythrocyte ChE activity. At termination, a brain sample was frozen for analysis of brain ChE activity. Blood and urine samples collected on day 29 were also tested for haematology plasma chemistry and urinalysis parameters. Terminal examinations included organ weight (adrenal, brain, epididymides, heart, kidneys, liver, lungs, spleen, testes or ovaries, thymus, thyroid with parathyroids, and uterus with cervix), and gross- and histo-pathology of tissues. In brief, statistical analysis was by one way anova, or in cases where significant heterogeneity was observed, and not corrected by logarithmic transformation, the Kruskal-Wallis analysis of ranks was used. Pairwise comparison was conducted by the Student's t-test and Williams test or non-parametric tests (not specified).

Results There were no deaths, and no clinical signs or local dermal responses. In the FOB, treatment was not associated with any behavioural changes which were considered indicative of neurotoxicity. Food consumption and body weight gain were not affected. Ophthalmoscopy did not reveal any treatment-related ocular changes.
Slight, but significant increases (up to 6% increase) in erythrocyte counts and haematocrit detected in males at 21.0, 31.5 or 63.0 mg (active constituent)/kg bw/d were within the background historic control range. Reduced plasma AST levels (up to 21% lower) in these male groups and lower urinary protein (up to 37% lower) in all treated female groups were not considered to be of toxicological relevance.

As shown in Table 27, plasma ChE activity was not affected at any dose level, while inhibition of erythrocyte ChE activity was observed at 63.0 mg (active constituent)/kg bw/d on Day 29 in males, but not in females. At termination, a dose-related, statistically significant ($p<0.01$) inhibition (8-17%) of brain ChE activity was observed from 21-63 mg (active constituent)/kg bw/d in both sexes. Although related to treatment, effects on brain ChE activity at 21.0 and 31.5 mg (active constituent)/kg bw/d were slight (8-10%) and considered of doubtful toxicological significance.

Table 27: ChE levels in plasma, erythrocytes & brain*

Dose (mg/kg bw/d)	Male					Female				
	0	**10.5**	**21**	**31.5**	**63.0**	**0**	**10.5**	**21**	**31.5**	**63.0**
Plasma ChE (U/L)										
Day 29	447	449	431	487	474	1834	1862	1659	1953	1800
Erythrocyte ChE (U/L)										
Day 29	983	958 (3)	870 (11)	933 (5)	788** (20)	890	825 (7)	853 (4)	908 (0)	838 (6)
Brain ChE [U/kg brain]										
Terminal	13010	12700	11835 ** (9)	11750 ** (10)	10765 ** (17)	12765	12310	11690 ** (8)	11435 ** (10)	10865 ** (15)

*Numbers in parentheses represent % inhibition *cf.* control values. **p<0.01 *cf.* control. n=6.

The dermal NOEL was 31.5 mg (active constituent)/kg bw/d based on inhibition of erythrocyte and brain ChE activity at 63.0 mg (active constituent)/kg bw/d.

Cheffings Y (1999). Dimethoate 400 g/L EC: Preliminary toxicity study by dermal administration to Han Wistar rats for 4 weeks. Huntingdon Life Sciences Ltd. Study period: January - March 1999. Guidelines: OECD 410 (1998); OPPTS-870.3200. GLP/QA: yes. DTF Doc No: '432-006' Ref: 3-10/Vol 3-5

Methods Dimethoate 400 g/L EC (Dimethoate Task Force; Lot No: 70917-00; 38% active constituent) was administered at 0, 5.25, 21, 42, 63 or 105 mg (active constituent)/kg bw/d to the clipped skin area of the dorsum between the limb griddles of Han Wistar rats (5/sex/dose) for 6 h per day, and 5 days per week, for 4 weeks under semi-occlusion. The rats (from Harlan UK Limited, Shaw's Farm, Blackthorn) were 10-12 weeks old, and weighed 232-289 g for males and 174-222 g for females, at the beginning of treatment. Clinical signs including skin reaction at the application site were observed daily. Food and water consumption and body weight were measured weekly. Blood samples were collected on Day 29 for haematology and plasma chemistry. Samples of venous blood were collected from all rats before dosing and on dosing Day 29 for analysis of erythrocyte ChE activity. At termination, a brain sample was frozen for analysis of brain ChE activity. Macroscopic pathology was examined at termination on Day 29. Statistical analyses for body and organ weight changes were conducted by a Behrens-Fisher test, otherwise Dunnett's test was used. Haematological and clinical chemisty data, as well as erythrocyte and brain ChE activity, were assessed using the Student's t-test.

Results There were no deaths, clinical signs or local dermal responses. Food consumption and body weight gain were not affected. Sporadic, non-dose related, but occasionally significant changes in haematology and blood chemistry were observed. These changes were considered to be non-treatment related.

Erythrocyte ChE activity was significantly ($p<0.01$) and dose-dependently (28-61%) decreased compared to pre-dose values in males from 42-105 mg (active constituent)/kg bw/d, whereas it was only marginally affected in females (11%; $p<0.05$) at 105 mg (active constituent)/kg bw/d (Table 28). A non-significant 21% decrease in erythrocyte ChE activity compared to concurrent control at 21 mg/kg bw/d as determined using CHCA, was not greater than 20% compared to pre-dose values and not observed using CCAA, and was therefore considered of doubtful toxicological significance. Statistically significant ($p<0.05$ or $p<0.01$) inhibition of brain ChE activity was observed in males (27-43%) and females (10-27%) at 63 and 105 mg (active constituent)/kg bw/d.

Table 28: ChE levels in plasma, erythrocytes and brain^

Dose (mg/kg bw/d)	Male						Female					
	0	**5.25**	**21**	**42**	**63**	**105**	**0**	**5.25**	**21**	**42**	**63**	**105**
Erythrocyte ChE [U/L(% reduction from pre-dose value)]												
CHCA	1035	1145	820 (14)	720 ** (28)	570 ** (36)	380 *** (61)	1180	1120 (6)	1045	1035	1045	880* (11)
CCAA	1060	1170	905 (12)	815 * (25)	700 ** (28)	530 *** (49)	1195	1120	1110	1100	1090	980 (4)
Brain ChE [U/kg brain (% reduction from control)]												
CHBA	13580	13190 (3)	12200 (10)	12210 (10)	9930** (27)	7810*** (42)	12210	12160	11670 (4)	11480 (6)	10950* (10)	8980*** (26)
CBAA	11490	11170 (3)	10220 (11)	10470 (9)	8430** (27)	6530*** (43)	10390	10380	9890 (5)	9670 (7)	9100** (12)	7580*** (27)

^Numbers in parentheses represent % inhibition *cf.* control values. n=6. *p<0.05; **p<0.01; ***p<0.001 compared with control. CHCA: using ,5-dithiobis (2-nitrobenzoic acid) as chromogen (DTNB). CCAA using 6,6-dithiodinicotinic acid as chromogen (DTNA). CHBA: using DTNB as chromogen. CBAA: using DTNA as chromogen.

At termination, no abnormalities were observed in organ weights or gross- pathology.
The dermal NOEL was 21 mg/kg bw/d based on inhibition of erythrocyte ChE activity in males at 42 mg (active constituent)/kg bw/d.

5 SUBCHRONIC STUDIES

5.1 Technical grade active constituent

Rats

Edson *et al* (1958). 6 month dietary study in rats. Fisons Ltd, Study No: Tox/52/14. February.
[Note: This study has been evaluated previously by OCSEH (Submission Nos. 150 and 415 and 1345)]

Dimethoate was fed to Wistar rats (20 males/group) at dose levels of 0, 0.5, 2, 10, 50, 200 and 800 ppm in their diets for 6 months. Animals were observed daily for signs of toxicity, morbidity and body weights and food consumptions were measured weekly. Blood samples were obtained by cardiac puncture from 6 animals/group after 1, 4, 9 and 16 weeks of treatment, and ChE activity determined.

Almost all of the 800 ppm animals developed severe toxic effects within a few days of treatment. Signs included fibrillation, weakness, apathy, loss of appetite and reduced weight gain. These effects were so severe as to warrant withdrawal of the test substance from the diet in this group. Recovery was apparent within 10-14 days. Animals receiving 200 ppm showed reduced weight gain and slight toxic effects typical of ChE inhibition. These toxic effects abated after several weeks but behavioural changes (aggressiveness) became evident after about 3 months of treatment. No changes in weight gain or clinical signs were seen in the other treatment groups.

Marked depression in erythrocyte and brain ChE activity was seen at 50 ppm and above. Histopathology examination was normal for all groups. The NOEL was 10 ppm (0.5 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 50 ppm (2.5 mg/kg bw/d) and above.

5.2 Metabolites

Rats

Löser E (1968b). Bay 45 432: Subchronic toxicological studies on rats. Bayer AG, Institut für Toxicologie, Wuppertal-Elberfeld. Study dates not provided. Guidelines: None QA/GLP: None. DTF Doc No: 533-001' Ref: 3-54/Vol 3-25.

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 (males) or 59 g (females). Rat bodyweights were measured weekly, and food consumption was determined 5 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 (ALT, AST, 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 erythrocytes) 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 AST, ALT, and total bilirubin in both sexes (Table 29), 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. At ≥2 ppm, ChE activity was inhibited in the erythrocytes 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 one sampling point. At macroscopic examination there were no findings that could be linked to treatment, and organ weights were also not affected.

Table 29: Liver function tests & % inhibition of ChE activity relative to control

Parameter	Dose(ppm):	0	0.5	1	2	4
AST (mU)	Males	11.01	9.35	13.14	15.65	15.83
	Females	10.70	11.54	9.94	12.68	15.87
ALT (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
Erythrocyte ChE	Males	0	7.7	10.7	46.8	59.1
1 week	Females	0	6.7	15.5	21.2	51.7
Erythrocyte ChE	Males	0	10.6	0	29.3	42.8
4 weeks	Females	0	6.0	10.7	21.2	48.0
Erythrocyte 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

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 AG Fachbereich Toxikologie, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal. Study duration: May 1990 – January 1991. Test guidelines: None GLP: not stated QA: Yes DTF Doc No: '537-002' Ref 3-70/Vol 3-30

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 erythrocyte ChE activity up to the time the present study was undertaken.

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

Results The test substance was demonstrated to be stable in the drinking water, and present at the appropriate concentrations. The test compound intake was (male/female) 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 Table 30, 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 erythrocyte ChE activity at a higher dose than those used in the present study (i.e. 500 ppb).

Table 30: Cholinesterase activity

Tissue	Males			Females		
	0 ppb	100 ppb	300 ppb	0 ppb	100 ppb	300 ppb
Plasma (kU/L)	0.52	0.52	0.60**	2.13	2.10	2.20
Erythrocyte (kU/L)	0.76	0.88*	0.92**	0.92	0.90	0.93
Brain (kU/g)	3.54	3.55	2.95	2.28	2.23	2.90**

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

Dogs

Ruf J & Mager H (1991). E 6876 Subchronic toxicity study on dogs (Thirteen-week stomach tube dosage test). Bayer AG, Fachbereich Toxikologie, Friedrich-Ebert-Strasse, D-5600 Wuppertal 1. Study duration: February – June 1989. Guidelines: None stated GLP/QA: yes DTF Doc No: '533-003' Ref: 3-56/Vol 3-26
(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.)

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. Cytochrome P450 and N-demethylase activities were determined in liver tissue. Plasma and erythrocyte 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 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.

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). American Cyanamid Company, Central Medical Department, Environmental Health Laboratory. Report date: 12 August 1968. Guidelines: None. DTF Doc No: '533-004' GLP/QA: No Ref: 3-55/Vol 3-25

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.

6 CHRONIC STUDIES

6.1 Technical Grade active constituent

Mice

Hellwig J, Deckhardt K & Mirea D (1986a). Report on the study of the toxicity of dimethoate in mice after 78 week administration in the diet. BASF Department of Toxicology, Germany. Guidelines: Based on 1978 USEPA guidelines; equivalent to FIFRA 83-2. GLP/QA: yes.

Methods Technical dimethoate (purity 96.7%) was fed to B6C3F1CrlBr mice (50/sex/group) at dose levels of 0, 25, 100 or 200 ppm in their diets for 18 months. Additional animals (10/sex/group) were also included in the study to assess haematological parameters and cholinesterase levels at an interim period of 51 weeks. These doses were estimated to be equivalent to approximately 0, 3.6, 13.7 and 31.1 mg/kg bw/d in males and 0, 5.2, 18.2 and 35.6 mg/kg bw/d in females at 0, 25, 100 and 200 ppm respectively. Animals were examined daily for signs of toxicity. Body weight and food consumption were determined weekly for the first 3 months of treatment and then every 2-4 weeks for the remainder of the study. Haematological and clinical chemistry parameters were determined and all animals were assessed for gross pathology. Histopathological examination was carried out upon termination of the study.

Results Intermittent decreases in body weight gain were seen in treated animals, especially at 200 ppm, during the first 50 weeks of treatment. During the latter stages of treatment, there was a subsequent increase in body weight gain in treated animals. There was no treatment-induced change in survival rate of the animals.

Dimethoate treatment significantly ($p<0.01$) inhibited plasma and erythrocyte ChE activity in a dose-related manner (Table 31). At 51 weeks of treatment, there were approximate 10%, 50% and 80% reductions in plasma ChE levels and 30%, 70% and 90% reductions in erythrocyte ChE levels at 25, 100 and 200 ppm dimethoate, respectively. Except for small increases in mean Hb content per red blood cell and mean corpuscular Hb concentration seen at 100 and 200 ppm, there were no treatment-induced changes in haematological parameters.

Table 31: Plasma & erythrocyte ChE activity

Dose (ppm)	Male				Female			
	0	25	100	200	0	25	100	200
Plasma ChE [µkat/L]								
51 weeks	74.43	66.73** (10)	38.31** (49)	16.40** (78)	101.56	90.67** (11)	49.09** (52)	22.90** (77)
Erythrocyte ChE [µkat/L]								
51 weeks	36.76	25.93** (29)	8.64** (76)	3.87** (89)	31.84	23.12** (27)	10.08** (68)	4.48** (86)

Numbers in parentheses represent % inhibition *cf.* control values. ** significant at $p<0.01$

There were slight decreases in ovary weights in the high dose females however no pathological changes in the ovaries were noted. A dose-related increase in liver weights was seen in animals from the 100 and 200 ppm groups. Histopathological examination revealed an increased incidence of hepatocytic vacuolization in the liver of these animals. Some increase was also seen in the incidence of extramedullary haematopoiesis in the spleen of animals from

the 100 and 200 ppm groups. Neoplasias were observed in both treated and control animals with similar incidence.

As shown in Table 32, microscopic examination revealed a higher incidence of neoplasm infiltration in hemolymphoreticular system of several organs and tissues, compared to control, these including in the jejunum, thymus and mesenteric lymph nodes of males at 200 ppm, and in the liver, bone marrow, thymus, lymph nodes and mesenteric lymph nodes of males in all treated groups. However, the changes were not statistically significant, and not dose-dependent. Furthermore, the lymphoma is a type of common tumours in mice and the incidences in this study are consistent with historical control (based on the US EPA 40 CFR Part 180, the data not provided by the applicant). In addition, there also was no evidence of precursor lesions to carcinogeniticy. Hence, the finding was not clearly related to the treatment, and dimethoate is not considered to be a carcinogen in mice under experimental conditions in this study.

Table 32: Incidence of neoplastic lesions in mice (n = 48-50 unless specified)

	Male				Female			
Dose (ppm)	**0**	**25**	**100**	**200**	**0**	**25**	**100**	**200**
Jejunum								
Neoplasm infiltrat. HLRS				**5 (10)**	1 (2)		1 (2)	1 (2)
Liver								
Neoplasm infiltrat. HLRS		1 (2)		1 (2)	**2 (4)**	**4 (8)**	**8 (16)**	**5 (10)**
Bone marrow (sternum)								
Neoplasm infiltrat. HLRS				1 (2)	**2 (4)**	**4 (8)**	**4 (8)**	**5 (10)**
Thymus								
Neoplasm infiltrat. HLRS				5 (10)	**5 (10)**	**9 (18)**	**11 (22)**	**10 (20.4)**
Lymph nodes (diverse)								
Neoplasm infiltrat. HLRS	0/4	1/3 (33.3)	0/1	1/3 (33.3)	**6/22 (27.3)**	**7/11 (63.6)**	**5/9 (55.6)**	**3/7 (42.9)**
Mesenteric lymph nodes								
Neoplasm infiltrat. HLRS	1 (2.0)	1 (2.0)	1 (2.0)	**7 (14)**	**5 (10)**	**11 (22)**	**10 (20)**	**9 (18)**

HLRS: hemolymphoreticular system.

Dimethoate is not considered to be a carcinogen in mice. The chemical produced dose-related reductions in erythrocyte ChE levels, therefore a NOEL was not established in this study. The LOEL was 3.6 mg/kg bw/d.

Rats

Hellwig J *et al* (1986b). Report on the study of the toxicity of dimethoate in rats after 24 month administration in the diet. BASF Department of Toxicology, Germany. Project No. 70 CO 326/8241 Guidelines: Based on USEPA guidelines 1978; equivalent to US-EPA FIFRA 83-5. GLP/QA: yes.

&

Squire RA (1988). An evaluation of vascular proliferative lesions in male Wistar rats from Project 70C0326/8241 (DTF Doc No: '437-003). Robert A. Squire Associates Inc. DTF Doc No: '437-004'

&

Squire RA (1988). Additional data for rat chronic feeding/oncogenicity study dimethoate (ACY prepared for registration in California). Robert A. Squire Associates Inc. DTF Doc No: '437-003'

Methods Technical dimethoate (purity 96.7%) was fed to Wistar rats (50/sex/group) at dose levels of 0, 5, 25 or 100 ppm in their diets for 2 years. Additional animals (15-20/sex/dose) were treated with 0, 1, 5, 25 or 100 ppm dimethoate and were used as satellite groups to determine haematological and clinicochemical parameters at interim periods of the study. These interim periods were at 4, 13, 26, 52 and 78 weeks of treatment. Doses were equivalent to approximately 0, 0.04, 0.23, 1.2 and 4.8 mg/kg bw/d in males and 0, 0.06, 0.3, 1.5 and 6.3 mg/kg bw/d in females at 0, 1, 5, 25 and 100 ppm respectively. Animals were examined daily for clinical signs. Body weight and food consumption were determined weekly for the first 3 months and then fortnightly. A total of 7 blood samplings and 2 urine collections (at 52 and 104 weeks) were carried out and clinicochemical, haematological and urinalysis parameters determined. All animals were killed at 104 weeks and assessed by gross pathology and extensive histopathological examination.

Results There was a transient depression in body weight gain in animals of the high dose group during the first 54-weeks of treatment. There was no change in food consumption, no ophthalmological changes and no clinical signs seen with dimethoate treatment.

As shown in Table 33, dimethoate-treatment led to an approximate 50% reduction ($p<0.01$) in plasma ChE activity at 1 month that persisted for the duration of treatment in animals at 100 ppm. Erythrocyte ChE activity was reduced ($p<0.05$ or $p<0.01$) at 1 month in a dose-related manner for females at 5, 25 and 100 ppm and for males at 25 and 100 ppm. In females, at 5 ppm, the erythrocyte ChE activity was inhibited up to 34% during the first 12 months of treatment; levels were unaffected at this dose after 18 and 24 months of treatment. In males, erythrocyte ChE activity was inhibited (18%, $p<0.01$) at 5 ppm at the 24-month time point only. At necropsy, a dose-related and toxicologically significant decrease in brain ChE activity was seen at 5, 25 and 100 ppm for males ($p<0.05$ or $p<0.01$; 23-62%) and at 25 and 100 ppm ($p<0.01$; 40-52%), for females.

There were signs of a slight anaemic process seen at 100 ppm with decreases in mean cell volume and Hb/RBC and an increase in corpuscular Hb concentration. In the last 52 weeks of treatment there was an increase in leukocytes at 100 ppm. No other haematological effects were noted. Clinicochemical analysis revealed slight, but significant decreases in total protein values in males at 100 ppm, increased ALT levels in females at 100 ppm and a decrease in potassium levels in females at 100 ppm. There were no treatment-related changes in urinalysis.

Dimethoate produced some changes in organ weights but only at 100 ppm; these consisted of increased liver weights in males and females, increased spleen weights and decreased ovary weights in females and increased adrenal weights in males.

Table 33: Plasma, erythrocyte & brain ChE activity

Dose	**Males**					**Females**				
ppm (mg/kg bw/day)	**0**	**1 (0.04)**	**5 (0.23)**	**25 (1.2)**	**100 (4.8)**	**0**	**1 (0.06)**	**5 (0.3)**	**25 (1.5)**	**100 (6.3)**
Plasma ChE [μkat/L]										
Pre-test	13.0	13.1	12.8	12.8	12.3	15.5	15.6	15.8	14.8	16.1

1 m	9.6	9.8	9.3	8.4	4.4** (54)	32.2	33.8	34.1	27.8* (14)	15.5** (52)
3 m	12.6	13.0	11.8	10.3	5.7** (55)	43.6	45.4	46.4	39.2	20.3** (53)
6 m	12.5	14.8	12.9	12.6	6.6** (47)	48.6	54.0	54.9	50.6	30.3** (38)
12 m	16.0	16.6	15.5	13.8	7.5** (53)	53.5	50.9	52.8	50.2	24.0** (55)
18 m	14.7	17.1	15.7	13.3	6.8** (54)	30.7	35.7	40.0	30.7	14.2** (54)
24 m	14.5	17.5	17.3*	15.2	7.6** (48)	29.1	26.6	31.3	25.6	12.9** (56)
Erythrocyte ChE [μkat/L]										
Pre-test	8.1	7.6	9.5	9.3	9.1	17.4	19.0	17.2	15.8	19.3
1 m	23.0	24.0	20.5	14.0** (39)	4.7** (80)	25.4	24.5	19.3** (24)	15.0** (41)	5.6** (78)
3 m	21.0	25.8	17.9	13.6** (35)	4.5** (79)	27.4	25.6	20.7* (24)	16.1** (41)	6.4** (77)
6 m	19.7	22.8	19.4	13.4** (32)	5.6** (72)	24.6	25.0	19.8** (20)	14.1** (43)	5.8** (76)
12 m	20.4	23.5	20.7	15.3 (25)	5.1** (75)	27.3	28.8	18.0** (34)	13.8** (49)	12.0** (56)
18 m	16.0	13.9	17.1	12.2 (24)	6.0** (62)	16.4	15.7	15.7	12.9 (21)	5.8** (65)
24 m	27.3	28.0	22.5** (18)	16.3** (40)	5.3** (81)	24.8	23.6	22.4	17.9** (28)	6.0** (76)
Brain ChE [μkat/L]										
24 m	0.48	0.44	0.37* (23)	0.33** (31)	0.18** (62)	0.50	0.50	0.45	0.30** (40)	0.19** (52)

Numbers in parentheses represent % inhibition *cf.* control values. Significant at * $p<0.05$, ** $p<0.01$.

As shown in Table 34, microscopic examination revealed some neoplastic and pre-neoplastic lesions. A higher incidence of hyperplasia of adrenal medulla was observed in males and females of all treated groups without a dose-relationship. Both islet-cell adenoma and exocrine adenoma in pancreas were increased at 100 ppm.

Numbers of malignant lymphomas in the hemolymphoreticular system were high in all groups with higher incidences in treated male groups compared to control, but a reversed pattern in female groups. In mammary glands, an increased incidence of fibroadenoma was shown in all treated female groups, and of carcinoma in the high dose group. However, it has been known that Wistar rats appear unusual susceptibility to the spontaneous development of vascular tumours in the mesenteric lymph nodes, as well as a high natural incidence of mammary fland tumours.

Hemangiosarcomas in the spleens occurred in all male groups, with an increased incidence and severity in all treated groups without a dose-relationship. Similarly, both the incidence and severity of hemangioma and hemangiosarcoma (alone or in combination) in the mesenteric lymph nodes, were increased in all treated male groups without proportion to the dose levels. The incidence of hemangiosarcoma in either spleen or mesenteric lymph nodes was above the up limit of historical control provided by the applicant. The incidence at each dose group becomes statistically significant when total angiogenic tumours at any site were counted.

However, these tumours were either with low incidence compared to concurrent and/or historical control (no statistically significant difference except fro the combined incidence of

total angiogenic tumours at any site), lacking a dose-response relationship, appearing in only one sex (angiogenic tumours in males but not females) and one species (rats only, but not mice). Along with the non-genetoxic nature of the chemical in vivo, the weight of evidence indicates that dimethoate is unlikely to be a carcinogen in rats.

Table 34: Incidence of neoplastic lesions in rats (n = 47-50 unless specified)

Dose (ppm)	Male				Female				Hist. control (%)[a]
	0	**5**	**25**	**100**	**0**	**5**	**25**	**100**	
Adrenal medulla									
Focal/multifocal unilateral/bilateral hyperplasia	3 (6)	**10 (20)**	**5 (10)**	**7 (14.3)**	2 (4)	**3 (6.1)**	**7 (14.3)**	**5 (10.6)**	
Pancreas									
Islet-cell adenoma	1 (2)		2 (4)	**4 (8)**					
Exocrine adenoma	4 (8)	4 (8)	1 (2)	**8 (16)**					
Mammary glands									
Fibroadenoma					7 (15.2)	**11 (22.5)**	**11 (22.0)**	**13 (26.5)**	
Carcinoma					2 (4.4)	2 (4.1)	1 (2.0)	**5 (10.2)**	
Hemolymphoreticular system									
Malignant lymphoma	6 (12)	**10 (20)**	**8 (16)**	**12 (24)**	17 (34)	16 (32)	14 (28)	10 (20)	
Spleen									
Hemangiosarcoma, n (%)	**1 (2)**	**3 (6)**	**2 (4)**	**5 (10)**	3 (6)			1 (2)	(0, 1)
Mesenteric lymph nodes									
Hemangioma, n (%)	1 (2)	**4 (8)**	**5 (10.2)**	**3 (6.1)**	1 (2)	1 (2.1)	1 (2.0)	1 (2.1)	(8, 16)
Hemangiosarcoma, n (%)		**4 (8.0)**	**3 (6.1)**	**3 (6.1)**	2 (4.0)	1 (2.1)		1 (2.1)	(0, 4)
Total angiogenic tumours in mesenteric lymph nodes, n (%)	1 (2)	8 (16)	9 (18.4)	6 (12.2)	3 (6)	2 (4.3)	1 (2)	2 (4.2)	
Total angiogenic tumours at any site	3 (6)	12* (24)	11* (22)	10* (20)	6 (12)	2 (4)	2 (4)	3 (6)	

*Significant increase by the Exact Fisher-Test.
[a]Historical control data derived from two 2-year studies in Wistar rats completed during 1987 at BASF, Ludwigshafen, FRG.
Endothelial proliferation: an intrasinosoidal proliferation of endothelial cells.
Angioma or hemangioma: a benign, neoplastic proliferation of endothelial cells.
Angiosarcoma or hemangiosarcoma: a malignant neoplastic proliferation of endothelial cells.

In conclusion, dimethoate is not considered to be a carcinogen in rats. The NOEL for male rats at the completion of this study (24 months) was 1 ppm (0.04 mg/kg bw/day) based on inhibition of brain cholinesterase. For female rats the NOEL was 1 ppm (0.06 mg/kg bw/day) based on dose related inhibition RBC cholinesterase at 12 months.

NCI (1977). Report No. 4. 80-week dietary study in rat and mouse
[Note: This study has been evaluated previously by OCSEH (Submission No. 1345)]

Osborne-Mendel rats (50/sex/group) were fed dimethoate technical in their diets for 80 weeks at dose levels of 0, 250 or 500 ppm (approximately 12.5 and 25 mg/kg bw/d at 250 and 500 ppm respectively, using a conversion factor of 20). Males did not tolerate the high dose well and after a 19 week treatment period doses were reduced to 0, 125 or 250 ppm (6.25 and 12.5 mg/kg bw/d), and treatment continued a further 61 weeks. Females had their

doses reduced by half after 43 weeks and treatment continued for a further 37 weeks at the lower level. At the end of the 80 week period, animals were fed a control diet for a further 34-35 weeks before being killed and necropsied.

There was a general dose-related reduction in bodyweight gain seen at the low and high dose in males and at the high dose in females. During the first weeks of the study, high dose males and females showed signs of dimethoate intoxication, including hyper-excitability, tremors and convulsions. During the 2nd year of the study all treatment groups exhibited adverse clinical signs, albeit in low incidence; these included diarrhoea, anaemia and epistaxis. Histopathologic examination revealed no significant increase in tumours associated with dimethoate treatment. However, several non-neoplastic lesions occurred more frequently in treated rats than in control animals. These included interstitial fibrosis of myocardium, focal cytomegaly of adrenal cortex, follicular cell hyperplasia in the thyroid gland and testicular atrophy.

Dogs

Burford P, McLean TA, Buist DP, Crook D, Gregson RL, Gopinath C (1990a). Dimethoate: 12-month dietary study in Beagle dogs (Final report – repeated daily dosage for 52 Weeks). Huntingdon Research Center. Study completion: 23 January, 1990. Guidelines: EPA-FIFRA 83-1, OECD 452 GLP/QA: yes. DTF Doc No: '437-011' Ref: 3-7/Vol 3-4.
&
Burford P, McLean TA, Buist DP, Crook D, Gregson RL, Gopinath C (1990b). Individual clinical observations. Supplement to MRID Number 41939801. Dimethoate 12-month dietary study in Beagle dogs (Repeated daily dosage for 52 Weeks). Huntingdon Research Center. DTF Doc No: '437-014' Ref: 3-8/Vol 3-4.

Methods Pure-bred Beagle dogs (6/sex/dose) received 0, 5, 20 or 125 ppm of dimethoate in the daily diet for 52 weeks. The dogs were supplied by Interfauna UK Ltd, Wyton, Huntingdon, were 25-32 weeks old, and weighed 7.3-13.5 kg at the beginning of the study. Dietary mixtures with the test substance (Source: Industria Predotti Chimici SpA; Batch no: 611A; Purity: 96.44%) were prepared and replaced weekly, and the concentration, homogeneity and stability of the test substance were analysed. Achieved intakes of the test substance in males/females were 0.18/0.19, 0.70/0.76 and 4.2/4.3 mg/kg bw/d at doses of 5, 20 and 125 ppm, respectively.

Dogs were observed daily for clinical signs and mortalities. Food consumption was measured daily and body weight twice per week. Ophthalmoscopic examinations were performed pre-dosing and during weeks 26 and 52. Blood samples were taken before treatment and after Weeks 13, 26 and 52 for assessment of standard haematological parameters and plasma chemistry, as well as plasma and erythrocyte ChE activity. Urine was collected at the same time for analysis of urinalysis parameters. Termination examinations included organ weight with the addition of lungs, pancreas, thymus, uterus and prostate. Gross- and histo-pathological examination was conducted on selected tissues, with only minor deviations. A sample of brain tissue was preserved for investigation of brain ChE activity. Statistical analyses were conducted by one way anova in cases of homogenous variance or in cases where heterogenous variances were detected (Bartlett's test), and not corrected for by logarithmic transformation, the Kruskal-Wallis analysis of ranks was employed. Pairwise

comparison was conducted by Student's t-test or non-parametric equivalents (not specified) of the t-test.

Results There were no clinical signs which were considered to be related to treatment. Food consumption and body weight changes were comparable among groups. Ophthalmoscopy revealed no treatment-related abnormalities.

For parameters in haematology, clinical chemistry and urinalysis, all individual values were within the normal range of variation. Whilst statistical analysis revealed significant differences between control and treated groups for a number of values, similar differences were also generally observed at the pre-dose investigations, and considered to be of no toxicological significance.

As shown in Table 35, inhibition ($p<0.05$ or $p<0.01$) of plasma ChE activity was observed at 125 ppm in males at weeks 13, 26 and 52, and in females at weeks 13 and 26. Erythrocyte ChE activity was significantly ($p<0.05$) decreased at 20 and 125 ppm in both sexes. The 20% decrease in erythrocyte ChE activity in males at 5 ppm at week 52, was not statistically significant or greater than 20% compared to pre-dose values, nor was it observed at other sampling times or seen in females, therefore it was considered of doubtful toxicological significance. Brain ChE activity was inhibited 14-18% ($p<0.01$) at 20 ppm and 55-56% at 125 ppm. A minimal (9-10%) statistically significant ($p<0.05$) decrease in brain ChE activity in males and females at 5 ppm was not considered of toxicological relevance.

Table 35: ChE levels in plasma, erythrocytes & brain

Dose (ppm)	Male				Female			
	0	**5**	**20**	**125**	**0**	**5**	**20**	**125**
Plasma ChE [µmol/mL/min]								
Pre-test	1.43	1.35	1.35	1.52	1.57	1.48	1.54	1.44
Week 13	1.64	1.50	1.53	1.28* (22)	1.84	1.83	1.65	1.18** (36)
Week 26	1.33	1.20	1.22	1.07* (20)	1.48	1.38	1.42	1.15* (22)
Week 52	1.45	1.49	1.51	1.15** (21)	1.70	1.72	1.68	1.47 (14)
Erythrocyte ChE [µmol/mL/min]								
Pre-test	2.18	2.13	2.26	2.66	2.42	2.32	2.48	2.27
Week 13	2.08	1.90	1.60* (23)	0.68** (67)	2.20	2.10	1.72* (22)	0.53** (76)
Week 26	2.23	1.99	1.62* (27)	0.70** (69)	2.25	1.97	1.80 (20)	0.59** (74)
Week 52	2.23	1.78 (20)	1.76 (21)	0.78** (65)	2.16	2.12	2.02	0.79** (63)
Brain ChE [µmol/mL/min]								
At termination	2.98	2.70* (9)	2.55** (14)	1.34** (55)	3.31	2.98* (10)	2.70** (18)	1.46** (56)

Numbers in parentheses represent % inhibition *cf.* control values. n=6. * $p<0.05$, ** $p<0.01$

Absolute and relative liver weights were lower in both sexes of the 125 ppm group (Table 36). Deposit of a brown, granular pigment was observed in isolated sinusoidal cells of the liver of treated groups without a dose-relationship. Staining by Perl's technique revealed positive liver sinusoidal cells in treated groups as well as control. These slight, non-dose related increases in pigmentation were considered of doubtful toxicological significance. No other abnormalities were detected at termination.

Table 36: Liver weight and pathology (n=6)

Dose (ppm)	Male				Female			
	0	5	20	125	0	5	20	125
Liver weight (g)	375	372	374	334*	347	342	331	303*
(% body)[@]	3.35	3.38	3.28	2.78	3.54	3.20	3.04	2.61
Brown pigment in liver sinusoidal cells			2	1		4	2	3
Perl's positive liver sinusoidal cells[a]	2+, 1+++	1+, 2++, 2+++	1+, 2++, 1+++	2+, 1++, 1+++	3+, 1++, 1+++	1+, 4++	4++, 1+++	2+, 1++, 2+++

[@]Not statistically analysed. * $p<0.05$ [a] numbers represent number of animals affected; + trace, ++ minimal, +++ moderate represent severity

The NOEL was 5 ppm (0.18/0.19 mg/kg bw/d) based on inhibition of erythrocyte and brain ChE activity at 20 ppm (0.70/0.76 mg/kg bw/d).

6.2 Metabolites

Mice

Schladt L (2001) E 6876 Oncogenicity study in B6C3F1 mice (administration in the drinking water over 24 months; T1032655). 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. Study duration: March 1989-March 1991. Guidelines: US EPA 83.2, 1984; OECD 451, 1981 GLP/QA: Yes. DTF Doc No: 555-001 Ref: 3-71/Vol 3-31 to 3-34.

Method 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, ALT, and AST; 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. 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 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).

As shown in Table 37, 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.

Table 37: Haematology findings

Parameter	Month	Males				Females			
		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	10.07	9.89	9.82*	9.88	10.00	10.41*	10.16
	103	10.57	9.92	9.10	9.06*	9.42	8.90	9.17	9.56
Hb (g/L)	51/52	148	149	150	149	147	152*	154*	150
	103	150	140	133 ns	134*	142	135	139	142
Hct (g/L)	51/52	0.485	0.476	0.467	0.463*	0.469	0.479	0.497*	0.475
	103	0.482	0.450	0.415	0.409**	0.441	0.430	0.433	0.444
MCHC (g/L RBC)	51/52	307	312	322**	322*	314	318	310	315
	103	311	311	319	328*	323	313**	320	320
Thrombocytes (10^9/L)	51/52	1508	1416	1357*	1474	1164	1145	1220	1330**
	103	1698	1769	1453	1561	961	1075	1130*	1006
Thromboplastin time (s)	51/52	18.9	18.9	19.0	19.0	19.8	19.3	18.7**	18.4**
	103	19.5	19.7	20.6*	20.3	19.9	20.2	19.7	20.0

*p<0.05; **p<0.01; n=10.

In males at the top two doses, AP, creatinine and urea were decreased in the serum (Table 38). 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.

Table 38: Clinical Chemistry

Parameter	Week	Males				Females			
		0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
AP (U/L)	51/52	101	101	104	96	181	171	206	201
	103	253	129	101	91*	323	330	281	254
Creatinine (µmol/L)	51/52	26	25	24	24	30	29	29	29
	103	39	28	26**	24**	25	27	26	26
Urea (µmol/L)	51/52	9.51	9.27	8.46	7.53**	9.33	8.54	8.39	7.38**
	103	20.69	11.58	10.07*	9.99*	10.08	9.33	9.69	8.93
Plasma ChE (kU/L)	52/53	5.25	4.71 (10)	4.57** (13)	1.82** (65)	6.15	6.21	5.69* (8)	2.88** (53)
	59	4.85	5.13	4.76 (2)	1.48** (69)	6.28	5.97* (5)	5.39** (14)	2.32** (63)
	104/105	8.31	6.50 (22)	5.04** (39)	1.09** (77)	-	-	-	-
	105	6.63	6.24 (6)	5.22 (21)	1.78** (73)	7.57	8.23	6.63 (12)	3.18** (58)
Erythrocyte ChE (kU/L)	52/53	0.51	0.38** (25)	0.22** (57)	0.09** (82)	0.41	0.31** (24)	0.25** (39)	0.08** (80)
	59	0.52	0.52 (0)	0.24** (54)	0.06** (88)	0.47	0.43 (9)	0.28** (40)	0.09** (81)
	104/105	1.11	0.83 (25)	0.56 (50)	0.08** (93)	0.57	0.79	0.46 (19)	0.16** (72)
Brain ChE (U/g)	52/53	2.21	1.84 (17)	1.68** (24)	0.71** (68)	2.00	2.05	1.55* (23)	0.68** (66)
	104/105	3.76	3.16 (16)	1.76** (53)	0.60** (84)	2.29	2.17 (5)	1.81* (21)	0.73** (68)

Numbers in parentheses represent % inhibition *cf.* control values. (n=10). *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. Erythrocyte ChE activity was strongly inhibited in both sexes at 32 ppm and at 4 ppm in males. In females at 4 ppm, erythrocyte 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, erythrocyte 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 erythrocyte ChE values at termination were highly variable, with 2 extremely high values. The apparent decrease in erythrocyte ChE activity in 0.5 ppm males is therefore not considered to have resulted from treatment. Therefore erythrocyte 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.

In males, absolute and relative liver weights were decreased at all doses (Table 39). 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.

Table 39: Organ weights at 24 months

Body/organ weight	Males				Females			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Body weight (g)	40	41	43**	42*	41	43	44*	45*
Liver: absolute	2664	2235	2185	2162	2081	2155	2064	2208
: relative	6887	5514**	5207**	5247**	5094	5055	4710	5011
Testes: absolute	220	234	222	219	-	-	-	-
: relative	562	574	525**	525**	-	-	-	-
Kidney: absolute	741	749	774	786*	549	536	527	535
: relative	1884	1826	1824	1884	1369	1260	1210**	1211**
Heart : absolute	251	252	242	261	223	222	229	216
: relative	644	617	572**	629	560	524	525	488*
Brain: absolute	505	499	503	508	517	511	514	523
: relative	1292	1224	1194**	1227*	1298	1214	1185*	1191

*p<0.05; **p<0.01. Organ weights are mg (absolute) or mg/100 g bw (relative).

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 (Table 40). 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.

Table 40: Incidence of histopathological findings possibly related to treatment (number/number examined)

Tissue	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.

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 two 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 (Table 46). The NOEL for this study was 0.5 ppm, equal to 0.1 mg/kg bw/d, based on inhibition of erythrocyte and brain ChE activities at doses ≥4 ppm.

Rats

Schladt L (1995). E6876 (Folimat®) Study for chronic toxicity and carcinogenicity in Wistar rats following two-year administration in drinking water. 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. Study duration: February 1989 - March 1991. Guidelines: US EPA 83-5, 1984 GLP/QA: Yes. DTF Doc No: '537-001' Ref: 3-69/Vol 3-27 to 3-30

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, erythrocyte 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 erythrocyte 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 (omitting gamma glutamyl transpeptidase, globulin, LDH and CPK). Organs were examined, except lungs were weighed and thyroid was not. 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.

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. Haematological findings at 24 months are shown in Table 41.

Table 41: Haematology findings at 24 months

Parameter	Males				Females			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Hb (g/L)	155	151	149	142**	149	149	147	145
Hct (g/L)	0.491	0.477	0.469	0.438**	0.474	0.469	0.459	0.448
MCV (fL)	53	54	52	52*	56	55	54	54
MCHC (g/L RBC)	317	317	317	324*	315	318	319	324*
Thrombocytes (10^9/L)	1088	1105	1107	1277**	982	826**	953	1077

*p<0.05; **p<0.01; n=10.

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 findings are listed in Table 42.

Table 42: Clinical Chemistry at 24 months

Parameter	Males				Females			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
ALT (U/L)	43.9	45.9	49.4	52.7*	76.4	62.2	71.0	76.3
Cholesterol (mmol/L)	5.13	5.99	4.31	4.48*	3.16	2.51	2.95	3.00
Creatinine (µmol/L)	46	60	45	46	59	47*	48	45**
Total bilirubin (µmol/L)	2.8	2.6	2.4	2.3*	2.0	1.9	2.0	2.0
Albumin (g/L)	30.6	28.6	29.8	31.9	36.4	37.9	39.5*	39.6*
K (mmol/L)	4.7	4.7	4.7	4.6	4.6	4.1*	3.8**	3.7**
Plasma ChE (kU/L)	1.00	0.82 (18)	0.79* (21)	0.32** (68)	1.93	2.05	1.71 (11)	0.56** (71)
Erythrocyte ChE (kU/L)	0.81	0.65** (20)	0.21** (74)	0.03** (96)	0.73	0.63 (14)	0.21** (71)	0.02** (97)
Brain Che (U/g)	2.02	1.91 (5)	1.28** (37)	0.51** (75)	1.86	1.87	0.98** (47)	0.34** (82)

Numbers in parentheses represent % inhibition *cf.* control values; n=10; *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.

At macroscopic examination, lens opacity was identified in (males/females) 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 (Table 43). 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.

Table 43: Body & adrenal weights at 24 months (mg or mg/100 g bw)

Parameter	Males				Females			
	0 ppm	0.5 ppm	4 ppm	32 ppm	0 ppm	0.5 ppm	4 ppm	32 ppm
Body weight (g)	476	461	455	433**	279	281	279	281
Adrenals: absolute	64	67	65	60	73	76	81*	86**
: relative	13	15	14	14	26	28	29	31**

*p<0.05; **p<0.01;

The numbers of animals in which retinal degeneration was observed was similar at all doses (Table 44). 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.

Table 44: Incidences of non-neoplastic lesions observed at 24 months

Tissue	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%)[1], 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

[1] 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.

A NOEL was not achieved in this study, due to inhibition of erythrocyte 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 erythrocyte, plasma or brain at doses up to 0.3 ppm, equal to 0.03 mg/kg bw/d, the highest dose tested. Inhibition of erythrocyte 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 erythrocyte ChE activity in males at the next highest dose of 0.5 ppm.

Dogs

Hoffmann K & Schilde B (1984). S 6876 (Omethoate) Chronic toxicity to dogs on oral administration (Twelve-month stomach tube study). Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld. Sponsor: Bayer AG. Study duration: April 1981-April 1982. Guidelines: None GLP/QA: None. DTF Doc No: '537-003' Ref 3-57/Vol 3-26.

Methods Due to the instability of the test compound in the feed, administration was via stomach tube. A stock solution of omethoate was prepared in water at a concentration of 0.0125%, adjusted to pH 3-4 with HCl, and stored for no longer than 3 days. The stock solution was diluted in water immediately prior to dosing. Analytical checks of omethoate concentration in the solutions were performed periodically. Dose levels of 0.025, 0.125 or 0.625 mg/kg bw/d were based on a pilot study that showed effects on ChE activity at 0.625 mg/kg bw/d, but no effects at 0.025 mg/kg bw/d. Controls were dosed with water only. The test animals were beagle dogs (6/sex/dose; 30-32 weeks of age and bodyweight 6.7-9.7 kg at study initiation; from Marshall Research Animals, North Rose, NY, USA). They were fed daily at 1-2 h post-treatment, any uneaten food remaining available for about 18 h. Tap water was provided *ad libitum*. The state of the dogs' health was assessed daily, and periodic examinations included temperature and pulse rate checks, reflex tests and ophthalmological examinations. These checks, as well as haematology, clinical chemistry, urinalysis and ophthalmology tests, were conducted 1-2 weeks prior to the start of the study (week 0) and at weeks 5, 12, 25, 39 and 51. Cholinesterase activity in the plasma and erythrocyte was determined at these times, and also at weeks 2 and 8. Organs were weighed and examined microscopically, with the weights of the lungs, prostate and pancreas also measured. Food consumption was determined daily, and bodyweights were recorded weekly.

Results There were no premature deaths or clinical signs that could be linked to treatment. Bodyweight gains were slightly reduced (11-16%) in females at 0.125 and 0.625 mg/kg bw/d relative to controls. These differences lacked statistical significance, and given the small group size and the extent of the reduction, are unlikely to represent a toxicologically significant effect. Bodyweight gain in males was not related to dose, with treated males gaining slightly more weight than controls overall. No treatment-related changes were observed in physiological parameters or haematology, urinalysis and ophthalmology tests.

The only changes in clinical chemistry were reductions in ChE activity in the plasma and erythrocytes (Table 45). Inhibition of plasma ChE activity was limited to the 0.625 mg/kg bw/d groups, ranging from 16 to 32% throughout the study. Erythrocyte ChE activity was also consistently inhibited in males at 0.125 mg/kg bw/d (average 20% across all seven time points), except in week 51 (no inhibition). Despite the lack of change in week 51, the overall consistency of this finding at previous sampling points suggests a treatment-related effect at this dose. The corresponding inhibition in females was more variable, and averaged <15%, the extent of this change not considered to be of biological significance. At 0.025 mg/kg bw/d, inhibition of erythrocyte ChE activity reached 20% on two occasions in males, but as the overall average was ~13%, these were considered to be incidental findings. Brain ChE activity was clearly inhibited in both sexes at 0.625 mg/kg bw/d, with 21% inhibition in males at 0.125 mg/kg bw/d, but no inhibition in females at this dose. Organ weights were unaffected by treatment, and macroscopic and microscopic examinations revealed no findings attributable to exposure to the test material. The NOEL was 0.025 mg/kg bw/d due to inhibition of brain and erythrocyte ChE activity in males at 0.125 mg/kg bw/d.

Table 45: Cholinesterase activity & percent inhibition relative at 51 weeks

Dose (mg/kg bw/d)	Males				Females			
	0	**0.025**	**0.125**	**0.625**	**0**	**0.025**	**0.125**	**0.625**
Plasma (kU/L)	1.81	1.59 (12)	1.51 (16)	1.33 (26)	1.96	1.83 (12)	1.56 (16)	1.38 (26)
Erythrocyte (kU/L)	2.06	1.94 (5)	2.00 (3)*	0.39 (32)	2.26	2.09 (7)	1.82 (19)	1.36 (40)
Brain (U/g)	0.61	0.58 (4)	0.48 (21)	0.37 (39)	0.62	0.60 (3)	0.63 -	0.44 (30)

Numbers in parentheses represent % inhibition *cf.* control values. *Average 20% inhibition across all time points.

7 REPRODUCTION STUDIES

7.1 Technical grade active constituent

Rats

Brooker AJ & Stubbs A (1991). Dimethoate: dietary range finding study in mature male and female rats and their juvenile offspring. Huntingdon Research Center. England. Study duration: February – June 1989. Guidelines: not stated. GLP/QA: not stated. DTF Doc No: '453-004' Ref: 3-21/Vol 3-7.

Methods F0 male and female rats (10/sex/dose) were treated with 0, 50, 75 or 100 ppm dimethoate (Batch No. and purity not stated) in the diet for 4 weeks prior to mating, throughout the 3-week mating period, and treatment continued until all F1 litters had weaned. Rats (Crl:CD (SD)BR VAF/Plus strain, from Charles River, St. Aubin les Elbeuf, France) were 9 weeks old and within a 40/20 g weight range for males/females. All F0 rats were regularly handled and observed daily for clinical signs. Food consumption and body weights were measured weekly, and for females, body weights were measured daily during the mating period, and for Gestation Days (GD) 0, 7, 10, 14, 17 and 20. Dams that littered were weighed on Days 0, 7, 14 and 21 post partum. Mating performance, pregnancy rate and litter data were recorded and assessed. Homogeneity and stability of test material in the diet was not measured. Achieved intakes of dimethoate were estimated to have ranged from 0, 2.7-3.2, 4.2-4.8 and 5.8-6.4 mg/kg bw/d respectively, for F0 males, and 0, 3.6-4.2, 5.6-6.1 and 7.2-7.9 mg/kg bw/d for respectively, for F0 females. In the F1 generation, achieved intakes were 0, 7.7-8.6, 12.3-13.7 and 16.0-18.1 mg/kg bw/d respectively, for F1 males, and 0, 7.2-8.3, 12.4-13.7 and 16.1 -18.4 mg/kg bw/d respectively, for F1 females during Weeks 5-6, in an ascending dose order.

All F0 males and remaining females were sacrificed on Day 13 post parturition. Two F1 males and 2 females per litter where possible, were selected to be retained on the diets to 6 weeks of age. All F1 rats were regularly examined for signs of toxicity, and food consumption and body weight were monitored from Week 4. Prior to terminal sacrifice of F0 adults and the retained weanlings, samples of whole-blood were withdrawn from the orbital sinus for haematology (erythrocytes, Hb, Hct, MCV, MCH and MCHC), and measurement of ChE activity in plasma and erythrocytes. At termination, parents and pups were sacrificed and examined externally and internally for abnormalities. Brains were weighed and collected for ChE determination. The uterus was inspected for implantation sites. Testes of F0 males which failed to inseminate females were weighed and examined histologically. Statistical analyses were not performed.

Results There were no mortalities, and no overall treatment-related changes in body weight or food consumption. However, females with live young lost weight in the first week post partum at 75 and 100 ppm (-5 g and -15.6 g, respectively), whereas the control and 50 ppm groups gained weight (7.3 g and 1.6 g, respectively) over the same period. In addition, intermittent body tremors were observed in 3 and 6 females at 75 and 100 ppm, respectively, mainly during the first 2 weeks post partum and bulging eyes were seen in 2 females at 100 ppm, on one or more occasions. No treatment-related macroscopic changes were noted in post mortem examinations on F0 rats.

Mating performance was not affected, and the pregnancy rate was greater than 90% in all groups. The implantation rate showed an apparent dose-related reduction in all treated groups which, coupled with an increase in pre-birth losses at 75 and 100 ppm, resulted in a lower litter size at birth (Table 46). Although there were no instances of total litter loss before or after parturition, pup loss from birth to weaning was greater than control at 75 and 100 ppm, resulting in an apparent dose-related decrease in litter size by weaning. From Day 4, mean pup weights were also reduced in all treated groups and remained lower through to termination.

Table 46: Pup/litter data

Dose (ppm)	Female			
	0	**50**	**75**	**100**
Implantations (no of animal)	16.1	15.7	15.2	14.4
Pre-birth loss, No (%)	0.7 (3.8)	0.8 (5.9)	1.4 (10.9)	1.6 (10.0)
Litter size at birth, total/live	15.4/15.3	15.2/15.2	14.0/14.0	13.2/13.2
Litter size at Day 4	15.2	14.9	13.9	13.1
Litter size at Day 21	15.2	14.8	12.6	12.2
Mean pup weight at birth, g	6.3	6.2	6.2	6.3
Mean pup weight at Day 4, g	10.9	10.5	10.0	9.9
Mean pup weight at Day 21, g	48.6	43.7	40.6	41.4

Note: Statistical analyses were not performed.

For F1 rats retained to 6 weeks of age, body weight gains after weaning were similar among groups, although overall body weight remained slightly lower for males at 100 ppm. Haematology parameters were within normal ranges. There were no effects on brain weights of either generation.

Plasma ChE activity was inhibited (24-49%) at all doses in F0 females and 25-64% at 75 and 100 ppm in F0 males (Table 47). Erythrocyte ChE activity was inhibited 56-67% and 53-67% at all doses in F0 males and females, respectively. Similarly, brain ChE activity was decreased 44-56% and 53-68% in all treated F0 male and female groups, respectively. In the F1 generation, plasma ChE activity was inhibited 29-49% in males and 30-47% in females at all doses. Erythrocyte ChE activity was inhibited 61-76% and 60-74% in males and females respectively at all doses. Likewise brain ChE activity was decreased markedly in males (62-72%) and females (59-72%) at all doses.

Table 47: ChE levels in plasma, erythrocytes and brain

Dose (ppm)	Male				Female			
	0	**50**	**75**	**100**	**0**	**50**	**75**	**100**
F0 generation								
Plasma (µmol/mL/min)	0.44	0.38 (14)	0.33 (25)	0.28 (64)	1.16	0.88 (24)	0.79 (32)	0.59 (49)
Erythrocyte (µmol/mL/min)	2.05	0.91 (56)	0.74 (64)	0.67 (67)	1.62	0.76 (53)	0.57 (65)	0.54 (67)
Brain (µmol/g/min)	7.45	4.20 (44)	3.61 (52)	3.31 (56)	7.89	3.70 (53)	3.17 (60)	2.55 (68)
F1 generation								
Plasma (µmol/mL/min)	0.45	0.30 (33)	0.32 (29)	0.23 (49)	0.70	0.49 (30)	0.46 (34)	0.37 (47)
Erythrocyte (µmol/mL/min)	2.37	0.92 (61)	0.84 (65)	0.71 (76)	2.33	0.93 (60)	0.64 (73)	0.60 (74)
Brain (µmol/g/min)	7.19	2.75 (62)	2.62 (64)	2.00 (72)	7.58	3.13 (59)	2.30 (70)	2.14 (72)

Note: statistical analyses were not performed.

() Numbers in parentheses represent percentage inhibition compared with the control value.

No NOEL could be established in this study. The LOEL for reproductive toxicity was 50 ppm (3.6 mg/kg bw/d) based on reduced implantation rate at higher doses. The LOEL for maternal toxicity was 50 ppm (equal to 3.6 mg/kg bw/d), based on the inhibition of plasma, erythrocyte and brain ChE activity at and above this dose. The LOEL for paternal toxicity was 50 ppm (equal to 2.7 mg/kg bw/d), based on the inhibition of erythrocyte and brain ChE activity at 50 ppm and above. The LOEL for pup toxicity was 50 ppm (equal to 3.6 mg/kg bw/d) based on decreased pup weight and inhibition of plasma, erythrocyte and brain ChE activity at all doses.

Brooker AJ, Homan AB, Parker CA, Offer JM, Anderson A & Dawe IS (1992). The effect of dimethoate on reproductive function of two generations in the rat. Volume I, II and III. Huntingdon Research Center. Study duration: September 1989 – November 1990. Guidelines: US EPA 83-4(1982); OECD 416. GLP/QA: Yes. DTF Doc No: '453-003' Ref: 3-22/Vol 3-7 & 3-8.

Methods Dimethoate (Dimethoate Task Force; Batch No: 611A; Purity: 96.44%) was administered to rats at dietary levels of 0, 1, 15 and 65 ppm throughout two generations. Rats (Crl:CD (SD)BR VAF/Plus strain, from Charles River France Limited, St. Aubin les Elbeuf, France) were 7 weeks old, and 109-155 g for males and 100-139 g for females. F0 male and female rats (28/sex/dose) were treated from 10 weeks prior to their first of two matings (for F1a and F1b litters) until all F1 litters had weaned. The F1 generation selected from the F1a litters (24/sex/dose) was directly treated with the test substance from approximately 4 weeks of age until all F2 litters had weaned. F1 rats were first mated at 16 weeks of age, and re-mated approximately 10 days after the weaning of F2a pups to produce the F2b litter. Rats that had not been successful at either of their first two pairings were remated with those of proven fertility in the same group in a partial third mating (the dams were allowed to rear any young to Day 4 post partum).

The test substance was dissolved in ethanol (removed by air evaporation later) and mixed well with the diet, then stored frozen until required. The concentration, homogeneity and stability of the test substance in the diet were analysed. Daily premating intakes of the test substance were (male/female) 0, 0.05-0.09/0.06-0.09, 0.74-1.40/0.83-1.41, 3.28-6.13/3.75-6.03 mg/kg bw/d, respectively, for the F0 generation, and 0, 0.05-0.14/0.06-0.13, 0.72-2.13/0.86-1.98, 3.29-9.90/3.99-9.86 mg/kg bw/d for the F1 generation respectively.

All rats were observed daily for mortality, clinical signs and water consumption. Food consumption and body weight were measured weekly in general. Body weight of females was measured daily during the mating period, on GD 0, 7, 10, 14, 17 and 20, and on days 0, 7, 14 and 21 post partum for dams that littered. Mating performance, pregnancy rate and litter data were recorded and assessed.

Erythrocyte and plasma ChE activity was determined 2 weeks prior to each mating of F0 rats; four weeks post weaning, twoweeks prior to the first mating and the week preceding the second mating of F1 rats, and prior to terminal sacrifice of F0 and F1 rats. At termination, parents and pups were sacrificed and examined externally and internally for abnormalities. Brains were weighed and collected for ChE determination. The uterus was inspected for implantation sites. Testes of males which failed to inseminate females were weighed and examined histologically.

Organ weight analyses (for adrenals, heart, kidneys, liver, lungs, ovaries, prostate/seminal vesicles, testes/epididymides and thymus) were conducted on all F0 and F1 adults. Histopathological examinations on a range of reproductive tract tissues (ovaries, pituitary, prostate, testes, uterus and vagina) were conducted on all control and 65 ppm rats, as well as any apparently infertile rats in other groups of both F0 and F1 generations.

Litter data were assessed using the Kruskal-Wallis test followed by Shirley's test, except where 75% of values for a given variable consisted of one value, in which case a Fisher's exact test was used. Mean body weight gains, food and water consumption and cholinesterase determinations were analysed by one way anova followed by Williams test for comparison with control. Otherwise, the Kruskal-Wallis test followed by Shirley's test was used as described above. Organ weights were analysed by analysis of covariance, using terminal body weight as a covariate, followed by the Williams test for comparison with control. If there was no significant relationship between organ weight and body weight, analysis was carried out as described as for body weight gains, and food and water consumption above.

Results There were no unscheduled deaths in F0 rats, while 7 mortalities (1 male each at 15 and 65 ppm, and 4 control females and 1 female 65 ppm) in F1 groups were not attributed to treatment. Treatment with dimethoate did not significantly alter body weight or feed consumption in F0 or F1 parental animals; body weight gain was slightly lower in high-dose F0 and F1 females during gestation and lactation, however the results did not achieve statistical significance. At 65 ppm, water consumption was significantly ($p<0.05$) reduced at week 2 in F0 males and weeks 5 and 6 in F1 females. For F1 offspring, body weights of males and females at 65 ppm were lower than controls at the formal commencement of dosing (Week 4), reflecting differences established during their pre-weaning phase (see Table below).

The pregnancy rate was reduced to 71.4% at 65 ppm at the second mating of the F0 generation (Table 48). In the first mate of the F1 generation, the pregnancy rate was lower in all treated groups (stated significantly; statistics not shown), however the results were within the range of historical control values. At the second mating of the F1 generation, there appeared to be a dose-related decrease in pregnancy rates. While the results at 15 and 65 ppm did not achieve statistical significance, they were below the historical control values supplied by the study author. It was considered that while results at 15 ppm were equivocal, on the basis of these data it cannot be ruled out decreased pregnancy rate at 15 ppm was related to dimethoate treatment.

Table 48: Reproduction data

Dose (ppm)	F0 (n=28)				F1 (n=23-24)			
	0	**1**	**15**	**65**	**0**	**1**	**15**	**65**
Mate 1								
Pregnancy rate, %	92.9	96.4	85.7	89.3	95.8	70.8	70.8	62.5
Historical control, %	88-100				61-96			
No young born	2	1	4	2	1	2	5	3
Non-pregnant (no implantation sites)				1		5	2	6
Total litter loss post partum				1			2	1
Rearing young to weaning	26	27	24	24	23	17	15	14
Mate 2								
Pregnancy rate, %	89.3	92.6	89.3	71.4	72.7	66.7	58.3	50.0
Historical control, %	82-96				67-75			
No young born	3	2	3	7	6	3	8	6

Non-pregnant (no implantation sites)				1		5	2	6
Total litter loss post partum							1	
Rearing young to weaning	25	26	25	20	16	16	13	12
Mate 3								
Pregnancy rate, %					100	0	42.9	9.1
Infertile mated with proven females						2	4	4
Males: No young born						2	2	3
Induced pregnancy in females							2	1
Infertile mated with proven males						6	3	7
Females: No young born						1		1
Non-pregnant						5	2	6
With live young Day 4 post partum							1	
Males unsuccessful in first 2 pairings	2	1	2	2		2	5	4
Males unsuccessful in all 3 pairings						2	2	3
Females unsuccessful in first 2 pairings				1		6	4	7
Females unsuccessful in all 3 pairings						5	2	6

Treatment at 65 ppm was associated with a reduction in litter size at birth which persisted through to Day 4 prior to cull in F2b litters (slightly lower values though not statistically significant were seen in F1b, F2a and F2b litters; Table 49). Since no data on implantation rate and hence pre-birth loss were available due to multiple matings, it was not clear whether the reductions were due to *in utero* losses. An apparent decrease in F2b litter size at 15 ppm on Day 4 (precull) was also observed but the results were not significant. Mean pup weight was decreased at 65 ppm at days 4 or 21 at 65 ppm for litters F1a, F1b and F2a.

Table 49: Pup/litter data

Parameter	**0**	**1**	**15**	**65**	**0**	**1**	**15**	**65**
Mate 1	**F1a**				**F2a**			
Total litter size at birth	16.4	15.3*	15.3*	14.2**	12.3	11.9	14.6	12.0
Live litter size at birth	16.2	15.1	15.3	14.2**	12.1	11.7	14.5	12.0
Litter size, Day 4, precull/postcull	15.3 /8.0	14.6 /8.0	15.0 /8.0	13.9 /7.7	11.7 /7.2	11.4 /7.4	12.9 /7.5	10.7 /7.1
Day 21	7.9	7.9	7.9	7.6	7.1	7.4	6.9	6.5
Mean pup weight (g), at birth	6.1	6.3	6.5*	6.3*	6.4	6.5	6.3	6.3
Day 4 pre-cull	10.1	10.4	10.6	9.7	10.4	10.5	9.7	9.0
Day 21	60.2	61.4	59.9	53.4**	58.6	58.8	58.1	47.0**
Total litter loss post partum				1			1	1
Mate 2	**F1b**				**F2b**			
Total litter size at birth	14.9	14.9	14.2	14.3	14.1	13.3	13.1	10.0 (*)
Live litter size at birth	14.8	14.6	14.0	14.1	13.9	13.3	12.7	10.0 (*)
Litter size, Day 4, precull/postcull	14.5 /8.0	14.3 /7.8	13.7 /7.7	13.8 /8.0	13.8 /8.0	13.1 /7.8	7.0 /7.5	9.8(*) /6.8
Day 21	8.0	7.7	7.6	7.7	7.9	7.8	6.6	6.7
Mean pup weight (g), at birth	6.7	6.5	6.7	6.6	6.4	6.2	6.6	6.8
Day 4 pre-cull	11.1	11.0	10.9	10.1*	10.7	10.2	10.5	11.0
Day 21	63.7	64.8	63.9	56.6**	58.5	57.2	57.6	54.0
Total litter loss post partum								1

The 65 ppm group of F0 Mate 1, the 15 and 65 ppm groups of F1 Mate 1, and the 65 ppm group of F1 Mate 2 each included 1 female showing total litter loss post partum.
*p<0.05; **p<0.01, by Kruskal-Wallis H-statistic followed by intergroup comparison with the control (Shirley's test), () unsupported by H-statistics, and Fisher's exact test for litter size.

The mean age of attainment of the startle reflex F1a and F2a pups at 65 ppm (F1a: 34.8 *vs* 34.3 days, $p<0.05$; F2a: 35.2 *vs* 34.5 days, $p<0.01$) was slightly but significantly delayed compared to control; given the slight nature of the delay and the fact that these effects were not seen across all matings the relationship to treatment is unclear. Post weaning development of the selected F1 generation, as assessed by the mean age of vaginal opening for females and cleavage of the balanopreputial skin fold in males, was similar in all groups. Among all F1 and F2 offspring at weaning, there were no treatment related increases in the incidence of macroscopic and organ weight changes. Histological examination revealed an increased incidence of testicular atrophy or reduction in spermatogenesis in F1 males at 65 ppm (4/23 vs 1/24 in control, this value was close to the upper historical range 4/24). These effects were seen in only one testis, and these animals successfully induced pregnancy in females. No effects were apparent when the examination was extended to 1 and 15 ppm groups. The relationship to treatment was considered unclear.

As shown in Table 50, plasma ChE activity was inhibited ($p<0.01$) in F0 males (28-30%) and females (31-32%) at 65 ppm. Erythrocyte ChE activity was inhibited ($p<0.01$) at ≥ 15 ppm in males (17-67%) and females (36-65%), and brain ChE activity was decreased ($p<0.01$) 18-60% in males and 32-62% in females. In F1 males and females, plasma ChE activity was inhibited 19-33% and 34-41%, respectively, at 65 ppm. Erythrocyte ChE activity was decreased ($p<0.01$) 24-65% in males, and 27-69% in females, at ≥ 15 ppm. Brain ChE activity in F1 males and females was inhibited ($p<0.01$) 28—61% and 30-71%, respectively. A statistically significant (13%) decrease in brain ChE activity was also seen in PND 4 pups at 65 ppm. Minor (11-14%) statistically significant reductions in plasma ChE activity at 15 ppm at single time points were not considered toxicologically significant in F0 and F1 animals.

Table 50: ChE in plasma, erythrocytes & brain [μmol/mL/min or μmol/g/min]

Dose (ppm)	Male				Female			
	0	1	15	65	0	1	15	65
F0 generation								
Plasma, 15 weeks of age (8 week dosing)	0.43	0.43 (0)	0.40 (7)	0.31** (28)	1.39	1.33 (4)	1.25 (10)	0.96** (31)
39 weeks (32 week dosing, termination)	0.56	0.54 (4)	0.50* (11)	0.39** (30)	1.56	1.52 (3)	1.34** (14)	1.06** (32)
Erythrocyte, 15 weeks	1.74	1.73 (1)	1.31** (25)	0.62** (64)	1.83	1.95 (7)	1.17** (36)	0.83** (55)
39 weeks	1.69	1.65 (2)	1.41** (17)	<0.56** (67)	1.65	1.58 (4)	0.95** (42)	<0.58** (65)
Brain, 39 weeks	5.89	5.63 (4)	4.83** (18)	2.38** (60)	5.94	6.02	4.03** (32)	2.24** (62)
F1 generation								
Plasma, 8 weeks of age	0.49	0.47 (4)	0.46 (6)	0.33** (33)	1.01	1.00	0.89* (12)	0.60** (41)
44 weeks	0.54	0.54	0.51 (6)	0.44** (19)	1.59	1.76	1.55 (3)	1.05** (34)
Erythrocyte, 8 weeks	1.58	1.61	1.18** (25)	0.68** (57)	1.65	1.71	1.20** (27)	0.51** (69)
44 weeks	1.70	1.92	1.29** (24)	0.59** (65)	1.70	1.74	1.40 (18)	0.56** (67)
Brain, 4 days	2.78	2.62 (6)	2.57 (8)	2.42** (13)	2.47	2.61	2.70	2.57
44 weeks	7.87	8.13	5.64** (28)	3.07** (61)	6.73	6.61 (2)	4.73** (30)	1.97** (71)

*p<0.05; **p<0.01, by analysis of variance followed by intergroup comparison with control (Williams' test).
() Numbers in parentheses represent percentage inhibition compared with the control value.

The NOEL for reproductive toxicity was 1 ppm (0.05 mg/kg bw/d) based on decreased pregnancy rate at 15 ppm and 65 ppm. The NOEL for maternal and paternal toxicity was 1 ppm (0.05/0.06 mg/kg bw/d, respectively) based on inhibition of erythrocyte ChE in males and erythrocyte and brain ChE activity in females, at 15 and 65 ppm. The NOEL for pup toxicity was 15 ppm (0.7 mg/kg bw/d) based on decreased body weight.

Mellert W, Hellwig J, Gembardt C, Deckardt K, van Ravenzwaay B (2003b). Dimethoate – two-generation reproduction toxicity study in Wistar rats administration in the diet. BASF Aktiengesellschaft. Study duration: November 2000 – May 2002. Guidelines: EC 87/302/EEC. OECD 416. EPA OPPTS 870.3800. GLP/QA: Yes. DTF Doc No: '453-007' Ref: 3-43/Vol 3-22 to 3-24.

Methods A daily dose of 0, 0.2, 1.0 and 6.5 mg/kg bw/d dimethoate (Source: Cheminova Agro A/S; Batch: 20522-00; purity: 99.1%) was administered to Wistar rats (F0, 25/sex/dose) in the diet throughout the study. The rats (CrlGlxBrlHan:WI, from Charles River Deutschland GmbH, Sulzfeld, Germany) were 35±1 days old and weighed 81-108 g for males and 78-101 g for females. At least 75 days after the beginning of treatment, F0 male and female pairs from the same dose group were mated to produce the first litter (F1a) and subsequently remated (after about 20 weeks of treatment or at least 10 days after weaning of F1a pups) to produce a second litter (F1b). F1a rats (25/sex/dose) selected post weaning for further breeding were continuously dosed for approximately 10 weeks at the same dose level as their parents, and the breeding schedules were repeated to produce F2a and F2b litters.

All parental animals were observed for mortality and morbidity twice daily, and clinical signs were monitored daily. Body weight was measured at weekly intervals until termination; assumed pregnant females were weighed on GD 0, 7, 14 and 20. Food consumption was determined weekly; for pregnant females food consumption was measured at days 0-7, 7-14, and 14-20 of gestation as well as for lacatation days 1-4, 4-7 and 7-14. Water consumption was determined once per week (over a period of 3 days) for F0 and F1 males and females. Additionally, water consumption was measured for females at GD 0-1, 6-7, 13-14 and 19-20 and during lactation at days 1-2, 4-5, 7-8, and 14-15. For both F0 and F1 generations, parental animals were examined for their mating and reproductive performances. Mating and fertility indices for males and females, and female reproduction and delivery parameters were determined. Estrous cycle was examined in F0 and F1 females over a 3-week period prior to mating until evidence of mating occurred. The estrous stage of each female was also determined on the day of scheduled sacrifice. Sperm motility was determined for all groups of F0 and F1 males, while sperm head count (cauda epididymis and testis) and morphology were assessed at termination for control and 6.5 mg/kg bw/d groups.

All F0 and F1 parents were sacrificed after weaning of their pups, and were assessed for organ weight (liver, kidneys, epididymides, testes, uterus with oviducts and cervix uteri, ovaries, seminal vesicles with coagulating glands and their fluids, prostate gland, brain, pituitary gland, adrenal gland, spleen, thyroid glands with parathyroid glands) and by gross-, and histo-pathological examination (left epididymis, seminal vesicles, coagulation glands and prostate gland from all dose groups; vagina, cervix uteri, uterus, ovaries, oviducts, left testis, pituitary gland and adrenal glands from all rats at 0 and 6 mg/kg bw/d and from those in other groups suspected of impaired fertility; brain, liver, kidneys and spleen, pituitary gland and thyroid glands with parathyroid glands from F1 rats of all dose groups; all other tissues with gross lesions), with special attention on reproductive organs. A quantitative assessment of

primordial and growing follicles in the ovaries was performed in all F1 parental females at 0 and 6.5 mg/kg bw/d.

F1 and F2 pups were sexed, and weighed on PNDs 0, 4, 7, 14 and 21, and their viability was recorded. All F1 and F2 litters were standardised on PND 4 to 4 pups/sex/litter, if possible. With the exception of F1a pups which were selected for F1 parents, all pups were sacrificed on PND 4 after culling or 21 after weaning. All pups, including stillborn pups and those that died during the rearing period, were examined macroscopically, and weights of brain, spleen and thymus were measured in 1 pup/sex/litter. Sexual maturation (days of preputial separation for males, and vaginal opening for females) of all F1 pups selected to become parents was also determined.

Blood samples were taken without anaesthesia from fasted F0 parents pre-dosing, and F0 and F1 parents prior to mating and before terminal sacrifice. Brain samples were taken at necropsy from all F0 and F1 parental rats, and from 1 male and female pup/litter of F1b and F2b pups in the control and the high dose group on PND 4. Measurement of brain, serum and erythrocyte ChE activity were conducted on these samples. The hematocrit values in the blood were determined in order to calculate erythrocyte ChE activities per litter.
Statistical analysis was conducted for various parameters using the Kruskal-Wallis test; if significant differences were detected, pairwise comparison was conducted. In some cases only pairwise comparison was conducted.

Results In general, the measured daily intakes of the test substance correlated well with the desired target concentrations (Table 51).

Table 51: Measured intake of the test substance

Dose (mg/kg bw/d)	F0			F1		
	0.2	**1.0**	**6.5**	**0.2**	**1.0**	**6.5**
Males	0.1-0.3	0.8-1.2	5.1-7.7	0.2	0.8-1.2	5.4-8.1
Females, premating	0.1-0.3	1.0-1.1	6.5-8.7	0.2	1.0-1.2	6.6-7.8
Gestation for F1a or F2a litter	0.2-0.3	1.0-1.1	6.5-7.4	0.2	0.9-1.1	5.9-7.1
Lactation for F1a or F2a litter	0.2-0.3	0.7-1.2	5.4-7.9	0.1-0.2	0.7-1.1	4.8-7.3
Gestation for F1b or F2b litter	0.2	0.9-1.1	6.1-6.9	0.2	0.9-1.1	6.0-7.1
Lactation for F1b or F2b litter	0.2	0.7-1.1	4.7-7.2	0.1-0.2	0.8-1.1	4.6-6.9

All F0 rats were killed as scheduled. In F1 parental rats, mortality occurred in 3 males and 1 female at 1.0 mg/kg bw/d, and 1 male and 1 female at 6.5 mg/kg bw/d, and all died during treatment days 44-193. Clinical examination, gross necropsy and histopathological findings suggested that the unscheduled deaths were not treatment-related. Similarly, there were no clinical signs in F0 and F1 parental rats that could be clearly attributed to treatment.
Body weight and food consumption were comparable between control and treated animals with the exception of a significantly decreased body weight gain (40% of the control; $p \leq 0.05$) in F2b dams at 6.5 mg/kg bw/d during lactation. Water consumption was unaffected by treatment; a significant ($p<0.05$) increase (16%) in water consumption was observed for F0 females during weeks 4-5 of treatment, but the effect was not dose related and considered incidental to treatment.

The test compound did not affect reproductive performance of the F0 and F1 males and females. Fertility indices for treated groups were mostly similar to, or greater than the control group for F0 and F1 males and females (Table 52). The scattered occurrence of a few non-breeding females throughout the different groups (4, 1, 0 and 2 for F1a litters; 1, 3, 4 and 0 for

F1b litters; 2, 1, 1 and 0 for F2a litters; and 1, 0, 3 and 0 for F2b litters at 0, 0.2, 1.0 and 6.5 mg/kg bw/d respectively) was not considered related to treatment, and no gross and histopathological findings accounted for the observed infertility.

Table 52: Fertility indices

Dose (mg/kg bw/d)	Male				Female			
	0	0.2	1.0	6.5	0	0.2	1.0	6.5
F1a litters	84	96	100	92	84	96	100	92
F1b litters	96	88	84	100	96	88	84	100
F2a litters	88	96	96	100	92	96	96	100
F2b litters	88	100	88	100	96	100	88	100

Other reproduction parameters including estrous cycle, mating behaviour, conception, gestation and parturition were similar between treatment groups and control. No effects were seen on sperm morphology, enumeration in the cauda epididymis or sperm motility in F0 or F1 animals.

Ten deaths out of a total of 251 F1b pups at 6.5 mg/kg bw/d during PNDs 1-4 led to a significantly reduced viability index (96% *vs* 99% in control, $p<0.05$). However, the mortality occurred primarily in just one litter (6/8 pups died/were cannibalised on PND 1), which was within the incidence range of the historical control (94-100%) provided by the sponsor. In addition, pup weight to weaning of this group was slightly but significantly ($p\leq0.05$) lower (7% lower than control) due to lower weight gain over the majority of the lactation period. Other F1 pup/litter data were comparable across dosing groups.

As shown in Table 53, treated groups in the F2 generation had slightly higher incidences of stillborn pups, but the live birth indices were within historical control range (96-100%). There was also a higher rate of F2b pup death at 6.5 mg/kg bw/d, and the lactation index of this group (94%) was out of the historical control range (95-100%). The increased rate of deaths, considered related to treatment, was attributed to a single litter, which were not properly nursed and were cannibalized between days 4 and 6 of lactation. There were no significant findings in organ weight and necropsy of pups, except slightly but significantly lower relative brain weight of F2b pups at 6.5 mg/kg bw/d on PND 4, but not on PND 21.

Table 53: Litter/pup data

Dose (mg/kg bw/d)	0	0.2	1.0	6.5
F1b pup death, PND 0-4	2	1	1	10
F1b viability index	99	100	100	96
F2a: Stillborn, pup/in litter	0/0	2/2	2/2	3/1
F2a Live birth index	100	99	99	99
F2b: Stillborn, pup/in litter	1/1	5/1	3/3	5/4
F2b Live birth index	100	98	99	98
F2b pup death, PND 4-21	0	3	1	12
F2b Lactation index	100	98	99	94
F2b brain weight, PND 4, g	0.42	0.43	0.44	0.41
% body	4.99	4.80	4.79	4.56**

**p<0.01 by Kruskal-Wallis + Wilcoxon-test.

As shown in Table 54, in F0 animals, serum ChE activity was inhibited only at the end of treatment at the high-dose in females. Erythrocyte ChE activity was decreased at 6.5 mg/kg bw/d in F0 males and females prior to mating and at study termination. A minor (9%), statistically significant decrease in erythrocyte ChE activity at 1.0 mg/kg bw/d in males was

not considered toxicologically significant. Brain ChE activity was decreased in F0 males at ≥ 1.0 mg/kg bw/d and in females at 6.5 mg/kg bw/d.

In the F1 generation, serum ChE activity was inhibited only in females at 6.5 mg/kg bw/d at the end of treatment. Erythrocyte ChE activity was inhibited in males and females at 6.5 mg/kg bw/d. Brain ChE activity was decreased at ≥ 1.0 mg/kg bw/d in females and 6.5 mg/kg bw/d in males. Minor statistically significant (10-13%) inhibitions of erythrocyte and brain ChE activity in males at 1.0 mg/kg bw/d were not considered toxicologically significant. High dose F1b female pups showed slight (9%) statistical significant decreases in brain ChE activity at PND 4. No decrease in ChE activity was seen in male F1b pups at PND 4 or F2b pups at PND 4. Pups at lower doses were not examined.

Table 54: ChE levels in serum, erythrocytes [Mykat/L(% control)] & brain [Mykat/g]

Dose (mg/kg bw/d)	Male				Female			
	0	**0.2**	**1.0**	**6.5**	**0**	**0.2**	**1.0**	**6.5**
F0, prior to mating								
SChE	10.4	11.0	11.7**	10.4	58.2	54.7 (6)	53.4 (8)	51.4 (12)
EChE	31.6	32.6	29.6 (6)	23.4*** (26)	32.2	32.4	30.1 (7)	17.4*** (46)
F0, end of treatment								
SChE	11.2	12.1	12.3**	10.9 (3)	46.4	43.3 (7)	42.7 (8)	36.6*** (21)
EChE	34.7	33.0 (5)	31.8** (9)	22.0*** (37)	32.0	33.1	29.8 (7)	12.9*** (60)
BChE	2.26@	2.26	1.80* (20)	1.09*** (52)	2.46	2.66	2.03 (17)	0.76*** (69)
F1, prior to mating								
SChE	10.6	11.4	11.2	10.1 (4)	43.5	44.3	46.2	41.4 (5)
EChE	32.4	31.8	30.4* (6)	20.8*** (36)	31.5	32.9	31.4	21.1*** (36)
F1, end of treatment								
Dose (mg/kg bw/d)	**Male**				**Female**			
	0	**0.2**	**1.0**	**6.5**	**0**	**0.2**	**1.0**	**6.5**
SChE	11.8	12.6	12.5	11.0 (7)	41.9	41.7	41.7	34.3* (18)
EChE	36.8	34.3	33.1** (10)	20.6*** (44)	31.8	32.0	30.4 (5)	13.4*** (58)
BChE	1.68	1.66	1.47** (13)	0.70*** (58)	1.62@	1.46 (10)	1.30*** (20)	0.52*** (68)
F1b pups PND 4, BChE	1.07	-	-	1.03 (4)	1.12	-	-	1.02** (9)
F2b pups PND 4, BChE	1.23	-	-	1.16 (6)	1.24	-	-	1.15 (7)

*p<0.05; **p<0.02; ***p<0.002. BChE, brain cholinesterase levels; EChR, erythrocyte levels; SChE, serum levels. @: Excluding 1 or 2 abnormal high values (2-3 times higher than most of the other values) in the control group.Numbers in parentheses represent % inhibition *cf.* control. – pups not examined at this dose.

There were no significant changes in absolute and relative organ weights of F0 rats. For F1 parental rats, slight but significant weight changes of the pituitary gland and thyroid were observed in males or females at 6.5 mg/kg bw/d and/or lower doses (see Table below). These changes were observed in a single sex, not associated with morphologic changes and did not exhibit clear dose response relationships, and were therefore not considered clearly attributable to treatment.

Reduced prostate weight in F1 males at 6.5 mg/kg bw/d, was associated with an increased incidence of male rats exhibiting diffuse atrophy of the glandular epithelium, graded moderate to severe, accompanied by an increased incidence and severity of reduced secretion. The morphologic characteristic of focal or diffuse atrophy of the epithelium of the dorso-lateral part of the prostate were changes in the shape of the epithelium from an upright cylindrical or cuboidal shape and vesicular, rounded to oval, upright nuclei to flattened, "endothelial-like" cell with inconspicuous cytoplasm and small, pale basophilic nuclei. Reduced secretion ranged from fading of fluid density over flocculated residues of fluid to absence of any fluid in the acini. Microscopic examination revealed minimal to moderate focal vacuolization of the epithelium of the corpus epididymidis in F0 male rats at 6.5 mg/kg bw/d. Similar findings in the epididymides were also observed in F1 males (Table 55).

Table 55: Selected organ weight & pathological findings

Dose	**F0**				**F1**			
(mg/kg bw/d)	**0**	**0.2**	**1.0**	**6.5**	**0**	**0.2**	**1.0**	**6.5**
Male								
Prostate, g	-	-	-	-	1.15	1.13	1.08	0.96**
% body	-	-	-	-	0.26	0.26	0.24	0.23*
Pituitary, g	-	-	-	-	9.88	9.24	9.78	8.88*
% body	-	-	-	-	0.002	0.002	0.002	0.002
Female								
Thyroid, g	-	-	-	-	16.9	21.4**	22.5**	19.0
% body	-	-	-	-	0.007	0.009**	0.009**	0.008
Pituitary, g	-	-	-	-	12.4	14.4**	14.6**	13.8*
% body	-	-	-	-	0.005	0.006**	0.006**	0.006**
Male								
Epididymides*, -vacuolisation, focal	-	-	-	1+, 1++, 5+++	-	-	-	8++, 4+++; 1++++
Dose	**F0**				**F1**			
(mg/kg bw/d)	**0**	**0.2**	**1.0**	**6.5**	**0**	**0.2**	**1.0**	**6.5**
Prostate*, - reduced secretion	2+, 4++, 2+++	3+, 1++, 1+++	1+, 8++, 2+++	3+, 5++, 2+++	1+, 7++, 4+++	3+, 5++, 4+++, 2++++	8++, 7+++	6++, 6+++, 7++++
- epithelial atrophy, diffuse	**4+++**	**1+++**	**2+++**	**4+++, 1++++**	6+++, 1++++	2+++, 2++++	1+++, 1++++	9+++, 6++++

Numbers in parentheses represent % inhibition *cf.* control.* Numbers represent the number of animals whereas + represents severity (+, grade 1; ++ grade 2; +++, grade 3; ++++, grade 4)

Under the conditions of this study, the NOEL for reproductive toxicity was 6.5 mg/kg bw/d. The NOEL for maternal toxicity was 0.2 mg/kg bw/d, based on the inhibition of brain ChE activity at 1 mg/kg bw/d. The NOEL for paternal toxicity was 0.2 mg/kg bw/d based on the inhibition of erythrocyte and brain ChE activity at 1 mg/kg bw/d or above. The NOEL for pup toxicity was 1.0 mg/kg bw/d, based on slightly increased pup deaths during lactation at 6.5 mg/kg bw/d.

7.2 Metabolites

Rats

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, Research and Consulting Company Ltd, PO Box CH 4452 Itingen, Switzerland. Study duration: April - July 1989. Guidelines: None stated GLP/QA: Yes. DTF Doc No: '553-001' Ref: 3-72/Vol 3-34.

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 dietary levels of 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, erythrocyte 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. 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 in mg/kg bw/d is summarised in Table56.

Table 56: Adult test compound intake

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

Values refer to ranges in mg/kg bw/d.

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 (Table 57). 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.

Table 57: 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 one 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.

Erythrocyte 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 (Table 58), this was considered to be due to treatment also.

Table 58: ChE activity in dams on postnatal day 21

Tissue	0 ppm	10 ppm	30 ppm	90/50 ppm
Plasma (µmol-SH/mL	1.49 ± 0.15	1.16 ± 0.55 (22)	1.01 ± 0.22 (32)	0.84* ± 0.12 (44)
Erythrocyte (µmol-SH/mL	1.95 ± 0.72	0.49** ± 0.29 (75)	0.59** ± 0.24 (70)	0.52** ± 0.10 (73)
Brain µmol-SH/g	4.31 ± 0.50	3.02** ± 0.16 (30)	2.53** ± 0.18 (41)	2.31** ± 0.13 (46)

Numbers in parentheses represent % inhibition *cf.* control. n = 5. *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 (Table 59), but this was considered secondary to lower body weight in these groups.

Table 59: Organ weights in pups

Parameter	0 ppm	10 ppm	30 ppm	90/50 ppm
Body weight (g) – males	41.8	38.9	33.9**	32.4, 32.3*
Body weight (g) – females	40.8	38.1	34.9**	26.4, 29.9
Brain – absolute (g) – males	1.41	1.37	1.37	1.37, 1.37
– relative to bw	3.39	3.55	4.08 **	4.23 **
Brain – absolute (g) – females	1.37	1.31	1.34	1.26, 1.32
– relative to bw	3.36	3.46	3.87 **	4.57 **
Testes – absolute (g)	0.22	0.19*	0.18*	0.15, 0.16*
– relative to bw	0.52	0.49	0.52	0.45, 0.50
– relative to brain wt	15.44	13.87	12.98*	10.71, 11.78*
Ovaries – absolute (g)	0.016	0.016	0.012	0.008, 0.011
– relative to bw	0.040	0.041	0.035	0.031, 0.036
– relative to brain wt	1.191	1.201	0.907	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 in mg/kg bw/d is summarised in Table 60.

Table 60: Subgroup test compound intake

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. values represent ranges in mg/kg/ bw/d.

Cholinesterase activities in erythrocytes 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 (Table 61). 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.

Table 61: ChE activity (µmol-SH/mL or µmol-SH/g)

Dose (ppm)	Males				Females			
	0	**10**	**30**	**90/50**	**0**	**10**	**30**	**90/50**
Plasma	0.57	0.53 (7)	0.45** (20)	0.45** (20)	2.00	1.43* (28)	0.96** (52)	0.65** (68)
Erythrocyte	1.84	0.67** (64)	0.56** (69)	0.53** (71)	1.90	0.80** (58)	0.44** (77)	0.66** (65)
Brain	4.95	3.78** (24)	2.85** (42)	2.66** (46)	4.65	3.24** (36)	3.02** (35)	2.79** (40)

Numbers in parentheses represent % inhibition *cf.* control value; n=5.

Effects were seen at all doses in this study. These included inhibition of plasma, erythrocyte 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. RCC Research and Consulting Company AG, Itingen, Switzerland. Study duration: September 1989 – July 1990. Guidelines: US EPA 83-4, OECD 416 GLP/QA: Yes. DTF Doc No. '553-002' Ref: 3-73/Vol 3-35 to 3-36

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, erythrocyte 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 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 in mg/kg bw/d is shown in Table 62.

Table 62: Amount (mg/kg bw/d) of omethoate ingested

	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

In the P generation, two 18 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 18 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 (Table 63).

Table 63: 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.

Plasma ChE activity was reduced in both sexes at 18 ppm, but this was not consistent for the two generations (Table 64 below). 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 erythrocyte 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 erythrocyte 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) (Table 64).

Table 64: Cholinesterase activity in plasma, erythrocyte & brain

Tissue	**Generation/sex**		**0 ppm**	**0.5 ppm**	**3 ppm**	**18 ppm**
Plasma	P	Male	0.65	0.60 (7)	0.60 (7)	0.49** (24)
(µmol-SH/mL)		Female	1.13	1.03 (9)	1.07 (6)	0.91 (20)
	F1	Male	0.67	0.75*	0.73	0.64
		Female	2.29	2.29	2.33	1.42** (38)
Erythrocyte	P	Male	1.73	1.60 (8)	0.91** (47)	0.31** (82)
(µmol-SH/mL)		Female	1.72	1.38** (20)	0.99** (42)	0.25** (85)
	F1	Male	1.84	1.70 (8)	1.11** (40)	0.34** (82)
		Female	1.48	1.33* (10)	0.60** (59)	0.35** (76)
Brain	P	Male	4.61	4.56	3.80** (18)	2.82** (39)

(µmol-SH/g)		Female	4.11	4.00 (7)	3.26** (21)	2.70** (34)
	F1	Male	7.55	6.84* (9)	5.32** (30)	3.35** (54)
		Female	6.75	5.68** (16)	4.72** (30)	3.04** (55)
	F1	Male pups	6.05	5.92	6.25	5.80
		Female pups	6.33	6.30	6.33	5.56** (12)
	F2	Male pups	7.77	7.57	7.60	6.44** (17)
		Female pups	8.08	7.67	6.86** (15)	6.45** (20)

Numbers in parentheses represent % inhibition *cf.* control value; n=10.
*p≤0.05, **p≤0.01

The parental NOEL for this study was 0.5 ppm (~0.04 mg/kg bw/d), due to inhibition of erythrocyte 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 Technical grade active constituent

Rats

Edwards JA, Leeming NM & Clark R (1984a). Effects of dimethoate on pregnancy of the rat. Huntingdon Research Centre. Guidelines: OECD 414 GLP/QA: yes
[Note: This study has been evaluated previously by OCSEH (Submission No. 1345)]

Dimethoate technical (Batch No: 611; Purity 97.3%) in 1% aqueous methylcellulose was administered to rats (GL:COBS CD (SD) BR strain, 25/group; 170-201 g) by gavage at doses of 0, 3, 6, and 18 mg/kg bw/d from day 6 up to and including day 15 of pregnancy. Animals were killed on day 20 of pregnancy and litter values determined and foetuses examined for visceral and skeletal abnormalities.

Significant signs of maternal toxicity (cholinergic effects) were observed at 18 mg/kg bw/d. These included hypersensitivity, tremors, abnormal gait and reduced weight gain and food intake. At 3 and 6 mg/kg bw/d transient salivation was seen post dosing. There were no instances of total litter loss. The mean incidence of skeletal anomalies was higher in all treated groups than in control, however similar anomalies occurred (at a similar incidence) in historical controls. A single foetus at 6 mg/kg showed retro-oesophageal aortic arch. No malformations were observed at 3 and 18 mg/kg bw/d. Dimethoate was not teratogenic under the conditions of the study.

Rabbits

Edwards JA, Leeming NM & Clark R (1984b). Effects of dimethoate on pregnancy of the New Zealand White Rabbit. Huntingdon Research Centre. Guidelines: OECD 414 GLP/QA: yes.
[Note: This study has been evaluated previously by OCSEH (Submission No. 1345)]

Methods Mated New Zealand white rabbits (16/group: 2.9-4.1 kg) received dimethoate technical (Batch No. 611A; Purity 97.3%) in 1% methylcellulose by gavage at doses of 0, 10, 20 and 40 mg/kg bw/d from day 7 to day 19 of gestation. On day 29, animals were killed, litter values determined and foetuses examined for visceral and skeletal abnormalities.

Results Significant signs of maternal toxicity including muscle tremors, unsteady gait and reduced body weight gain were observed in does at 40 mg/kg bw/d. Reduction in foetal body weight gain was observed at this dose. There were no obvious adverse effects of dimethoate on litter size, upon post implantation losses, on incidence of malformations, visceral and skeletal anomalies or on skeletal variants at any of the treatment levels. Dimethoate was not teratogenic in rabbits.

8.2 Metabolites

Rats

Holzum B (1990a). E 6876 (common name omethoate): Study for embryotoxic effects on rats following oral administration. Department of Toxicology, Bayer AG, Wuppertal, FRG. Study duration: January 1989 – December 1989. Guidelines: US EPA 83-3, OECD 414 GLP/QA: Yes. DTF Doc No: '551-001' Ref: 3-74/Vol 3-36

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 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 muzzle. 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 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.

Rabbits

Holzum B (1990b). E 6876 (common name: omethoate) Study for embryotoxic effects on rabbits following oral administration. Department of Toxicology, Bayer AG, Wuppertal, FRG Study duration: May 1989–January 1990. Guidelines: US EPA 83-3, OECD 414 QA/GLP: Yes. DTF Doc No. '551-002' Ref 3-75/Vol 3-36.

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, erythrocyte 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 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 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. Additionally, 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 erythrocyte ChE activities were reduced on gestation days 14 and 19 (Table 65). Plasma ChE activity was inhibited at 5 mg/kg bw/d, while inhibition of erythrocyte and brain ChE activities at 1 and 5 mg/kg bw/d was dose-related. The NOEL for maternal and foetal effects was 0.2 mg/kg bw/d. This is based on inhibition of erythrocyte and brain ChE activity in the does, and foetal malformations at ≥1 mg/kg bw/d.

Table 65: Maternal cholinesterase activity in plasma, erythrocyte and brain in kU/L

Dose (mg/kg bw/d)		0	0.2	1	5
GD 14	Plasma	0.55	0.64**	0.53	0.31 (43)***
	Erythrocyte	0.73	0.68	0.53 (27)**	0.19 (74)***
GD 19	Plasma	0.47	0.46	0.42 (10)	0.29 (38)**
	Erythrocyte	0.68	0.64	0.40 (41)***	0.09 (87)***
	Brain	3.77	3.12 (17)	2.39 (36)**	1.31 (65)***

Numbers in parentheses represent % inhibition *cf.* control value; n=5. **p≤0.01, ***p≤0.001

8.3 Formulations

Khera KS (1979). Teratogenicity evaluation of commercial formulation of dimethoate (Cygon 4E) in the cat and rat. *Toxicol. App. Pharmacol.* 48, A34.

Khera KS, Whelan C, Trivett G & Angers (1979). Teratogenicity studies on pesticidal formulations of dimethoate, diuron, and lindane in rats. *Bull. Environ. Contam. Toxicol.* 22, 522-529.
[Note: This study has been evaluated previously by OCSEH (Submission No. 1345)]

Mated female Wistar rats (20/group) received, by gavage, doses of dimethoate formulation at levels of 0, 3, 6, 12 or 24 mg/kg bw/d on days 6 to 15 of gestation. The formulation used, Cygon 4E, contained 47.3% dimethoate; the non-active constituents were unknown. The animals were necropsied on day 22. Eight dams of the high dose group showed signs of toxicity including clonic spasms and muscular tremors. No signs of maternal toxicity were observed in the other treated groups. There were no changes in reproduction parameters with dimethoate treatment. A significant increase in the incidence of skeletal anomalies (way ribs, extra ribs) was seen in foetuses of the 12 and 24 mg/kg groups. The NOEL in foetuses was 6 mg/kg bw/d of the formulation (2.8 mg/kg bw/d of dimethoate).

Mated female cats received single daily doses of dimethoate formulation (Cygon 4E containing 47.3% dimethoate) in gelatin capsules at levels of 0, 3, 6 or 12 mg/kg bw/d from day 14 to day 22 of gestation. Animals were necropsied on day 43 of gestation. There were no signs of maternal toxicity in any of the treatment groups. In the high dose group, polydactyly was observed in 8 of 39 foetuses. This was significantly different from controls. The NOEL was 6 mg/kg bw/d for the formulation or 2.8 mg/kg bw/d of dimethoate.

9 GENOTOXICITY STUDIES

9.1 Active constituent

In vitro studies

A summary of findings from *in vitro* dimethoate genotoxicity studies is shown in Table 101.

Engelhart G (1993). Ames salmonella/mammalian-microsome mutagenicity test and Escherichia coli/mammalian microsome reverse mutation assay. BASF Aktiengesellschaft, Department of Toxicology, Z 470, D-67056 Ludwigshafen/Rhein, FRG. Study duration: February – March 1992. Guidelines: OECD/EEC/US FIFRA. GLP/QA: yes. BASF. DTF Doc No: '457-010' Ref: 3-14/Vol 3-6.

Methods In the Ames test and in the *Escherichia coli* – reverse mutation assay, Dimethoate Technical (Source: Not stated; Batch No: 611 A; Purity: 98.45%) was tested for its mutagenic potential at concentrations: 20 – 8000 μg/plate for *Salmonella typhimurium* TA100 and *E. coli* WP2 uvrA; and 20 – 5000 μg/plate for TA1535, TA1537 and TA98, in the presence and absence of metabolic activation (S9 mix).

Results Bacteriotoxic effects, evidenced as a reduction in bacterial background lawn, were not observed at any dose, in the presence or absence of metabolic activation. There were no increases in the number of his+ revertants on tester strains TA1535, TA1537 and TA98 (Table 66). In two independent tests on TA100, a slight but dose-related increase in the number of mutant colonies was observed from 2000 – 2500 μg/plate onward with and without S9 mix (Table 68). Repeated tests also detected a dose-related increase in the number of trp+ revertants of *E. coli* WP2 uvrA induced by high doses of dimethoate (from 500 μg/plate or higher) with or without S9 mix.

Table 66: Findings in the reverse mutation assays [Revertants/plate (quotient)]

Conc (μg/plate)	0	20	100	500	2000	2500	4000	5000	6000	8000	Positive*
TA 100 (- S9) – Test 1	101 (1.0)	97 (1.0)	96 (0.9)	117 (1.2)	-	155 (1.5)	-	197 (1.9)	-	-	612 (6.0)
TA 100 (+ S9) – Test 1	116 (1.0)	128 (1.1)	127 (1.1)	143 (1.2)	-	175 (1.5)	-	243 (2.1)	-	-	713 (6.1)
TA 100 (- S9) – Test 2	100 (1.0)	-	-	-	163 (1.6)	-	189 (1.9)	-	195 (1.9)	287 (2.9)	756 (7.5)
TA 100 (+ S9) – Test 2	142 (1.0)	-	-	-	174 (1.2)	-	254 (1.8)	-	216 (1.5)	291 (2.1)	1293 (9.1)
E.coli WP2 uvrA (- S9) – Test 1	14 (1.0)	15 (1.0)	19 (1.3)	24 (1.6)	-	28 (2.0)	-	37 (2.6)	-	-	148 (10.3)
E.coli WP2 uvrA (+ S9) – Test 1	45 (1.0)	43 (1.0)	35 (0.8)	46 (1.0)	-	57 (1.3)	-	61 (1.4)	-	-	43 (1.0)

E.coli WP2 uvrA (- S9) – Test 2	28 (1.0)	28 (1.0)	29 (1.1)	31 (1.1)	-	62 (2.2)	-	88 (3.2)	-	-	507 (31)
E.coli WP2 uvrA (+ S9) – Test 2	35 (1.0)	39 (1.1)	39 (1.1)	51 (1.5)	-	64 (1.8)	-	78 (2.2)	-	-	364 (10.4)
E.coli WP2 uvrA (- S9) – Test 3	23 (1.0)	-	-	-	35 (1.5)	-	51 (2.2)	-	56 (2.4)	89 (3.8)	437 (18.7)
E.coli WP2 uvrA (+ S9) – Test 3	48 (1.0)	-	-	-	57 (1.2)	-	85 (1.8)	-	80 (1.7)	122 (2.6)	117 (2.4)

*positive control: N-methyl-N'-nitro-N-nitroso-guanidine for TA100 (-S9); N-ethyl- N'-nitro-N-nitroso-guanidine for *E.coli* WP2 uvrA (-S9); and 2-aminoanthracene for TA100 (+S9) and E.coli WP2 uvrA (+S9). – not tested at this concentration

There was a dose related increase in revertants in test strains TA100 (approximately 2-3 fold at 4000-8000 μg/plate) and E.coli WP2 uvrA (approximately 2-4 fold at 2500-8000 μg/plate) under the experimental conditions used.

Fautz R (1990a). Unscheduled DNA synthesis in primary hepatocytes of male rats in vitro with dimethoate technical (LSC). Cytotest Cell Research GmbH & Co. KG. Study duration: July 1989. Guidelines: OECD 482; EEC 87/302; US EPA 40. GLP/QA: yes. DTF Doc No: '457-007' Ref: 3-15/Vol 3-6.

Methods Dimethoate was assessed in the UDS assay for its potential to induce DNA repair synthesis using primary hepatocytes of male rats *in vitro*. In two independent tests, the freshly isolated hepatocytes were exposed to dimethoate technical (Source: not specified; Batch No: 611 A; Purity: 96.38%) at 0, 23, 76, 229, 763 or 2290 μg/mL (~ 10 mM) for 3 h in the presence of ^{3}HTdR. The incorporated radioactivity into the DNA was estimated by LSC. 2-Acetylaminofluorene (2-AAF; 22.32 μg/mL medium) was used as positive control.

Results In a pre-experiment test, cell viability was decreased at the high-dose (31%) as determined by exclusion of Trypan blue. In the two independent tests with dimethoate technical, a reproducible increase in the incorporation of ^{3}HTdR into the hepatocytes was evident compared to the corresponding solvent control (Table 67). Since the induction of ^{3}HTdR incorporation due to DNA repair-synthesis was statistically significant at all concentrations used in Test 1, and the increase was also confirmed and dose-dependent in the high dose range of Test 2, dimethoate technical was considered to be an UDS-inducing agent.

Table 67: Findings in the UDS assay

Conc (μg/mL)	0	23	76	229	763	2290	Positive control
Test 1 (dpm/μg DNA)	136	168*	148*	169*	218*	180*	432
Test 2 (dpm/μg DNA)	104	106	109	116*	186*	292*	659

*$p<0.05$ by the non-parametric Mann-Whitney test.

Under the experimental conditions reported, the test substance induced DNA damage and led to repair synthesis in the hepatocytes used.

Fautz R (1990b). Unscheduled DNA synthesis in primary hepatocytes of male rats in vitro with dimethoate technical (Autoradiography). Cytotest Cell Research, GmbH & Co. KG. Study duration: December 1989 – January 1990. Guidelines: OECD 482; EEC 88/302. GLP/QA: yes. DTF Doc No: '457-008' Ref: 3-16/Vol 3-6.

Methods Dimethoate was assessed in the UDS assay for its potential to induce DNA repair synthesis using primary hepatocytes of male rats *in vitro*. In two independent tests, the freshly isolated hepatocytes were exposed to dimethoate technical (Source: not specified; Batch No: 611 A; Purity: 96.38%) at 0, 7.63 22.9, 76.3, 229.0, 763.3 µg/mL (~ 10 mM) for 18 h in the presence of ^{3}HTdR. 2-Acetylaminofluorene (2-AAF) was used as positive control. The incorporation of ^{3}HTdR into the DNA of mammalian cells which are not in the S-phase of the cell cycle can be determined by autoradiography.

Results A reproducible statistically significant increase in the net gains that exceeded the range of biological variation was observed at the highest dose 763 µg/mL in the two independent tests, as well as at 229 µg/mL in Test 1 (Table 68). Slight but significant increases were also seen in other concentrations in Test 2. It was noted that in Test 1, 763 µg/mL elicited toxic effects evidenced by a reduction of neutral red uptake.

Table 68: Findings in the UDS assay

Conc (µg/mL)	0	7.63	22.9	76.3	229.0	763.3	Positive control
Test 1 (net gr/nucleus)	-0.15	0.07	0.30	-0.35	6.95*	5.30*	31.2
Test 2 (net gr/nucleus)	-2.94	-3.57	-1.40*	1.84*	0.79*	8.12*	24.1

*$p<0.05$ by the non-parametric Mann-Whitney test.

The test substance induced DNA damage leading to repair synthesis in the hepatocytes used, and is hence considered to be positive in the UDS test system.

Table 69: 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 TA1535 TA 1537 TA 100 *E. coli* WP2	20-5000 µg/plate (±S9); 20-8000 µg/plate (±S9)	611A / 98.45%	+, - +, - +, - +, - +, -	-, - -, - -, - +, + +, +	Engelhart (1993) [GLP, QA]
Reverse mutation in bacteria [a]	*S. typhimurium* (TA 100, TA 98, TA 1535, TA 1537, TA 1538) *E. coli* (WP2 hcr)	0-10,000 µg/plate	NR	+, -	+ TA100 + WP2 hcr	Moriya *et al* (1983)
Reverse mutation in bacteria [b]	*E. coli* WP2 uvr A-WP67 *S. typhimurium*	5-10 µL of concentrate	NR	NR	+ *E. coli* - *S. typhimurium*	Hanna & Dyer, (1975)
Forward mutation test system	*S. pombe* (SP-198 ade 6-60/rad 10-198 h)	1.3-131 mM	NR	+, -	-	Gilot-Delhalle *et al* (1983)
5-methyltryptophan resistance mutation	*E. coli* K-12/galrs18	1-6 x 10^{-3} mol/L	NR	NR	+	Mohn, (1973)
CHO/HPRT	CHO	1000-3500 µg/mL	611A/ 97.3%	+, -	-	Johnson & Allen (1985) [GLP]
DNA Damage and Repair						
Unscheduled DNA synthesis	Rat hepatocytes	23-2290 µg/mL	611A /96.38%	-	+	Fautz (1990a) [GLP, QA]
Unscheduled DNA synthesis	Rat hepatocytes	8-763 µg/mL	611A / 96.38%	-	+	Fautz (1990b) [GLP, QA]
Comet assay	Human lymphocytes	10-200 ug/mL	98%	-	+	Undeger and Basaran, (2005)
Chromosomal Effect Assays						
Sister Chromatid Exchange	Chinese hamster V79 cells	0, 10, 20, 40, 80 µg/mL	94%	-	+	Chen *et al* (1981)
Sister Chromatid Exchange	Chinese hamster V79 cells	0, 20, 80 µg/mL	94%	+	+	Chen *et al* (1982)

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation. [a] Pesticide screening assay, limited methodology and results available, [b] Pesticide screening assay, limited methodology and results available; required incubation of tester plates for 72 h, rather than 48 h, before induced revertants were detected.

In vivo studies

A summary of findings from *in vivo* dimethoate genotoxicity studies is shown in Table 70. Evaluations of submitted *in vivo* genotoxicity studies are included following the summary table.

Table 70: Summary of the genotoxicity studies

Assay	Species (Strain)	Dose	Batch / Purity	Result	Reference
Gene Mutation					
Dominant lethal mutation	Mouse	0, 5, 10 and 20 mg/kg bw/d for 5 days	611A/96. 89%	-	Becker (1985) [GLP, QA]
Dominant lethal mutation	Mouse	10 mg/kg ip 0.6 ppm in drinking water (5 d/w for 7 w)	> 99%	-	Degraeve and Moutschen (1983), Degraeve *et al* (1984)
Host mediated mutagenicity; reverse mutation assay	Mouse *S. typhimurium*	155 mg/kg bw (given as three equal doses over 3 days).	NR	+	Usha Rani *et al* (1980)
Recessive lethal mutation	*Drosophila*	10-20 ppm: adult feeding	97%	+/-	Velazquez, (1986)
Recessive lethal mutation	*Drosophila*	1 mg/kg feeding	NR	-	Woodruff *et al* (1983)
Recessive lethal mutation[f]	*Drosophila*	LD50 and ½ LD50 feeding	NR	+	Tripathy, (1988)
Chromosomal Effect Assays					
Micronucleus (marrow cells)[e]	Mouse	51.7 mg/kg (two oral doses with 24 hr interval)	NR	+	Usha Rani *et al* (1980)
Micronucleus (marrow cells)	Mouse	(55 mg/kg bw, ip) or in two doses (55 mg/kg bw) 24 hours apart	611A /97.3%	-	Sorg (1985) [GLP]
Bone marrow cytogenetic assay	Rat	(0, 15, 75 and 150 mg/kg bw, ip)	611A/ 97.3%	-	San Sebastian (1985)[GLP]
Bone marrow cytogenetic assay	Mouse	10 mg/kg ip 0.6 ppm in drinking water (5 d/w for 7 w)	> 99%	-	Degraeve and Moutschen (1983), Degraeve *et al* (1984)
Assay	**Species (Strain)**	**Dose**	**Batch /**	**Result**	**Reference**

			Purity		
Bone marrow cytogenetic assay	Mouse	20, 60 mg/kg ip	-	+/-	Nehez *et al* (1983)
Bone marrow cytogenetic assay[b]	Rat	0, 7.00, 9.33 and 14 mg/kg bw/d, 5 days per week by gavage for 6 weeks	98%	+/-	Nehez *et al* (1994)
Bone marrow cytogenetic assay[c]	Rat	0, 7.0, 9.33 and 14 mg/kg bw/d, 5 days per week by gavage for 4 generations	98%	+/-	Nehez *et al* (1996)
Bone marrow cytogenetic assay[d]	Hamster	16-160 mg/kg bw ip	NR	+/-	Dzwonkowska and Hubner, (1986)
DNA Damage and Repair					
Unscheduled DNA synthesis (hepatocytes)	Rat	50, 100 or 200 mg/kg bw	611A/ 96.41%	-	Jackh (1991); Engelhardt (1997) [GLP, QA]

Results are expressed as +, positive; -, negative; +/-, equivocal. [a] No effect was seen at 20 mg/kg bw dimethoate. Increased incidences of numerical aberrations (type not defined), breaks and chromosome aberrations were observed at 60 mg/kg bw. There was no investigation of mitotic index, and only 20 mitoses were scored per animal, use of colchicine was not mentioned; no positive control was included. It was not entirely clear whether the increases were related to the number of cells, or the total number of aberrations. Purity was not recorded; but it was stated that no impurities were detected by TLC. [b] There was no investigation of mitotic index, and only 20 mitoses were scored per animal, use of colchicine was not mentioned; no positive control was included. There was an increase in the number of numerical chromosome aberrations (type not defined), there was no clear effect on the incidence of structural chromosome aberrations. [c] The number of cells with less than 42 chromosomes was decreased in a dose-related and statistically significant manner in the F0 and F1 generations, but not in the F2 and F3 generations: there was no clear effect on the number of structural aberrations. There was no investigation of mitotic index, and only 20 mitoses were scored per animal, use of colchicine was not mentioned; no positive control was included. [d] a formulation Bi 58 EC, containing 37% dimethoate. Slight increase in chromosomal and chromatid breaks, but no relationship to dose; top dose unaffected. [e] The source and purity of the test substance was not reported, there was no positive control. [f] Test substance defined as Rogor (obtained from Rallis Insia Ltd, Bombay) with 30% active constituent.

Jackh R (1991). UDS and S-phase response in primary rat hepatocytes after in vivo exposure (in vitro labelling). BASF, Toksikologie. Study duration: September 1990 – June 1991. Guidelines: American Standard for the Testing of Materials. GLP/QA: yes. DTF Doc No: '457-009' Ref: 3-17/Vol 3-7.
&
Engelhardt (1997). Re-evaluation of the BASF project no: 82M0505/904270. Addendum to report BASF Project no: 82M0505/904270 BASF. DTF Doc No: '457-009' BASF. Ref: 3-18/Vol 3-7.

Methods Dimethoate technical (Source: not specified; Batch No: 611A; Purity: 96.41%) dissolved/suspended in 0.5% aqueous carboxymethylcellulose was administered at 50, 100 or 200 mg/kg bw to male Wistar rats (3/dose/time point) by gavage. Liver perfusion was started at 4 and 12 h post dosing. The hepatocytes were isolated, incubated in culture with ^{3}H-thymidine, and subsequently examined for UDS by autoradiography. Negative controls (solvent, and corn oil) and positive controls [(2-acetaminofluorene for UDS; 50 mg/kg bw) and (4-acetaminofluorene for S-phase response; 1,000 mg/kg bw) were used for comparison.

Results All positive and negative controls had adequate responses in the study. No dose level of dimethoate revealed UDS induction in the hepatocytes of the treated rats as compared to the current vehicle controls. Neither the nuclear grains, nor the resulting net grains, were distinctly enhanced due to the *in vivo* treatment of the animals with the test substance for 4 or 12 h respectively. The net grain values obtained after treatment with the test article were consistently negative. In addition, no substantial shift to higher values was obtained in the percentage distribution of the nuclear grain counts.

Dimethoate technical did not induce an increase in repair synthesis in the hepatocytes of dimethoate treated rats.

9.2 Metabolites

In vitro studies

A summary of findings from submitted *in vitro* genotoxicity studies for omethoate is shown in Table 71. Evaluations of studies that resulted in positive findings are provided after the table.

Table 71: 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	775-12,400 µg/plate	unknown	+, - +, - +, - +, -	-/- +/+ +/+ -/- (see evaluation below)	Herbold (1988a) [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]
Unscheduled DNA synthesis	Primary rat hepatocytes	0.513 – 5130 µg/mL	97.4%	-	+ (see evaluation below)	Cifone (1989) [GLP]
Sister Chromatid Exchange	CHO cells	Up to 5.0 mg/mL	96%	+/-	+/+ (see evaluation below)	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 BA (1988a). E 6876 c.n. Omethoate. Salmonella/microsome test to evaluate for point mutagenic effects. Bayer AG Fachbereich Toxicology, Friedrich-Ebert-Straße 217-333 D-5600 Wuppertal 1, FRG. Sponsor: Bayer AG. Study duration: 16 – 25 March 1988. Guidelines: None stated GLP/QA: Yes. DTF Doc No: '557-003' Ref 3-59/Vol 3-26.

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.

Lehn H (1989). E 6876 (c.n. omethoate): mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro. Department of Toxicology, Bayer AG, Wuppertal, FRG. Study duration: November-December 1988. Guidelines: None stated. GLP/QA: Yes. DTF Doc No: '557-006' Ref 3-61/Vol 3-26.

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. The study authors considered that the results of this study indicated that omethoate was mutagenic in the CHO-HGPRT forward mutation assay, 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 72).

Table 72: Mutation frequencies

Omethoate conc. (µg/mL)	Mutation frequency							
	-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.

Cifone MA (1989). Mutagenicity test on E 6876 in the rat primary hepatocyte unscheduled DNA synthesis assay. Hazelton Laboratories America, Maryland, USA. HLA study no. 10419-0-447. Study duration: August-September 1988. Guidelines: None stated. GLP/QA: Yes. DTF Doc No: '557-005' Ref 3-63/Vol 3-26.

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 (Table 73). The positive control produced a strong response. This study shows that omethoate can induce DNA damage in mammalian cells *in vitro*.

Table 73: Mutagenicity in the rat primary hepatocyte UDS assay

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. Hazelton Biotechnologies, The Netherlands. Study duration: March – June 1988. Guidelines: None stated GLP/QA: Yes. DTF Doc No: '557-002' Ref 3-62/Vol 3-26.

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) (Table 74).

Table 74: *In vitro* effects of dimethoate in CHO cells

Omethoate conc.	SCEs/diploid (mean ± SD)			
	-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.

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. Therefore, omethoate was considered positive for the induction of SCE in this assay, both in the presence and absence of metabolic activation.

In vivo studies

A summary of findings from submitted *in vivo* genotoxicity studies for omethoate is shown in Table 107.

Table 75: Summary of *in vivo* Genotoxicity Studies

Assay	Species (Strain)	Dose	Batch / Purity	Result	Reference
Gene Mutation					
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 (bone marrow)	Mouse (NMRI SPF Han Wistar)	22.5 mg/kg bw; gavage, single dose	96.0%	-	Herbold (1988b) [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 foetuses to evaluate for induced somatic changes in the genes of the coat pigment cells. Bayer report no. 19017, Study duration: July-November 1989 - 24 November 1989. Guidelines: none stated GLP/QA: Yes DTF Doc No: '557-009' Ref 3-68/Vol 3-26.

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 10^{th} 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 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. 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 76).

Table 76: Number & percentage of F1 mice with/without spots

Dose (mg/kg bw)	Without spots		With spots			
			WMVS		RS	
	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 Technical grade active constituent

Rats

Lamb IC (1993a). A range-finding acute study of dimethoate in rats. Wil Research Laboratories, Inc. Study duration: October 1992. Guidelines: 81-8-SS. GLP/QA: Yes. DTF Doc No: '421-012' Ref: 3-26/Vol 3-10.

Methods In a range-finding acute neurotoxicity study, rats (Sprague-Dawley Crl:CD BR, from Charles River Breeding Laboratories; approximately 34 days of age) were given a single oral dose of 2, 20, 25, 50, 100, 200, 300, 500, 750 or 1000 mg/kg bw of dimethoate (Source: Cheminova Agro A/S; Batch No: 20522-00; Purity: 99.1%) in 0.5% aqueous carboxymethylcellulose. Dose volumes of 10 – 33.4 mL/kg bw were used. Animals were observed for clinical signs frequently on the dosing day (15, 30, 45, 60, 75, 90, 105, 120, 135, and 150 mins, and 3, 4, 5, 6, 7 and/or 8 hrs), and for 6-7 days after the dosing day. Body weight was measured on Days -1, 0, 7/8. A macroscopic examination was carried out on rats found dead. All survivors were euthanized on Day 7/8 and discarded. No statistical analyses were carried out.

Results Deaths occurred at 200 mg/kg bw and higher doses within 24 h except one male each at 250 or 300 mg/kg bw/d that died on Day 2 (Table 77).

Table 77: Mortality following single doses of dimethoate

Dose (mg/kg bw)	Rat (n/sex)	Mortality	
		M	F
2	2+2	0	0
20	3	0	0
25	2	0	0
50	2	0	0
100	2	0	0
200	6	2	0
250	2	1	0
300	2	2	0
500	2	2	2
750	1	1	1
1000	1	1	1

Clinical signs prior to deaths included gait alterations (rocking, lurching or swaying or prostration), constricted pupils, salivation, tremors (whole body and/or forelimbs/hindlimbs), absent forelimb/hindlimb grasp, laboured and/or shallow respiration and impaired/absent righting reflex. The signs first occurred within 15-90 mins depending on the dose; hypothermia (body cool to touch), lacrimation and staining on various body surfaces appeared later, and all signs persisted (often increasing in severity) through to the time of death. Macroscopic examination revealed dark red lungs in 1 male and opacity of one eye in the other male at 200 mg/kg bw.

The main clinical signs and the time of peak effect for all groups are shown in the Table below. The occurrence of clinical signs peaked at approximately 2 h post dosing, and several

persisted at 3 h and 8 h. All survivors were clinically normal by Day 4 at 200 and 250 mg/kg bw, and by Day 6 at 300 mg/kg bw (Table 78).

Table 78: Clinical signs in rats approximately 2 hours after exposure to dimethoate

Main signs	Dose start*	Time of peak effect
Gait alterations (rocking, lurching, swaying or prostration	25	105 min – 4 h
Tremors (whole body and/or forelimb/hindlimb	25	90-105 min
Constricted pupils	50	30 min – 2 h
Salivation	50	1 – 3 h
Reduced/absent forelimb/hindlimb grasp reflex	200	1 – 4 h
Laboured respiration, lacrimation and hypothermia	300	4 – 5 h
Body surface staining	100	-

* Dose at which the effect was first observed.

Reduced body weight gain was seen in males at 200 mg/kg bw and in females at 250 mg/kg bw on Day 7; body weights were not assessed at higher doses due to mortality.
There were no treatment related effects observed at 2 and 20 mg/kg bw. Dose levels of 2, 20 and 200 mg/kg bw were selected for the acute neurotoxicity study in rats, with an estimated time of peak effect at 2 h post dosing.

Lamb IC (1993b). An acute neurotoxicity study of dimethoate in rats. Wil Research Laboratories, Inc. Study duration: November 1992 – February 1993. Guidelines: US EPA 81-8-SS. GLP/QA: Yes. DTF Doc No: '468-005' Ref: 3-27/Vol 3-11 to 3-13.

Methods A single oral dose of 0, 2, 20 or 200 mg/kg bw of dimethoate (Source; Battelle, Columbus, Ohio; Lot No: 20522-00; Purity: 99.1%) in deionised water was given to Sprague-Dawley rats (15/sex at 200 mg/kg bw, and 12/sex/dose for other groups) by gavage. Rats (Crl:CD BR, from the Charles River Breeding Laboratories, Inc., Portage, Michigan) were 43 days old, 157-270 g for males and 126-182 g for females. A dose volume of 10 mL/kg bw was used for all groups. Rats were observed for viability, clinical signs and body weights. FOB and motor activity evaluations were performed during pretest, at 2 h (the estimated time of peak effects) and on Days 7 and 14 post dosing. At necropsy on Day 15, brain weights and brain dimensions were measured for all animals. In addition, all rats were perfused in situ, and 5/sex/dose at 0 and 200 mg/kg bw/d were randomly selected for subsequent neuropathological evaluation. Statistical analyses of body weight, body weight changes and brain weight was conducted by one-way ANOVA followed by Dunnett's test for pairwise comparison. Histopathological findings were analysed by the Kolmogorov-Smirnov test. FOB data were analysed by two way repeated measures anova. If significant treatment or treatment-time interactions occurred, a one way anova was conducted at each time point, followed by Dunnett's multiple T-test to determine significant differences from control. FOB observations that yielded ordinal or descriptive data were analysed using the repeated measures SAS CATMOD procedure. In cases of significant effects, Fisher's Exact test or Dunnett's test were used to establish differences from control.

Results There were no mortalities. Clinical signs were observed at 200 mg/kg bw, with gait alterations (rocking, lurching or swaying in 15/30 rats), tremors (whole body or forelimbs/hindlimbs in 13/30) and constricted pupils (11/30) generally limited to Days 1-2.

Body surface staining (the majority of rats), decreased defecation (18/30) and hunched body (in 1 male on Day 2) were also observed in this group. Males at 200 mg/kg bw showed

significantly lower body weight gain (38%, p< 0.01) than controls during the week following treatment, resulting in an overall lower body weight gain for Days 0-14.

The FOB and motor activity evaluations revealed various alterations at 200 mg/kg bw. Responses at the estimated time of peak effect (approximately 2 h post dosing) on Day 0 are shown in Table 79. Except gait alterations, tremor and constricted pupils which persisted to Days 1 and/or 2, other responses were transient in nature, and were not apparent on Days 7 and 14. Treatment-related absence of pupil response was also observed at 20 mg/kg bw.

Table 79: Findings in FOB on Day 0 [Incidence]

Dose (mg/kg bw)	**Male**				**Female**			
	0	**2**	**20**	**200**	**0**	**2**	**20**	**200**
Number of rats tested	12	12	12	15	12	12	12	15
Home cage observations								
Posture, flattened, limbs may be extended	0	0	0	**2**	0	0	0	**3**
Lying on side, limbs in air	0	0	0	**1**	0	0	0	**1**
Convulsions, clonic, whole body tremors	0	0	0	**15 ***	0	0	0	**15 ***
tonic, opisthotonos	0	0	0	**1**	0	0	0	**0**
Tremors, moderately, markedly coarse (3, 4.5 mm)	0	0	0	**2++, 13+++***	0	0	0	**15+++ ***
Feces consistency, unformed, diarrhoea	0	0	0	**2**	0	0	0	**1**
Handling observations								
Lacrimation, slight, severe	0	0	0	**3+**	0	0	0	**2+, 2+++**
Salivation, slight, severe	0	0	0	**4+++**	0	0	0	**1+, 2+++**
Fur appearance, slightly soiled, very soiled, crusty	0	0	0	**2+, 1+++**	0	0	0	**1+**
Red/crusty deposits in eyes, nose or mouth	0	0	1	**1-2**	0	0	0	**1**
Eye prominence, exophthalmos	0	0	0	**1**	0	0	0	**0**
Open field observations								
Time to first step, second	0.7	0.6	0.6	**1.2**	0.5	0.5	0.5	**1.4#**
Impaired mobility, slightly, moderately	0	0	0	**4+, 11++***	0	0	0	**3+, 12++**
Gait, walks on tiptoes	0	0	0	**2**	0	0	0	**5***
ataxia, excessive sway, rocks or lurches as proceeds forward	0	0	0	**13***	0	0	0	**10***
Convulsions, clonic, whole body tremors	0	0	0	**15***	0	0	0	**15***
Tremors, moderately, markedly coarse	0	0	0	**3++, 12+++***	0	0	0	**1++,14 +++***
Gait impairment, slight, considerable	0	0	0	**2+, 13++***	0	0	0	**15++***
Bizarre/stereotypic behaviour, rearing (counts)	6.8	6.8	5.8	**0##**	6.1	6.0	8.8#	**0.55##**
Dose (mg/kg bw)	**Male**				**Female**			
	0	**2**	**20**	**200**	**0**	**2**	**20**	**200**
Sensory observations								
Approach response	12+	12+	11+, 1++	**6-, 9+***	12+	12+	12+	**7-, 8+***
Touch response	12+	12+	11+, 1++	**4-, 11+**	12+	12+	12+	**6-, 9+***

Startle response	6+, 6++	4+, 8++	3+, 9++	**3-, 5+, 7++**	4+, 8++	5+, 7++	4+, 8++	**6-, 3+, 6++**
Tail pinch response	7+, 5++	7+, 4++, 1+++	8+, 4++	**12-, 3+***	4+, 8++	5+, 7++	4+, 8++	**11-, 4+***
Pupil response, (absence, present)	12+	1-, 12+	**5-, 7+***	**14-, 1+***	2-, 10+	2-, 10+	**6-, 6+**	**15-***
Air righting reflex, slightly uncoordinated	0	0	1	**10***	0	0	0	**7***
lands on side	0	0	0	**2**	0	0	0	**3**
lands on back	0	0	0	**1**	0	0	0	**1**
Neuromuscular observation								
Hindlimb extensor strength, (Present, reduced, absent)	12+	12+	12+	**5+, 9±, 1-**	12+	11+,1 ±	12+	**2+, 5±, 8-***
Grip strength, forelimb (g)	607	673	657	**462#**	586	582	556	**405##**
Rotarod performance (s)	74.1	79.4	97.8	**12.7##**	95.0	65.4	84.3	**18.7##**
Hindlimb footsplay (mm)	71.8	75.1	81.9	**58.3**	65.3	66.0	66.8	**53.5**
Motor activity counts								
Total activity (counts)	1122	1512 #	1208	**674#**	1372	1238	1752	**599#**
Ambulatory activity	644	808	697	**282#**	718	673	964	**313#**

Numbers = the number of rats: - no reaction; +, Slight/slow; ++, moderate/more; +++severe/fast.
*p<0.05 by Fisher's Exact test. #p<0.05 by Dunnett's test.

Brain weights or dimensions were comparable across all groups. No treatment-related microscopic lesions were observed in the central or peripheral nervous system of animals that received 200 mg/kg bw dimethoate.

There were no treatment-related alterations at 2 mg/kg bw. The NOEL for acute neurotoxicity was 2 mg/kg bw/d, based on treatment-related absence of pupil response at 20 mg/kg bw.

Schaefer GJ (1999a). An acute dietary neurotoxicity study of dimethoate technical in rats. MPI Research, Inc. Study duration: January - February. Guidelines: US EPA. GLP/QA: yes. DTF Doc No: '421-016' Ref: 3-28/Vol 3-14.
&
Schaefer GJ (1999b). An acute dietary neurotoxicity study of dimethoate technical in rats: Supplementary information for MRID 44818901. MPI Research, Inc. DTF Doc No: None. Ref: 3-29/Vol 3-14.

Methods Sprague-Dawley rats (24/sex/dose; Charles River Breeding Laboratories, Inc., Portage, Michigan; 8 to 9 weeks of age; 167-240 g for males, 159 to 218 grams for females) were given 0, 1, 2, 3, or 15 mg/kg bw of dimethoate technical (Source: Cheminova Agro A/S, Denmark; Lot No: 20522-00; Purity: 99.1%) in the diet for 3 h on Day 1. Homogeneity, stability and concentrations of the test substance in the diet were analysed before and during the study. The average concentrations ranged from 82 to 95% of the nominal doses. Rats were clinically examined daily for 14 days; food consumption was measured daily, and body weight weekly. Blood samples were taken from 8 rats/sex/dose and the rats were then sacrificed at the peak time of effect on Day 1 (2.5-3 h following removal of diet, determined in MPI Study No: 827-003) for plasma, erythrocyte and brain ChE determinations. FOB and

locomotor activity (MA) determinations were conducted on 16 rats/sex/dose at the peak time of effect on Day 1, and on Day 15. Following FOB and MA tests on Day 15, blood samples were taken from 8 rats/sex/dose and the rats were then sacrificed for determination of ChE activity in plasma, erythrocytes and different brain regions. The remaining 8 rats/sex/dose were euthanized and necropsied, and any abnormal tissues were retained for histopathology. For each endpoint, Levene's test was used to analyse the homogeneity of variance. In cases of homogenous variances, Dunnett's test was used for pairwise comparison, otherwise comparisons were made using Welch's t-test with a Bonferroni correction. In addition, each endpoint was assessed using a chi-square test with a Bonferroni correction.

Results There were no mortalities and no treatment related findings for clinical signs, food consumption or body weight. Open field measurements revealed mild tremors in 1 male at 15 mg/kg bw on Day 1, and in 1 male at 2 mg/kg bw on Day 15; ataxia in 1 female each at 3 and 15 mg/kg bw on Day 1; and bizarre behaviour (digging in corners of box) in 1 female at 15 mg/kg bw on Day 1; these findings were not clearly related to treatment. There were no other abnormal findings in cageside observations, FOB evaluations or locomotor activity.

As shown in Table 80, on Day 1, males and females at 15 mg/kg bw showed significant reductions in ChE activity in the plasma, erythrocytes, hippocampus, cortex and striatum (up to 50%, 58%, 33%, 36% and 41% reduction for males, and 40%, 65%, 38%, 43% and 47% reduction for females respectively, compared to control). At day 15 at the high dose, statistically significantly lower (15-16%) ChE activity was only detected in hippocampus of male and female rats. Significant inhibition also appeared at 3 mg/kg bw, in erythrocyte ChE activity from males (29% reduction) and in cortex from females (11% reduction) on Day 1. At 2 mg/kg bw on Day 1, plasma and erythrocyte ChE activity was decreased greater than 20% compared to controls in males.

Table 80: ChE activities in plasma and erythrocyte (nM/L/min) & brain tissues (nM/g/min) (n=8)

Dose (mg/kg bw)	Male					Female				
	0	1	2	3	15	0	1	2	3	15
Plasma, D1	0.4	0.4	0.3 (25)	0.3 (25)	0.2** (50)	0.5	0.5	0.6	0.5	0.3** (40)
D15	0.3	0.3	0.3	0.3	0.3	0.6	0.6	0.6	0.8	0.5 (17)
Erythrocyte, D1	2.4	2.0 (17)	1.8 (25)	1.7* (29)	1.0** (58)	2.0	1.8 (10)	1.7 (15)	1.7 (15)	0.7** (65)
D15	2.2	1.8 (18)	1.9 (14)	1.8 (18)	1.9 (14)	1.7	1.9	2.0	1.9	1.5 (12)
Hippocampus, D1	0.49	0.47	0.50	0.47	0.33** (33)	0.50	0.49	0.47	0.46	0.31** (38)
D15	0.86	0.82	0.88	0.89	0.72* (16)	0.85	0.82	0.81	0.86	0.72** (15)
Cortex, D1	1.54	1.48	1.48	1.42	0.99** (36)	1.57	1.57	1.49	1.40** (11)	0.89** (43)
D15	1.73	1.73	1.72	1.76	1.53 (12)	1.75	1.66	1.65	1.71	1.57 (10)
Striatum, D1	0.58	0.58	0.64	0.72	0.34 (41)	0.53	0.50	0.46 (13)	0.52	0.28** (47)
D15	0.97	0.94	0.95	0.98	0.84 (13)	0.94	0.95	0.96	0.87	0.82 (13)

*p<0.05; **p<0.01 by a chi-square test. D: Day. Results expressed as mean values.
() Numbers in parentheses represents percentage inhibition compared to control values (10% or higher).

Organ weights including weights of brain tissues were not affected. No treatment related macroscopic findings were observed at necropsy.

The NOEL for acute neurotoxicity was 1 mg/kg bw, based on inhibition of plasma and erythrocyte ChE activity at 2 mg/kg bw.

Lamb IC (1994). A subchronic (13 week) neurotoxicity study of dimethoate in rats. Wil Research Laboratories, Inc. Study duration: January 1993 – February 1994. Guidelines: FIFRA 82-7. GLP/QA: yes. DTF Doc No: '468-006' Ref: 3-30/Vol 3-15 to 3-18.

Methods Sprague-Dawley rats (10/sex/dose except 12 males at 125 ppm) received 0, 1, 50 or 125 ppm dimethoate (Cheminova Agro A/S; Lot No: 20522-00; Purity: 99.1%) in the diet for 91 - 94 consecutive days. Achieved daily intakes of the test substance were 0, 0.06/0.08, 3.2/3.8 and 8.1/9.9 mg/kg bw/d, respectively, for males/females. Rats were observed for viability, clinical signs, body weights, food consumption and in FOB and Motor Activity evaluations. Blood samples were collected from non-fasted rats (5/sex/dose; except 7 males at 125 ppm) at pretest, Weeks 3, 7 and 13 for plasma and erythrocyte ChE activity determinations. Weights of brain and brain regions, and brain region ChE activity were evaluated on these rats at study termination. *In situ* tissue perfusion was performed on the remaining 5 rats/sex/dose for brain weight and brain dimensions. Any gross changes, abnormal coloration or lesions of the brain and spinal cord were recorded. The central (brain – forebrain, centre of cerebrum, midbrain, cerebellum and pons, and the medulla oblongata; spinal cord – at cervical swellings C3-C8 and at lumbar swellings T13-L4; gasserian ganglion/trigeminal nerves; lumbar dorsal root ganglion and fibers at T13-L4; lumbar ventral root fibers at T13-L4; cervical dorsal root ganglion and fibers at C3-C8; cervical ventral root fibers at C3-C8; optic nerves and eyes) and peripheral nervous system tissues (sciatic nerve mid-thigh region and at sciatic notch, sural nerve, tibial nerve, peroneal nerve, forelimbs and tail) from 0 and 125 ppm groups were collected for subsequent neuropathological evaluation. Statistical analysis was by one way analysis of variance for body weight, body weight changes, food consumption, cholinesterase determinations, brain weight data and brain dimensions. Where significant differences were indicated, Dunnett's test was used for pairwise comparison. For FOB measurements and Locomotor activity, analysis was conducted by two-way repeated measures anova. If significant treatment or treatment-time interactions were seen, a one way anova was conducted at each time point. Dunnett's multiple T-test was conducted to determine significant differences from the control group.

Results There were no mortalities. Hairloss and dried tan staining on forelimbs, and/or small faeces were seen in all groups, but were more frequent at 50 and 125 ppm. Mean body weight gain was significantly ($p<0.05$) reduced (16%) at 125 ppm in male rats over the study period (weeks 0-13). Body weight gain was not significantly affected by treatment in females. Sensory observations revealed that in one or more weeks, rats at 50 and 125 ppm responded more energetically during tests for the approach response, tail pinch response or touch response than concurrent control (Table 81). Neuromuscular observations detected lower forelimb grip strength in males and females at 125 ppm, and a reduced rotarod performance in males at 50 and 125 ppm at week 12. The study author argued that the majority of alterations listed below were within the ranges of WIL historical control data which were provided by the sponsor. However, it has to be pointed out there were limited information for these studies available (eg. no species and study durations were provided), which reduces the value of the data for comparison. Significantly ($p<0.01$, $p<0.05$) lower body temperature was detected in

males of all treated groups (37.3-37.5 *vs* 38.1) in Week 12 only, without a dose-relationship. These changes were not considered to be related to treatment. No other findings were detected by FOB or locomotor activity examinations.

Table 81: Functional observation battery findings

Dose (ppm)	Male					Female				
	0	**1**	**50**	**125**	**Hist. range**	**0**	**1**	**50**	**125**	**Hist range**
Number of rats tested	10	10	10	12		10	10	10	12	
Sensory observations (values for weeks 3, 7, 12)										
Approach response, % animals	0, 0, 0	0, 0, 0	0, 0, 0	40, 0, 10	2.4-6.3	1, 0, 0	0, 0, 0	0, 0, 0	0, 17, 17	19-20
Touch response, % animals	0, 0, 0	0, 0, 0	10, 0, 0	30, 10, 30	12.5 (Wk 3 only)	1, 0, 1	1, 1, 1	20, 20, 30	42, 25, 33	16-20
Tail pinch response, % animals	0, 10, 44	0, 10, 20	30, 10, 40	40, 30, 60	30-60	4, 2, 4	5, 3, 4	20, 40, 60	50, 42, 58	30-47
Neuromuscular observation (values for weeks 3, 7, 12)										
Grip strength, forelimb (g)	1047, 1273, 1438	1042, 1369, 1388	1012, 1315, 1422	930, 1181, 1262	758-1023	870, 1078, 1332	870, 1135, 1321	846, 1073, 1272	738, 968, 1029**	670-893
Rotarod performance (s)	63, 49, 49	56, 55, 22	38, 29, 19	41, 22, 10	57-105	60, 76, 108	100, 110, 120	91, 67, 76	69, 89, 71	52-94

WIL FOB historical control data provided by the sponsor. The data summarised the similar study intervals (Weeks 3, 6 and 13). Study duration for the data was not stated. ** $p<0.01$ by Dunnett's test.

According to the study author, the ChE activity data were derived from two laboratories. The blood samples for pretest, Weeks 3 and 7 (as 7a) were analysed at WIL Research Laboratories, Inc. The data from this laboratory were inconsistent with previous findings in subchronic and chronic studies. Hence, duplicate blood samples for Week 7 (7b), and blood samples for week 13 were sent to Battelle, Columbus, Ohio for analysis.

Data presented in Table 82 include results of blood samples at weeks -2, 3 and 7a, and of brain tissues by WIL, and those of blood samples at Weeks 7b and 13 by Battelle. The differences shown between the two data sets (Wk 7a and 7b) on the same set of blood samples indicate different sensitivities between the laboratory methods. The data from Battelle for blood samples from Weeks 7 and 13 are more consistent with previous observations in other studies. Plasma ChE activity was significantly ($p<0.05$ or $p<0.01$) decreased (24-48%) at ≥ 50 ppm in males and also decreased approximately 50% (n.s) compared to controls at 125 ppm in females. Erythrocyte ChE activity was inhibited in males and females at 50 and 125 ppm. A slight, but significant (11%; $p<0.05$) reduction of erythrocyte ChE activity seen in males at 1 ppm only at week 7 was not considered toxicologically significant. ChE activity was decreased in various brain regions (olfactory, midbrain (with striatum), brainstem, cerebellum and cortex) at the high dose in males and/or females.

Table 82: ChE activities in plasma and erythrocyte (U/L) and brain regions (U/g)

Dose (ppm)	Male				Female			
	0	**1**	**50**	**125**	**0**	**1**	**50**	**125**
Plasma, Wk -2	760	778	740	873	990	1122	1189	1017
Wk 3	648	624 (4)	526 (19)	449** (31)	2600	2724	2643	1234 (53)
Wk 7a	583	600	554 (5)	469 (20)	3231	3241	3403	1623 (50)
Wk 7b	**364**	**355 (2)**	**276* (24)**	**202** (45)**	**1974**	**2100 (6)**	**2321 (18)**	**952 (52)**
Wk 13	**382**	**355 (7)**	**241** (37)**	**199** (48)**	**2458**	**2144 (13)**	**2526**	**1235 (50)**
Erythrocyte, Wk -2	6137	6931	6839	6428	6519	6428	6700	6387
Wk 3	6935	6660 (4)	6610 (5)	6946 (0)	7459	7453	6735 (10)	7407 (1)
Wk 7a	7422	7075 (5)	6660 (10)	7082 (5)	7001	7719	7229	7432
Wk 7b	**2045**	**1811* (11)**	**1082** (47)**	**808** (60)**	**2065**	**2316**	**1358** (34)**	**922** (55)**
Wk 13	**1762**	**1638 (7)**	**895** (49)**	**83** (53)**	**1648**	**1724**	**962* (42)**	**705** (57)**
Hippocampus	2.95	3.05	2.70 (8)	2.76 (6)	2.98	3.09	2.79 (6)	2.89 (3)
Olfactory	3.22	3.22	2.96 (8)	2.63** (18)	2.99	3.03	2.92 (2)	2.69 (10)
Midbrain (with striatum)	3.39	3.38	3.24 (4)	2.89** (15)	3.54	3.53	3.25 (8)	2.91** (18)
Brainstem	3.39	3.60	3.09 (9)	2.83** (17)	3.57	3.66	3.34 (6)	2.87* (20)
Cerebellum	2.93	3.09	2.85 (3)	2.79 (5)	3.02	3.12	3.18	2.66 (12)
Cortex	3.13	2.99 (4)	2.85 (9)	2.76** (12)	3.09	3.23	2.80 (9)	2.78 (10)

*p<0.05; **p<0.01 by Dunnett's test. Wk: week. (n=5 except n=7 for males at 125 ppm). Data presented includes results of blood samples at weeks -2, 3 and 7a, and brain tissues by WIL, and those of blood samples at weeks 7b and 13 by Battelle. Numbers in parentheses represent % inhibition *cf*. control.

There were no treatment related abnormalities in absolute and relative brain and brain region weights, and in brain length and width measurements. No treatment related neuropathological lesions were observed at the microscopic examination of perfused tissues.

The NOEL was 1 ppm (0.06/0.08 mg/kg bw/d in males/females) based on reduction of ChE activity in plasma and erythrocytes at 3.2 mg/kg bw/d.

Myers DP (2001a). Dimethoate: dose range finding study in CD rats by oral gavage administration preliminary to developmental neurotoxicity study. Huntingdon Life Sciences Ltd. Ltd. DTF Doc No: '468-009' Ref: 3-31/Vol 3-19.

Methods A preliminary study was conducted to assess the influence of dimethoate on pregnant/lactating CD rats and their offspring and to select doses for both ChE activity determinations and developmental neurotoxicity studies. Female rats (Crl:CD BR strain, from Charles River UK Limited, Margate, Kent, England) were mated with stock males from the same strain and source, and were 10-11 weeks of age, and in the weight range of 215 to 277 g at GD 0. Mated female rats (15/dose, F0) were dosed by gavage at 0, 0.2, 3 or 6 mg/kg bw/d of dimethoate (Source: Cheminova A/S; Batch no: 20522-00, in water) from GD 6. Five of the F0 female rats per dose were treated to GD 20, and killed 3 h after the last dosing, and

litter data were assessed, and ChE activity determined in maternal and foetal plasma, erythrocytes and brain. The remaining 10 F0 females per dose were treated from GD 6 to Post Natal Day (PND) 10 and selected F1 offspring from these litters (2/sex/litter where possible) were dosed from PND 11 to PND 21 inclusive, in order to assess effects on survival, weight gain, and plasma, erythrocyte and brain ChE activity. Detailed observations on F0 females were performed daily, and food consumption and body weight were recorded every 3-4 days during gestation and lactation. Dams and undosed F1 offspring were killed on, or shortly after PND 21, the latter served as within-litter "control". Four untreated F1 offspring were killed on PND 21, perfused and the brain embedded, sectioned and subjected to light microscopy examination. At termination, blood and brain were collected from dams and foetuses killed on GD 20, and from pups killed on PND 21 for measurement of plasma, erythrocyte and brain ChE activity. Statistical analyses were conducted only for gestation body weight and body weight change data using one way analysis of variance followed by the Williams test.

Results Post dosing salivation was observed in two dams at 3 mg/kg bw/d and three dams at 6 mg/kg bw/d. Body weight gain was significantly ($p<0.05$) lower (12% lower from GD 6-20) at 3 and 6 mg/kg bw/d during gestation, but not during lactation, and food consumption was not affected. Macroscopic examinations of dams on GD 20 revealed no abnormalities, and brain weights of dams and foetuses were unaffected.

There was no effect on post-implantation survival up to birth (Table 83). Mean numbers of corpora lutea and implantations, the growth and survival of the foetuses, mean litter weights and foetal weights at birth were comparable across groups. However, there was an increase in post-natal pup mortality at 6 mg/kg bw/d, with a total loss of 2 litters (14 and 12 pups) by PND 2, while 4 out of 14 pups from another dam died by PND 4. Underactivity, hypothermia and lack of feeding (no milk in stomach at necropsy) were seen in these pups soon after birth. The incidence of total pup loss resulted in reductions in live litter size, as well as birth and viability indices of this group, compared with control.

Table 83: Percentages of offspring survival indices

Dose (mg/kg bw/d)	Male			
	0	0.2	3	6
Post-implantation survival index	94.4	92.7	93.1	91.0
Live birth index	98.7	100	99.3	87.2
Viability index	97.8	99.3	96.8	77.5
Lactation index, PND 7	100	100	98.7	88.9
PND 11	100	100	98.7	88.9

Group mean body weight for both male and female offspring at 6 mg/kg bw/d was noticeably lower at PND 1 and remained lower through to PND 11 (Table 84). Direct dosing (PNDs 11-21) of pups did not alter body weight gain, though the pattern of lower bodyweight at 6 mg/kg bw/d established prior to PND11 persisted through to PND 21.

Table 84: Pup weights

Dose (mg/kg bw/d)	Male				Female			
	0	0.2	3	6	0	0.2	3	6
Pup weight on PND 1, g	6.9	6.4	6.6	5.7	6.6	6.0	6.2	5.5
on PND 11, g	25.3	24.4	25.2	20.8	24.7	23.1	24.2	20.1
on PND 21 (dosed pup), g	52.7	49.4	51.0	44.4	49.5	46.4	49.0	44.5
on PND 21 (undosed pup), g	52.7	50.7	49.6	45.1	51.0	50.5	46.9	47.0

Necropsy revealed no treatment related macroscopic changes in dams, and no findings in dosed and undosed offspring killed on PND 21. The marginally higher relative brain weight of male pups at 6 mg/kg bw/d probably reflected the lower body weight of this group. Histopathological examination revealed no treatment-related lesions.

Statistical analyses were not conducted on ChE activity data. Plasma, erythrocyte and brain ChE activity was decreased ≥20 % at doses of ≥ 3 mg/kg bw/d in F0 females (Table 85). In GD 20 male foetuses, ≥20 % reductions in plasma ChE activity were seen following maternal doses of ≥ 0.2 mg/kg bw/d, and erythrocyte and brain ChE activity were inhibited at ≥ 3 mg/kg bw/d. In GD 20 females, erythrocyte ChE activity was inhibited at doses of ≥ 0.2 mg/kg bw/d and erythrocyte and brain ChE activity were inhibited at ≥ 3 mg/kg bw/d. F1 pups directly dosed from PND 11-21 showed inhibited plasma, erythrocyte and brain ChE activity at ≥ 3 mg/kg bw/d.

Table85: Cholinesterase activities

Dose (mg/kg bw/d)	Male				Female			
	0	0.2	3	6	0	0.2	3	6
F0 females on GD 20								
Plasma (U/L)	-	-	-	-	1255	1374	939 (25)	545 (57)
Erythrocyte (U/L)	-	-	-	-	1245	1205 (3)	275 (78)	190 (85)
Brain (U/kg)	-	-	-	-	12710	12680	3240 (75)	1580 (88)
F1 foetuses on GD 20								
Plasma (U/L)	233	186 (20)	58 (75)	48 (79)	239	210 (12)	81 (66)	65 (73)
Erythrocyte (U/L)	930	1000	280 (70)	120 (87)	1165	820 (30)	215 (82)	50 (96)
Brain (U/kg)	2150	2320	1670 (22)	1390 (35)	1970	2100	1500 (24)	1140 (42)
F1 pups dosed PND 11-21								
Plasma (U/L)	535	554	329 (39)	213 (60)	494	529	294 (40)	198 (60)
Erythrocyte (U/L)	1504	1475 (2)	608 (60)	447 (70)	1464	1426 (3)	511 (65)	288 (80)
Brain (U/kg)	10555	9942 (6)	5835 (45)	4720 (55)	9338	9886	5414 (42)	3186 (66)

Numbers in parentheses represent percentage inhibition compared with the control value.

The results suggest that 6 mg/kg bw/d is unsuitable for use in a developmental neurotoxicity study based on lower pup birth weight and increased early post-natal pup death. A NOEL was not established in this range-finding test, since 0.2 mg/kg bw/d, the lowest dose tested induced decreased plasma ChE activity in male and erythrocyte ChE activity in female F1 foetuses. The LOEL was 0.2 mg/kg bw/d.

Myers DP (2001b). Dimethoate effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage administration. Huntingdon Life Sciences Ltd. Study duration: July – October 2000. Guidelines:. GLP/QA: yes. DTF Doc No: '468-007' Ref: 3-32/Vol 3-19.

Methods The effects of acute and/or repeated dosing with dimethoate was tested on plasma, erythrocyte and brain ChE activity during gestation in female rats and their pre-term foetuses, in pre-weaning offspring, and in young rats.

Female rats (Crl:CD BR strain, from Charles River UK Limited, Margate, Kent, England) were mated with stock males from the same strain and source, and were 10-11 weeks of age, and in the weight range 216 to 260 g at commencement of the study. The mated F0 female rats (19/dose) were dosed by gavage at 0, 0.1, 0.5 or 3 mg/kg bw/d of dimethoate (Source: Cheminova A/S; Batch no: 20522-00, purity: 99.1%, dissolved in water) from GD 6. Nine F0 females per dose were treated to GD 20, and killed 3 h after the last dose for necropsy and examination of uterine contents and ovaries (corpora lutea, implantation sites, early or late resorption sites, foetuses). The remaining 10 F0 females/dose were treated from GD 6 to PND 10 and selected F1 litters (8 litters/dose group) were dosed from PND 11-21 inclusive.

The mean concentrations of dimethoate in test solutions analysed were within $\pm$2% of nominal values. Detailed observations on F0 females were performed daily, and food consumption and body weight were recorded once every 3-4 days during gestation and lactation. Gestation index, gestation length, litter data, foetal and litter weights, litter size after birth and survival indices, brain weights for GD 20 dams and foetuses, all other adult rats, and all offspring on PNDs 4, 11, 21 and 60 were measured. Blood and brain were collected at termination from dams and foetuses, all other adult rats, as well as all offspring for the measurement of plasma, erythrocyte and brain ChE activity. Statistical analyses was carried out by analysis of variance followed by Williams' test or in cases of heterogenous variance using the Kruskal-Wallis test followed by Shirley's test. Where 75% or greater of the values of a given variable were the same, a Fischer's exact test was used.

A number of additional experiments were conducted as described below:

- A group of 8 pregnant females was undosed throughout the study. Selected offspring from each litter (8/sex/dose, 1/sex from each litter) were treated with dimethoate at 0, 0.1, 0.5 or 3 mg/kg bw/d by gavage on PND 11, and killed 2 h after dosing. ChE activity was determined in plasma, erythrocyte and brain.

- Naïve young adult rats were dosed with dimethoate 0, 0.1, 0.5 or 3 mg/kg bw/d by gavage for one day (8/sex/dose) or 11 consecutive days (8/sex/dose), and killed 2 h after dosing, and ChE activity was determined for plasma, erythrocyte and brain. The rats were approximately 7-8 weeks of age, and in the weight range 221-286 g for males and 166-210 g for females on the day before treatment commenced.

- Five dosed offspring were killed on PND 61, perfused with fixative and the brain embedded, sectioned and subjected to light microscopic examination.

Results For F0 parent females and offspring, there were no deaths, and clinical signs were generally restricted to staining of the coat and hairloss which was observed at a similar frequency in all groups. There was no effect of treatment on body weight or body weight gain during gestation or lactation. Gestation length and gestation index were not affected. All F0 females killed at GD 20 were confirmed to be pregnant with live foetuses. Treatment did not affect the mean numbers of corpora lutea, implantations or the growth and survival of the foetuses and sex ratio. Litter weights and foetal weights of treated groups were comparable to control. All F0 females dosed to PND 10 were pregnant and gave birth to living young. The number of implantation sites, litter size at PND 1, sex ratio, survival of the offspring through to PND 11, pup body weight at birth, and body weight gain during PNDs 1-11 for all treated groups were similar to the control. No macroscopic findings were observed at necropsy on females killed on GD 20 or PND 21.

For all directly dosed and undosed male and female offspring, there were no treatment-related clinical signs or mortalities. Body weight and body weight gain up to PND 60 were comparable between treated groups and control. Necropsy of offspring at PNDs 4, 11, 21 and 60 revealed no abnormalities that were considered to be related to treatment. Brain weight for offspring of all treated groups was similar to that of controls. For the young adults treated with a single dose or 11 consecutive doses, there were no mortalities, and no clinical signs which were considered to be related to treatment. All groups treated with 11 doses, except males at 0.5 mg/kg bw/d, showed slight but not statistically significant reduction in body weight gains (up to 22% lower than control). Brain weights were not affected. Necropsy did not reveal treatment related findings.

Inhibition of ChE activity was the only treatment-related effect observed in this study (see Table 86). The results are summarized as follows:

- Dams killed on day GD 20 showed decreased plasma, erythrocyte and brain ChE activity at 3 mg/kg bw/d; a minor (10%; $p<0.05$) decrease in brain ChE activity at 0.5 mg/kg bw/d was not considered toxicologically significant.

- In GD 20 foetuses, plasma, erythrocyte and brain ChE activity was decreased at 3 mg/kg bw/d; brain ChE activity was statistically significantly reduced at 0.1 mg/kg bw/d (12%), but not at 0.5 mg/kg bw/d (10%; $p>0.05$).

- PND 4 male pups showed decreased (17%, $p<0.01$) erythrocyte ChE activity at 3 mg/kg bw/d; plasma ChE activity was slightly (10%) and significantly decreased in females at the same dose. In addition, slight (8-13%) statistically significant decreases in brain ChE activity were observed in males at doses of ≥ 0.1 mg/kg bw/d; there was no effect on brain ChE activity of females at the same time point. Taking into account the small magnitude (8-13%) of brain ChE inhibition, and the absence of clear dose responses, these effects were considered of doubtful toxicological significance.

- In pups that received dosing directly (PNDs 11-21), inhibition of plasma, erythrocyte and brain ChE activity was seen at 3 mg/kg bw/d on PND 21; statistically significant inhibition of erythrocyte (23%) and brain (12%) ChE activity was observed in females at 0.5 mg/kg bw/d. A slight (4%) but statistically significant inhibition of brain ChE activity in males at the low-dose was not considered toxicologically relevant. Recovery was essentially complete by 60 days, though slight (4%) statistically significant effects were observed for brain ChE activity at ≥ 0.5 mg/kg bw/d in females.

- Male pups given single doses of dimethoate at 3 mg/kg bw on PND 11 exhibited decreased plasma and brain ChE activity. Females at the same dose showed inhibited erythrocyte and brain ChE activity. However, inhibition was statistically significant but below 20% and is therefore of doubtful toxicological significance.

- After single doses of dimethoate in young adult rats, statistically significant inhibition of plasma (12-19%), erythrocyte (17-27%; toxicologically significant) and brain ChE activity (12-14%) were seen at 3 mg/kg bw in males and females.

- After 11 doses of dimethoate in young adult rats, plasma, erythrocyte and brain ChE activity was inhibited in males and females at 3 mg/kg bw/d. Slight (10-13%), statistically

significant inhibition of brain ChE activity at 0.5 mg/kg bw/d was not considered toxicologically significant.

Table 86: Plasma, erythrocyte & brain ChE activity

Parameter	0	0.1	0.5	3	0	0.1	0.5	3
	Dosed F0 females on GD 20				F1 foetuses (from dosed F0) on GD 20			
Plasma (U/L)	1381	1216 (12)	1184 (14)	776** (44)	258	257	239 (7)	147** (43)
Erythrocyte (U/L)	1669	1563 (6)	1459 (13)	709** (58)	1213	1225	1181 (3)	834** (31)
Brain (U/L)	12838	13044	11563* (10)	5094** (60)	1781	1569* (12)	1600 (10)	1188** (33)
	Males				Females			
Undosed F1 pups (from dosed F0) on PND 4								
Plasma (U/L)	612	607 (1)	588 (4)	566 (8)	640	605 (5)	591* (8)	576** (10)
Erythrocyte (U/L)	1291	1403	1254 (3)	1071** (17)	1260	1261	1352	1088 (14)
Brain (U/L)	3137	2817* (10)	2889* (8)	2744** (13)	2823	2914	2650 (6)	2638 (7)
@Dosed F1 pups (from dosed F0) on PND 21								
Plasma (U/L)	506	535	478 (6)	307** (39)	487	507	463 (5)	304** (38)
Erythrocyte (U/L)	1638	1659	1494 (9)	669** (59)	1900	1619 (15)	1466* (23)	663** (65)
Brain (U/L)	10375	9944* (4)	9044 (13)	5675** (45)	10275	9906 (4)	9019** (12)	5956** (42)
@Dosed F1 pups (from dosed F0) on PND 60								
Plasma (U/L)	373	369 (1)	340 (9)	337 (10)	907	915	945	846 (7)
Erythrocyte (U/L)	1075	1100	1100	1038	1109	1119	991 (11)	1044 (6)
Brain (U/L)	13000	13100	12988	13044	13275	12950 (2)	12738* (4)	12744* (4)
Pups (from undosed female) dosed and killed on PND 11								
Plasma (U/L)	756	748 (1)	688 (9)	614** (19)	742	700 (6)	720 (3)	609 (18)
Erythrocyte (U/L)	1663	1634 (2)	1597 (4)	1544 (7)	1997	1647 (18)	1894 (5)	1475 (26)
Brain (U/L)	6475	6363 (2)	6144* (5)	5375** (17)	6256	6350	6125 (2)	5144** (18)
Young adult rats after 1 dose								
Plasma (U/L)	375	387	364 (3)	305* (19)	688	657 (5)	729	602 (12)
Erythrocyte (U/L)	1122	1247	1131	928* (17)	1209	1128 (7)	1106 (9)	881** (27)
Brain (U/L)	13794	13544 (2)	13294* (4)	12131** (12)	14150	13625 (4)	13850 (2)	12106** (14)
Young adult rats after 11 doses								
Plasma (U/L)	343	327 (5)	302 (12)	215** (37)	790	949	770 (3)	624 (21)
Erythrocyte (U/L)	1094	1169	903 (17)	456** (58)	1019	991 (3)	950 (7)	375** (63)
Brain (U/L)	14100	13988 (1)	12700* (10)	7469** (47)	14869	13913 (6)	12881** (13)	6188** (58)

@dosed F1 pups: dosed GDs 11-21 inclusive.
*p<0.05; **p<0.01
() Numbers in parentheses represent percentage inhibition compared with the control value.

Histopathological examination on the 5 dosed offspring allocated to gravity perfusion on PND 61, revealed no significant lesions in the brain or peripheral nerves.

The maternal NOEL was 0.5 mg/kg bw/d based on inhibition of plasma, erythrocyte and brain ChE activity at 3.0 mg/kg bw/d. The NOEL for foetotoxicity was 0.5 mg/kg bw/d based on inhibition of plasma, erythrocyte and brain ChE activity at 3.0 mg/kg bw/d. The NOEL for pup toxicity was 0.1 mg/kg bw/d on the basis of inhibition of erythrocyte ChE activity in pups directly dosed with 0.5 mg/kg bw.

Myers DP (2001c). Dimethoate: developmental neurotoxicity study in the CD rat by oral gavage administration. Huntingdon Life Sciences Ltd. Study duration: October 2000 – June 2001. Guideline: US EPA Subdivision F, OPPTS 870.6300. GLP/QA: yes. DTF Doc No: '468-008' Ref: 3-33/Vol 3-20 to 3-21.
&
Reiss R & Gaylor D (2002). Statistical analysis of selected endpoints in the dimethoate developmental neurotoxicity study. Sciences International, Inc. DTF Doc No: None. Ref: 3-34/Vol 3-21.
&
Myers DP (2003). Dimethoate: developmental neurotoxicity study in the CD rat by oral gavage administration. Huntingdon Life Sciences Ltd. DTF Doc No: None. Ref: 3-35/Vol 3-21.

Methods The potential of dimethoate to cause functional or morphological changes in the nervous system of offspring was assessed following maternal exposure during pregnancy and early lactation combined with direct exposure of the offspring during PNDs 11-21.
Female rats (Crl:CD BR strain, from Charles River UK Limited, Margate, Kent, England) were mated with stock males from the same strain and source, and were 10-11 weeks of age, and in the weight range 219-315 g on GD 0. Dimethoate was administered to pregnant F0 female rats (24/dose) by gavage at 0, 0.1, 0.5 or 3 mg/kg bw/d (Source: Cheminova A/S, Denmark; Batch no: 20522-00, purity: 99.1%, dissolved in water) from GD 6 to PND 10, and to the offspring from PND 11-21 inclusive (except on the day of necropsy, PND 11 or 21). Observations for deaths and clinical signs of dams (autonomic function including lacrimation, salivation, piloerection, exophthalmos, urination, defecation, pupil size and palpebral closure; convulsions, tremors or other abnormal movements; posture and gait abnormalities; any unusual or abnormal behaviour, stereotypies, emaciation, dehydration, hypotonia/hypertonic, altered appearance of fur, red or crusty deposits around the eyes, nose or mouth) were made daily. Arena observations (palpebral closure, posture, gait, tremor, twitches, convulsion, activity count, rearing count, grooming, urination, defecation) and in the hand observations (removal from cage, salivation, lacrimation, piloerection, exophthalmos, pupil closure reflex, reactivity to handing) on 10 dams/dose were performed on GDs 12 and 18, and PNDs 4 and 10. Body weight and food consumption of F0 females were measured every 3 to 4 days during gestation, and lactation, and the duration of gestation was recorded. Observations on F1 litters included clinical signs, foetal and litter weights, litter size, at and after birth, survival indices and sex ratio. Brain weights were determined for dams and foetuses at GD 20 and offspring on PNDs 4, 11, 21 and 60. On PNDs 4, 11, 21, 35, 45 and /or 60, arena observations included surface righting reflex, pupil closure reflex (PND 35 only), physical condition, locomotor co-ordination and abnormal behaviours, in the hand observations and standard arena observations. On PND 4, F1 offspring from each litter were allocated for assessment of motor activity at PNDs 13, 17, 22 and 59, auditory startle response habituation and pre-pulse inhibition of startle at PNDs 23/24 and 60/61, learning and memory at PNDs 23/24 and 61/62.

In all litters except two at 3 mg/kg bw/d, a different pup was allocated to each behavioural test. F1 pup body weights were measured weekly from PND 28 to termination at PND 65. Sexual maturation was assessed from PND 28 onwards for males on completion of balano-preputial separation and from PND 38 onwards for females on vaginal opening.

F0 females were killed on PND 21 and subjected to a detailed macroscopic necropsy. Selected F1 offspring (1 pup/litter, 10/sex/dose/time point) were killed on PND 11 or PND 21 for macroscopic pathology and neuropathology of brain sections (coronal sections: olfactory lobes, forebrain, cerebrum, hippocampus, thalamus, hypothalamus, cerebrum, tectum, tegmentum, medulla oblongata. Mid-sagittal sections: cerebellum, pons from all groups). Histological processing of tissues was conducted on PND 21 animals [brain sections as above, eye, thyroid/parathyroid, thymus, lungs, heart, liver, kidneys, adrenals, pancreas, spleen, gastrointestinal (GI) tract (stomach, duodenum, jejunum, ileum, caecum, colon, rectum), ovaries, uterus, testes, epididymis, prostate, pituitary, abnormalities, lymph nodes – mandibular and mesenteric control and the high dose group)]. On day 65 rats, brain sections and nerve tissues (spinal cord, dorsal root ganglia/fibres, ventral root fibres, eyes, optic nerves, skeletal muscle, sciatic and tibial nerves from control and the high dose group) were examined. Other unallocated F1 pups were killed on PNDs 11, 21 or 65 for macroscopic pathology, brain weight and brain measurements. F1 offspring allocated to behavioural assessments and unallocated offspring were retained and killed on PND 65±2.

Results Two F0 dams at 3 mg/kg bw/d and 5 dams at 0.5 mg/kg bw/d showed salivation after dosing on one or two occasions during gestation. Seven F0 females at 3 mg/kg bw/d lost weight (mean weight loss 8 g) during PNDs 1-4, compared with 2 females (mean weight loss 4 g) in control, without a corresponding change in food consumption. Gestation length, parturition and gestation index were not affected. Treatment did not affect brain weight of F0 females. There was no evidence of neurotoxicity during FOB assessment of the dams.

Three F0 females at 3 mg/kg bw/d and 1 female at 0.5 mg/kg bw/d lost their litters during early lactation (PNDs 2-4) and were killed on the day of litter loss for reasons of animal welfare; their mammary tissue was found to be pale and inactive at necropsy. Following direct dosing of F1 pups during PNDs 11-21, offspring in 3 additional litters at 3 mg/kg bw/d died or were killed, before or after weaning. All above deaths led to increased pup mortality at 0.5 and 3 mg/kg bw/d, and lower survival indices at 3.0 mg/kg bw/d (See Tables below). Therefore, up to weaning (PND 21), the total pup deaths were 15, 11, 24 and 44 respectively at 0, 0.1, 0.5 and 3 mg/kg bw/d when excluding pups in the totally lost litters (1 and 3 litters at 0.5 and 3 mg/kg bw/d respectively), and 15, 11, 42 and 88 when including those. The affected litters/pups at 0.5 and 3 mg/kg bw/d appeared to be small in size and apparently unfed, exhibited opaque eyes, slow respiration, were cold to touch with little food apparent in the stomach, and/or underactive with the dam paying minimal attention to the litter during lactation. Weight gain of offspring was slightly, but not significantly, reduced at 3 mg/kg bw/d during lactation; this was attributed to the low pup weights of two litters in this group.

As shown in Table 87, different statistical analyses were used by the study author. The study author argued that the best method for data analysis in this case was using the non-parametric Mann-Whitney U test for comparison of percentage mortality in the litters (Part IV of Table 91). This resulted in marginally significant differences at 3 mg/kg bw/d (p values 0.02-0.05 for PNDs 1-4, 5-11 and 1-21), but a lack of significance at 0.5 mg/kg bw/d. Historical control data (5 studies) were available from the same facility, using the same rat strain, during

October 2000 and September 2002. The range of pup mortality was 11-23 pups in 11-28 litters excluding those in the totally lost litters, and 11-45 including those. The mortality at 0.5 mg/kg bw/d, with or without including pups in the lost litters, will fall into the range of historical control. However, since the concurrent control was within the normal range (not extremely low), and the mortality at 0.1 mg/kg bw/d comparable, thereby also supporting its rationality, more weight should be put on the concurrent control rather than historical control when the comparison is made.

Table 87: Statistical analysis on litter or pup mortality (Death No/Total)

Group	PNDs 1-4	PNDs 5-11	PNDs 12-21	PNDs 1-21	PNDs 1-4	PNDs 5-11	PNDs 12-21	PNDs 1-21
	I. Total pup mortality				III. Total litters with mortality			
Concurrent controls	10/359	3/192	2/189	15/359	7/24	3/24	2/24	10/24
Combined controls@	29/728	6/384	3/378	38/728	17/48	6/48	3/48	22/48
0.1 mg/kg bw/d	8/343	3/184	0/181	11/343	5/23	2/23	0/23	6/23
0.5 mg/kg bw/d	32/360 *	9/184	1/175	42/360 *	9/24	3/23	1/23	10/24
3 mg/kg bw/d	70/366 *	15/169 *	3/154	88/366 *	13/24	7/22	2/21	10/24
	II. Total pup mortality (excluding sacrificed pups/litters)				IV. Mean percentage mortality in litters (including sacrificed litters)			
Concurrent controls	10/359	3/192	2/189	15/359	2.7	1.6	1.1	4.2
Combined controls	29/728	6/384	3/378	38/728	3.9	1.6	0.9	5.3
0.1 mg/kg bw/d	8/343	3/184	0/181	11/343	2.4	1.6	0.0	3.4
0.5 mg/kg bw/d	17/354	9/184	1/175	27/345	8.0	4.9	0.6	10.5
3 mg/kg bw/d	42/338 *	12/166 *	3/154	57/335 *	18.9*	11.9*	3.2	23.6*

I & II. by Chi-square test for independence. *Statistical significance defined as P<0.017 (or 0.05/3) using a 5% probability value with a Bonferroni correction for three separate comparisons.
III. By Fisher Exact Test.
IV. By non-parametric Mann-Whitney U test. A significance level of 0.017 was used for tests.
@: combined with control from a similarly designed study on conducted on malathion at the same laboratory.

In addition, the increase in pup mortality in another developmental study, a two generation reproduction study, and a cross-fostering study suggests that it is inappropriate to exclude pups from dams that lost their entire litters from analysis. Hence, the significant increase in mortality at 0.5 and 3 mg/kg bw/d cannot be discounted (Table 88). The pup survival rate at 0.1 mg/kg bw/d was not affected. Pup survival after PND 28 and the sex ratio for all groups were normal.

Table 88: F1 Offspring data

Parameter	0	0.1	0.5	3
Live birth index, %	99.7	98.7	98.7	97.9*
Viability index, %	97.6	98.8	92.9	82.7
Lactation index, Day 7, %	99.0	98.9	96.7	93.0
Day 11, %	98.4	98.4	95.1	88.2
Day 21, %	86.2	87.2	87.0	84.0
Pup weight at PND 1, m/f, g	6.5/6.2	6.4/6.0	6.5/6.0	6.5/6.1
at PND 4, m/f, g	9.0/8.5	8.5/8.1	9.2/8.7	8.1/7.7
at PND 21, m/f, g	52.3/51.5	50.9/48.9	52.8/51.0	48.3/47.1

*p<0.05.

During arena observations on F1 offspring, male and female offspring at 3 mg/kg bw/d tended to be slightly less active than control as shown by consistently, but not significantly, lower values for maximum pivoting angle, maximum distance travelled and number of sections

entered in the arena on PND 4 (Table 89). Slightly lower values of activity count and/or surface righting reflex were also observed in males and/or females at 3 mg/kg bw/d on PNDs 11 and 21, but not at latter stages to PND 60. These observations, together with lower body weight/body weight gain of F1 pups during lactation, suggest a slight, reversible developmental delay.

Table 89: Mean arena observations on F1 offspring

Dose mg/kg bw/d	**0**	**0.1**	**0.5**	**3**	**0**	**0.1**	**0.5**	**3**
PND 4								
Maximum pivoting angle (°)	81.0	58.5	27.0	36.0	103.5	40.5	81.0	40.5
Maximum distance travelled (cm)	1.2	0.8	1.4	0.4	1.2	0.4	0.6	0.4
Activity (number of sections)	2.6	2.0	1.3	0.7	2.6	1.3	1.9	0.9
PND 11								
Surface righting reflex (1-3)	8(1), 2(2)	5(1), 5(2)	7(1), 3(2)	2(1), 8(2)	6(1), 4(2)	6(1), 4(2)	7(1), 3(2)	5(1), 3(2), 2(3)
Activity count	2.5	1.8	2.6	2.9	3.0	1.3*	3.3	1.3*
PND 21								
Activity count	7.1	4.7	4.5	2.7**	7.4	7.8	6.3	4.4

*p<0.05, ** p<0.01

Motor activity data on F1 pups showed considerable inter- and intra-group variation, and only females at 3 mg/kg bw/d had a significant decrease in high beam score (46, 20, 26, 4.5** at 0, 0.1, 0.5 and 3 mg/kg bw/d respectively) on PND 17. There were no abnormalities in other observations, including Morris water maze for learning and memory, auditory startle response – habituation, auditory startle response – pre-pulse inhibition. Sexual maturation for F1 males and females was not affected. At necropsy on PNDs 11, 21 and 65, there were no effects of treatment on brain weight and morphometry. On PNDs 21 and 65, single cases of a minimal focus of degeneration of the granular layer of the cerebellum, or malformation of the cerebellar folia at 3 mg/kg bw/d as well as in control, were not considered to be related to treatment.

The NOEL for maternal toxicity was 0.5 mg/kg bw/d based on inhibition of plasma, erythrocyte and brain ChE activity at 3.0 mg/kg bw/d in a previous study (Myers, 2001b). The NOEL for foetotoxicity/pup toxicity was 0.1 mg/kg bw/d, based on increased pup mortality at 0.5 and 3.0 mg/kg bw/d. The NOEL for developmental neurotoxicity was 0.5 mg/kg bw/d based on reduced activity and responses during arena observations at 3 mg/kg bw/d.

10.2 Metabolites

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. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Study duration: March 11 – 13, 2002. Guidelines: None GLP/QA: None DTF Doc No: '0-541-002' Ref: 3-77/Vol 3-37.

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 erythrocyte ChE activities. Brain ChE activity was also determined.

Results 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 90).

Table 90: Cholinesterase activity in serum (µkat/L), erythrocytes (µkat/L erythrocyte) and brain (µkat/g protein) at 8 or 24 hours post-treatment

Dose (mg/kg bw):	Males				Females			
	0	5	10	15	0	5	10	15
Serum 8 h	11.9	5.75** (51)	4.90** (58)	4.25** (64)	27.6	26.4 (4)	13.2** (52)	14.96** (47)
Serum 24 h	10.8	7.34** (32)	5.62** (47)	5.06** (53)	25.0	24.6	12.9** (48)	11.1** (55)
Erythrocyte 8 h	27.3	12.1** (55)	9.64** (64)	7.02** (74)	28.4	11.7** (58)	9.04** (68)	8.71** (69)
Erythrocyte 24 h	31.6	17.6** (44)	11.8** (62)	8.9** (71)	44.3	22.7** (48)	16.2** (63)	13.4** (69)
Brain 24 h	1.33	0.79 (40)	0.59 (55)	0.54* (59)	1.56	0.93 (40)	0.88* (43)	0.69** (55)

Numbers in parentheses represent percent inhibition relative to the corresponding concurrent control
*p<0.05, ** p<0.01

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. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Study duration: 10-11 June 2002 Guidelines: None GLP/QA: None DTF Doc No: '0-541-003' Ref: 3-78/Vol 3-37.

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 erythrocyte ChE activities. Brain ChE activity was also determined.

Results Cholinesterase activity was inhibited in erythrocyte 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 erythrocyte ChE activity was only present at 1.5 mg/kg bw to an extent considered likely to be treatment-related (Table 91). 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. As the inhibition of erythrocyte 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 91: Cholinesterase activity in serum (µkat/L), erythrocytes (µkat/L erythrocyte) and brain (µkat/g protein) at 3 days before treatment, & 2.5 or 24 hours post-treatment

Dose (mg/kg bw)	Serum Day -3	Serum 2.5 h	Serum 24 h	Erythrocyte Day -3	Erythrocyte 2.5 h	Erythrocyte 24 h	Brain 24 h
0	12.1	12.4	11.9	32.3	32.5	33.3	3.20
0.25	12.1	11.6	11.3	32.6	28.3** (12)	33.9	3.23
0.50	12.1	10.8	11.0	34.7	23.8** (26)	30.8	2.8 (13)
0.75	12.5	10.0* 19%	11.3	32.9	20.1** (37)	28.7** (13)	2.85 (10)
1.50	12.4	8.03** (35)	10.8	33.1	14.2** (56)	25.0** (24)	2.21 (30)

() Numbers in parentheses represent percent inhibition relative to the corresponding concurrent control
p<0.05, ** p<0.01

Mellert W, Deckardt K, Kaufmann W & van Ravenzwaay B (2003a). Omethoate – acute oral neurotoxicity study in Wistar rats; single administration by gavage. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Study duration: October – December 2002. Guidelines: OECD 424 and OPPTS 870.6200) GLP/QA: Yes. DTF Doc No: O-541-004' Ref: 3-79/Vol 3-38.

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 erythrocytes of 10 rats/sex prior to administration and in the serum, erythrocytes and brain at 2.5 h post-treatment. Another 10 rats/sex were assessed for

FOB and motor activity on days -7, 0 (about 2 h post-treatment), 7 and 14 as well as for serum and erythrocyte 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 There were no deaths. General clinical signs (apathy, increased respiration, frequent chewing, 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, erythrocyte 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 92). 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 92: Cholinesterase activity in serum (µkat/L), erythrocyte (µkat/L erythrocytes) and brain (µkat/g protein) at 2.5 hours post-treatment

Dose (mg/kg bw)	Males			Females		
	Serum	Erythrocyte	Brain	Serum	Erythrocyte	Brain
0	11.7	31.9	3.87	28.7	33.8	4.47
0.20	11.8	31.5	3.62 (6)	27.7 (6)	31.4 (7)	3.83 (14)
0.25	11.6	27.9 (13)	3.57 (8)	29.9 (8)	31.1 (8)	4.03 (10)
0.35	10.7 (9)	26.8* (16)	3.14 (19)	27.2 (19)	29.3 (13)	3.25* (27)
5	5.4** (54)	7.22** (77)	0.75** (81)	16.9 (81)	8.3** (75)	0.89** (80)

Numbers in parentheses represent percentage inhibition relative to the corresponding concurrent control
*p≤0.05, **p≤0.01

Hens

Bomann W & Sykes AK (1993) E 6876 (c.n. omethoate) Study for delayed neurotoxicity following acute oral administration to the hen. Bayer AG, Wuppertal, Institute of Toxicology Agriculture, Fachbereich Toxikologie, Friedrich-Ebert-Str. 217-333. Study duration September – October, 1989. Guidelines: US EPA 81-7 GLP/QA: Yes DTF Doc No: '541-001' Ref: 3-76/Vol 3-36.

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 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.

11 HUMAN STUDIES

11.1 Oral

Sanderson DM & Edson EF (1964). Toxicological properties of the organophosphorus insecticide dimethoate. Br. J. Industr. Med. 21: 52-64.
Twenty subjects ingested 2.5 mg of laboratory grade dimethoate in aqueous solution, corresponding to about 0.04 mg/kg bw/d for 4 weeks. No signs of toxicity were observed, and there was no significant change in blood cholinesterase activity. The same results were found in 2 subjects that ingested 9 mg (0.13 mg/kg) and 18 mg (0.26 mg/kg bw) dimethoate respectively, for 21 days. It was also reported that five males ingested single doses of 0.25 mg/kg bw dimethoate, without toxic effect or cholinesterase inhibition.

Edson EF, Jones KH & Watson WA (1967). Safety of dimethoate insecticide. Br. Med. J. M 5578: 554-555.

Dimethoate (purity not specified) was given as a flavoured aqueous solution to male and female volunteers, five days per week, at doses that resulted in average daily doses of 5, 15, 30, 45 and 60 mg for periods of 14 to 57 days as indicated in Table 93. Venous blood samples were taken twice prior to dosing and two times per week during the dosing period. There were no localized gastrointestinal effects and no other noted clinical signs. It was reported that no significant changes in ChE activity were seen in groups A and B, while values in Group C, showed a 'downward trend' by day 20, which continued until day 57 (interim data not shown). It was also reported that erythrocyte ChE activity paralleled that of whole blood ChE activity in Groups C and D (data not shown).

Table 93: Safety of dimethoate in human volunteers

Group	No. subjects	Daily dose (mg)	Duration	Mean Whole Blood ChE[b]		Mean weight (kg)	Daily intake[a]
				Pretest	End		
A	12	5	28	114	110	74	0.067
B	9	15	39	128	124	75	0.200
C	8	30	57	121	92 (24)	71	0.423
D	6	45	45	113	74 (35)	77	0.584
E	6	60	14	121	96 (21)	60	1.00

[a] mg/kg bw/d, [b] defined as 'cholinesterase units' (Δ pH/h x 100). Numbers in parentheses represent percentage inhibition compared to pretest values.

The NOEL was 0.2 mg/kg bw/d based on decreased whole blood ChE activity at higher doses.

***Reviewer's note*:** Volunteers received dimethoate 5 days per week, and were not treated for the other 2 days per week. A steady state of plasma level of the chemical was likely not achieved, and toxicological findings under this circumstance might be different from that under the normal dosing regime (7 days/week) in a study.

Krieger RI & Thongsinthusak T (1993). Metabolism and excretion of dimethoate following ingestion of overtolerance peas and a bolus dose. *Fd Chem. Toxic*. 31(3): 177-182.

Methods A 150 kg adult male volunteer consumed eight 135 g samples of sugar peas (approx 1.1 kg) estimated to contain 17 ppm total dimethoate during a five hour period. The consumed dose of dimethoate was estimated to be 18.7 mg or approximately 0.12 mg/kg bw. Urine samples were collected for 41 h. Erythrocyte ChE activity was determined 24 hours after exposure via ingestion of the sugar peas. About 15 days later, the same volunteer ingested a 20.2 mg dose (0.13 mg/kg bw) of dimethoate (stated to be purified technical grade; 98% pure) in a gelatin capsule. Urine samples were collected for 50 h. In both tests, urine was analysed for dimethoate, dimethoxon and corresponding dimethylphosphates.

Results Following exposure to dimethoate as a residue on peas, the major phosphate ester was dimethylphosphorothioate, with lesser amounts of dimethylphosphorodithioate and dimethylphosphate detected in the urine. Small amounts of dimethoate and dimethoxon were also recovered, but these were estimated to account for less than 0.3% of the dose. Erythrocyte ChE activity did not differ markedly from the pre-dose value. Similarly, dimethylphosphorothioate was the most prominent of the dimethylphosphates in the urine, two hours after the bolus dose. Whereas 30 μmol of dimethylphosphorothioate was recovered, the levels of dimethylphosphorodithioate and dimethylphosphate were 22 and 11 μmol respectively. Dimethoate and dimethoxon detected in the urine accounted for approximately 0.4% of the administered dose.

11.2 Dermal

Edson & Stroude (1958). Dermal absorption in human. Fisons Ltd, Project No: Tox/52/24.
[Note: This study has been evaluated previously by OCSEH (Submission No. 437)]

A 40% (ai) formulation of dimethoate was applied, undiluted, to the forearms of human volunteers at a rate of 0.5 mL/100 cm^2 skin. The application time was 2 h, after which the skin area was uncovered, washed and dried. Blood samples were taken 24 h prior to and post exposure and ChE activity determined. Areas of 50 to 200 cm^2 were treated on one forearm, both arms were used for areas of 250 to 500 cm^2. An average dosage unit of 0.25 mLs/50 cm^2 was used. Local skin responses were absent or negligible in all but one subject. In this subject, a fine punctate on irritant rash appeared and lasted for 2 weeks. There was no treatment-related decrease in plasma ChE activity in any of the subjects tested.

11.3 Occupational Exposure

Al-Jaghbir MT, Salhab AS, and Hamasheh FA (1992). Dermal and inhalation exposure to dimethoate. *Arch. Environ. Contam. Toxicol.* 22: 358-361.

Dermal and respiratory exposure to dimethoate was measured for six workers applying dimethoate (40% EC; no further details on the formulation supplied) to tomato plants under plastic houses in the Central Jordan Valley during April-May 1989. The study was conducted in six plastic houses which were 19 metres long, 9 metres wide and 3.2 metres high, and covered with a polyethylene transparent plastic sheet. The average temperatures in the houses were 33.7 ± 2.1 ºC and 36.2 ± 1.3 ºC during the first and second sprayings, respectively. The

spraying was carried out by six volunteers, aged between 21 and 26 years, typically wearing long-trousers, a short sleeved open neck shirt and tennis shoes. Available gloves, hats and masks were not used. Each volunteer applied two sprays, 0.5 h in duration, with an interval of 15 days in between. The sprayers used a Lurmark-PTP 20 knapsack sprayer, giving a working pressure of about 40 psi, and a liquid capacity of about 20 L. A hollow cone spray nozzle was used, and approximately 25 mL of dimethoate was diluted to make about 20 L.

Ten gauze sponges (12 ply, 10 cm^2) were applied to body sites on each sprayman. At the end of each spraying period, dimethoate was extracted from the sponge three times using dichloromethane. The exposure rate, calculated as $\mu g/cm^2/h$ at the various body sites is shown in Table 94. The daily exposure rate was calculated as (mg dimethoate/cm^2/hr) x surface area of body part x time (assuming a working day of 4 h). The mean total dermal exposure, except hand exposures (not determined), was 914 mg/4 h day.

Table 94: Recovery of dimethoate from sponges applied to various body sites of spraymen applying dimethoate as a 40% EC

Sprayman	Mean dimethoate recovery from gauze sponges ($\mu g/cm^2/hr$)										
	Front	**Back**	**RS**	**LS**	**URA**	**ULA**	**LRA**	**LLA**	**URL**	**ULL**	**Total**
1	2.78	1.81	19.7	12.2	9.3	15.4	83.8	38.4	44.4	37.2	265
2	5.67	2.65	19.3	12.9	18.8	53.0	51.3	39.7	50.9	45.2	299
3	2.25	1.52	10.4	8.38	21.1	37.5	50.7	31.2	57.4	35.9	256
4	9.16	1.64	14.5	11.4	17.3	40.1	82.1	42.3	50.7	31.6	301
5	6.56	4.33	16.2	13.3	19.8	25.7	50.9	27.9	46.4	36.0	247
6	8.17	1.57	16.6	11.2	17.6	28.7	54.2	28.9	48.1	46.8	262
Mean	**5.77**	**2.25**	**16.2**	**11.6**	**17.3**	**33.4**	**62.2**	**34.7**	**49.7**	**38.8**	**272**

Values represent the mean of two applications. RS = Right shoulder, LS = left shoulder, URA = upper right arm, ULA = upper left arm, LRA = lower right arm, LLA = lower left arm, URL = upper right leg, ULL = upper left leg. Total = all pads

An air sampling pump, consisting of a battery-powered air pump fitted with a glass impinger filled with 15 mL of ethylene glycol, was fitted to each sprayman. Daily respiratory exposure (mg/d) was calculated by multiplying the concentration of dimethoate in air by the average ventilation rate of a man conducting light work (1,740 L/h), by a working time of 4 h. The calculated respiratory exposure (mg/d) of the spraymen is shown in Table 95. The mean total respiratory exposure was 17 mg/4h day.

Table 95: Estimated respiratory exposure to spraymen applying dimethoate 40% EC

Sprayman	Respiratory exposure to dimethoate (mg/d)		
	Test 1	**Test 2**	**Average (mg/d)**
1	16.5	15.9	16.2
2	17.2	18.2	17.7
3	14.6	16.1	15.3
4	16.4	17.4	16.9
5	18.0	16.9	17.5
6	18.9	17.6	18.2
Mean			**17.0**

Plasma ChE activity was measured before spraying and at 0.5 h and 24 h after spraying. These values were compared with those obtained 36 days prior to exposure. As shown in Table 96, plasma ChE activity was inhibited ≥20% compared to pretest (0 time) values at both time points for all subjects, with the exception of a 17% inhibition at 14 h in subject 4.

Table 96: Plasma ChE activity in spraymen exposed to dimethoate 40% EC

Sprayman	Plasma ChE activity expressed as a percentage of values 36 days prior to exposure		
	Pretest (0 time)	**0.5 h**	**24 h**
1	96.3	62.3 (34)	71.4 (24.9)
2	97.9	59.5 (38.4)	66.7 (31.2)
3	93.7	61.2 (32.5)	70.3 (23.4)
4	98.5	54.4 (44.1)	81.6 (16.9)
5	96.7	63.1 (33.6)	75.4 (21.3)
6	98.2	58.4 (39.8)	63.8 (34.4)
Mean	**96.9**	**59.8 (37.1)**	**71.5 (25.4)**

Values are percentages of values obtained 36 days pretest. Each represents a mean of two samplings. () Numbers in parentheses represent the difference in percentage between pretest (0 time) and the percentage obtained 0.5 h or 24 h after exposure.

Aprea C, Terenzoni B, De Angelis V, Sciarra G, Lunghini L, Borzacchi G, Vasconi D, Fani D, Quercia A, Salvan A & Settimi L (2004). Evaluation of skin and respiratory doses and urinary excretion of alkylphosphates in workers exposed to dimethoate during treatment of olive trees. *Arch. Environ. Contam. Toxicol*. 48: 127-134.

Dimethoate was sprayed on olive trees in the Viterbo province in central Italy from July to September 2001, using a tractor powered atomizer; only in one case was a tractor cabin used. The duration of the spray treatments lasted several hours, but were not prolonged greater than 1 day on any occasion. Mean estimates for the duration of spraying ranged from 91 to 115 minutes. Eighteen male workers, aged 34 to 66 years, underwent biological monitoring, and respiratory and skin exposure was investigated in 9 subjects. Personal protective equipment used by the subjects was reported to be extremely variable, ranging from nothing to mask and helmet with filters; hand protection ranged from nothing to neoprene gloves. Cotton garments, waterproof, nondisposable overalls, disposable Tyvek overalls, shoes or boots and cloth caps were also used. Personal air sampling was carried out to measure the amount of dimethoate in inhalable airborne particulate and vapour. Skin contamination was estimated using 16 cm^2 pads of α-cellulose placed on the face; 49 cm^2 pads were placed on the skin of the chest, back, right forearm, left arm, right anterior thigh, left posterior thigh, right shin and left calf. Hand exposure to dimethoate was evaluated by washing hands at the end of the work shift with ethanol. A model was used to estimate daily skin contamination (excluding hands) and absorbed doses were calculated assuming 10% skin penetration. Biological monitoring was carried out using assays of urinary dimethoate metabolites. Urine samples were collected before spraying and up to 24 hours after the shift. Estimated dimethoate exposures of the workers are shown in Table 97. Large standard deviations in the data precluded any conclusions regarding urinary dimethoate excretion in workers and a general population group.

Table 97: Exposure (µg/d) in workers applying dimethoate to olive trees

Body area	Mean ± SD
Potential respiratory dose	9.94 ± 9.22
Exposed skin dose (face and neck)	68.69 ± 95.95
Unexposed skin dose	
All data (n=9)	118.9 ± 213.4
Cotton garments (n=5)	196.2 ± 271.7
Nondisposable waterproof garments (n=2)	35.57 ± 30.54
Disposable Tyvek overalls (n=2)	9.09 ± 10.16
Hand dose	
All data (n=9)	73.21 ± 67.73
Leather gloves (n=1)	ND
Rubber gloves (n=3)	76.43 ± 64.41
Neoprene gloves (n=5)	83.16 ± 77.93
Total skin contamination	260.8 ± 239.0
Total potential dose	3.56 ± 3.44
Total absorbed dose	**0.375 ± 0.370**

ND =No data

Matos E, & Larripa I (1982). Effect of an accidental exposure to dimethoate and derivatives. Medicina 52: 381-384.

In 1979, an accident occurred at a plant where dimethoate was being formulated resulting in the exposure of 26 men to the insecticide and derivatives formed during an unintentional exothermic reaction. Blood samples were taken two months after the accident to investigate the effects of exposure on sister chromatid exchange in peripheral blood lymphocytes. The authors concluded that the results showed a significant increase in SCE mean values in the exposed population compared to control groups [10 firemen (from a different locality) and 10 ordinary people]. The relevance of these findings to dimethoate exposure is unclear because of the undefined exposure and poor study design.

11.4 Poisoning incidents

Eddleston M, Eyer P, Worek F, Mohammed F, Senarathna L, von Meyer L, Juszczak, E, Hittarage A, Azher S, Dissanayake W, Revzi Sheriff MH, Szinicz L, Dawson AH & Buckley NA (2005). Differences between organophosphorus insecticides in human self-poisoning: a prospective cohort study Lancet, 366(9495): 1452-1459.

Eddleston *et al.* (2005) conducted a prospective study of 802 patients admitted to Sri Lankan hospitals following self poisoning with chlorpyrifos, dimethoate, or fenthion. The authors concluded that the mode of death differed between the three OPs, with many deaths in dimethoate-poisoned patients (35/60) occurring 12-48 hours after ingestion as a result of hypotensive shock. The majority of these patients presented with poor respiratory function requiring mechanical ventilation, and hypotension requiring vasopressors and atropine. No dimethoate patients with mild symptoms on arrival died from delayed respiratory arrest and deaths later than 5 days after admission were uncommon. There were differences in butyrylcholinesterase (BuChE) enzyme activity in the groups of patients exposed to the three organophosphate compounds. In particular BuChE activity was not inhibited to as large extent following exposure to dimethoate. The authors also reported differences in ageing of erythrocyte ChE, with significant ageing (72%) reported on admission for dimethoate patients and only 19% for chlorpyrifos. Complete ageing had occurred by 24 hours making oxime treatment thereafter ineffective. In dimethoate exposed patients treated with pralidoxime,

erythrocyte ChE activity was slightly below admission values at 12 hours. Overall, the authors concluded that dimethoate produced a 'different clinical syndrome' to other organophosphates whereby some patients were deeply unconscious despite having erythrocyte ChE activities in the range of 10-20% of normal. Furthermore, severely poisoned patients were hypotensive and it was suggested that the patients died from hypotension while being ventilated.

Jovanovic D, Randjelovic S, & Joksovic D (1990). A case of unusual suicidal poisoning by the organophosphorous insecticide dimethoate. *Human & Experimental Toxicology* 9: 49-51.

A 20-year old male was admitted to hospital 16 hours following sc injection of 10 mL of Sistemin (40% dimethoate) into his left forearm. The patient reported dizziness, blurred vision, muscular fibrillations as well as abdominal cramps. The patient was initially treated with atropine and oxime HI-6, and diazepam and antibiotics. Plasma and erythrocyte ChE activities were low upon admission and decreased further over the next few days, before partial recovery during days 6-11. Plasma and erythrocyte ChE activities remained low on day 24 (estimated to be 50-60% of normal), when the patient was discharged.

LeBlanc FN, Benson, BE, & Gilg AD (1986). A severe organophosphate poisoning requiring the use of an atropine drip. *J. Toxicol. Clin. Toxicol.* 24(1): 69-76.

A 68 year old male ingested approximately 90 mLs of Cygon 2-E® reported to contain 23.4% dimethoate. The patient was initially treated with ipecac, activated charcoal, magnesium citrate and several bolus doses of atropine. The patient's condition deteriorated over the following 30 minutes resulting in a loss of consciousness and drop in blood pressure. Over the next 24 hours the patient's condition 'stabilized' but within 8 hours of transfer to another ward he suffered a second bout of hypotension, bradycardia and hypoxia. Cholinergic signs were observed. The patient was treated with atropine for the next 14 days after which sensorium and respiratory parameters improved. Two days later he suffered a third respiratory and cardiac event, this time progressing to asystole. The patient was then maintained on atropine for 35 days, at which time the drip was removed and no further relapses were seen. The patient was reported to have had a 'great deal' of spastic rigidity, which began to resolve with time. The patient was discharged 42 days following admission. The man was reported to be suffering from a hearing deficit and a 'non-specific personality change.'

De Bleeker J, Van Den Neuker K, & Willems J (1992). The intermediate syndrome in organophosphate poisoning: presentation of a cases and review of the literature. *Clinical Toxicology* 30(3): 321-329.

A 44 year-old healthy woman was admitted to hospital 8 hours following the ingestion of approximately 150 mL of Roxion 40® stated to contain dimethoate at 400 g/L and cyclohexanone at 540 g/L. The patient was treated with atropine, oral activated charcoal and mannitol. At 36 hours following admission, the patient exhibited chills, bronchial hypersecretion and hyperlacrimation. Pupils were miotic, reacted poorly to light and the patient complained of double vision. The patient could not sit unaided because of hypotonia. Atropine dosage was increased and pralidoxime in IV perfusion was initiated. On the third day, the patient suddenly developed progressive dyspnea and subsequently carbonarcosis requiring artificial respiration. Prior to this, the patient reported double vision, ptosis, and general weakness. While the patient was ventilated, no fasciculation of muscarinic symptoms

were noted. Proximal limbs muscles and neck flexors were weak. On the fourth day, similar signs were reported and the patient was treated with diazepam for tonic clonic seizures. The patient was extubated on day 7, but exhibited rapid breathing and became confused and required further intubation. On day 9, the patient was extubated successfully and released from hospital on day 13. Examination 7 weeks after admission revealed no signs of polyneuropathy.

Fonseka MMD, Medagoda K, Tillakaratna Y, Gunatilake, SB, & de Silva, HJ (2003). Self-limiting cerebellar ataxia following organophosphate poisoning. *Human & Experimental Toxicology* 22: 107-109.

A 21 year-old male ingested about 250 mL of dimethoate at a reported concentration of 50 g/L. The patient was admitted exhibiting pinpoint pupils and bradycardia. The patient received gastric lavage and intravenous atropine and pralidoxime. After three days, the patient developed respiratory insufficiency evidenced by low tidal volume and vital capacity and was treated with intermittent positive ventilation for 3 days. At day 8 after admission, and increasing in severity for two days, the patient complained of difficulty walking and unsteadiness. It was reported that he exhibited normal reflexes and no sensory deficit. The patient exhibited marked gait ataxia with poor coordination in the lower limbs. The patient improved over 6 days and made a complete recovery.

11.5 Sensitization

Haenen C, De Moor A, & Dooms-Goossens A. (1996). Contact dermatitis caused by the insecticides omethoate and dimethoate. *Contact Dermatitis* 35(1): 54-55.

Haenen *et al*. (1996) reported a case of a 38-year-old woman with chronic eczema of the fingertips, that had spread to the hands, neck and shins, and which had persisted for 3 years. It was reported that the woman sprayed roses, in a greenhouse, once a week with omethoate and that her husband, sprayed the roses with dimethoate, but less frequently. The woman came into contact with the roses about five hours after spraying, and wore leather gloves which reportedly rapidly became wet. A patch test was conducted and positive results were observed for omethoate and dimethoate. Control tests in ten other subjects were negative. The purity of the omethoate and dimethoate were not established.

Schena D, & Barba A. (1992). Erythema-multiforme-like contact dermatitis from dimethoate. *Contact Dermatitis* 27: 116-117.

A 41-year old warehouse worker presented with fixed erythematous oedematous lesions showing centrifugal extension with a depressed violet centre. The lesions had appeared the day after he had been exposed to dimethoate as a result of accidental breakage of a container. The lesions were reported to be located symmetrically on the palms and backs of the hands and on the wrists and spread to the rest of the skin over the next few days. After resolution of the lesions the subject was patch tested with a series of pesticides and showed positive reactions to dimethoate 1% pet.

12 OTHER STUDIES

12.1 Mechanistic Studies

Afifi NA, Ramadan A, Abd El-Aziz, & Saki EE. (1991). Influence of dimethoate on testicular and epididymal organs, testosterone plasma level and their tissue residues in rats. *Dtsch. Tierarztl. Wschr.* 98: 419-423.

Male albino rats (25/group; 12-15 months old, weighing 175-185 g; obtained from the Laboratory Animal Colonies, Ministry of Public Health, Helwan, Egypt) were given dimethoate orally (Cygon®, Egyptian Seed Oil Chemical Co. Opera-Cairo, Egypt) at 6.25 and 12.5 mg/kg bw/d for 65 days. Animals were observed for sex organ weight, sperm number and quality, and serum testosterone concentration.

Mortality, body weight and clinical signs were not reported. Relative weights of the testis, seminal vesicles and prostate gland were decreased at 6.25 and 12.5 mg/kg bw/d (Table 98).

Table 98: Relative weights of testicles, seminal vesicles and prostate gland in rats given a dimethoate formulation orally for 65 days

Organ	Relative organ weight (g/100 g bw)		
	Control	6.25 mg/kg bw	12.50 mg/kg bw
Testicles	1.45	1.28 ***	1.09 ***
Testicles (21 d recovery)	1.45	1.30 ***	1.14 ***
Seminal vesicles	0.19	0.15 ***	0.11 ***
Seminal vesicles (21 d recovery)	0.19	0.16 ***	0.12 ***
Prostate gland	0.15	0.12 ***	0.09 ***
Prostate gland (21 d recovery)	0.16	0.13 ***	0.11 ***

***significant at $p<0.001$, n=5

Decreases in sperm concentration, sperm motility and percentage live sperm as well as an increase in total sperm abnormalities were seen following both doses of dimethoate (Table 99).

Table 99: Spermatogenic parameters in rats given a dimethoate formulation orally for 65 days

Parameter	Control	6.25 mg/kg bw	12.50 mg/kg bw
Sperm cell concentration (10^8/mL)	1.64	1.28 ***	0.84 ***
Sperm cell concentration (10^8/mL); 21 d recovery	1.63	1.46 **	1.32 ***
Live sperm	97.20	85.60 ***	81.60 ***
Live sperm; 21 d recovery	97.00	92.40 ***	88.00 ***
Motility (%)	95.20	17.40 ***	7.00 ***
Motility (%); 21 d recovery	95.00	39.60 ***	33.80 ***
Total sperm abnormalities (%)	1.40	15.40 ***	27.60 ***
Total sperm abnormalities (%); 21 d recovery	1.60	7.80 ***	15.00 ***

** significant at $p<0.01$, *** significant at $p<0.001$, n=5

A significant ($p<0.001$) decrease in plasma testosterone was seen in treated groups at all measured time points (Table 100).

Table 100: Serum testosterone concentration in rats given a dimethoate formulation orally for 65 days

Timepoint	Serum testosterone concentration		
	Control	6.25 mg/kg bw	12.50 mg/kg bw
0	4.15 ± 0.050	4.14 ± 0.017	4.14 ± 0.044
14 d	4.07 ± 0.036	3.55 ± 0.116***	3.17 ± 0.054***
28 d	3.97 ± 0.023	3.35 ± 0.114***	3.02 ± 0.046***
42 d	3.84 ± 0.053	3.22 ± 0.072***	2.72 ± 0.036***
65 d	3.84 ± 0.043	3.10 ± 0.033***	2.01 ± 0.064***
Recovery 21 d	3.92 ± 0.034	3.70 ± 0.047***	3.49 ± 0.107***

***p<0.001, n=5

Histopathological examination revealed vacuolization of primary and secondary spermatocytes, irregular arrangement of spermatogenic cells, with a decreased number of spermatids and spermatozoa. Interstitial tissue was thickened by oedematous fluid and showed signs of haemorrhage. Eosinophilic material was seen in the alveoli of seminal vesicles and prostate glands. In the liver, the central veins and hepatic sinusoids were described as dilated and filled with blood, with haemorrhagic areas. Hyperplastic proliferation of the bile duct and connective tissue proliferation in the portal tracts was seen. Cytoplasm's of hepatic parenchyma were reported to be vacuolated with nuclear pyknosis and karyorrhexis. TLC of dimethoate concentrations revealed similar concentrations in liver, testis and skeletal muscle; concentrations declined slightly 21 days after removal of dimethoate from the diet.

***Reviewers note*:** These findings, while appearing to indicate an affect on male reproductive organs weights and sperm quality, were conducted on a 40% formulation of dimethoate with unknown non-active constituents. A large proportion of the findings, including altered reproductive organs weights, similar dimethoate levels in liver and testes, and histopathological effects in the liver, were not consistent with results obtained in the majority of studies in the database.

Kumar Maiti P & Kar A. (1997). Dimethoate inhibits extrathyroidal 5′-monodeiodination of thyroxine to 3,3′,5-triiodothyronine in mice: the possible involvement of lipid peroxidative process. *Toxicology Letters* 91: 1-6.

Dimethoate (purity, source unspecified) in corn oil was given by ip injection to male Swiss mice (8/group; 30 ± 2 grams) at doses of 0, 2, 4, and 8 mg/kg bw/d for 30 days. Serum concentrations of TSH, thyroxine (T4) and triiodothyronine (T3) and liver levels of type-I iodothyronine 5′-monodeiodinase were measured. In addition, hepatic lipid peroxidation, and the activity of superoxide dismutase and catalase were investigated. Presentation of results was in graphical form, therefore ranges of alterations in biochemical values are approximate only. There was no effect on body weight and no clinical signs were recorded. At 4 and 8 mg/kg bw/d, serum T4 concentration was significantly increased (10-35%, p<0.02 or 0.01) whereas serum T3 was decreased (30-40%, p<0.01 or p<0.001). At the same doses, hepatic type-I iodothyronine 5′-monodeiodinase was significantly decreased (25-40%, p<0.01 or p<0.001) compared to control. Hepatic lipid peroxidation (20-60%) and the activity of superoxide dismutase (60-80%) and catalase (20-50%) were significantly (P<0.05, p<0.02, p<0.01, p<0.001) increased compared to control in all treated groups.

REFERENCES

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

Afifi NA, Ramadan A, Abd El-Aziz & Saki EE (1991). Influence of dimethoate on testicular and epididymal organs, testosterone plasma level and their tissue residues in rats. *Dtsch. Tierarztl. Wschr.* **98**: 419-423.

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Aprea C, Terenzoni B, De Angelis V, Sciarra G, Lunghini L, Borzacchi G, Vasconi D, Fani D, Quercia A, Salvan A & Settimi L (2004). Evaluation of skin and respiratory doses and urinary excretion of alkylphosphates in workers exposed to dimethoate during treatment of olive trees. *Arch. Environ. Contam. Toxicol*. **48**: 127-134.

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APPENDICES

APPENDIX I: Australian registered products containing dimethoate

APVMA Product Code	Product Name	Product Registrant	Product Description (Use & Pack Sizes)	Content & formulation type
32953 HG	Chemspray Rogor Insecticide	Envirogreen Pty Ltd	For control of fruit fly, aphids, thrips, leaf miners, bean fly & other pests on fruit trees, vegetables, potatoes & ornamentals; 500 & 100 mL.	300 g/L EC
32962	Nufarm Dimethoate Systemic Insecticide	Nufarm Australia Limited	For control of a wide range of pests on fruit, vegetables, pastures, cotton, lucerne, peanuts & ornamentals; 500 mL, 5 & 20 L.	400 g/L EC
32963 HG	Garden King Rogor 100 Systemic Insecticide	Envirogreen Pty Ltd	Controls aphids, fruit fly, jassids, two-spotted mite & thrips in the home garden; 200 mL	100 g/L EC
39239	Farmoz Dimethoate 400 Systemic Insectide	Farmoz Pty Limited	For the control of certain insects (including aphids, thrips, jassids, lucerne flea, red-legged earthmite, Queensland fruit fly, leaf hoppers & wingless grasshopper) in field crops, fruit crops, oilseed & fibre crops, vegetables, ornamentals, forest trees. To use as a post harvest fruit dip & as seed dressing for lupins, peas, sub-clover, lucerne, linseed & canola; 5, 20 & 200 L.	400 g/L EC
41070 HG	CRG Systex Insectide	Chemical Recovery Co Pty Ltd	For the control of aphids, thrips & other suckling insects in ornamentals, beans & vegetables; 100, 200 & 500 mL.	300 g/L EC
48956 HG	Richgro Garden Products Rogor Insecticide	A Richards Pty Ltd T/A Richgro Garden Products	For the control of fruit fly, aphids, thrips, leaf miners, bean fly & other insects on fruit trees, vegetables, potatoes & ornamentals; 250 mL.	100 g/L EC
49167	Summit Dimethoate Systemic Insecticide	Summit Agro Australia Pty Ltd	Controls pests including Queensland fruit fly, redlegged earthmite, thrips, jassids, lucerne flea, leaf hoppers wingless grasshoppers; 5, 20 & 200 L.	400 g/L EC
49600	Saboteur Systemic Insecticide	Crop Care Australasia Pty Ltd	For control of insect pests on fruit, vegetables, citrus, pastures, cotton, lucerne, peanuts & ornamentals; 20 & 200 L.	400 g/L EC
49833	Rotam Romethoate Systemic Insecticide	Rotam Australasia Pty Ltd	For control of pests on crops including bananas, berry fruits, cereals, citrus, cotton, legume crops and lucerne; 20 & 200 L.	400 g/L EC
50342	Dimethomax Systemic Insecticide	Nufarm Australia Ltd	For control of a wide range of pests on fruit, vegetables, pastures, cotton, lucerne, peanuts & ornamentals; 5, 20 & 200 L.	400 g/L EC
51545	Chemag Dimethoate Insecticide	Chemag Pty Ltd	For control of pests on bananas, berry fruits, cereals, citrus, cotton, grapes legume crops, lucerne & medic pastures, peanuts, pome & stone fruits, oil seeds, ornamentals, tropical fruit & vegetables. Product can be used as a seed treatment for certain crops & as post harvest dip for quarantine purposes; 10, 20, 200, 500 & 1000 L.	400 g/L EC

APPENDIX I: Australian registered products containing dimethoate (Cont'd)

APVMA Product Code	Product Name	Product Registrant	Product Description	Dimethoate content
51658	Sipcam Rogor Systemic Insecticide	Sipcam Pacific Australia Ltd	For control of pests on fruits & vegetable crops, pastures, cotton, lucerne, oil seed crops & ornamentals; 500 mL, 5 & 20 L.	400 g/L EC
52673 HG	Garden King Rogor Garden Insect Spray	Envirogreen Pty Ltd	Controls aphids, thrips, mealy bug, azalea lace bug, fruit fly & other insect pests on fruit trees, vegetables & ornamental plants in the home garden; 300 g	0.3 g/kg AE
53045	Agcare Biotech Dimethoate 400 EC Systemic Insecticide	Agcare Biotech Pty Ltd	For the control of a wide range of pests on fruit, vegetables, pastures, cotton, lucerne, peanuts & ornamentals; 500 mL, 5 & 20 L.	400 g/L EC
55272 HG	Superway Dimethoate 300 Systemic Insecticide	Superway Garden Products Pty Ltd	For the control of fruit fly, aphids, thrips, leaf miners, bean fly and other pests on fruit trees, vegetables, potatoes and ornamentals in the home garden; 125, 250 & 500 mL & 1 L.	300 g/L EC
55441	4 Farmers Dimethoate 400 Systemic Insecticide	4 Farmers Pty Ltd	For control of pests on fruit, vegetables, citrus, pastures, cotton, lucerne, peanuts & ornamentals; 20 & 200 L.	400 g/L EC
55495	Superway Dimethoate 400 Systemic Insecticide	Superway Garen Products Pty Ltd	For control of insect pests on fruits, vegetables, pastures, cotton, lucerne, oil seed crops, peanuts & ornamentals; 500 mL, 1, 2.5, 5, 10 & 20 L.	400 g/L EC
55704	Conquest Dimethoate 400 Systemic Insecticide	Conquest Agrochemicals Pty Ltd	For the control of pests on some fruit, vegetables, citrus, pastures, cotton, lucerne, peanuts & ornamentals; 5, 20 & 200 L.	400 g/L EC
56454	Danadim Insecticide	Cheminova Australia Pty Limited	For the control of a wide range of insect pests on fruit trees, vegetables, citrus, pastures, cotton, lucerne, peanuts & ornamentals; 5, 20 & 200 L.	400 g/L EC
56887	United Farmers Unidime 400 Insecticide	United Farmers cooperative Company Ltd	For control of pests on some fruit, vegetables, citrus, pastures, cotton, lucerne, peanuts & ornamentals. Pack size; 5, 20 & 200 L.	400 g/L EC
57860	Halley Dimethoate 400 Systemic Insecticide	Halley International Enterprise (Australia) Pty Ltd	For control of insect pests on fruits & vegetable crops, pastures, cotton, lucerne, oil seed crops, peanuts & ornamentals; 500 mL; 1, 2.5, 5, 10, 20, 100 & 200 L.	400 g/L EC

APPENDIX II: NOHSC classification

Hazard classification

Dimethoate is listed in the National Occupational Health and Safety Commission (NOHSC) Hazardous Substances Information System (NOHSC, 2004).

According to the toxicology data available and applying the NOHSC (ASCC) Approved Criteria, dimethoate should be classified as follows:

The overall revised classification (for dimethoate) recommendation (to ASCC) should be as follows:

≥ 25%	**R43, R23/R24/25, R48/25**
10% ≤ conc < 25%	**R43, R20/R21/22, R48/25**
3% ≤ conc < 10%	**R43, R20/R21/22, R48/22**
1% ≤ conc < 3%	**R43, R48/22**

All codes above translate into the following risk phrases:

R20 Harmful by inhalation
R21 Harmful in contact with skin
R22 Harmful if swallowed
R23 Toxic by inhalation
R24 Toxic in contact with skin
R25 Toxic if swallowed
R36 Irritating to eyes
R38 Irritating to skin
R43 May cause sensitisation by skin contact
R48 Danger of serious damage to health by prolonged exposure

All formulations of dimethoate are classified as hazardous when they contain > 1% dimethoate.

In accordance with Commonwealth/State/Territory Hazardous Substances legislation, the following control measures must be instituted, where applicable.

1. Induction and training - Appropriate induction and on-going training of all workers with the potential for exposure to dimethoate products, in relation to those substances in the workplace and commensurate with the risk identified by the workplace assessment process.

It is recommended that appropriate training courses (eg. Farm Chemical User Course or recognised equivalent) be identified for all workers involved in the use of dimethoate products.

2. Workplace assessment – A suitable and sufficient assessment of the risks to health created by work involving potential exposure to dimethoate.

3. Control - As far as practicable, the prevention or adequate control of exposure of workers to hazardous substances should be secured by measures other than the provision of PPE. Control measures should be implemented in accordance with the NOHSC hierarchy of controls.

It is recommended that industry-based standard operating procedures (including safe work practices) be developed, where appropriate.

The use of PPE for exposure mitigation should be limited to situations where other control measures are not practical or where PPE is used in conjunction with other measures to increase protection. Where PPE is used, it should be selected and used in accordance with the relevant Australian Standards. Protective equipment should be properly selected for the individual and task, be readily available, clean and functional, correctly used and maintained.

4. Record keeping – Records should be maintained in accordance with the NOHSC Control of Workplace Hazardous Substances (NOHSC, 1994a).

APPENDIX III: Results of the Wilcoxon Signed Ranks Test on endpoints of ChE inhibition across dimethoate studies

The Wilcoxon matched pairs signed ranks test is a nonparametric test to compare two paired groups. Non-parametric methods provide an alternative series of statistical methods that require no or very limited assumptions to be made about the data. This allows for the use of small sample sizes as normal distribution does not need to be assumed. The Wilcoxon signed ranks test analyses the median of differences between the matched pairs and does not determine the magnitude of difference between matched groups (Motulsky, H, 1999). The differences between the LOEL for erythrocyte and brain ChE inhibition for each study was measured across dimethoate studies. If any observations are exactly equal they are ignored and dropped from the sample size. The Wilcoxon signed ranks test for the dimethoate studies is presented in appendix XII.

The Wilcoxon signed ranks test tests the null hypothesis that there are no systematic differences within pairs (LOELs for brain and erythrocyte ChE inhibition) when the test statistic (W^-) is a long way from its mean. The alternative hypothesis was that LOELs are systematically different between the 2 pairs (brain and erythrocyte ChE inhibition). The significance level chosen was 5% ($\alpha = 0.05$).

Wilcoxon signed ranks test for matched pairs of erythrocyte and brain ChE endpoints

	Brain LOEL	RBC LOEL	Difference	Absolute difference (bold = positive)	Order of absolute difference	Rank	Signed rank
Rat 4 week (f)	2.6	2.6	0	0	NA	NA	NA
Dog 4 week	2.2	2.2	0	0	NA	NA	NA
6 month rat study	2.5	2.5	0	0	NA	NA	NA
Dog 1 year (m)	0.7	0.7	0	0	NA	NA	NA
Dog 1 year (f)	0.76	0.76	0	0	NA	NA	NA
Repro rat 15 weeks (f)	0.83	0.83	0	0	NA	NA	NA
Rats 20 weeks repro (f)	6.5	6.5	0	0	NA	NA	NA
Rat 2 year (m)	0.23	1.2	-0.97	0.97	1	1	-1
Rat 2 year (f)	1.5	0.3	1.2	**1.2**	2	2	2
Rat 15 weeks repro (m)	3.28	0.74	2.54	**2.54**	3	3	3
Rat 13 week (m)	8.1	3.2	4.9	**4.9**	4	4	4
Rats 20 weeks repro (m)	1	6.5	-5.5	5.5	5	5	-5
Rat 13 week (f)	9.9	3.8	6.1	**6.1**	6	6	6
Rat 4 week (m)	2.48	10.4	-7.92	7.92	7	7	-7

The statistical analysis has been performed using a two-tailed statistical test. The null hypothesis and alternative hypothesis are:

H0: LOELs have the same distribution between the 2 groups (brain and RBC) across all studies.
H1: LOELs are systematically different between the 2 groups.

The Wilcoxon's test statistic is calculated by summing the positive ranks (positive differences in bold) (W^+) and the negative ranks (W^-) and taking the minimum of the two.

$W^+ = 15$ and $W^- = 13$, the Wilcoxon test statistic = 13.

The sample size is 7 (discounting zero differences), n = 7

The mean of W^- is = $\frac{n_1(n+1)}{4} = \frac{7(8)}{4} = 14$ and standard deviation = $\sqrt{\frac{n(n+1)(2n+1)}{24}} = \sqrt{\frac{7(8)(15)}{24}} = \sqrt{35} = 5.92$. A normal approximation using the mean and standard deviation can be employed to determine a p – value for statistical analysis. However, due to the small sample size n = 7, critical T values for Wilcoxon's Signed-Ranks and Matched-Pairs Signed-Ranks tests have been used to determine significance. The level of significance chosen for this analysis is significance > 95% ($\alpha = 0.05$).

Table for critical values adapted from Sheskin, D, 2004

Critical Values for T in the Wilcoxon Signed Mixed-Pairs Signed-Rank Test

The values below are the approximate critical values of T for two-tailed tests at level P. For a signicant result, the calculated T must be less than or equal to the tabulated value.

(Values of P are halved for one-tailed tests using R_- and R_+.)

n	$P = 0.10$	$P = 0.05$
5	2	-
6	2	0
7	3	2
8	5	3
9	8	5
10	10	8
11	14	10
12	17	13
13	21	17
14	26	21
15	30	25
16	36	29
17	41	34
18	47	40
19	53	46
20	60	52
21	67	58
22	75	65
23	83	73
24	91	81
25	100	89

In order to be significant, the obtained Wilcoxon test statistic must be equal or less than the tabled critical T value at the prescribed level of significance (0.05). The Wilcoxon test statistic = 13. Figure 3 gives a critical value for n = 7 and $\alpha = 0.05$ of 2. Since 13 is greater than 2, the null hypothesis cannot be rejected at the 5% significance level.

In conclusion, there is not enough evidence at the 5% level to conclude that the distribution of erythrocyte and brain ChE inhibition LOELs is different across animal studies.