Pesticide residues in food – 2016

Toxicological evaluations

Sponsored jointly by FAO and WHO

**Special Session of the Joint Meeting of the
FAO Panel of Experts on Pesticide Residues
in Food and the Environment
and the
WHO Core Assessment Group on Pesticide Residues**

Geneva, Switzerland, 9–13 May 2016

Food and Agriculture
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World Health
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* Evaluated within the periodic review programme of the Codex Committee on Pesticide Residues

2016 Special Session of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

Geneva, 8–13 May 2016

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Abbreviations used

AChE	acetylcholinesterase
ACP	acid phosphatase
ADI	acceptable daily intake
AFC	antibody-forming cell
AHS	Agricultural Health Study
AhR	aryl hydrocarbon receptor
ALP	alkaline phosphatase
AMPA	aminomethylphosphonic acid
aOR	adjusted odds ratio
AP	apurinic/apyrimidinic
APG	alkyl polyglucoside
AR	androgen receptor
ARfD	acute reference dose
aRR	adjusted risk ratio
ASDN	androstene-4-ene-3,17-dione
AST	aspartate aminotransferase
AUC	area under the plasma concentration–time curve
AUC_t	area under the concentration versus time–curve calculated up to the last detectable sample
BChE	butyrylcholinesterase
B_{max}	maximum amount of binding
BfR	German Bundesinstitut für Risikobewertung
BMD	benchmark dose
BMD_{10}	estimated benchmark dose for a 10% inhibition
BMD_{15}	estimated benchmark dose for a 15% inhibition
BMD_{20}	estimated benchmark dose for a 20% inhibition
BMD_{30}	estimated benchmark dose for a 30% inhibition
BoNT	botulinum neurotoxin
BUN	blood urea nitrogen
bw	body weight
CA	chromosomal aberrations
CAS	Chemical Abstracts Service
CCPR	Codex Committee on Pesticide Residues
CEBS	Chemical Effects in Biological Systems
cfu	colony-forming unit
ChE	cholinesterase
CHO	Chinese hamster ovary
Ci	curie (1 Ci = 3.7×10^{10} becquerel [Bq])
CI	confidence interval
C_{max}	maximum concentration
CYP	cytochrome P450
CMC	carboxymethylcellulose
CYP	cytochromes P450
2,4-D	2,4-dichlorophenoxyacetic acid
DEL	yeast deletion (assay) DEP diethylphosphoric acid
DETP	diethylphosphorothioic acid
DMSO	dimethyl sulfoxide
DMDTP	dimethyl dithiophosphate
DMP	dimethyl phosphate

DMTP	dimethyl thiophosphate
DNA	deoxyribonucleic acid
DPRA	direct peptide reactivity assay
DSB	double strand break
EDSP	Endocrine Disruptor Screening Program
ELISA	enzyme-linked immunosorbent assay
ENDO	endonuclease
EPSPS	5-enolpyruvylshikimate 3-phosphate synthase
eq	equivalent
ER	estrogen receptor
ERTA	estrogen receptor transcriptional activation
F	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
F_{2A}	second filial generation, first litter
F_{2B}	second filial generation, second litter
FAO	Food and Agriculture Organization of the United Nations
Fpg	formamidopyrimidine-DNA-glycosylase
FSH	follicle-stimulating hormone
FSTRA	fish short-term reproduction assay
GD	guideline
GGT	gamma-glutamyltransferase
GIT	gastrointestinal tract
GLP	good laboratory practice
GSH	glutathione
Hb	haemoglobin
Hct	haematocrit
Hep2	epidermoid cancer
HepG2	hepatocellular carcinoma
HESS	Hazard Evaluation Support System
HIC	highest ineffective concentration
HPLC	high-performance liquid chromatography
HPLC-EC	high pressure liquid chromatography-electrochemical¬-electrochemical detection
HPLC/MS-MS	high-performance liquid chromatography with mass spectrometry
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HTC	hepatoma cell
IARC	International Agency for Research on Cancer
IC_{50}	median inhibitory concentration
IEDI	international estimated daily intake
IL	interleukin
IP	intraperitoneal
IM	isomalathion
IU	International Unit
IV	intravenous
ISS	Istituto Superiore di Sanità
IW-LED	intensity-weighted lifetime-exposure days
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_d	dissociation constant
ke/fd	killed in extremis or found dead
LABC	levator ani plus bulbocavernosus muscle complex
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LEC	lowest effective concentration

LED	lifetime-exposure days
LH	luteinizing hormone
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
M	male
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MDCA	malathion dicarboxylic acid
MIC	minimum inhibitory concentration
MMC	minimum microbicidal concentration
MMCA	malathion monocarboxylic acid
MN	micronuclei
MN-PCE	micronucleated polychromatic erythrocytes
MOA	mode of action
mRNA	messenger ribonucleic acid
N	sample size
N/A	not applicable
NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NB	*nota bene*
NCE	normochromatic erythrocyte
ND	not determined
NHL	non-Hodgkin lymphoma
NI	not investigated
no.	number
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NP	not provided
NR	not reported
N/S	not stated
NS	not specified
NS	not significant
NTE	neuropathy target esterase
NTP	National Toxicology Program
OASIS	Organization for the Advancement of Structured Information Standards
OECD	Organisation for Economic Co-operation and Development
8-OHdG	8-hydroxy-2'-deoxyguanosine
OPPTS	Office of Prevention, Pesticides & Toxic Substances
OR	odds ratio
8-Oxo-dG	8-hydroxy-2'-deoxyguanosine
2-PAM	2-pyridinealdoxime methiodide (in Jenkins, 1988)
2-PAM	pyridine-2-aldoxime methochloride (in Frick et al., 1987, from the 1993 JMPR)
PCE	polychromatic erythrocyte
PDII	primary dermal irritation index
PEG	polyethylene glycol
PHA	phytohaemagglutinin
PND	postnatal day
POE	polyoxyethylene ether
POE-APE	polyoxyethylene ether phosphates – polyoxyethylene alkyl ether phosphate
POEA	polyoxyethyleneamine
POES	polyethoxylated tallow amine
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion

ppm	parts per million
PWG	Pathology Working Group
PXR	pregnane X receptor
Q	quartile
QSAR	quantitative structure–activity relationships
ref.	reference
RBA	relative binding affinity
RfD	reference dose
rhCG	recombinant human chorionic gonadotrophin
RNA	ribonucleic acid
ROS	reactive oxygen species
RPC_{max}	maximum level of response
RR	risk ratio
rRNA	ribosomal ribonucleic acid
RR	relative risk
rtER	rainbow trout estrogen receptor
S9	$9000 \times g$ supernatant fraction from liver homogenate
SCE	sister chromatid exchange
SCSA	sperm chromatin structure assay
SD	standard deviation
SDH	succinate dehydrogenase
SDS	sodium dodecyl sulfate
SI	Stimulus Index
SN2	bimolecular nucleophilic substitution
SSB	single strand breaks
StAR	steroidogenic acute regulatory protein
T4	thyroxine
TEPP	tetraethyl pyrophosphate
TK	thymidine kinase
T_{max}	time to reach the maximum concentration
TAF	toxicity adjustment factor
TG	test guideline
Tk	terminal kill
TLC	thin-layer chromatography
TOCP	triorthocresyl phosphate
TP	testosterone propionate
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TSH	thyroid-stimulating hormone
U	enzyme unit
UDS	unscheduled DNA synthesis
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	ultraviolet
VTG	vitellogenin
v/v	volume per volume
WHO	World Health Organization
w/w	weight per weight

Introduction

The toxicological monographs contained in this volume were prepared by a WHO Core Assessment Group on Pesticide Residues that met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in a Joint Meeting on Pesticide Residues (JMPR) in Geneva, Switzerland, on 9–13 May 2016.

The three compounds (diazinon, glyphosate and malathion) were evaluated following the recommendation of an electronic task force of the WHO Core Assessment Group on Pesticide Residues that the compounds be re-evaluated due to public health concerns identified by International Agency for Research on Cancer (IARC) and the availability of a significant number of new studies. Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting has been published by the FAO as *FAO Plant Production and Protection Paper 227*. That report contains comments on the compounds considered, acceptable daily intakes and acute reference doses established by the WHO Core Assessment Group. As no residue data were requested, maximum residue levels previously established by the FAO Panel of Experts for these compounds remain unchanged and no monographs on residues were prepared.

The toxicological monographs contained in this volume are based on working papers that were prepared by WHO experts before the 2016 Joint Meeting. A special acknowledgement is made to those experts and to the experts of the Joint Meeting who reviewed early drafts of these working papers.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological properties or toxicity of the compounds included in this volume should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Meeting on Pesticide Residues, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva, Switzerland.

Methodology

Literature search methodology

For each of the 3 compounds under review, the information was collected from 3 sources.

• The individual publications considered by IARC were provided to JMPR.

• The dossiers provided by industry for registration of the compounds in the European Union, the United States of America and Japan were submitted.

• The JMPR experts performed an update of the literature search done by IARC for "cancer", "genotoxicity" and "epidemiological data".

For the articles related to cancer and cancer-mechanisms, the literature search strategy involved performing targeted searches on the agents or major metabolites in the following databases:

1) Google Scholar (http://scholar.google.com/);

2) PubMed (http://www.ncbi.nlm.nih.gov/pubmed);

3) WEB OF SCIENCE (https://apps.webofknowledge.com);

4) BioOne (http://www.bioone.org/); and

5) ScienceDirect (http://www.sciencedirect.com/).

A keyword searching strategy was employed, using the keywords and the Boolean Operators (AND, or , and NOT). ([mh] = mesh term; PubMed's controlled vocabulary, [tiab] = text word to be searched in the title or abstract

"Comet Assay"[mh] OR "Germ-line-mutation"[mh] OR "Mutagenesis"[mh] OR "Mutagenicity tests"[mh] OR "Sister-chromatid exchange"[mh] OR "Mutation"[mh] OR

Ames-Assay[tiab] OR Ames-test[tiab] OR Bacterial-Reverse-Mutation-Assay[tiab] OR Clastogen*[tiab] OR DNA-Repair*[tiab] OR Genetic-toxicology[tiab] OR hyperploid[tiab] OR micronucleus-test[tiab] OR tetraploid[tiab] OR Chromosome-aberrations[tiab] OR DNA damage[tiab] OR Mutation[tiab] OR chromosome-translocations[tiab] OR DNA protein crosslinks[tiab] OR DNA-damag*[tiab] OR DNA-inhibit*[tiab] OR Micronuclei[tiab] OR Micronucleus[tiab] OR Mutagens[tiab] OR Strand-break*[tiab] OR Unscheduled-DNA-synthes*[tiab] OR chromosomal-aberration[tiab] OR chromosome-aberration[tiab] OR chromosomal-aberrations[tiab] OR chromosomal-abnormalit*[tiab] OR chromosome-abnormalit*[tiab] OR genotoxic*[tiab] OR Comet- assay[tiab] OR Mutagenic[tiab] OR Mutagenicity[tiab] OR mutations[tiab] OR chromosomal- aberration-test[tiab] OR Sister-chromatid-exchange[tiab]

The search resulted in 157 references for Diazinon–Cancer; 99 for Diazinon–Genotox; 251 for Glyphosate–Cancer; 269 for Glyphosate–Genotox; 227 for Malathion–Cancer; and 182 for Malathion–Genotox.

For epidemiological literature the search was restricted to identifying articles published after the three IARC Monographs were published. The search strategy and results are summarized in the table below.

Search terms	Search engine	Number of hits	Hits after screening for relevance
(diazinon OR glyphosate OR malathion) AND cancer	PubMed (limited to humans; published in the last 5 years)	31	*N* = 2
Scopus (limited to 2014–2016)		28	Koutros et al. (2015); Lerro et al. (2015)
(diazinon OR glyphosate OR malathion) AND (NHL OR lymphoma OR leukemia OR "lung cancer" OR "prostate cancer")	PubMed (limited to humans; published in the last 5 years)	11	
	Scopus (limited to 2014–2016)	9	

Methodology of epidemiological studies

The pre-agreed evaluation process and Tier 1 screening criteria used to evaluate epidemiological studies on diazinon, glyphosate and malathion are described in "Section 2.2: Methods for the evaluation of epidemiological evidence for risk assessment" of the JMPR meeting report[1].

Evaluation process of epidemiological evidence for risk assessment for glyphosate, malathion and diazinon

The evaluation process and Tier 1 screening criteria are shown in Fig. 1 below.

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[1] Pesticide residues in food 2016: Special session of the joint FAO/WHO meeting on pesticide residues May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/)

Fig. 1. Pre-agreed evaluation process and Tier 1 screening criteria
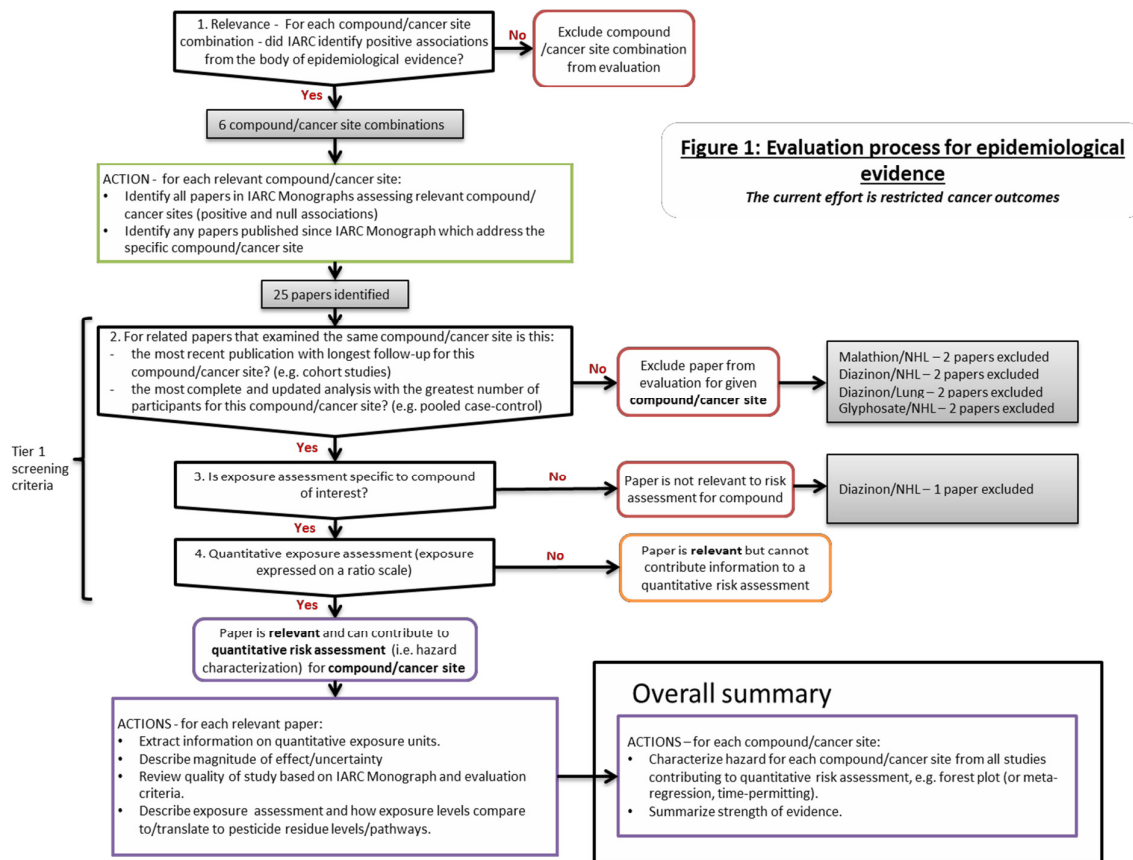
(a)   Identification of compound/cancer sites and screening of papers

This assessment was restricted to studies of cancer outcomes. The body of epidemiological evidence for non-cancer outcomes was not evaluated; numerous studies have assessed risks for neurodevelopmental, neurodegenerative or reproductive outcomes, among other health outcomes. Restricting the assessment to non-cancer outcomes was partly driven by feasibility reasons: a clinically relevant adverse effect size (or an acceptable level of risk) for a non-cancer outcome must be defined, and the methodologies for hazard identification and characterization based on observational epidemiological findings of non-carcinogenic adverse effects are less well-established than those for cancer (see, for example, Clewell & Crump, 2005; Nachman et al., 2011).

The International Agency for Research on Cancer (IARC) monographs on diazinon, glyphosate and malathion refer to a total of 45 epidemiological studies. Two more recently published studies evaluated at least one of malathion, diazinon or glyphosate in relation to cancer outcomes (Lerro et al., 2015; Koutros et al., 2015). An additional study on prostate cancer (Mills & Yang, 2003), which was not included in the IARC monographs, was also identified.

The 45 publications referred to in the IARC monographs and the three publications since (Mills & Yang, 2003; Lerro et al., 2015; Koutros et al., 2015) covered 48 compound/cancer site combinations. The current evaluation focuses on the 6 compound/cancer site combinations for which IARC identified positive associations from the body of epidemiological evidence, that is, those associations noted in section 6.1 of the monographs, and which underpin IARC's evaluation of limited evidence in humans for the carcinogenicity of malathion, diazinon and glyphosate. The definition for limited evidence of carcinogenicity used by IARC is as follows: "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is

considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence" (IARC, 2015). The 6 compound/cancer site combinations are:

A.   Malathion / non-Hodgkin lymphoma (NHL)

B.   Malathion / prostate cancer

C.   Diazinon / NHL

D.   Diazinon / leukaemia

E.   Diazinon / lung cancer

F.   Glyphosate / NHL

When identifying relevant publications it was noted that there were stand-alone analyses for specific subtypes of NHL (of which there are many). Evaluations of risk for subtypes of NHL were not undertaken separately as there was insufficient evidence (too few studies or small numbers of cases); nor were evaluations of risk undertaken for other haematopoietic and lymphoid tumours, as the positive associations identified by IARC were for total NHL.

There were 26 publications for these 6 compound/cancer site combinations. Seven studies were excluded from at least one evaluation for a given compound/cancer site during Tier 1 screening, either because they were not specific to the pesticide in question; because the publication had been superseded by a later publication on the same cohort and this later publication included longer follow- up time; or because there was a more complete analysis on the same study population with a greater number of participants.

(b)   Overview of studies included in evaluation

The IARC monograph on malathion (IARC, 2015) provided an overview of the epidemiological studies which have assessed pesticide exposures and cancer risk. Therefore, only a brief summary (largely based on the IARC monograph) of the studies contributing to the current evaluation is provided here for context.

The Agricultural Health Study is a prospective cohort study of pesticide applicators (predominantly farmers; $n \approx 52\,000$) and their spouses ($n \approx 32\,000$) from Iowa and North Carolina, United States of America, enrolled in 1993–1997. The Study has examined a range of cancer outcomes and published analyses with longer periods of follow-up (e.g. De Roos et al., 2005; Beane Freeman et al., 2005; Koutros et al., 2013; Alavanja et al., 2014; Jones et al., 2015; Lerro et al., 2015). Information on participants' use of 50 pesticides and other determinants of exposure was gathered retrospectively via baseline and two follow-up questionnaires. Cumulative lifetime exposure estimates were calculated. Validation studies have been conducted to assess the reliability and accuracy of exposure intensity scores (a component of the exposure assessment) (Coble et al., 2005; Hines et al.,
2008; Thomas et al., 2010). The impact of exposure misclassification in this study was to bias risk estimates towards null (Blair et al., 2011).

The United States Midwest case–control studies are three population-based case–control studies of cancer conducted in Nebraska (Zahm et al., 1990), Iowa and Minnesota (Brown et al., 1990; Cantor et al., 1992) and Kansas (Hoar et al., 1986) that have been pooled (748 cases/2236 controls) to analyse NHL in white males only (Waddell et al., 2001; De Roos et al., 2003; Lee et al., 2004). Information on participants' occupational use of pesticides was gathered retrospectively via a questionnaire. There were some differences in case ascertainment and exposure assessment methods between the three studies. For 39% of the pooled study population, proxy respondents were used (Waddell et al., 2001), for whom recall of specific pesticide use could be problematic and subject to recall bias that may differ for cases and controls. De Roos et al. (2003) used the same study population as Waddell et al. (2001) to perform an extensive evaluation and adjustment for other pesticides.

The Cross-Canada Study of Pesticides and Health (CCSPH) is a population-based case–control study of haematopoietic cancers in men diagnosed in 1991–1994 across six Canadian provinces (McDuffie et al., 2001). It includes 517 NHL cases and 1506 controls. A questionnaire was administered by post, followed by a telephone interview for those that reported pesticide exposure of 10 hours/year or more and for a 15% random sample of the remainder. The study was not restricted to pesticide exposure experienced by a specific occupational group (McDuffie et al., 2001). Further analyses stratified by asthma/allergy status – to assess possible effect modification by immune system modulation – have been conducted (Pahwa et al., 2012). The study has a large sample size and detailed information of pesticide exposures; however, the proportion exposed to pesticides was low.

The three sets of studies above were deemed as high quality and highly informative by the IARC Working Group (IARC, 2015).

A number of other case–control studies of pesticide exposure and cancer risk were included in this evaluation: the Florida Pest Control Worker study (Pesatori et al., 1994); nested case–control studies within the United Farm Workers of America cohort study (Mills & Yang, 2003; Mills, Yang & Riordan, 2005); a population-based case–control study of prostate cancer in British Columbia, Canada (Band et al., 2011); and case–control studies of NHL/haematopoietic cancers from Sweden (Hardell et al., 2002; Eriksson et al., 2008) and France (Orsi et al., 2009). The IARC Working Group (IARC, 2015) noted substantial limitations in these studies, either in relation to exposure assessment, scope for and variation in exposure misclassification, lack of detail in the publication, which hindered interpretation, lack of specificity due to high correlations between use of different pesticides, and limited power.

(c)   Strengths and limitations of studies included in evaluation

The included studies predominantly examined the occupational pesticide exposures of farmers and other pesticide applicators, with the vast majority of research being on males only. None of the studies assessed exposure via food consumption or ambient exposure from agriculture (e.g. spray drift). The scientific evidence available is therefore limited in its generalizability and the extent to which it can be translated to general population exposure scenarios and levels that would be associated with pesticide residues. Nonetheless, these observational epidemiological studies provide insight into real-world exposure scenarios and allow for observation of the species of interest (humans) over the long follow-up periods relevant to cancer.

The number of high quality studies is relatively small. Typically the number of exposed cases in studies is small, particularly when evaluating specific pesticides, which limits study power.

Relatively few studies have assessed exposure quantitatively, meaning the epidemiological evidence available to inform/establish dose–response relationships is very limited. Exposure misclassification is a potential issue for all studies. This is expected to be largely non-differential for cohort studies (i.e. the Agricultural Health Study), resulting in attenuation of risk estimates. All except one of the studies included are case–control studies, and these may be affected by recall bias, that is, cases and controls recall past pesticide exposure with differing accuracy, leading to differential exposure misclassification that can bias risk estimates either towards or away from the null. As a cohort study, the Agricultural Health Study avoids recall bias.

Given that studies focused on occupational exposures among farmers/pesticide applicators, it is unlikely that they were exposed to only one specific pesticide, so confounding, possible effect modification and additive/multiplicative effects due to coexposures are all concerns. However, many studies were able to adjust risk estimates for other pesticide coexposures, which yields more accurate risk estimates.

There are some issues in terms of comparing studies and evaluating the consistency of evidence overall. Results of studies may appear heterogeneous, but usually there are too few studies to

really assess consistency and heterogeneity. Exposure assessment methods and referent groups vary between studies.

Finally, changes in disease classifications (particularly that of NHL) or screening/diagnosis rates (prostate cancer) over time, may limit comparability between studies.

(d)   *Publication bias*

A formal analysis of publication bias was not undertaken because the number of studies (risk estimates from non-overlapping study populations) available were few and it is advised that funnel plot tests for asymmetry be used only where there are at least 10 studies to allow sufficient statistical power to distinguish true asymmetry from chance (Higgins & Green, 2011; Sterne et al., 2011). Other formal objective statistical tests require a larger number of studies, typically at least 30, to achieve sufficient statistical power (Lau et al., 2006). As a result, publication bias cannot be fully excluded. However, given the very considerable resources invested in these types of (large, difficult exposure assessment) studies, it is unlikely that results would go unpublished.

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MALATHION

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Explanation

Malathion is the International Organization for Standardization–approved common name for *S*-1,2-*bis*(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorothioate (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service (CAS) number 121-75-5. The chemical structure of malathion is shown in Fig. 1.

Malathion is a non-systemic organophosphorus insecticide whose mode of pesticidal action is the inhibition of cholinesterase activity. It is used to control insects on agricultural crops and stored commodities and for vector control.

The toxicity of malathion was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1963, 1965, 1966, 1997 and 2003. Malathion was listed in the periodic review programme of the Codex Committee on Pesticide Residues (CCPR) but was not yet scheduled for review. The compound was reviewed by the present Meeting following the recommendation of an electronic task force of the World Health Organization Core Assessment Group on Pesticide Residues that it be re-evaluated due to public health concerns identified by the International Agency for Research on Cancer (IARC) and the availability of a significant number of new studies.

The current Meeting evaluated all previously submitted toxicological data in addition to new published and unpublished toxicological studies and published epidemiological studies on cancer outcomes. All critical unpublished studies contained certificates of compliance with good laboratory practice (GLP), unless otherwise specified. Human volunteer studies were conducted according to the Declaration of Helsinki or equivalent ethical standards.

Fig. 1. Chemical structure of malathion
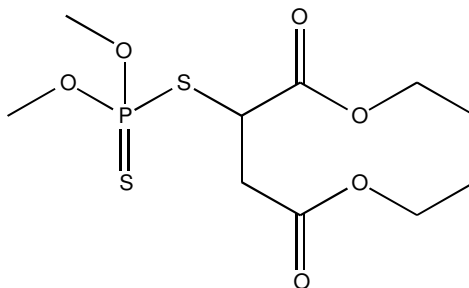
Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

In an absorption, distribution, metabolism and excretion study, Reddy, Freeman & Cannon (1989) administered [2,3-^{14}C]malathion (> 98% radiochemical purity) in corn oil as a single gavage dose of 40 or 800 mg/kg body weight (bw), or 15 daily gavage doses of unlabelled malathion (purity 94.6%) at 40 mg/kg bw followed by a single gavage dose of 40 mg/kg bw radiolabelled malathion, to groups of five Sprague Dawley (Crl:CD [BR]) rats per sex. Urine and faeces were collected at 4, 8, 12, 24, 48 and 72 hours after dosing. The rats were terminated after 72 hours, and blood and tissues collected. Expired air was not collected during the definitive phase of the study because excretion of radioactivity via exhaled carbon dioxide was less than 1% of the administered radioactive dose in a preliminary study. Radioactivity was quantified in excreta, blood and tissues by liquid scintillation counting. Metabolites were analysed in excreta by high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry.

The cumulative mass balance of radioactivity is summarized in Table 1. Recovery of radioactivity was greater than 90%, with the majority (76–88%) detected in urine and relatively low

levels detected in faeces (6–14%). Based on the concentration of radioactivity in urine and remaining in the carcass, gastrointestinal absorption was estimated to be at least 77% in males and 86% in females. The majority of radioactivity was excreted in urine within 24 hours of dosing. Less than 1% of radioactivity was detected in tissues, with the highest proportions detected in the liver, skin, fat and gastrointestinal tract (GIT) (Table 2).

Table 1. Cumulative mass balance of radioactivity in rats following oral dosing with [^{14}C]malathion

| | Mean % of administered radioactivity | | | | | |
| | 40 mg/kg bw (single dose) | | 800 mg/kg bw (single dose) | | 40 mg/kg bw (repeated dose) | |
Sample	Males	Females	Males	Females	Males	Females
Urine (including cage wash)						
0–4 h	12.4	44.9	14.7	25.3	33.8	47.7
0–8 h	68.0	69.2	33.0	49.4	66.8	69.4
0–12 h	77.4	81.0	48.3	67.1	75.8	79.3
0–24 h	81.4	85.4	62.5	81.2	82.3	86.6
0–48 h	83.2	87.5	73.6	84.1	83.9	87.9
0–72 h	83.8	88.0	76.2	85.2	84.6	88.4
Faeces						
0–4 h	–	–	0.005[a]	–	–	–
0–8 h	–	–	0.005[a]	–	–	–
0–12 h	2.8	1.6	2.2	1.9	0.3	0.0
0–24 h	9.4	4.8	10.5	5.3	5.5	4.2
0–48 h	10.7	5.8	12.9	6.4	6.5	5.7
0–72 h	11.0	5.9	13.7	6.6	6.8	5.8
Total						
0–4 h	12.4	44.9	14.7	25.3	33.8	47.7
0–8 h	68.0	69.3	32.7	49.4	66.8	69.3
0–12 h	80.2	82.7	50.5	69.1	75.9	79.3
0–24 h	90.8	90.3	73.0	86.5	87.8	90.9
0–48 h	93.9	93.3	86.5	90.5	90.5	93.5
0–72 h	94.8	94.0	89.9	91.9	91.4	94.2
Carcass	0.36	0.46	0.67	0.50	0.46	0.24

bw: body weight; (–) indicates no faeces produced

[a] Mean of two rats.

Source: Reddy, Freeman & Cannon (1989)

Table 2. Levels of radioactivity in tissues 72 hours after oral dosing with [^{14}C]malathion

Tissue	Mean % of administered radioactivity					
	40 mg/kg bw (single dose)		800 mg/kg bw (single dose)		40 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
Blood	0.042	0.032	0.033	0.020	0.046	0.034
Plasma	0.025	0.015	0.019	0.011	0.026	0.200
Erythrocytes	0.011	0.012	0.011	0.008	0.011	0.010
Liver	0.204	0.127	0.193	0.106	0.252	0.110
Kidneys	0.016	0.012	0.015	0.009	0.020	0.015
Lungs	0.002	0.002	0.002	0.002	0.002	0.002
Brain	0.007	0.005	0.003	0.002	0.006	0.005
Heart	0.001	0.001	0.001	0.000	0.001	0.001
Spleen	0.002	0.001	0.002	0.001	0.002	0.002
Testes	0.008	–	0.004	0.000	–	–
Ovaries	–	0.001	–	0.000	0.006	0.000
Uterus	–	0.001	–	0.000	–	0.001
Adrenals	0.000	0.000	0.000	0.000	0.000	0.000
Fat	0.110	0.080	0.084	0.039	0.074	0.044
Skin	0.102	0.091	0.134	0.062	0.123	0.065
Muscle	0.039	0.031	0.037	0.020	0.048	0.023
Bone	0.055	0.038	0.046	0.028	0.059	0.036
GIT	0.053	0.062	0.158	0.058	0.044	0.021

bw: body weight; GIT: gastrointestinal tract

Results expressed as means.

Source: Reddy, Freeman & Cannon (1989)

In a human volunteer study conducted by Wester at al. (1983), 20 µL undiluted [^{14}C]malathion (purity not specified) was applied to the ventral forearm of five males (4.6 cm^2 total area; 5 mg/cm^2) followed by repeated administration of unlabelled malathion. This procedure was repeated eight days later when radioactivity was first detected in urine. The absorption of radioactivity was 4.48% of the applied dose after the first application and 3.53% after the second application; there was no statistically significant difference between these two estimates of dermal absorption.

An in vitro study undertaken by de Ligt (2004) to examine the absorption of [^{14}C]malathion by human and rat skin used flow-through diffusion cells. The test material comprised an undiluted formulation containing 440 g/L malathion or an aqueous field-strength dilution of this formulation containing 1.5 g/L malathion. The exposure time was 8 hours, reflecting a typical day's work, and the post-exposure period was 16 hours. The mean flux constant through human skin was 0.281 µg/cm per hour for the undiluted formulation and 0.081 µg/cm per hour for the diluted formulation. The flux constant through rat skin was 3.372 and 0.765 µg/cm per hour, respectively. Estimated dermal absorption through human skin was 1.44% and 8.74% for the undiluted and diluted formulation, respectively. Estimated dermal absorption through rat skin was 3.05% and 56.89% for the undiluted and diluted formulation, respectively.

In an in vitro dermal absorption study by Moody et al. (2007), samples of human breast or leg skin ($n = 5$) were exposed to [^{14}C]malathion (purity > 95%) at concentrations of 2 mmol/L,

20 mmol/L or 200 mmol/L for 30 minutes. This was followed by a 6.5-hour collection period. The mass balance of radioactivity is shown in Table 3. Estimates of dermal absorption ranged from 8–20.7%.

Table 3. Mass balance of radioactivity following dermal exposure to [^{14}C]malathion

Parameter	% of administered radioactivity		
	2 mmol/L	20 mmol/L	200 mmol/L
Total absorption into receiver (%)	11.6	3.0	0.7
Total absorption into skin depot (%)	9.1	5.0	9.6
Total absorption (%)	20.7	8.0	10.3
Skin wash (%)	60.2	85.8	67.4
Charcoal trap (%)	0.8	0.2	0.2
Donor chamber wash (%)	1.8	2.8	1.8
Total mass balance	83.5	96.9	79.7

Results expressed as means.

Source: Moody et al. (2007)

1.2 Biotransformation

In the study by Reddy, Freeman & Cannon (1989) described in section 1.1 of this monograph, the major metabolites detected in urine (> 80% of urinary radioactivity) were malathion α-monocarboxylic acid, and malathion β-monocarboxylic acids (MMCA) and malathion dicarboxylic acid (MDCA). The remaining urinary radioactivity comprised desmethyl malathion, *O,O*-dimethyl phosphorothioic acid, fumaric acid, 2-mercaptosuccinic acid, *O,O*-dimethyl phosphorodiothioic acid, monoethyl fumarate and malaoxon. Malaoxon was found only in urine samples and accounted for less than 2% of total urinary radioactivity. The metabolite profile in faeces was qualitatively comparable to urine.

In a published study, Buratti et al. (2005) characterized the metabolism of malathion (10–500 µmol/L) by human liver microsomes and the role of cytochrome P450 (CYP) enzymes in generating malaoxon. At low malathion concentrations (≤ 50 µmol/L), CYP1A2 catalysed malaoxon formation, whereas CYP2B6 and 3A4 played a more significant role at higher concentrations of malathion (≥ 400 µmol/L).

In a published study, Buratti & Testai (2005) determined that human microsomes efficiently metabolized malathion to malathion monocarboxylic acid (MMCA) via hepatic carboxylesterase activity and that isomalathion was a potent non-competitive inhibitor of this process (inhibitory constant [K_i] = 0.6 µmol/L).

Takeuchi et al. (2006) screened 200 pesticides for in vitro peroxisome proliferator–activated receptor (PPAR)α and PPARγ activity using an in vitro reporter gene assays in CV-1 monkey kidney cells transiently transfected with the expression plasmid for mouse PPARα or PPARγ. Takeuchi et al. (2008) also screened 200 pesticides for in vitro aryl hydrocarbon receptor activity using an in vitro luciferase reporter gene assay in mouse hepatoma Hepa1c1c7 cells stably transfected with a dioxin-responsive element (DR-EcoScreen cells). Malathion (purity >95%) showed no activity in either assay.

Groups of three female Holtzmann rats were administered a single gavage dose of 73 µmol/kg bw malathion (unspecified purity), MMCA (purity 89.4%), MDCA (purity 99.6%), dimethyl dithiophosphate (purity 97.9%), dimethyl thiophosphate (purity 98.7%) or dimethyl phosphate (purity 98%) in corn oil. Urine and faeces were collected every 24 hours for eight days. The results are summarized in Table 4. The authors concluded that low-level human dietary and non-occupational urine biomonitoring studies will be confounded by preformed pesticide biomarkers used to infer potential human pesticide exposure. This has profound implications for epidemiology studies where biomarker excretion is used as a surrogate for organophosphate exposures that cannot be related to a particular insecticide residue (Chen et al. 2013).

Table 4. Proportion of metabolites in urine following administration of a single dose of malathion or malathion metabolites to rats

Treatment	% of urinary metabolite					Total
	MMCA	MMDA	DMP	DMTP	DMDTP	
Malathion	11.0	45.5	0.78	6.0	6.8	70.2
MMCA	8.7	15.1	0.53	2.9	4.8	32.0
MDCA	0.0	36.3	0.05	5.5	3.8	45.6
DMP	0	0	84.5	0	0	84.5
DMTP	0	0	8.3	46.7	0	55.0
DMDTP	0	0	1.1	3.9	40.8	45.9

DMDTP: dimethyl dithiophosphate; DMP: dimethyl phosphate; DMTP: dimethyl thiophosphate; MDCA: malathion dicarboxylic acid; MMCA: malathion monocarboxylic acid

Results expressed as mean % of administered dose; $n = 3$.

Source: Chen et al. (2013).

Human volunteer studies described in section 3.1 of this monograph have analysed metabolites in urine and blood. In the study by Gillies & Dickson (2000), where male and female volunteers received a single oral dose of 0.5–15 mg/kg bw, blood samples were collected from high-dose and control participants prior to dosing and then at various times up to 72 hours after dosing. Similarly, urine was collected from all participants to analyse malathion metabolites. No malathion or malaoxon were detectable in plasma (limit of detection = 102 and 99.8 ng/mL, respectively). Supplementary analysis of urinary metabolites by Aston (2000) indicated that the majority of metabolites were excreted within 12 hours of dosing, with total clearance by 24 hours. MMCA was the main urinary metabolite followed by dimethyl thiophosphate, MDCA, dimethyl phosphate and dimethyl dithiophosphate; the total concentration of metabolites in urine was proportional to the dose.

In a second human study (Jellinek, Schwartz & Connolly Inc., 2000), where volunteers also received a single oral dose of malathion up to 15 mg/kg bw, plasma was analysed for malaoxon and other major metabolites. Urine was collected prior to dosing and at 0–12, 12–24 and 24–48 hours. No malathion or malaoxon was detected in plasma (limit of detection = 100 ng/mL). Approximately 90% of the dose was excreted in urine within 12 hours, with excretion completed by 24–48 hours. The major urinary metabolites were MMCA and MDCA (30% and 12% of the administered dose, respectively). Other urine metabolites accounting for approximately 30% of the administered dose, included dimethyl thiophosphate, dimethyl phosphate and dimethyl dithiophosphate (Aston, 2000).

The disposition kinetics of malathion and its metabolites in humans (MMCA, MDCA, dimethyl dithiophosphate, dimethyl thiophosphate and dimethyl phosphate) following oral, dermal or intravenous exposure was estimated using a heuristic toxicokinetic model, based on data from

Feldmann & Maibach (1974) and Jellinek, Schwartz & Connolly Inc. (2000). The time taken to recover 50% of the absorbed dose of malathion as urinary metabolites was about 4 hours following oral administration, 11.8 hours following dermal administration and 3.2 hours following intravenous administration. When simulating a single oral exposure, approximately 80–89% of the absorbed dose was eliminated from the body within 12 hours; after a single dermal application, 29–53% of the absorbed dose was estimated to be excreted during the same period. Assuming a continuous 8-hour dermal exposure scenario, the model estimated that 52–80% of the absorbed dose would be eliminated from the body within 24 hours and 84–98% within 48 hours (Bouchard et al., 2003).

The proposed metabolic pathway of malathion in rats is shown in Fig. 2:

- oxidative desulfuration of malathion in the liver to generate malaoxon, which may be excreted in urine or further metabolized by phosphatases;

- hydrolysis of malaoxon by phosphatases to yield *O,O*-dimethyl phosphorothioic acid or hydrolysis of malathion to yield *O,O*-dimethyl phosphorodithioate;

- hydrolysis of the carboxyester by tissue or plasma carboxylesterases, resulting in α- and β-monocarboxylic acid or dicarboxylic acid (major pathway);

- dealkylation, probably by glutathione-*S*-transferases;

- glutathione-dependent demethylation to yield *S*-methyl-glutathione and the corresponding desmethyl phosphate compound.

Fig. 2. Proposed metabolic pathway of malathion in rats
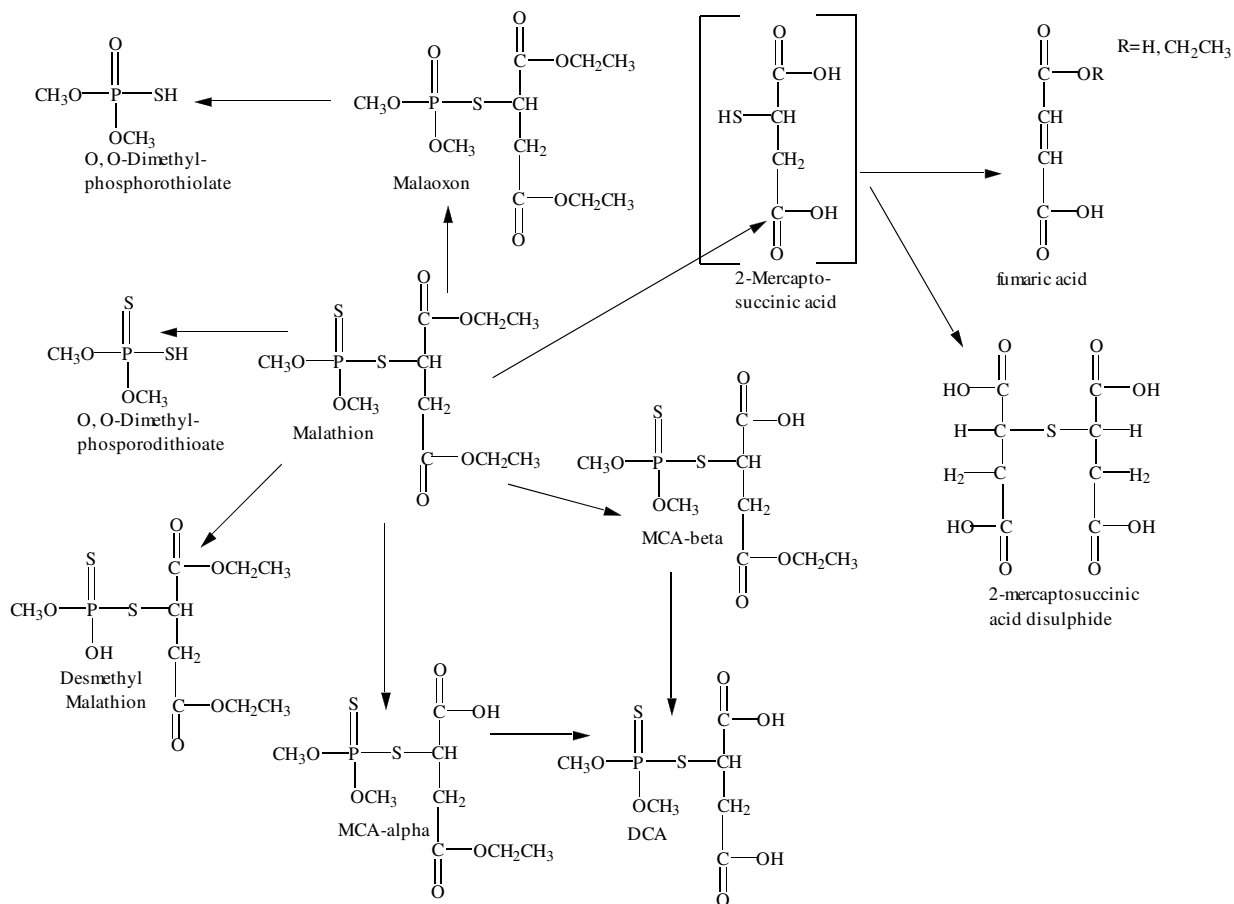
2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity tests on malathion, including skin and eye irritation and skin sensitization studies, are summarized in Table 5. In acute oral dosing studies in rats, a range of clinical signs were observed consistently at doses above 1260 mg/kg bw: piloerection, decreased locomotor activity, ataxia, comatose condition, ptosis, respiratory depression, sedation, tremors, clonic convulsions, salivation, lacrimation and red staining around the eyes and mouth (Terrell, 1978; Terrell,1979a,b; Kynoch, 1986a; Fischer, 1991a,b; Kuhn, 1996; Moore, 2002, 2003). These clinical signs started after 1 to 24 hours, with survivors recovering 1–5 days after dosing (Fischer, 1991a,b; Moore, 2003). In acute dermal toxicity studies in rats, no clinical signs or dermal irritation occurred at 2000 mg/kg bw (Kynoch, 1986b; Bollen, 2003a). In an acute dermal study in rabbits, decreased locomotor activity and salivation, and irritation at the application site occurred at and above 5000 mg/kg bw (Parke, 1978). In a whole-body inhalation study in rats, partial closing of the eyes, excessive salivation, abnormal respiration and body posture occurred at 5.2 mg/L (Jackson, 1986). Nose-only exposure to malathion aerosols resulted in ruffled fur, hunched posture, salivation and a red secretion from the nose at 5.2 mg/L; rats recovered by one hour after exposure (Decker, Knuppe & Ullrich, 2003).

Table 5. Results of studies of the acute toxicity of malathion

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD_{50} (mg/kg bw) or LC_{50} (mg/L)	Reference
Rat	SD	M + F	Oral	NS	Undiluted	5 000	Terrell (1978)[a]
Rat	SD	M + F	Oral	NS	Undiluted	3 800 (males) 4 400 (females)	Terrell (1979a)[a]
Rat	SD	M + F	Oral	NS	Undiluted	3 200 (males) 3 700 (females)	Terrell (1979b)[a]
Rat	SD (CD)	M + F	Oral	96–98	Undiluted	5 400 (males) 5 700 (females) 5 500 (combined)	Kynoch (1986a)
Rat	Crl:CD[SD] BR	M + F	Oral	94.6	Undiluted	1 768 (males) 1 539 (females) 1 649 (combined)	Fischer (1991a)
Rat	Crl:CD[SD] BR	M + F	Oral	96.8	Undiluted	6 156 (males) 4 061 (females) 5 000 (combined)	Fischer (1991b)
Rat	HSD:SD	M + F	Oral	99.1	Undiluted	8 227 (combined)	Kuhn (1996)
Rat	SD	M + F	Oral	92.2 (0.44 IM)	Undiluted	1 857	Moore (2002)
Rat	SD	M + F	Oral	96.0	Undiluted	2 382	Moore (2003)
Rat	SD (CD)	M + F	Dermal	96–98	Undiluted	> 2 000	Kynoch (1986b)
Rat	BrlHan:WIST@Mol	M + F	Dermal	92.2 (0.44 IM)	Sesame oil	> 2 000	Bollen (2003a)

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD$_{50}$ (mg/kg bw) or LC$_{50}$ (mg/L)	Reference
Rabbit	New Zealand White	M + F	Dermal	NS	Undiluted	8 790	Parke (1978)[a]
Rat	COBS Wistar	M + F	Inhalation (whole body)	96–98	NS	> 5.2	Jackson (1986)
Rat	HanBrl:WIST(SPF)	M + F	Inhalation (nose only)	96.3 (0.43 IM)	Undiluted	> 5.20	Decker, Knuppe & Ullrich. (2003)
Rabbit	New Zealand White	NS	Dermal irritation	96–98	Undiluted	Slightly irritating	Liggett & Parcell (1985a)
Rabbit	New Zealand White	F	Dermal irritation	92.2 (0.43 IM)	Undiluted	Not irritating	Bollen (2003b)
Rabbit	New Zealand White	NS	Eye irritation	96–98	Undiluted	Slightly irritating	Liggett & Parcell (1985b)
Rabbit	New Zealand White	F	Eye irritation	92.2 (0.43 IM)	Undiluted	Slightly irritating	Bollen (2003c)
Guinea-pig	Hartley/Dunkin	F	Skin sensitization (Buehler)	96–98	75% w/v in acetone	Not sensitizing	Kynoch & Smith (1986)
Guinea-pig	CBA/Jlbm	F	Skin sensitization (Maximization)	92.2 (0.43 IM)	50% w/v in sesame oil or undiluted	Sensitizing	Bollen (2003d)
Mice	CBA/Jlbm	F	Skin sensitization (LLNA)	96.3 (0.44 IM)	25, 50 and 100% in acetone olive oil (4:1 v/v)	Not sensitizing	Wang-Fan (2003)
Mice	CBA/J	F	Skin sensitization (LLNA)	96.0	10–100% in acetone olive oil (4:1 v/v)	Not sensitizing	Lowe (2011a)

bw: body weight; GLP: good laboratory practice; F: female; IM: isomalathion; LC$_{50}$: median lethal concentration; LD$_{50}$: median lethal dose; LLNA: local lymph node assay; M: male; v/v: volume per volume; NS: not specified; w/v: weight per volume

[a] Study predates GLP and modern test guidelines.

2.2 Short-term studies of toxicity

Rats

In a 14-day range-finding study (Barnett Jr, 2011a), malathion (purity 96%) in corn oil was administered by gavage to groups of eight Crl:CD[SD] adult rats per sex at 0, 800 or 1000 mg/kg bw per day for up to 10 days. Observations for death and clinical signs were made throughout the study, with body weight and feed consumption recorded daily. Female rats were terminated on day 4 and male rats on day 11. The rats were necropsied and their kidney and liver weights recorded. There were no deaths or treatment-related clinical signs. Body-weight gain was reduced at 800 and 1000 mg/kg bw per day in males (−9.8% and −19.8%, respectively) and at 1000 mg/kg bw per day in females (−44.4%). A slight reduction in feed consumptions was noted at these same doses (−4.7% and −7.1%, respectively, in males; −5.9% and −8.5%, respectively, in females). Terminal body weights were comparable across all groups. Liver weight was increased at 800 and 1000 mg/kg bw per day in males

(+24.4% and +25.0%, respectively) and females (+8.3% and +11%, respectively). Some variations in kidney weights were noted but these were not clearly treatment related.

Barnett Jr (2012c) undertook a 14-day range-finding study in juvenile rats to determine suitable doses for a subsequent pubertal endocrine disruptor screening assay (Barnett, 2012d). Groups of eight Crl:CD[SD] rats per sex were administered malathion (purity 96.0%) in corn oil at 0, 250, 450 or 600 mg/kg bw per day from postnatal days 23–36 in males and 22–35 in females. There were no deaths. Salivation (graded as slight to extreme) was observed in at least one male at every dose and in all females at 450 and 600 mg/kg bw per day. There were no effects on body weight, feed consumption, the occurrence of macroscopic abnormalities, brain or liver weights. In males, erythocyte and brain acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at every dose (−67.4% to −79.6% and −9.3% to −16.0%, respectively), while in females erythrocyte and brain acetylcholinesterase activity was significantly lower than the control at 450 and 600 mg/kg bw per day (−56.5% to −77.7% and −6.7% to −14.6%, respectively).

In a 28-day repeat-dose toxicity study by Barnett Jr (2012a), malathion (purity 95.8%) was admixed in the diet and fed ad libitum to groups of Crl:CD[SD] rats (15/sex) at concentrations of 0, 100, 500, 5000 or 10 000 parts per million (ppm) (equal to 0, 9.2, 46.1, 457.5 and 947.8 mg/kg bw per day in males and 0, 9.4, 47.4, 461.3 and 910.1 mg/kg bw per day in females). Rats were observed daily for deaths and clinical signs. Body weight and feed consumption were recorded daily. Following termination on day 29, the rats were necropsied and blood and brain tissue collected to analyse acetylcholinesterase activity. In addition, the nasal passages, the nasal cavity and the neck and associated organs and tissues were examined. The liver and kidneys were weighed and, along with the nasal cavity, processed and retained for possible histopathological examination. There were no deaths or treatment-related clinical signs. Absolute body weight was 9% to 11% lower than the control ($P < 0.01$) at 10 000 ppm in males throughout the exposure period, while overall body-weight gain was 20% lower ($P < 0.01$) than the control. In high-dose males, feed consumption was significantly lower than the control during the first week of exposure (−11%; $P < 0.01$). Overall feed conversion efficiency was 14% lower than the control ($P < 0.1$) in high-dose males. Body weight was unaltered in females, while feed consumption was significantly lower than the control during the last week of exposure in high-dose females (−11%; $P < 0.05$).

Erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$) than the control at 500, 5000 and 10 000 ppm in males (−22.3%, −82.6% and −88.6%, respectively) and at every dose in females (−13.8%, −29.0%, −82.9% and −88.6% at 100, 500, 5000 and 10 000 ppm, respectively). Brain acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at 500, 5000 and 10 000 ppm in males (−7.2%, −21.5% and −21.2%, respectively) and at 5000 and 10 000 ppm in females (−25.0 and −47.8%, respectively; $P < 0.01$); the significant reduction in brain acetylcholinesterase in males at 500 ppm was not considered toxicologically significant as it was less than 10%. Benchmark dose (BMD) modelling was applied to the erythrocyte acetylcholinesterase data using an exponential model. The estimated dose for a 20% inhibition (BMD_{20}) was 45.6 mg/kg bw per day in males and 42.9 mg/kg bw per day in females. The BMD_{10} for brain acetylcholinesterase inhibition was 215.8 mg/kg bw per day in males and 159.2 mg/kg bw per day in females.

There were no treatment-related macroscopic abnormalities. Absolute and relative liver weights were significantly increased ($P < 0.01$ or 0.05) at 5000 (males: 23 and 30%, respectively; females 12% and 10%, respectively) and 10 000 ppm (males: 31% and 48%, respectively; females: 21% and 27%, respectively). Histopathological examination revealed minimal and mild hepatocellular degeneration, a combination of cellular hypertrophy and clumping of basophilic material in the cytoplasm in the livers of two males at 10 000 ppm. The study authors proposed that this finding may have been related to the increases in organ weight, but due to the small number of male rats affected, this change was considered equivocal. At 5000 and 10 000 ppm, relative paired kidney weights were significantly increased ($P < 0.01$; +15 to +20%) but were not accompanied by any microscopic changes. Histopathological examination of nasal tissue revealed goblet cell depletion on the nasal

septum (graded as minimal to marked) and minimal to moderate hyperplasia of the olfactory epithelium (consisting of increased numbers of nuclei) at 10 000 ppm (Table 6). The authors proposed that these findings were the result of continued nasal exposure to malathion in the diet.

Table 6. Histopathological findings in nasal tissue in rats exposed to malathion in the diet for 28 days

| | No. of findings per dietary concentration | | | |
| | Males | | Females | |
Finding	0 ppm	10 000 ppm	0 ppm	10 000 ppm
No. of animals	15	15	15	15
Nose, Level 2 – Goblet cell depletion				
Minimal	0	1	0	4
Mild	0	3	0	6
Moderate	0	9	0	4
Marked	0	2	0	0
Total	0	15	0	14
Nose, Level 3 – Hyperplasia of the olfactory epithelium				
Minimal	0	3	0	6
Mild	0	12	0	9
Total	0	15	0	15
Nose, Level 4 – Hyperplasia of the olfactory epithelium				
Minimal	0	0	0	2
Mild	0	3	0	9
Moderate	0	12	0	4
Total	0	15	0	15
Nose, Level 5 – Hyperplasia of the olfactory epithelium				
Minimal	0	0	0	1
Mild	0	2	0	5
Moderate	0	12	0	8
Total	0	14	0	14

no.: number; ppm: parts per million

Results expressed as the absolute number of rats with the finding.

Source: Barnett Jr (2012a)

The no-observed-adverse-effect level (NOAEL) was 500 ppm (equal to 46.1 mg/kg bw per day in males and 47.4 mg/kg bw per day in females) based on the inhibition of erythrocyte and brain acetylcholinesterase activity at 5000 ppm (equal to 457.5 mg/kg bw per day in males and 461.3 mg/kg bw per day females).

Malathion (purity 96.4%) was admixed in the diet at concentrations of 0, 50, 100, 500, 10 000 or 20 000 ppm and fed ad libitum to CDF®(F-344)/CrlBr rats (5/sex per dose) for 29 or 30 days (equal to 0, 5.1, 10.4, 51.9, 1036 and 2008 mg/kg bw per day in males and 0, 5.7, 11.6, 57.6, 1134 and 2193 mg/kg bw per day in females, respectively). Rats were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly. Blood was sampled pretreatment and at

termination to analyse haematology and clinical chemistry parameters, including analysis of plasma cholinesterase (ChE) and erythrocyte acetylcholinesterase activities. Ophthalmoscopy was performed pretreatment and prior to termination. Following termination, the rats were necropsied and selected organs weighed (adrenals, brain, kidneys and liver) and histopathology performed on tissues from control and high-dose rats. In the remaining groups, histopathology was performed on the kidneys, liver and lungs. Brain acetylcholinesterase activity was analysed in all the rats.

There were no deaths and no treatment-related clinical signs. In high-dose males, absolute body weight was 12% lower ($P < 0.05$) than the control during the first week of exposure, while in females it was 10% and 8% lower ($P < 0.05$) than the control during the first and second weeks of exposure. In males at the highest dose, body-weight gain was 29–64% lower than the control ($P < 0.01$ or 0.05) during the first three weeks of exposure, while in females, body-weight gain was 27–72% lower than the control ($P < 0.01$ or 0.05) over the entire exposure period.

There were no treatment-related ophthalmological findings. At 10 000 and 20 000 ppm, mean corpuscular volume (males only), mean corpuscular haemoglobin (both sexes) and mean corpuscular haemoglobin concentration (females only) were significantly lower ($P < 0.01$ or 0.05) than the control. However, as the magnitude of these reductions was small (2–5%) and within the normal range, they are not considered treatment related. Alkaline phosphatase (ALP) was significantly lower than the control at 10 000 and 20 000 ppm (−24% and −27%, respectively, in males; −31% and −43%, respectively, in females); however, reduced ALP alone is not toxicologically relevant. At these same doses, total protein was 13–15% ($P < 0.01$) higher than the control and total albumin was 14–19% ($P < 0.05$) higher than the control. However, these increases are unlikely to be toxicologically relevant. In males, plasma cholinesterase activity was 28% and 59% lower ($P < 0.01$) than the control at 10 000 and 20 000 ppm, respectively, while in females it was 45% and 78% lower ($P < 0.05$). Although erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at these same doses, the magnitude (< 20%) would not normally be considered toxicologically significant. In males, brain acetylcholinesterase activity was 11% and 26% lower ($P < 0.05$ or 0.01) than the control at 10 000 and 20 000 ppm, while in females it was 17% and 28% lower ($P < 0.01$), respectively.

Relative kidney weight was significantly higher ($P < 0.01$) than the control at 10 000 ppm (males only; +18%) and 20 000 ppm (both sexes; +32% in males and +27% in females); there was no difference in absolute kidney weight. In males, absolute and relative liver weight was significantly higher ($P < 0.01$) than the control at 20 000 ppm (+28% and +26%, respectively). In females, absolute and relative liver weight was significantly higher ($P < 0.01$) than the control at 10 000 (+30% and +29%, respectively) and 20 000 ppm (+38% and +52%, respectively). There were no treatment-related macroscopic findings. Histopathology revealed centrilobular hypertrophy of hepatocytes at 10 000 (four rats/sex per group) and 20 000 ppm (all rats).

The NOAEL was 500 ppm (equal to 51.9 mg/kg bw per day in males and 57.6 mg/kg bw per day in females) for the inhibition of brain acetylcholinesterase activity at 10 000 ppm (equal to 1036 mg/kg bw per day in males and 1134 mg/kg bw per day in females) (Daly, 1993a).

In a 90-day toxicity study by Daly (1993b), malathion (purity 96.4%) was admixed in the diet at concentrations of 0, 100, 500, 5000, 10 000 or 20 000 ppm and fed ad libitum to CDF(F-344)/CrlBr rats (10/sex per dose) (equal to 0, 7, 34, 340, 680 and 1390 mg/kg bw per day, respectively, in males and 0, 8, 39, 384, 784 and 1597 mg/kg bw per day in females, respectively). The rats were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly. Blood was sampled at termination and analysed for haematology and clinical chemistry parameters, including plasma and erythrocyte cholinesterase activities. Following termination, the rats were necropsied, organs weighed and tissues histopathologically examined. The brains were also sampled to analyse acetylcholinesterase activity.

One high-dose male was found dead on day 20, following emaciation, laboured breathing, anogenital staining, decreased feed consumption and tremors; autopsy was unremarkable. There were

no other deaths. Anogenital staining was also observed in four males and six females at the highest dose. Adverse effects on body weight and feed consumption were confined to the highest dose. Absolute body weight was 10–12% lower than the control throughout most of the study, reaching statistical significance ($P < 0.01$ or 0.05) during week 1 (both sexes), weeks 8–13 (males) and 6–13 (females). With the exception of reduced feed consumption in high-dose females during week 1 (−17%; $P < 0.01$), feed consumption was significantly elevated ($P < 0.01$ or 0.05) throughout most of the exposure period.

Key haematological, clinical chemistry, organ weight and histopathological findings are presented in Table 7. There was a significant ($P < 0.01$) dose-related reduction in mean corpuscular volume and mean corpuscular haemoglobin at and above 5000 ppm (4–10%) of the control. Plasma cholinesterase and erythrocyte and brain acetylcholinesterase activities were significantly lower ($P < 0.01$ or 0.05) than the control at and above 5000 ppm (up to 17–91%, 58–72% and 9–44% of the control, respectively), with the reduction in erythrocyte acetylcholinesterase in 500 ppm males less than 20% and therefore not considered toxicologically significant. Gamma-glutamyltransferase (GGT) was significantly increased ($P < 0.01$) in males at 20 000 ppm and in females at 10 000 and 20 000 ppm.

At the highest dose, terminal body weight was approximately 10% lower than the control ($P < 0.01$ or 0.05) and thus relative brain (both sexes) and testes (males) weights were increased. Absolute and relative kidney weights were significantly increased ($P < 0.01$ or 0.05) at and above 10 000 and 5000 ppm, respectively. Microscopic examination of the kidneys revealed an increase in the severity but not the incidence of chronic nephropathy in males at and above 5000 ppm. In males, absolute and relative liver weights were significantly higher ($P < 0.01$ or 0.05) than the control at and above 5000 ppm (28–37% and 26–44%, respectively), while in females increases occurred at 10 000 and 20 000 ppm (26% and 11–28%, respectively). Periportal hypertrophy of hepatocytes was observed microscopically at 10 000 and 20 000 ppm in males and at and above 5000 ppm in females. Hypocellularity of the femur bone marrow was observed microscopically in three high-dose females and in the bone marrow of the sternum of four high-dose females.

The NOAEL was 500 ppm (equal to 34 mg/kg bw per day in males and 39 mg/kg bw per day in females) for the inhibition of erythrocyte and brain acetylcholinesterase activity at 5000 ppm (equal to 340 mg/kg bw per day in males and 384 mg/kg bw per day in females).

Table 7. Effect of 90 days of dietary exposure to malathion in rats

Parameter	Measure per dietary concentration					
	0 ppm	100 ppm	500 ppm	5 000 ppm	10 000 ppm	20 000 ppm
MCV (pg)						
Males	50.0	49.5	48.9	47.4** (−5%)	46.6** (−7%)	44.8** (−10%)
Females	54.5	53.1	53.1	53.3	52.1** (−4%)	50.1** (−8%)
MCH (g/dL)						
Males	17.3	17.1	17.0	16.6** (−4%)	16.2** (−6%)	15.8** (−9%)
Females	19.2	18.8	18.8	18.5** (−4%)	18.3** (−5%)	17.6** (−8%)
Plasma ChE (IU/L)						
Males	0.575	0.571	0.562	0.478* (−17%)	0.387** (−33%)	0.201** (−65%)
Females	0.339	0.337	0.328	0.190* (−44%)	0.0971** (−71%)	0.0311** (−91%)
Erythrocyte AChE (IU/mL)						
Males	1.1	1.0	0.9** (−18%)	0.4** (−64%)	0.4** (−64%)	0.3** (−72%)
Females	1.2	0.9	0.9	0.5** (−58%)	0.4** (−67%)	0.4** (−67%)
Brain AChE (IU/g)						

Parameter	Measure per dietary concentration					
	0 ppm	100 ppm	500 ppm	5 000 ppm	10 000 ppm	20 000 ppm
Males	11.4	10.8	11.2	10.4* (−9%)	9.9** (−13%)	9.1** (−20%)
Females	11.5	11.7	11.2	10.3* (−10%)	9.5** (−17%)	6.4** (−44%)
GGT (IU/L)						
Males	0	0	0	0	1	5*
Females	0	0	0	1	3**	10**
Terminal bw (g)						
Males	264.9	262.2	264.3	268.8	253.2	234.0**(−12%)
Females	159.9	155.1	154.2	151.5	153.2	144.4* (−9%)
Brain weight – relative (%)						
Males	6.79	6.85	6.76	6.69	7.06	7.50** (+10%)
Females	10.51	10.63	10.88	10.98	10.67	11.33** (+8%)
Kidney weight – absolute (g)						
Males	2.05	2.05	2.10	2.22	2.33* (+4%)	2.55** (+24%)
Females	1.32	1.29	1.30	1.37	1.40	1.50** (+13%)
Kidney weight – relative (%)						
Males	7.74	7.84	7.93	8.26** (+7%)	9.22** (+19%)	10.88** (+29%)
Females	8.27	8.32	8.46	9.02** (+9%)	9.13** (+10%)	10.35** (+26%)
Liver weight – absolute (g)						
Males	7.43	7.39	7.83	9.49** (+28%)	9.93** (+34%)	11.71** (+37%)
Females	4.81	4.48	4.53	4.77	5.10	6.08** (+26%)
Liver weight – relative (g)						
Males	2.80	2.82	2.96	3.53** (+26%)	3.92** (+29%)	5.00** (+44%)
Females	3.01	2.89	2.94	3.14	3.33** (+11%)	4.20** (+28%)
Testes weight – relative (%)						
Males	1.47	1.48	1.45	1.46	1.52	1.63* (+11%)
Histopathology – periportal hypertrophy of hepatocytes ($n = 10$)						
Males	0	0	0	0	1	9
Females	0	0	0	1	4	10
Histopathology – hypocellularity of bone marrow ($n = 10$)						
Males	0	0	0	0	0	0
Females	0	0	0	0	0	4

AChE: acetylcholinesterase; bw: body weight; ChE: cholinesterase; GGT: gamma-glutamyltransferase; IU: International Unit; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Source: Daly (1993b)

In a 90-day repeat-dose toxicity study by Barnett Jr (2012b), malathion (purity 95.8%) was admixed in the diet and fed ad libitum to groups of Crl:CD[SD] rats at concentrations of 0, 100, 500, 5000 or 10 000 ppm. The study was divided into two parts: (1) groups of 10 rats per sex allocated to the subchronic phase of the study; and (2) groups of 15 rats per sex allocated to the analysis of acetylcholinesterase activity. The doses achieved in the subchronic phase of the study were 0, 7.2,

35.0, 353.6 and 733.8 mg/kg bw per day in males and 0, 7.5, 35.9, 363.1 and 719.0 mg/kg bw per day in females, while the doses achieved in the acetylcholinesterase phase were 0, 6.2, 31.4, 311.8 and 635.3 mg/kg bw per day in males and 0, 6.6, 33.8, 335.5 and 680.3 mg/kg bw per day, respectively. Rats were observed regularly for deaths and clinical signs. Body weight and feed consumption were recorded daily during the first week of exposure and weekly thereafter. Ophthalmological examinations were performed prior to exposure and in the week prior to termination. On day 91, the 10 rats per sex per group in the subchronic phase of the study were terminated and their blood haematology and clinical chemistry parameters analysed. Rats were necropsied and selected organs weighed. Tissues, including bone marrow and tissue from the nasal cavity and turbinates, were histopathologically examined. The remaining 15 rats per sex per group were processed to analyse erythrocyte and brain acetylcholinesterase activity.

Subchronic phase: There were no deaths or treatment-related clinical signs. In males, overall (day 1–85) body-weight gains were 17% and 15% lower than the control ($P < 0.05$) at 5000 and 10 000 ppm, respectively, while in females overall body-weight gain was significantly lower ($P < 0.05$) than the control at 10 000 ppm (−25%). At 10 000 ppm, feed consumption was significantly lower than the control ($P < 0.01$) in both sexes during the first week of dosing (−20% in males, −13% in females). Overall feed conversion efficiency was significantly lower ($P < 0.01$ or 0.05) than the control at 5000 and 10 000 ppm in males (−8% at both doses) and 10 000 ppm in females (−15%). There were no treatment-related ophthalmological findings. In males, there were no significant intergroup differences in haematological parameters, while in females, significant changes in platelet volume (+12%, $P < 0.05$), MCV (−4%, $P < 0.05$) and MCH (−6%, $P < 0.05$) at the highest dose were within the normal range for age- and sex-matched rats and therefore not considered adverse. At 10 000 ppm, GGT was significantly higher than the control ($P < 0.05$) in both sexes, while cholesterol was increased at 5000 and 10 000 ppm in males (+50% and +84%, respectively; $P < 0.05$) and 10 000 ppm in females (+68%; $P < 0.05$). Slight though significant ($P < 0.05$) increases in total protein (+9%), albumin (+7%) and globulin (+12%) occurred at 5000 and 10 000 ppm without a change in the albumin to globulin ratio in males.

There were no treatment-related macroscopic abnormalities. In males, absolute liver weight was 28% higher than the control at 10 000 ppm ($P < 0.01$), while relative liver weight was increased at 5000 (+20%, $P < 0.01$) and 10 000 ppm (+44%, $P < 0.01$). At 10 000 ppm, absolute and relative paired kidney weights were significantly increased by 23% and 38%, respectively ($P < 0.01$). Absolute paired epididymides weights were significantly reduced at 5000 (−12%, $P < 0.01$) and 10 000 ppm (−10%, $P < 0.05$). Absolute prostate weights were decreased at 5000 (−20%, $P < 0.05$) and 10 000 ppm (−22%, $P < 0.01$). Relative paired testes weight was 19% higher than the control ($P < 0.01$) at 10 000 ppm. None of these changes in organ weights were accompanied with any corroborating microscopic changes. In females, organ weight changes were confined to the highest dose and included increased relative liver weight (+29%, $P < 0.01$) and relative paired kidney weight (+29%, $P < 0.01$). At and above 500 ppm, minimal to mild depletion of goblet cells in the nasal cavity occurred (Table 8). Small numbers of cells with abundant non-staining cytoplasm were interspersed where goblet cells were depleted. Minimal to moderate hyperplasia of olfactory epithelium was also noted at these same doses consisting of increased numbers of nuclei (Table 8). There were no treatment-related effects in the bone marrow.

Table 8. Histopathological findings in the nasal tissue of rats exposed to malathion in the diet for 90 days

	No. of findings per dietary concentration								
		Males				Females			
Finding	0 ppm	100 ppm	500 ppm	5 000 ppm	10 000 ppm	0 ppm	100 ppm	500 ppm	5 000 ppm	10 000 ppm
No. of animals examined	10	10	10	10	10	10	10	10	10	10
Goblet cell depletion – nose Level 2										
Minimal	0	0	5	1	3	0	0	3	4	1
Mild	0	0	0	7	2	0	0	2	3	2
Moderate	0	0	0	2	4	0	0	0	1	6
Marked	0	0	0	0	0	0	0	0	0	1
Total	0	0	5	10	9	0	0	5	8	10
Hyperplasia of olfactory epithelium – nose Level 3										
Minimal	0	0	0	6	7	0	0	0	5	3
Mild	0	0	0	3	3	0	0	0	4	6
Moderate	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	9	10	0	0	0	9	10
Hyperplasia of olfactory epithelium – nose Level 4										
Minimal	0	0	0	0	0	0	0	0	1	0
Mild	0	0	0	7	7	0	0	0	4	5
Moderate	0	0	0	3	3	0	0	0	5	5
Total	0	0	0	10	10	0	0	0	10	10

No.: number; ppm: parts per million

Results expressed as the number of rats with the finding.

Source: Barnett Jr (2012b)

Cholinesterase phase: There were no treatment-related deaths. Treatment-related clinical signs were confined to high-dose females, where a significant increase ($P < 0.01$) in urine-stained abdominal fur occurred. Absolute body weight was 9–12% lower than the control ($P < 0.01$) throughout the study at 10 000 ppm in males, with overall (day 1–91) body-weight gain 16% ($P < 0.01$) lower than the control. Absolute body weights were unremarkable in females, while body-weight gain was significantly lower than the control during the first week of exposure (−32%, $P < 0.01$). Feed consumption was significantly lower ($P < 0.01$ or 0.05) than the control at various times (−10%) in males at 10 000 ppm, with overall feed consumption significantly lower than the control (−8%, $P < 0.05$). Overall feed conversion efficiency was also significantly lower than the control in high-dose males (−9%, $P < 0.01$). In females at 10 000 ppm, feed consumption was reduced only during the first week of exposure (−13%, $P < 0.01$), while feed conversion efficiency was also reduced during the first week (−24%, $P < 0.01$). There were no treatment-related macroscopic findings.

In males, erythrocyte acetylcholinesterase activity was significantly lower than the control at and above 500 ppm ($P < 0.01$), while toxicologically significant inhibition occurred at 5000 and 10 000 ppm (−16.3%, −72.9% and −85.5% at 500, 5000 and 10 000 ppm, respectively). In females, erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at every dose but was toxicologically significant at and above 500 ppm (−10.2%, −19.6%, −79.2% and −85.6% at 100, 500, 5000 and 10 000 ppm, respectively). Brain acetylcholinesterase activity was

significantly lower ($P < 0.01$) than the control at 5000 and 10 000 ppm in both sexes (-17.3% and -18.1% in males and -21.7% and -49.5% in females, respectively). BMD modelling was applied to these data using an exponential model recommended by the United States Environmental Protection Agency (USEPA). The estimated BMD_{20} of erythrocyte acetylcholinesterase was 48.7 mg/kg bw per day for males rats and 42.0 mg/kg bw per day for females. The estimated BMD_{10} for brain acetylcholinesterase activity was 174.6 mg/kg bw per day for male rats and 118.4 mg/kg bw per day for female rats.

The NOAEL was 100 ppm (equal to 7.2 mg/kg bw per day in males and 7.5 mg/kg bw per day in females) for goblet cell depletion in the nasal cavity and the inhibition of erythrocyte acetylcholinesterase activity at 500 ppm (equal to 35.0 mg/kg be per day in males and 35.9 mg/kg bw per day in females). The lower NOAEL in this study compared to other 90-day studies of toxicity is considered an outlier due to the likelihood that it resulted from inhalational exposure to the test compound in feed.

In a 13-week inhalational toxicity study by Beattie (1994), groups of 15 Crl:CD[SD]BR rats per sex were exposed (whole-body) to aerosols of malathion (purity 94%) for six hours per day, five days per week for 13 weeks at nominal concentrations of 0, 0.24, 1.10 or 4.94 mg/L (analytical concentrations of 0, 0.1, 0.45 and 2.0 mg/L, respectively). These concentrations were based on the results of a 2-week range-finding study (Beattie 1993) that tested nominal concentrations of 0, 1.56, 6.29 or 13.81 mg/L (analytical concentrations of 0.56, 1.58 and 4.23 mg/L, respectively). The rats were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly. Ophthalmology was performed pretreatment and during week 13. Blood and urine were sampled during week 13 to analyse haematology, clinical chemistry and urine analysis parameters, including plasma and erythrocyte cholinesterase activities. Following termination, the rats were necropsied, organs weighed and tissues histopathologically examined, with attention paid to effects on the nasal cavities, bronchi, lungs, skin and eyes. Brains were also sampled to analyse acetylcholinesterase activity.

There were no deaths. Treatment-related clinical signs included excessive salivation and oily fur, which occurred at all doses and affected up to 2, 5 and 15 rats, at 0.1, 0.45 and 2.0 mg/L, respectively, in both sexes. Red staining of the muzzle, lower jaw, periorbital region, cranial region and ventral cervical region were observed at every concentration, but there is uncertainty about whether these finding were due to malathion exposure or to high concentrations of aerosols. There was no treatment-related effect on body weight or feed consumption. Ophthalmology and haematology were unremarkable. Toxicologically significant inhibition of erythrocyte acetylcholinesterase occurred at 0.45 (-22% in males, $P > 0.05$; -27% in females, $P < 0.05$) and 2.0 mg/L (-43% in males, $P > 0.05$; -44% in females, $P < 0.05$). Brain acetylcholinesterase activity was significantly lower than the control only in high-dose females (-41%, $P < 0.01$). In high-dose males and females, serum cholesterol was 33% and 31% higher than the control ($P < 0.01$), respectively. There were some changes in urine analysis parameters at the highest dose, including increased white blood cells and epithelial cells in males and lower urine pH (pH 6 or 6.5 versus pH 6–7 in the control) and increased uric acid crystals in females.

There were no treatment-related macroscopic findings. Absolute liver weight was significantly higher ($P < 0.05$) than the control at the highest dose (+14% in males and +15% in females), with a corresponding increase ($P < 0.05$) in relative liver weight (+20% in males and +14% in females). Absolute kidney weight was 9% higher than the control ($P < 0.05$) in high-dose females. As the magnitude of these differences was small and, in the absence of any treatment-related microscopic changes in these organs, these differences in liver and kidneys weights were not considered adverse. Histopathology revealed a high incidence of laryngeal hyperplasia and degeneration and/or hyperplasia of the olfactory epithelium in the nasal cavity, at all doses, with a dose-related increase in severity (Table 9). A NOAEL was not determined because treatment-related changes in the respiratory tract occurred at all doses.

Table 9. Histopathological findings in rats exposed to aerosols of malathion for 13 weeks

Parameter	No. of findings per aerosol concentration			
	0 mg/L	0.1 mg/L	0.45 mg/L	2.0 mg/L
Laryngeal hyperplasia				
Males				
Incidence	0/15	13/15	15/15	15/15
Severity[a]	–	1.1	2.4	2.9
Females				
Incidence	0/15	15/15	15/15	15/15
Severity[a]	–	1.4	2.7	2.5
Degeneration and/or hyperplasia of the olfactory epithelium				
Males				
Incidence	1/15	15/15	15/15	14/15
Severity[a]	0.1	1.6	1.7	2.6
Females				
Incidence	1/15	10/15	15/15	14/15
Severity[a]	0.1	0.7	1.6	2.6

No.: number

Results expressed as number of animals with the finding / number of animals examined.

Severity expressed as the mean histopathological grading: 1 – slight, 2 – mild, 3 – moderate, 4 – severe.

Source: Beattie (1994)

Rabbits

In a dermal toxicity study by Moreno (1989), groups of six New Zealand White rabbits per sex were exposed to malathion (purity 94%) for six hours per day, five days per week for three weeks at doses of 0, 50, 300 or 1000 mg/kg bw per day. Other than irritation at the application site, the only treatment-related effect was reduced cholinesterase activity. At 300 mg/kg bw per day, erythrocyte acetylcholinesterase activity was 26% lower than the control ($P < 0.01$) in females, while at the highest dose, plasma, erythrocyte and brain cholinesterase activities were significantly lower than the control in both sexes (plasma: −57% in males, −48% in females; erythrocytes: −74% in males, −66% in females; cerebrum: −66% in males, −53% in females; cerebellum: −41% in males, −49% in females. The NOAEL was 300 mg/kg bw per day for the inhibition of erythrocyte acetylcholinesterase activity and 1000 mg/kg bw per day for the inhibition of brain acetylcholinesterase activity.

A 21-day dermal toxicity study was conducted in Hra:NZW(SPF) rabbits by Barnett (2006d) to generate cholinesterase data suitable for BMD modelling. Groups of 10 rabbits per sex were exposed to malathion (purity 96%) for 6 hours per day via clipped skin under an occlusive dressing at doses of 0, 75, 100, 150 or 500 mg/kg bw per day. Following the exposure period, the application site was washed with water. The rabbits were observed daily for mortality and clinical signs. The application site was examined for signs of irritation immediately after washing. Ophthalmological examinations were performed prior to commencing the study and at termination in rabbits from the control and high-dose groups. The rabbits were terminated on day 22, necropsied and blood and brain acetylcholinesterase activity analysed. Blood haematology and clinical chemistry parameters was also analysed. Organs were weighed, and tissues from the control and high-dose groups examined histopathologically.

There were no treatment-related deaths or clinical signs. Irritation was observed at the application site: erythema (every dose), flaking (every dose), oedema (grade 1) and scabs (500 mg/kg

bw per day). There was no treatment-related effect on body weight, feed consumption, haematology or clinical chemistry parameters, or on the occurrence of macroscopic or microscopic findings. Absolute and relative spleen weights were significantly higher than the control at 500 mg/kg bw per day but were unaccompanied by any other treatment-related effects. Plasma cholinesterase activity was significantly lower ($P < 0.01$) than the control at 500 mg/kg bw per day (−32% in males and −37.1% in females). In males, erythrocyte acetylcholinesterase was significantly lower ($P < 0.01$) than the control at every dose but only the reduction at the highest dose was considered toxicologically significant (−17.5%, −18.4%, −19.4% and −60.9% at 75, 100, 150 or 500 mg/kg bw per day, respectively). In females, statistically and toxicologically significant inhibition of erythrocyte acetylcholinesterase occurred at 150 and 500 mg/kg bw per day (−24.2% and −71.2%, respectively; $P < 0.01$). Brain acetylcholinesterase activity was inhibited only at 500 mg/kg bw per day (−21.9% in males and −23.0% in females; $P < 0.01$). The NOAEL was 150 mg/kg bw per based on the inhibition of brain acetylcholinesterase activity at 500 mg/kg bw per day.

Dogs

In a 28-day range-finding study by Fischer et al. (1988), malathion (purity 92.4%) in gelatine capsules was administered orally to groups of three beagle dogs of each sex at doses of 0, 125, 250 or 500 mg/kg bw per day. Dogs were observed daily for mortality and clinical signs. Body weight was recorded weekly and feed consumption daily. Blood was sampled pretreatment and on days 15 and 19 (termination) to analyse haematology, clinical chemistry parameters and plasma and erythrocyte cholinesterase activities. Ophthalmoscopy was not performed. Following termination, dogs were necropsied, organs weighed and histopathology performed. Brain acetylcholinesterase was not analysed.

One high-dose male died on day 24, following anorexia and listlessness. Clinical signs occurred in 1 to 4 dogs at every dose and included increased diarrhoea, loose stools and mucoid faeces. In addition, at the highest dose emesis, anorexia and depression occurred sporadically. These clinical signs began from day 1 and generally persisted throughout the dosing period. At 500 mg/kg bw per day, body weight was 18% ($P < 0.05$) lower than the control during week 3, with body-weight gain lower during weeks 1–2 and 2–3 (−4 g versus 0 and +2 g, respectively, in the control). Also at 500 mg/kg bw per day, feed consumption was significantly lower ($P < 0.05$) than the control during week 1 (−26%), 2 (−42%) and 3 (−26%). There were no treatment-related haematology findings.

Plasma and erythrocyte cholinesterase activities were significantly lower ($P < 0.05$) than the control at 250 and 500 mg/kg bw per day (plasma: −17% at termination at 250 mg/kg bw per day and −20% on day 15 at 500 mg/kg bw per day; erythrocytes: −32% on day 15 at both 250 and 500 mg/kg bw per day). At termination, serum albumin was significantly lower than the control ($P < 0.05$) at 250 and 500 mg/kg bw per day (−11% and −32%, respectively). At 500 mg/kg bw per day, absolute uterus and ovary weight was 58% lower ($P < 0.05$) than the control. There were no treatment-related macroscopic or histopathological findings.

In a one-year toxicity study by Shellenberger & Billups (1987), malathion (purity 95%) in gelatine capsules was administered orally to groups of six beagle dogs per sex at doses of 0, 62.5, 125 or 250 mg/kg bw per day. Dogs were observed daily for mortality and clinical signs. Body weight was recorded weekly and feed consumption daily. Blood and urine were sampled pretreatment and after 6 weeks, 3 months and 6 months to analyse haematology, clinical chemistry or urine analysis parameters, including plasma and erythrocyte cholinesterase activities. Ophthalmoscopy was performed pretreatment and prior to termination. Following termination, dogs were necropsied, organs weighed and histopathology performed and brain acetylcholinesterase analysed.

There were no treatment-related deaths and clinical signs. There were no significant intergroup differences in body weight although high-dose dogs had body weights approximately 0.5–1 kg lower in the second half of the exposure period. In high-dose males, feed consumption was

significantly lower ($P < 0.05$, 10–23%) than the control at various times starting at week 11. There were no treatment-related ophthalmological effects.

Significant reductions ($P < 0.05$) in erythrocyte counts (−9% to −18% in males and −14% to −17% in females), haemoglobin (−10% to −16% in both sexes) and haematocrit (−12% to −15% in both sexes) occurred consistently at the highest dose, with erythrocytes also significantly lower ($P < 0.05$), than the control at 125 mg/kg bw per day in females at 3 and 6 months (−11% or −10%, respectively). Platelet counts were 21–44% higher than the control ($P < 0.05$) throughout the study and at every dose in males at 6 weeks and in females at termination, but still within the normal range for beagle dogs (200–500 × 10^9/L). Albumin was 10–12% lower than the control ($P < 0.05$) at the highest dose in males and 13–19% lower in females at every sampling point. In females, albumin was 8% or 9% lower than the control ($P < 0.05$) at 62.5 and 125 mg/kg bw per day during week 6 only. In high-dose females, the albumin to globulin ratio was 21% and 15% lower than the control ($P < 0.05$) at 6 weeks and 6 months, respectively. Lactate dehydrogenase was approximately threefold higher than the control ($P < 0.05$) in high-dose males at 3 months and in high-dose females at termination. Serum calcium was 7–10% lower than the control ($P < 0.05$) in high-dose males at 6 months and high-dose females at every sampling point. There was no treatment-related effect on urine analysis parameters.

The results of cholinesterase analysis are summarized in Table 10. Plasma and erythrocyte cholinesterase activities were significantly lower ($P < 0.05$) than the control at every dose. While the inhibition of erythrocyte acetylcholinesterase activity was greater than 20%, there was a flat dose-related increase in the level of inhibition and no significant reduction in brain acetylcholinesterase activity; on this basis the inhibition of erythrocyte acetylcholinesterase activity is considered to be of marginal toxicological significance.

Table 10. Effects of one-year of capsular exposure of dogs to malathion on cholinesterase activity

Parameter	Measure of cholinesterase activity per dietary concentration			
	0 mg/kg bw per day	62.5 mg/kg bw per day	125 mg/kg bw per day	250 mg/kg bw per day
Plasma ChE (mU/mL)				
Males				
6 weeks	1 918	1 401* (−27%)	1 480* (−23%)	1 348* (−30%)
3 months	1 850	1 348* (−27%)	1 384* (−25%)	1 321* (−29%)
6 months	1 907	1 421* (26%)	1 442* (−24%)	1 421* (_26%)
12 months	1 837	1 361 (−26%)	1 333 (−27%)	1 474 (−20%)
Females				
6 weeks	1 995	1 551* (−22%)	1 489* (−25%)	1 254* (−37%)
3 months	1 912	1 468* (−23%)	1 326* (−31%)	1 244* (−35%)
6 months	2 098	1 629* (−22%)	1 545* (−26%)	1 304* (−38%)
12 months				
Erythrocyte AChE (mU/mL)				
Males				
6 weeks	4 090	3 273* (−18%)	3 257* (−20%)	3 163* (−23%)
3 months	4 053	3 181* (−22%)	2 987* (−26%)	2 978* (−27%
6 months	3 727	2 950* (−21%)	2 900* (−22%)	2 753* (−26%)
12 months	3 790	2 793* (−26%)	2 860* (−25%)	2 810* (−26%)
Females				

	Measure of cholinesterase activity per dietary concentration			
Parameter	0 mg/kg bw per day	62.5 mg/kg bw per day	125 mg/kg bw per day	250 mg/kg bw per day
6 weeks	3 960	3 113* (−21%)	3 077* (−22%)	3 023* (−24%)
3 months	3 915	3 047* (−22%)	2 987* (−24%)	2 913* (−26%)
6 months	3 717	2 913* (−22%)	2 760* (−26%)	2 550* (−31%)
12 months	3880	2 820* (−27%)	2 813* (−28%)	2 800* (−28%)
Brain AChE – cerebrum (mU/mL)				
Males	1 050	997 (−5%)	983 (−6%)	1 004 (−4%)
Females	888	885	823 (−7%)	885
Brain AChE – cerebellum (mU/mL)				
Males	2 602	2 831	2 677	2 190 (−16%)
Females	2 390	2 704	2 383	2 123 (−11%)

AChE: acetylcholinesterase; bw: body weight; ChE: cholinesterase; *: $P < 0.05$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Source: Shellenberger & Billups (1987)

There were no treatment-related macroscopic findings. In males, absolute kidney weight was 25% ($P < 0.05$) and 47% ($P < 0.05$) higher than the control at 125 and 250 mg/kg bw per day, respectively, with a corresponding increase in relative kidney weight (23% and 58%, respectively). Relative liver weight increased by 33% ($P < 0.05$) in high-dose males. In females, relative kidney weight increased ($P < 0.05$) by 17% and 70% at 125 and 250 mg/kg bw per day, respectively. Relative liver weight increased ($P < 0.05$) at every dose (34%, 31% and 51% at 62.5, 125 and 250 mg/kg bw per day, respectively). These increases in organ weights were not accompanied by any histopathological findings.

The NOAEL was 125 mg/kg bw per day for reduced body weight and haematological changes at 250 mg/kg bw per day.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a pre-GLP study conducted by the National Cancer Institute (NCI, 1978), malathion (purity > 95%) was admixed in the diet at concentrations of 8000 or 16 000 ppm and fed ad libitum to groups of B6C3F1 mice (50/sex per group) for 80 weeks (equivalent to doses of 1200 and 2400 mg/kg bw per day, respectively). Concurrent control groups comprised 10 untreated mice per sex, while matched controls from similar bioassays comprised 40 mice per sex for statistical comparisons. Following the exposure period, the mice were observed for a further 14 or 15 weeks. There was no treatment-related effect on survival. There were no treatment-related clinical signs observed during the first year of exposure, while alopecia, rough and discoloured fur, reduced feed consumption, hyperexcitability and abdominal distension occurred with increasing frequency in treated mice. Five high-dose females displayed generalized body tremors from weeks 71–79. Graphically presented data indicated that mean body weight was lower than the control at both doses throughout the exposure period. In males, the incidence of hepatocellular carcinoma was consistent across all groups (2/10 [20%], 7/48 [15%] and 11/49 [22%] at 0, 8000 and 16 000 ppm, respectively) while there was a slight increase in neoplastic nodules in the liver of high-dose males (6/49 versus 3/49 in the pooled control). While combining the incidence of hepatocellular carcinoma and nodules resulted in a significant linear trend when either the matched control ($P = 0.019$) or pooled control ($P = 0.019$) was used, pairwise comparisons of either neoplasm were not statistically significant. In addition, the incidence of these findings was consistent with historical control data from the same laboratory where the incidence of

spontaneous liver tumours in males was 19%. On the basis of these findings, the authors concluded that malathion was not carcinogenic in mice. Rueber (1985) re-examined the slides from this study and concluded that malathion caused an increase in neoplasms in the liver of male mice. However, this conclusion is not considered reliable because of the absence of methodological detail on how the slides were re-examined. The United States National Toxicology Program (NTP) (Huff et al., 1985) also re-evaluated the same slides and confirmed the conclusion of the original study authors. A NOAEL for chronic toxicity was not determined because clinical signs during the second year of exposure and reduced body weight occurred at both doses. Overall this study was considered not acceptable for the evaluation of carcinogenicity because of the small number of concurrent control animals.

Malathion (purity 96.4%) was admixed in the diet at concentrations of 0, 100, 800, 8000 or 16 000 ppm and fed ad libitum to groups of 65 B6C3F1 (BR) mice per sex. Ten mice per sex per group were terminated after 12 months, and all the survivors after 18 months. The doses achieved over 18 months were, respectively, 0, 17, 143, 1476 and 2978 mg/kg bw per day in males and 0, 21, 167, 1707 and 3448 mg/kg bw per day in females. Mice were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly for 14 weeks, twice weekly to week 26 and monthly thereafter. Blood was sampled at 12 and 18 months to analyse haematology and clinical chemistry parameters, including the analysis of plasma and erythrocyte cholinesterase activities. In mice assigned for termination after 12 months, blood was also collected at 9 months to analyse erythrocyte acetylcholinesterase activity. Following scheduled termination at 12 and 18 months, the mice were necropsied, organs weighed and tissues histopathologically examined. Brain acetylcholinesterase was analysed in all mice.

Survival was comparable across all groups and there were no adverse, treatment-related clinical signs. At 8000 and 16 000 ppm, absolute body weight was significantly lower than the control ($P < 0.01$) over the entire period of exposure; at the end of the study, absolute body weight was 3%, 3%, 14% and 20% lower than the control in males and 0%, 0%, 10% and 16% lower than the control in females at 100, 800, 8000 and 16 000 ppm, respectively. At these same doses, feed consumption was also significantly lower than the control ($P < 0.01$ or 0.05), most consistently from week 30 (mean feed consumption over the exposure period was 2% and 6% lower than the control in males and 5.4% and 12.5% lower than the control in females at 8000 and 16 000 ppm, respectively).

There was no treatment-related effect on haematological parameters. The results of cholinesterase analyses are presented in Table 11. Plasma cholinesterase was significantly lower ($P < 0.01$ or 0.05) than the control at 8000 and 16 000 ppm in males, and at and above 800 ppm in females. Statistically and toxicologically significant inhibition of erythrocyte acetylcholinesterase activity occurred in both sexes at and above 800 ppm. Brain acetylcholinesterase activity was inhibited by more than 20% at 8000 and 16 000 ppm; however, only inhibition at the highest dose at termination was statistically significant.

Table 11. Effects of 18 months of dietary exposure to malathion on cholinesterase activity in mice

Parameter	Measure of cholinesterase activity per dietary concentration				
	0 ppm	100 ppm	800 ppm	8 000 ppm	16 000 ppm
Plasma ChE (µmol/mL per min)					
Males					
12 months	8.8	8.3	6.8 (−23%)	1.1* (−88%)	0.6* (−93%)
18 months	2.1	2.3	1.6 (−24%)	0.2** (−91%)	0.1** (−95%)
Females					
12 months	10.8	10.8	8.9* (−18%)	1.2** (−89%)	0.6** (−94%)
18 months	2.5	2.2 (−12%)	1.6* (−36%)	0.2** (−92%)	0.1** (−96%)
Erythrocyte AChE (µmol/mL per min)					

Parameter	Measure of cholinesterase activity per dietary concentration				
	0 ppm	100 ppm	800 ppm	8 000 ppm	16 000 ppm
Males					
9 months	2.4	2.6	1.5 (−28%)	0.7* (−71%)	0.7* (−71%)
12 months	4.9	4.5 (−8%)	3.1* (−37%)	1.7** (−65%)	1.5** (−69%)
18 months	3.9	3.3 (−15%)	2.2 (−44%)	0.4** (−90%)	0.3** (−92%)
Females					
9 months	1.8	1.8	1.1** (−39%)	0.7* (−61%)	0.6** (−67%)
12 months	4.8	4.9	3.1 (−35%)	1.7** (−65%)	1.5** (−69%)
18 months	3.6	2.5 (−31%)	1.5* (−58%)	0.3** (−92%)	0.3** (−92%)
Brain AChE (µmol/L per g per min)					
Males					
12 months	15.6	17.2	17.4	14.9 (−4%)	11.9 (−24%)
18 months	16.2	16.3	15.1 (−7%)	12.4 (−23%)	10.2** (−37%)
Females					
12 months	16.2	16.8	15.8	17.7	13.0 (−20%)
18 months	15.2	13.7 (−10%)	14.8 (−3%)	12.2 (−20%)	8.7** (−43%)

AChE: acetylcholinesterase; ChE: cholinesterase; min: munite; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % decrease (−) relative to the control in parentheses.

Source: Slauter (1994)

Selected macroscopic and microscopic findings are summarized in Table 12. No treatment-related macroscopic abnormalities were observed in mice terminated after 12 months. Necropsy revealed an increased incidence of liver masses, liver nodules and tan or yellow foci (mainly graded as slight) at the highest dose. In mice terminated after 12 and 18 months, absolute and relative liver weights were increased in males at 8000 and 16 000 ppm, and in females at 16 000 ppm. In high-dose mice terminated after 18 months, absolute heart weight was significantly lower than the control in both sexes (−21% in males and −19% in females). Hypertrophy of hepatocytes occurred microscopically at 8000 and 16 000 ppm in mice terminated after 12 and 18 months. In mice terminated after 12 months, the hypertrophy was graded as trace to moderate, while in mice terminated after 18 months, it was graded as mild to trace at 8000 ppm and moderate to severe at 16 000 ppm. The incidence of hypertrophy was outside of the performing laboratory's historical control range (0–4.76% over 18 months in both sexes).

The incidence of hepatocellular adenomas was increased at 8000 and 16 000 ppm in both sexes, which was outside the performing laboratory's historical control range (14.29–21.74% in males and 0–10.24% in females over 18 months). In males, the occurrence of liver carcinomas was significantly higher in the test mice than in the controls ($P < 0.05$) at the lowest dose and second-highest dose; however, these increases were not considered treatment related for the following reasons:

- There was no dose–response relationship.

- The non-dose-related increase in carcinomas was not corroborated in females where equivalent increases in liver hypertrophy and liver adenomas occurred as in males.

- Liver adenomas and carcinomas are common age-related tumours observed in dietary studies in untreated control B6C3F1 male mice. Published data (Haseman, Hailey & Morris, 1998) indicate that the combined incidence of these tumours is 10–68%, with the range for adenomas 4–60% and for carcinomas 6–29%.

In females terminated after 12 months, early disappearance of the x-zone of the inner cortex of the adrenals occurred in all mice at 8000 and 16 000 ppm. In females terminated after 18 months, multifocal mineralization in the cortex of the kidney occurred at 8000 and 16 000 ppm (1/55, 6/52, 8/52, 32/53 and 36/51 at 0, 100, 800, 8000 and 16 000 ppm, respectively). Due to severe nasal toxicity in a 24-month rat study (Daly, 1996a), the USEPA requested that the nasal turbinate tissue in the current study be histopathologically examined. This examination by Swenberg (1999c) detected no neoplastic lesions. Non-neoplastic lesions identified at 8000 and 16 000 ppm consisted of degeneration and loss of cellularity of the olfactory epithelium, loss of olfactory nerves in the submucosa, increased glandular secretion due to the retention of mucus and atrophy of the olfactory epithelium adjacent to the retained mucus. This nasal toxicity occurred at both 12 and 18 months.

The NOAEL was 800 ppm (equal to 143 mg/kg bw per day in males and 167 mg/kg bw per day in females) for the inhibition of brain acetylcholinesterase activity at 8000 ppm (equal to 1476 mg/kg bw per day in males and 1707 mg/kg bw per day in females). The NOAEL for carcinogenicity was 800 ppm (equal to 143 mg/kg bw per day in males and 167 mg/kg bw per day in females) for the occurrence of liver adenomas at 8000 ppm (equal to 1476 mg/kg bw per day in males and 1707 mg/kg bw per day in females) (Slauter, 1994).

Table 12. Macroscopic and microscopic findings in mice after dietary exposure to malathion

Parameter	Measure per dietary concentration				
	0 ppm	100 ppm	800 ppm	8 000 ppm	16 000 ppm
Liver masses – 18 month necropsy [a]					
Males	0/50	8/51 (16%)	4/48 (8%)	5/54 (9%)	18/50 (36%)
Females	1/55 (2%)	0/52	3/52 (6%)	2/53 (4%)	10/51 (20%)
Liver nodules - 18 month necropsy [a]					
Males	5/50 (10%)	2/51 (4%)	3/48 (6%)	10/54 (19%)	19/50 (38%)
Females	1/50 (2%)	2/51 (4%)	0/48	9/54 (17%)	29/50 (58%)
Tan or yellow liver foci - 18 month necropsy [a]					
Males	0/50	0/51	1/48 (2%)	2/54 (4%)	18/50 (36%)
Females	0	0	0	2/54 (4%)	9/50 (18%)
Absolute liver weight (g) – 12 months [b]					
Males	1.62	1.71	1.78	1.98** (+22%)	2.38** (+47%)
Females	1.55	1.68	1.56	1.66	1.92** (+24%)
Relative liver weight (%) – 12 months [b]					
Males	5.15	5.19	5.42	6.95** (+35%)	8.30** (+61%)
Females	5.22	5.39	5.22	6.21** (+19%)	7.56** (+45%)
Absolute liver weight (g) – 18 months [b]					
Males	1.90	2.90	1.96	2.26** (+19%)	2.66** (+40%)
Females	1.93	1.77	1.96	1.92	2.18
Relative liver weight (%) – 18 months [b]					
Males	5.59	6.15	5.82	7.51** (+34%)	9.38** (+68%)
Females	6.19	5.76	6.26	6.90	8.51** (+37%)
Hypertrophy of hepatocytes – 12 months [a]					
Males	0/10	0/10	0/10	7/10	10/10
Females	0/10	0/10	0/10	5/10	10/10
Hypertrophy of hepatocytes – 18 months [a]					

	Measure per dietary concentration				
Parameter	0 ppm	100 ppm	800 ppm	8 000 ppm	16 000 ppm
Males	0/50	1/51	0/48	1/54	3/50
Females	0/55	0/52	0/52	53/53	51/51
Hepatocellular adenoma – 18 months [a]					
Males	1/50	6/51	2/48	13/54*	49/50**
Females	0/55	1/52	0/52	9/53*	42/51**
Hepatocellular carcinoma – 18 months [a]					
Males	0/50	6/51*	2/48	6/54*	1/50
Females	1/55	0/52	2/52	1/53	2/51

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] Results expressed as the number of animals with the finding / number of animals examined per group, with the % increase (+) or decrease (−) relative to the control in parentheses, or as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

[b] Results expressed as absolute weight (g) or relative weight (%) with the % increase (+) relative to the control in parentheses.

Source: Slauter (1994)

Rats

In a pre-GLP study conducted by the National Cancer Institute (NCI, 1978), malathion (purity > 95%) was admixed in the diet at concentrations of 4700 or 8150 ppm and fed ad libitum to groups of Osborne–Mendel rats (50/sex per group) for 80 weeks (equivalent to doses of 1200 and 2400 mg/kg bw per day, respectively). Concurrent control groups comprised 15 untreated rats per sex, while matched controls from similar bioassays comprised 40 rats per sex. Following the exposure period, the rats were observed for a further 30 weeks and then terminated after 109 weeks. There was no treatment-related effect on survival. During the second year of treatment, an increase in clinical signs, including rough hair coats, pale mucous membranes, dermatitis, ataxia, alopecia and haematuria, was reported. Graphically presented data showed that body weights of treated females were lower than the control. The treated rats had an increase in proliferative lesions of the thyroid (Table 13). The Cochran–Armitage test or pairwise comparisons using Fisher's exact test found no significant increase in tumours in males. In females, the Cochran–Armitage test found a significant dose-related increase in thyroid follicular cell adenomas and carcinomas ($P = 0.026$), but pairwise comparisons with Fisher's exact test found no significant differences. On the basis of these findings, the authors concluded that malathion was not carcinogenic in rats. Rueber (1985) re-examined the slides from this study and concluded that the incidence of neoplasms across all tissues was increased in the treated rats. However, this conclusion is not considered reliable because of the absence of methodological detail on how the slides were re-examined. The NTP (Huff et al., 1985) also re-evaluated the same slides and confirmed the original interpretation that malathion is not carcinogenic in rats. Overall this study was considered not acceptable for the evaluation of carcinogenicity because of the small number of concurrent control animals.

Table 13. Thyroid lesions in rats after dietary exposure to malathion

| | No. and incidence per dose | | | | | |
| | Males | | | Females | | |
Lesion	Control	Low dose	High dose	Control	Low dose	High dose
C-cell hyperplasia	0/14	1/41 (2.4%)	3/47 (6.4%)	0/15	5/48 (10.4%)	3/49 (6.1%)
C-cell adenoma	0/14	1/41 (2.4%)	3/47 (6.4%)	0/15	1/48 (2.1%)	2/49 (4.1%)
Follicular cell hyperplasia	1/14 (7.1%)	7/41 (17%)	8/47 (17%)	0/15	3/48 (6.3%)	0/49
Follicular cell adenoma	1/14 (7.1%)	1/41 (2.4%)	1/47 (2.1%)	0/15	0/48	1/49 (2%)
Follicular cell carcinoma	0/14	2/41 (4.9%)	6/47 (12.8%)	0/15	0/48	3/49 (6.1%)

No.: number

Results expressed as the number of rats with the lesion / number of rats examined, with the incidence (%) in parentheses.

Source: NCI (1978)

In a subsequent pre-GLP study by the National Cancer Institute (NCI, 1979a), malathion (purity > 95%) was admixed in the diet at concentrations of 2000 or 4000 ppm and fed ad libitum to groups of F344 rats (49 or 50/sex per group) for 103 weeks (equivalent to doses of 100 and 200 mg/kg bw per day, respectively). The rats were then observed for a further 2 or 3 weeks. Concurrent control groups comprised 50 untreated rats per sex. No signs of toxicity were observed in females. There was a slight reduction in survival in males at the highest dose (88%, 86% and 80% survival at 0, 2000 and 4000 ppm, respectively), with a significant ($P = 0.001$) dose-related trend. There were no treatment-related neoplastic lesions. Treatment-related non-neoplastic lesions included chronic inflammation of the stomach in both sexes (4.1%, 13% and 23% in males and 0%, 4.5% and 8.5% in females at 0, 2000 and 4000 ppm, respectively), gastric ulcers in males (2%, 20% and 32% at 0, 2000 and 4000 ppm, respectively) and fatty metamorphosis of the liver in females (0, 12 and 19% at 0, 2000 and 4000 ppm, respectively). The authors concluded that malathion was not carcinogenic. Rueber (1985) re-examined the slides and concluded that the total incidence of benign and malignant neoplasms across all tissues increased in treated males. However, this conclusion is not considered reliable because of the absence of methodological detail on how the slides were re-examined. The NTP (Huff et al., 1985) also re-evaluated the same slides and confirmed the original interpretation that malathion is not carcinogenic in rats.

In a pre-GLP study, malathion (purity 92.1%) was admixed in the diet at concentrations of 0, 100, 1000 or 5000 ppm and fed ad libitum to groups of 50 Sprague Dawley rats per sex for 24 months (doses estimated to be equal to 0, 5, 50 and 250 mg/kg bw per day, respectively). The rats were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded weekly to week 13 and then at 24, 53, 79 and 103 weeks. Blood and urine were sampled at 3, 6, 12 and 24 months to analyse haematology and clinical chemistry or urine analysis parameters, including plasma and erythrocyte cholinesterase activities. Brain acetylcholinesterase was not analysed. Following scheduled termination, the mice were necropsied, organs weighed and tissues histopathologically examined.

There were no treatment-related deaths or clinical signs. At 1000 and 5000 ppm, body weight was significantly lower ($P < 0.05$) than the control throughout the study (up to 10% lower than the control in males and 5% in females at both doses). There was no treatment-related effect on feed consumption or haematology and urine analysis parameters. Erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.05$) than the control at 1000 and 5000 ppm (−6% to −18%, −25% to

−42 and −45% to −82% in males and −7% to −13, −20% to −45 and −37% to −71% in females at 100, 1000 and 5000 ppm, respectively). There were no significant intergroup differences in plasma cholinesterase activity. There were no treatment-related macroscopic findings including any increase in palpable masses in treated groups; the incidence of palpable masses was 12%, 6%, 14% and 12% in males and 30%, 48%, 38% and 20% in females at 0, 5, 50 and 250 mg/kg bw per day, respectively. Absolute and relative liver weights were 14% and 26% higher, respectively, than the control ($P < 0.05$) in high-dose males; this was accompanied by periportal hepatocellular hypertrophy and cystic hepatocellular degeneration observed microscopically. There was no evidence of a treatment-related increase in neoplastic or non-neoplastic lesions although there was a slight increase in the occurrence of benign fibroadenomas of the mammary gland in low- and mid-dose females (Table 14). However, there was no increase at the highest dose (where the incidence was reduced) and neither the study authors nor Seely (1991) determined the findings to be statistically significant. Further, mammary gland fibroadenomas are a common age-related tumour in female Sprague Dawley rats and can occur in approximately 50% of control rats (Dinse et al., 2010).

Table 14. Incidence of mammary neoplasms in female Sprague Dawley rats after dietary exposure to malathion

Neoplasm	No. and incidence per dietary concentration			
	0 ppm	100 ppm	1 000 ppm	5 000 ppm
Adenoma	2/50 (4%)	4/50 (8%)	1/50 (2%)	22/50 (4%)
Adenocarcinoma	2/50 (4%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Fibroadenoma	8/50 (16%)	9/50 (18%)	15/50 (30%)	5/50 (10%)
Mixed tumour	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)

No.: number; ppm: parts per million

Results expressed as the number of rats with the finding / number of female rats examined, with the incidence (%) in parentheses.

Source: Seely (1991).

The NOAEL was 100 ppm (estimated to be equal to 5 mg/kg bw per day) for the inhibition of erythrocyte acetylcholinesterase activity at 1000 ppm (estimated to be equal to 50 mg/kg bw per day) (Rucci, Becci & Parent, 1980; Seely, 1991). The NOAEL for carcinogenicity was 5000 ppm (estimated to be equal to 250 mg/kg bw per day), the highest tested dose.

Malathion (purity 96.4%) was admixed in the diet at concentrations of 0, 100, 500, 6000 or 12 000 ppm and fed ad libitum to groups of CDF®(F-344)/CrlBr rats (90/sex per dose) for 24 months. The low-dose group was reduced to 50 ppm from day 113 because of the inhibition of erythrocyte acetylcholinesterase activity. Thirty-five rats per sex per group were assigned to the chronic phase of the study and terminated after 12 months, while 44 rats per sex per group were assigned to the oncogenicity phase of the study and terminated after 24 months. The rats were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded at regular intervals. Ophthalmology was performed pretreatment and at 3, 6 and 12 months. Electroretinograms and fundic photographs were recorded at 3, 6 and 12 months (chronic phase) and at 24 months (oncogenicity phase). Blood was sampled at 6 and 12 months (chronic phase – 10 rats/sex per group) and 18 and 24 months (chronic phase – all rats) to analyse haematology and clinical chemistry parameters, including cholinesterase activity. Urine was collected at 6, 12, 18 and 24 months for urine analysis. Following death or scheduled termination, the rats were necropsied and their organs weighed, tissues histopathologically examined and brain acetylcholinesterase activity analysed.

The doses achieved over 24 months were 0, 7, 29, 359 and 729 mg/kg bw per day in males and 0, 8, 35, 415 and 868 mg/kg bw per day in females at 0, 100, 500, 6000 or 12 000 ppm,

respectively. From day 113, the achieved low dose was 2 mg/kg bw per day in males and 3 mg/kg bw per in females.

Survival was adversely affected by treatment at 6000 and 12 000 ppm from 14 months in males and towards the end of the study in females; after 24 months, survival was 67%, 75%, 53%, 26% and 0% in males and 69%, 74%, 75%, 62% and 36% in females at 0, 100, 500, 6000 and 12 000 ppm, respectively. The most common apparent cause of death was chronic nephropathy or mononuclear cell leukaemia. The only treatment-related clinical sign was yellow anogenital staining in high-dose females. Body weight was significantly lower ($P < 0.05$) than the control at 6000 and 12 000 ppm (up to 11.1% and 16.8 % lower in males and 5.1% and 16.8% lower in females, respectively), while feed consumption increased (up to 9.7% and 19.6% in males and 8.8% and 25.6% in females, respectively). There were no treatment-related ophthalmological or urine analysis findings.

At 6000 and 12 000 ppm, significantly reduced haemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin and increased platelet counts, cholesterol and GGT occurred over the majority of the 24-month exposure period (Table 15). At 6000 and 12 000 ppm, significant reductions ($P < 0.01$ or 0.05) in aspartate aminotransferase (males only; −50% to −58% at 12 and 18 months), alanine transaminase (females only; −26% at 12 months) and alkaline phosphatase (both sexes at 6, 12 and 18 months; −24% to −36% and −25% to −45% at 6000 and 12 000 ppm, respectively) were of uncertain toxicological significance. Plasma cholinesterase was significantly lower than the control ($P < 0.01$ or 0.05) over 24 months at 6000 and 12 000 ppm (males: −17% to −64% and −43% to −53%, respectively; females: −38% to −61% and −70% to −89%, respectively). Acetylcholinesterase activity was significantly lower than the control ($P < 0.01$ or 0.05) over 24 months at 6000 and 12 000 ppm (males: −43% to −48% and −48% to −58%, respectively; females: −44% to −58% and −51% to −66%, respectively). Toxicologically significant inhibition of erythrocyte acetylcholinesterase activity occurred in females at 3 months (−30%, $P < 0.01$). Brain acetylcholinesterase activity was significantly lower than the control ($P < 0.01$ or 0.05) at 6000 and 12 000 ppm (males: −12% to −31% and −16% to −19%, respectively; females: −12% to −18% and −28% to −67%, respectively).

Table 15. Effects of 24 months of dietary exposure to malathion on haematology and clinical chemistry parameters in rats

Parameter	Measure per dietary concentration				
	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
Hb (g/dL)					
Males					
6 months	15.5	15.5	15.5	14.7** (−5%)	14.6* (−6%)
12 months	15.6	15.6	15.8	14.6** (−6%)	13.8** (−12%)
18 months	15.1	15.2	15.4	14.2	12.7** (−16%)
24 months	14.5	14.5	14.0	12.3* (−15%)	No survivors
Females					
6 months	14.9	14.7	14.9	14.3* (−4%)	14.3** (−4%)
12 months	15.6	15.4	15.6	15.0** (−4%)	15.2
18 months	15.2	14.8	15.6	14.9	15.2
24 months	14.0	13.5	13.6	12.1	13.4
Hct (%)					
Males					
6 months	43.4	43.5	43.4	41.7* (−4%)	41.4* (−5%)

Parameter	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
			Measure per dietary concentration		
12 months	44.2	44.6	54.2	42.3* (−4%)	40.2** (−9%)
18 months	44.4	44.3	45.1	42.0	37.8**
24 months	40.2	40.2	38.9	34.5	No survivors
Females					
6 months	40.9	40.5	40.9	39.4* (−4%)	39.5* (−3%)
12 months	44.3	43.7	44.1	42.7	43.3
18 months	43.6	42.7	44.7	42.6	43.3
24 months	39.6	38.3	37.8	34.5	38.6
Erythrocytes (10^6/µL)					
Males					
12 months	9.15	9.30	9.28	8.92	8.52** (−7%)
Females					
12 months	8.24	8.09	8.43	8.21	8.43
Platelets (10^3/µL)					
Males					
6 months	590	612	595	679	721** (+22%)
12 months	584	578	538	637	694** (+19%)
18 months	553	579	610	688* (+24%)	830** (+50%)
24 months	621	526	556	764	No survivors
Females					
6 months	627	635	624	630	640
12 months	560	529	569	555	615** (+10%)
18 months	478	533	489	573** (+20%)	596** (+25%)
24 months	509	460	455	512	631* (+24%)
MCV (fL)					
Males					
6 months	47.8	47.2	47.7	46.0** (−4%)	45.7** (−4%)
12 months	48.2	48.0	48.8	47.4	47.1* (+2%)
18 months	53.0	51.4	51.9	50.3	50.8
Females					
6 months	51.1	51.3	51.1	50.3** (−2%)	49.5** (−3%)
12 months	52.2	52.1	52.0	51.0** (−2%)	50.2** (−4%)
18 months	53.0	52.7	53.1	51.9** (−2%)	51.4** (−3%)
MCH (pg)					
Males					
6 months	17.1	16.8*	17.0	16.3** (−5%)	16.1** (−6%)
12 months	17.0	16.7	17.1	16.4** (−4%)	16.2** (−5%)
18 months	18.1	17.6	17.7	17.1** (−6%)	17.0** (−6%)
24 months	18.5	18.7	19.1	18.5	No survivors
Females					

Parameter	Measure per dietary concentration				
	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
6 months	18.6	18.6	18.6	18.3** (−2%)	17.9** (−4%)
12 months	18.4	18.3	18.4	18.0** (−2%)	17.7** (−4%)
18 months	18.4	18.1	18.6	18.1	18.0
24 months	19.3	19.8	20.3	19.1	17.9** (−7%)
Cholesterol (mg/dL)					
Males					
6 months	78	80	81	129** (+65%)	163** (+109%)
12 months	94	90	93	134* (+44%)	211** (+124%)
18 months	153	144	137	224	500** +227%)
24 months	218	222	263	522** (+139%)	No survivors
Females					
6 months	99	99	102	121** (+22%)	147** (+48%)
12 months	130	123	127	151** (+16%)	172** (+32%)
18 months	126	142	157	231** (+83%)	286** (+127%)
24 months	263	162	284	341	430** (+63%)
GGT (IU/L)					
Males					
6 months	0	0	0	2	7
12 months	0	0	0	1*	4**
18 months	1	1	1	5**	13**
24 months	3	2	6	15**	No survivors
Females					
6 months	1	0	1	2**	6**
12 months	1	1	1	2	4**
18 months	1	1	1	3*	1
24 months	0	1	0	8**	3

Hb: haemoglobin; Hct: haematocrit; GGT: gamma-glutamyltransferase; IU: International Unit; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Source: Daly (1996a)

Necropsy revealed emaciation and irregular kidney surface in high-dose decedents. Absolute and relative liver and kidney weights in rats terminated after 12 and 24 months were significantly increased ($P < 0.01$) in both sexes at 6000 and 12 000 ppm (Table 16). Absolute and relative spleen weights were significantly increased in high-dose males terminated after 12 months; in the absence of similar changes at other times or in females, or any microscopic changes, these increases was not considered treatment related. Changes in thyroid/parathyroid weight occurred inconsistently over time and between sexes and is therefore unlikely to be treatment related (Table 16).

Table 16. Effects of 24 months of dietary exposure to malathion on organ weights in rats

Parameter	Measure per dietary concentration				
	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
Kidney weight (g)					
Males					
12 months	2.539	2.587	2.565	2.884** (+14%)	3.282** (+29%)
24 months	3.767	3.245	3.570	4.193** (+11%)	No survivors
Females					
12 months	1.653	1.561	1.675	1.795** (+9%)	1.864** (+13%)
24 months	2.262	2.286	2.409	2.760** (+22%)	3.090** (+37%)
Relative kidney weight (%)					
Males					
12 months	6.99	7.19	7.17	8.35** (+10%)	10.14** (+45%)
24 months	1.10	0.98	1.05	1.34	No survivors
Females					
12 months	7.85	7.77	8.15	8.79** (+12%)	9.78** (+25%)
24 months	0.94	0.91	0.98	1.20** (+32%	1.62** (+72%)
Liver weight (g)					
Males					
12 months	11.798	11.422	11.613	14.440** (+22%)	16.056** (+36%)
24 months	15.297	14.530	16.569	20.428** (+34%)	No survivors
Females					
12 months	7.096	7.096	6.810	7.644	8.255** (+16%)
24 months	10.168	10.295	10.921	13.187** (+30%)	13.315** (+31%)
Relative liver weight (%)					
Males					
12 months	3.25	3.17	3.23	4.18** (+29%)	4.96** (+53%)
24 months	4.44	4.33	4.89	6.52** (+47%)	No survivors
Females					
12 months	3.37	3.29	3.30	3.74** (+11%)	4.32** (+28%)
24 months	4.23	4.08	4.42	5.59** (+32%)	6.82** (+61%)
Spleen weight (g)					
Males					
12 months	0.711	0.709	0.711	0.773	0.877* (+23%)
24 months	2.077	2.530	2.575	1.456	No survivors
Females					
12 months	0.568	0.507	0.536	0.538	0.549
24 months	0.974	1.001	1.413	8.890	0.903
Relative spleen weight (%)					
Males					
12 months	1.96	1.97	1.99	2.24*	2.70** (+38%)
24 months	6.06	7.55	7.62	4.47	No survivors

Parameter	Measure per dietary concentration				
	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
Females					
12 months	2.71	2.37	2.61	2.64	2.89
24 months	4.07	4.12	5.95	3.83	4.73
Thyroid/parathyroid weight (g)					
Males					
12 months	0.0211	0.0227	0.0226	0.0242* (+15%)	0.0264** (+25%)
24 months	0.0354	0.0401	0.0640	0.0420** (+19%)	No survivors
Females					
12 months	0.0178	0.0174	0.0179	0.0198	0.0181
24 months	0.0381	0.0276	0.0302	0.0349** (−8%)	0.0281
Relative thyroid/parathyroid weight (%)					
Males					
12 months	5.82	6.31	6.34	7.00** (+20%)	8.15** (+40%)
24 months	1.04	1.25	1.89	1.34** (+29%)	No survivors
Females					
12 months	8.49	8.16	8.74	9.68* (+14%)	9.51
24 months	1.61	1.11	1.21	1.51** (−6%)	1.48** (−8%)

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Source: Daly (1996a)

Non-neoplastic findings: Table 17 summarizes the non-neoplastic histopathological findings in nasoturbinal and nasopharyngeal tissues. A range of treatment-related changes occurred at 6000 and 12 000 ppm in both sexes, including dilated mucosal glands (the majority graded as slight), subacute or chronic inflammation of the nasal mucosa (the majority graded as slight to moderate), degeneration of the epithelium (the majority graded as moderate to moderately severe), epithelium cysts in the nasal mucosa (mainly graded as minimal to slight) and glandular and epithelium hyperplasia (mainly graded as slight). Chronic nephropathy occurred with similar frequency across all dose groups, including the control, with a slight increase in the severity of nephropathy at the highest dose

Table 17. Histopathological findings in the nasal tissue of rats exposed to malathion in the diet for 24 months

Parameter	No. per dietary concentration				
	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
No. of animals	90	90	90	90	90
Nasal mucosa (olfactory) – glands dilated					
Males	2	1	0	31	27
Females	2	1	0	38	33
Nasal mucosa (olfactory) – subacute/chronic inflammation					
Males	6	1	7	52	35
Females	0	3	2	42	20
Nasal mucosa (olfactory) – epithelium degeneration					

Parameter	No. per dietary concentration				
	0 ppm	100 or 50 ppm	500 ppm	6 000 ppm	12 000 ppm
Males	4	2	5	66	69
Females	2	2	1	69	66
Nasal mucosa (olfactory) – epithelium cysts					
Males	0	0	0	43	55
Females	0	0	0	58	62
Nasal mucosa (olfactory) – glandular hyperplasia					
Males	0	0	0	17	18
Females	0	0	0	24	14
Nasal mucosa (olfactory) – epithelium hyperplasia					
Males	0	0	0	42	51
Females	0	0	0	57	54
Nasal mucosa (olfactory) – olfactory epithelium replaced by ciliated and non-ciliated columnar epithelial cells					
Males	6	1	7	43	43
Females	2	2	1	50	25
Nasal mucosa (olfactory) – hyperplasia of ciliated and non-ciliated columnar epithelial cells					
Males	3	1	4	18	22
Females	2	1	0	33	21
Nasal mucosa (respiratory) – subacute/chronic inflammation					
Males	10	2	12	41	21
Females	7	4	5	34	10
Nasal mucosa (respiratory) – glands dilated					
Males	18	0	13	28	24
Females	8	4	6	14	20
Nasal mucosa (respiratory) – hyperplasia					
Males	13	2	12	44	41
Females	7	3	7	44	33
Nasal lumen – cell/cell debris/metachromatic basophilic amorphous material					
Males	15	5	22	69	63
Females	10	7	9	64	58
Nasopharynx – epithelial hyperplasia					
Males	10	0	15	22	14
Females	4	1	14	26	21

No.: number; ppm: parts per million

Results expressed as the number of rats with the finding.

Source: Daly (1996a).

Neoplastic findings: Neoplasms observed microscopically in nasoturbinal tissue included an adenoma in one male at 6000 ppm and a carcinoma in one male at 12 000 ppm. Spontaneous neoplasms are uncommon in nasoturbinal tissue of F344 rats; this occurrence has not been observed by the performing laboratory in six previous studies (0/238 males and 0/241 females). Published historical control data (Haseman, Arnold & Eustis, 1990) indicated that the incidence of nasal adenomas is 0–2% in control males (in gavage studies only) and of nasal carcinomas is also 0–2% (dietary studies).

In males, liver adenomas and carcinomas occurred with similar frequency across all groups (adenomas: 2.9%, 3.6%, 5.5%, 3.6% and 1.4% at 0, 100/50, 500, 6000 and 12 000 ppm, respectively; carcinoma: 1.4%, 3.6%, 1.8%, 1.8% and 0% at 0, 100/50, 500, 6000 and 12 000 ppm, respectively). In females, the incidence of liver adenomas was significantly increased ($P < 0.01$ or 0.05) at 6000 and 12 000 ppm (0%, 1.8%, 1.8%, 5.5% and 4.3% at 0, 100/50, 500, 6000 and 12 000 ppm, respectively), while the incidence of liver carcinomas was significantly increased ($P < 0.05$) at the highest dose (0%, 1.8%, 1.8%, 0% and 4.3% at 0, 100/50, 500, 6000 and 12 000 ppm, respectively). The occurrence of liver adenomas in females was within the performing laboratory's historical control range (0–5.4%), while the occurrence of carcinomas was outside its historical control range (0–2.4%).

A number of subsequent independent histopathological re-evaluations closely examined the microscopic findings in the liver (females), pituitary and uterus (females), and nasal tissue (both sexes).

- Hardisty (2000) confirmed the treatment-related increase in hepatocellular adenomas at 6000 and 12 000 ppm but determined that no hepatocellular carcinomas were present at any dose in females.

- Swenberg (1999a) confirmed that there were no treatment-related neoplasms in the uterus or pituitary glands.

- Swenberg (1999b) confirmed that dietary exposure to malathion at 6000 or 12000 ppm caused significant nasal toxicity characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, oedema and inflammation. The pattern of distribution of these changes was noted to be unusual for a dietary study and was more consistent with inhalational exposure. No treatment-related increases in neoplasms were apparent in the nasoturbinal and nasopharyngeal tissues. A total of four nasal epithelial cell tumours were observed, one in each of the two highest doses of each sex; all were well-differentiated adenomas and each occurred at a different site.

Swenberg (1999b) noted a large squamous cell carcinoma arising from the alveolus of the tooth in one low-dose female; the absence of a similar neoplasm at substantially higher doses in females (up to two orders of magnitude higher) or at any dose in males indicates that this finding was not treatment related. Swenberg (1999b) stated that there was no relationship between the tumour of the tooth or any other neoplasm, including a squamous cell carcinoma in the palate of one high-dose female and a small squamous cell papilloma of the palate, because these neoplasms arise from different tissues and cannot be combined. The published historical range for squamous cell carcinoma or papilloma of the palate in control female F344 rats in dietary studies is 0–4% (Haseman, Hailey & Morris, 1998).

Bolte (1999a,b) examined additionally prepared slides and concluded that the carcinoma originally observed in the respiratory epithelium of one high-dose male was in fact an adenoma of the respiratory epithelium.

The NOAEL was 500 ppm (equal to 29 mg/kg bw per day in males and 35 mg/kg bw per day in females) for reduced erythrocyte parameters, inhibition of brain acetylcholinesterase activity and the occurrence of nasal toxicity at 6000 ppm (equal to 359 mg/kg bw per day in males and 415 mg/kg bw per day in females) (Daly, 1996a). The NOAEL for carcinogenicity was also 500 ppm based on the increase in nasal adenomas at 6000 ppm (Daly, 1996a, 1999).

In a published study, Cabello et al. (2001) examined the potential of malathion to cause mammary tumours in an experimental rat tumour model. In the first experiment, groups of five female Sprague Dawley rats (16 days old) were administered eserine (0.33 mg/kg bw twice per day), parathion (2.25 mg/kg bw twice per day), malathion (170 mg/kg bw twice per day), atropine (2.25 mg/kg bw twice per day), serine plus atropine or malathion (twice per day at the doses already described) plus atropine (twice per day at the doses already described) either subcutaneously or intraperitoneally for five days. The rats were terminated 16 hours after the final injection. Mammary

tissue was histopathologically examined. There were no treatment-related effects on the density of terminal end buds or alveolar buds per mm^2.

In a second experiment, the protocol was repeated using slightly older female rats (39 days old). Acetylcholinesterase was analysed in blood sampled from each rat although no results were presented. Rats were terminated 16 hours after the final injection. Mammary tissue was histopathologically examined. In malathion-treated rats there was a significant increase ($P < 0.05$) in the density of terminal end buds (12.04 versus 3.30/mm^2 in the control), while the density of alveolar buds decreased from 20.80 to 2.50/mm^2.

In a third experiment, 70 rats per group (39 days old) received subcutaneous injections twice daily for five days of the treatments at the doses described. Rats were palpated weekly for tumours and terminated after 28 months. Mammary tissue was histopathologically examined. There were no deaths over the 28 months of the study. The incidence of mammary tumours increased in malathion-treated rats (17/70 versus 0/70 in the control). The tumours were described as adenocarcinomas and "grossly nodular and encapsulated, with areas of cribiform or papillary patterns". As mammary tumours are a common age-related neoplasm in this particular rat strain, the absence of any tumours in controls, the lack of any effect on survival and the absence of further details of the classification of the tumours makes it difficult to interpret these findings. Overall this study is not considered relevant to the risk assessment of malathion because it used dose routes not relevant to possible human exposures.

2.4   Genotoxicity

An extensive range of in vitro and in vivo genotoxicity tests have been conducted on malathion and its metabolites or impurities. General details of the mythology used to evaluate these studies (along with those on diazinon and glyphosate) are described in "Section 2: General Considerations" of the JMPR meeting report[12].

(a) In vitro studies

Genotoxicity tests in prokaryotes and lower eukaryotes (Table 18)

In a cell-free assay using DNA isolated from *Escherichia coli* K-12, malathion at 0.1 mg/mL induced DNA breakage (Griffin & Hill, 1978). Testing was in the absence of metabolic activation only.

In multiple studies, malathion and malathion formulations were reported negative for mutagenicity, with or without metabolic activation, in multiple strains of *Salmonella typhimurium* (generally some combination of TA97a, TA98, TA100, TA102, TA1535, TA1536, TA1537, TA1538) and/or various strains of *E. coli* (K-12, WP2 uvr) (Dean, 1972; Mohn, 1973; Hyun & Lee, 1976; USEPA, 1977, 1990; Haworth et al., 1983; Pednekar, Gandhi & Netrawali, 1987; Traul, 1987; Wong et al., 1989; USEPA, 1990; Bowles, 2005; Taylor, 2008a,b,c; Beevers 2009; Thompson, 2013; Schreib, 2015b). In contrast, Shiau et al. (1980) reported positive mutagenic activity at 300 µg/plate in *S. typhimurium* strain 1535 and in *Bacillus subtilis* strain TKJ6321 but not TKJ5211 in the absence of metabolic activation only. Shiau et al. (1980) also reported positive results for a DNA damage assay with and without metabolic activation in various strains of *B. subtilis*, while Venkat et al. (1995) reported positive results for the SOS test (a DNA damage response test) in *E. coli*; the test was conducted without metabolic activation only. In contrast, USEPA (1977) reported malathion as negative in the *B. subtilis* rec assay and for differential survival in DNA repair–deficient *E. coli*, both of which were tested without metabolic activation only.

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[12] Pesticide residues in food 2016: Special session of the joint FAO/WHO meeting on pesticide residues. May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/).

In lower eukaryotes, malathion was negative, with and without metabolic activation, in a mutational assay in *Schizosaccharomyces pombe* (Gilot-Delhalle et al., 1983) and for mitotic recombination in *Saccharomyces cerevisiae* (USEPA, 1977).

Malaoxon was negative for mutagenicity in *S. typhimurium* strains TA97, TA98, TA100 and TA1535 (Zeiger et al., 1988) as well as TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA (Schreib, 2015a); both studies were conducted with and without metabolic activation. Isomalathion, *O,O,O*-trimethyl phosphorothioate, *O,O,S*-trimethyl phosphorothioate and *O,S,S*-trimethyl phosphorodithioate were negative for mutagenicity in *S. typhimurium* strains TA97, TA98 and TA100, with and without metabolic activation (Imamura & Talcott, 1985).

Table 18. In vitro genotoxicity tests in prokaryotes and lower eukaryotes

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
Malathion							
DNA breakage (Acellular)	DNA isolated from *E. coli* K-12	0.1 mg/mL in hexane	NS	Positive	Not tested	Inadequate due to a lack of reporting detail (e.g. exposure duration)	Griffin & Hill (1978)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1.6, 8, 40, 200, 1 000 & 5 000 µg/plate in DMSO	Batch/lot no. 1050-OSJ-15A, 99.1% w/w malathion, 0.25% w/w MeOOSPS-triester, 0.3% w/w isomalathion, 0.08% w/w malaoxon	Negative	Negative	GLP & TG compliant	Beevers (2009)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	50, 150, 500, 1 500 & 5 000 µg/plate in DMSO	Batch/lot no. 9010501, 96.0% w/w malathion, 0.25% isomalathion	Negative	Negative	GLP & TG compliant	Bowles (2005)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1538	10 µL/plate in DMSO	NS	Negative	Negative	Inadequate Only maximum concentration tested reported and quantitative results not provided	Hyun & Lee. (1976)
Reverse mutation (Prokaryote)	*E. coli* WP2 uvr	NS	97.4%	Negative	Not tested	Inadequate Semiquantitative paper disk method; details not provided	Dean (1972)
Reverse mutation (Prokaryote)	*S. typhimurium* TA100, TA1535, TA1537, TA1538 *E. coli* WP2	1, 5, 10, 50, 100 & 500, 1 000 µg/plate	Technical grade from America Cyanamid; lot 40216006.300	Negative	Not tested	Solvent not specified but likely DMSO	USEPA (1977)

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
Rec assay (Prokaryote)	*B. subtilis* H17 Rec⁺, M45 Rec⁻	1 mg/disk	Technical grade from America Cyanamid; lot 40216006.300	Negative	Not tested	Solvent not specified but likely DMSO	USEPA (1977)
Differential survival due to DNA damage (Prokaryote)	*E. coli* W3110, P3478	1 mg/disk	Technical grade from America Cyanamid; lot 40216006.300	Negative	Not tested	Solvent not specified but likely DMSO	USEPA (1977)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1537	1, 3.3, 10, 33, 100, 333, 1 000, 3 333 & 10 000 µg/plate	NS	Negative	Negative	Solvent not specified but likely DMSO S9 from liver of induced male rats plus Syrian hamster	Haworth et al. (1983)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1537	10, 100 & 1 000 µg/plate	NS	Negative	Negative	–	Hyun & Lee (1976)
Reverse mutation (Prokaryote)	*S. typhimurium* TA97a, TA98, TA100, TA1535	10, 100, 1 000 & 5 000 nL/plate in DMSO	Technical grade 1 103 g/L	Negative	Negative	–	Machado (1996)
Forward mutation (Prokaryote)	*E. coli* K-12/galRS18	200 mmol/L	NS	Negative	Not tested	Inadequate Resistance to 5-methyl-DL-tryptphan, solvent not provided, data not provided	Mohn (1973)
Reverse mutation (Prokaryote)	*S. typhimurium* TA97a, TA98, TA100	33 & 1 650 mg/L in DMSO	NS	Negative	Negative	Metabolic activation by S9 or caecal microbial extract (0.1 mL per plate), compound added in 0.2 mL	Pednekar, Gandhi & Netrawali (1987)

End-point	Test object	Concentration	Purity	Results		Comments	Reference
				−S9	+S9		
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Experiment I except TA102 – 0.033, 0.10, 0.33, 1.04, 2.6 & 5.2 µL/plate Experiment I TA 102 and Experiment II - 0.031 6, 0.100, 0.316, 1.0, 2.5 & 5.0 µL/plate in DMSO	Batch/lot no. 9010501/ME+H2, 95.7% w/w malathion, 0.19% w/w MeOOOPS-triester, 0.83% w/w MeOOSPS-triester, >0.34% w/w isomalathion, <0.02% malaoxon	Negative	Negative	GLP & TG compliant	Schreib (2015b)
Forward mutation (Prokaryote)	*B. subtilis* TKJ5211, TKJ6321	5–300 µg/plate in DMSO	NS. Sample obtained from American Cyanamid	Positive: (300 µg/plate), TKJ6321 Negative (300 µg/plate), TKJ4211	Negative	Inadequate Semiquantitative disk assay measuring His[+], Met[+] mutations	Shiau et al. (1980)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1536, TA1537, TA1538	5–300 µg/plate in DMSO	NS Sample obtained from American Cyanamid	Positive (300 µg/plate) in TA1535 only	Negative	Inadequate Semiquantitative disk assay Quantitative data not presented	Shiau et al. (1980)
DNA damage (Prokaryote)	*B. subtilis* (15 strains)	5–300 µg/plate in DMSO	NS Sample obtained from American Cyanamid	Negative	Not tested	Inadequate Semiquantitative disk assay Quantitative data not presented	Shiau et al. (1980)
Rec assay (Prokaryote)	*B. subtilis* H17 Rec[+], M45 Rec[-]	NS	NS. Sample provided by Japanese government	Negative	Not tested	Inadequate Semiquantitative disk assay Quantitative data not presented	Shirasu et al. (1976)

End-point	Test object	Concentration	Purity	Results −S9	Results +S9	Comments	Reference
				−S9	**+S9**		
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1537 *E. coli* WP2 uvrA	1.5, 5, 15, 150, 500, 1 500, 5 000 μg/plate in DMSO	Batch/lot no. D2014-OSJ-MLT-01-S, 95.8% w/w malathion, 0.50% w/w MeOOOPS-triester, 1.72% w/w MeOOSPS-triester, 0.39% isomalathion, 0.073% w/w malaoxon	Negative	Negative	GLP & TG compliant	Thompson (2013)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA	100, 500, 1 000, 2 500 and 5 000 μg/plate in DMSO	Batch/lot no. AC 4870-54B, 95.2 % w/w malathion	Negative	Negative	GLP & TG compliant	Traul (1987)
DNA damage - SOS test (Prokaryote)	*E. coli* PQ37	NS. DMSO or sodium taurocholate micells as vehicles	Reference grade provided by USEPA	Positive	Not tested	Inadequate due to lack of details. Started with stock solution at 1:100; activity was 2708 units/umol in DMSO, 3765 units/umol in taurocholate, 5th most active of 47 pesticides	Venkat et al. (1995)
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA102, TA1535, TA1537	80–400 ppm, vehicle not specified	Technical grade from American Cynamid	Negative	Negative	Inadequate due to lack of details. No quantitative data; vehicle not specified	Wong et al. (1989)
Mitotic recombination (Yeast)	*S. cerevisiae* D3	5% w/v or v/v	Technical grade from America Cyanamid; lot 40216006.300	Negative	Negative	Solvent not specified but likely DMSO	USEPA (1977)
Forward mutation (Yeast)	*S. pombe (ade6)*	30, 91 and 182 mmol/L in 5% ethanol	99%	Negative	Negative	S9 from induced male mice	Gilot-Delhalle et al. (1983)

End-point	Test object	Concentration	Purity	Results		Comments	Reference
				−S9	+S9		
Malaoxon							
Reverse mutation (Prokaryote)	*S. typhimurium* TA97, TA98, TA100, TA1535	100, 333, 1 000, 3 333 and 10 000 µg/plate in DMSO	94.4%	Negative	Negative	Summary paper. Activation using S9 from rat and Syrian hamster	Zeiger et al. (1988)
Isomalathion							
Reverse mutation (Prokaryote)	*S. typhimurium* TA97, TA98, TA100	10, 100 and 1 000 µg/plate in DMSO	> 99%	Negative	Negative	However, alkylates nitrobenzylpyridine, which is a measure of compound reactivity	Imamura & Talcott (1985)
MDCA							
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1.6, 8, 40, 200, 1 000 and 5 000 µg/plate in DMSO	Batch No. 621-Bse-81A, 98.8% purity	Negative	Negative	GLP & TG compliant	Taylor, 2008b
Desmethyl malathion							
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Exp 1: 1.6, 8, 40, 200, 1 000, 5 000 µg/plate; Exp 2: 31.25, 62.5,125, 250, 500, 1 000, 2 000, 5 000 µg/plate in water	Batch 972-OSJ-41C, 45.9% malathion as free acid	Negative	Negative	GLP & TG compliant	Taylor, 2008c
Desmethyl-malathion monocarboxylic acid, potassium salt							
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA	31.6, 100, 316, 1 000, 2 500 and 5 000 µg/plate in DMSO	77.6% w/w, Batch 676-Bse-16A	Negative	Negative	GLP & TG compliant	Donath (2012)
Desmethyl-malaoxon dicarboxylic acid, trisodium salt							
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvrA	0.031 6, 0.100, 0.316, 1.0, 2.5 and 5.0 µL/plate in distilled water	23.9% w/w, Batch No. P1334-CSO-15-filtered	Negative	Negative	GLP & TG compliant	Schreib (2015a)

End-point	Test object	Concentration	Purity	Results		Comments	Reference
				−S9	+S9		
MMCA							
Reverse mutation (Prokaryote)	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	1.6, 8, 40, 200, 1 000 and 5 000 μg/plate in DMSO	92.2%, Batch No. 676-BSe-8A, mixture of alpha and beta isomers	Negative	Negative	GLP & TG compliant	Taylor (2008a)
O,O,O-trimethyl phosphorothioate							
Reverse mutation (Prokaryote)	*S. typhimurium* TA97, TA98, TA100	10, 100 & 1 000 μg/plate in DMSO	> 99%	Negative	Negative	However, alkylates nitrobenzylpyridine, which is a measure of compound reactivity	Imamura & Talcott (1985)
O,O,S-trimethyl phosphorothioate							
Reverse mutation (Prokaryote)	*S. typhimurium* TA97, TA98, TA100	10, 100 & 1 000 μg/plate in DMSO	> 99%	Negative	Negative	However, alkylates nitrobenzylpyridine, which is a measure of compound reactivity	Imamura & Talcott (1985)
O,S,S-trimethyl phosphorodithioate							
Reverse mutation (Prokaryote)	*S. typhimurium* TA97, TA98, TA100	10, 100 & 1 000 μg/plate in DMSO	> 99%	Negative	Negative	However, alkylates nitrobenzylpyridine, which is a measure of compound reactivity	Imamura &Talcott (1985)

DMSO: dimethylsulfoxide; GLP: good laboratory practice; MDCA: malathion dicarboxylic acid; MMCA: malathion monocarboxylic acid; NS: not specified; ppm: parts per million; S9: 9000 × *g* supernatant from induced male rat; −S9: without metabolic activation; +S9: with metabolic activation; TG: test guideline; USEPA, United States Environmental Protection Agency; v/v: volume per volume; w/w: weight per weight

Genotoxicity tests in non-human mammalian cells (Table 19)

The results of in vitro genotoxicity studies conducted on malathion, metabolites and impurities in non-human mammalian cells are summarized in Table 19.

Malathion was reported to induce DNA–protein crosslinks in isolated rat lymphocytes when tested in the absence of metabolic activation only (Ojha & Gupta, 2014).

Malathion was reported as positive for the induction of DNA damage, as measured by the alkaline and neutral versions of the comet assay, in isolated rat lymphocytes in the absence of metabolic activation only (Ojha & Gupta, 2014; Ojha & Srivastava 2014) and as measured by the alkaline comet assay in metabolically competent rat hepatoma cells (Bianchi et al., 2015) or in rat adrenal gland PC12 cells tested in the absence of metabolic activation only (Lu et al., 2012). Two of these positive studies concluded that reactive oxygen species were involved in the induction of damage (Lu et al., 2012; Ojha & Srivastava, 2014). Supportive of this hypothesis, malathion appears to selectively induce markers of oxidative stress in Tox21/ToxCast high-throughput screening assays (USEPA interactive Chemical Safety for Sustainability Dashboard, 2016).

Malathion was reported to induce DNA damage as measured by the induction of sister chromatid exchanges in Chinese hamster ovary (CHO) and V79 cells tested in the absence of metabolic activation only (Chen et al., 1981; Nishio & Uyeki, 1981), and positive for inducing sister chromatid exchanges in CHO cells in the absence and presence of metabolic activation (Galloway et al. 1987). Szekely, Goodwin & Delaney (1992) reported negative sister chromatid exchange results for malathion tested in V79 cells but did report a concentration-related increase in polyploidy. A malathion formulation was negative for the induction of unscheduled DNA synthesis in rat primary hepatocytes (Pant, 1989).

Malathion was reported to induce chromosomal aberrations in CHO cells in the presence but not the absence of metabolic activation (Galloway et al., 1987). A malathion formulation was positive for the induction of micronuclei in Chinese hamster lung cells, tested in the absence of metabolic activation only (Ni et al., 1983) but negative for micronuclei induction in metabolically competent rat hepatoma cells (Bianchi, Mantovani & Marin-Morales, 2015).

Malathion and malathion formulations were positive for the induction of small (clastogenic response) and large (mutagenic response) colonies in the mouse lymphoma (L5178Y) assay, with and without metabolic activation (Edwards, 2001b).

In studies with malaoxon, Ivett et al. (1989) reported a significant increase in sister chromatid exchanges but not chromosomal aberrations in CHO cells, with or without metabolic activation (the sister chromatid exchange response was much weaker with metabolic activation). Nishio & Uyeki (1981) also reported an increase in sister chromatid exchanges when testing in the absence of metabolic activation only; these investigators also reported that malaoxon was more potent than malathion in this assay.

Malaoxon was also reported to induce DNA damage as measured by the alkaline comet assay in rat adrenal gland PC12 cells when tested in the absence of metabolic activation only (Lu et al., 2012). It was also mutagenic in mouse lymphoma (L5178Y) cells in the absence but not the presence of metabolic activation (Myhr & Caspary, 1991). In this study, there seemed to be a preference for the induction of small colonies, generally considered to be indicative of chromosome damage rather than gene mutations.

Table 19. In vitro genotoxicity tests in non-human mammalian cells

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
Malathion							
DNA damage (alkaline comet assay)	Hepatoma tissue culture (rat HTC cells)	0.000 9, 0.009 &, 0.09 mg per 5 mL in distilled water	50% (500 CE from Dipil Chemical Industry Ltd - Massaranduba, SC, Brazil)	Not applicable	Positive (0.009 mg/5 mL)	Decreasing damage with increasing concentration Benzo(a)pyrene used as positive control to show that cells were metabolically competent	Bianchi, Mantovani & Marin-Morales (2015)
Chromosome damage (micronuclei)	Hepatoma tissue culture (rat HTC cells)	0.000 9, 0.009 & 0.09 mg per 5 mL in distilled water	50% (500 CE from Dipil Chemical Industry Ltd - Massaranduba, SC, Brazil)	Not applicable	Negative	Criteria for concentration selection not specified Cytokinesis block assay Benzo(a)pyrene used as positive control to show that cells were metabolically competent	Bianchi, Mantovani & Marin-Morales. (2015)
DNA damage (SCE)	Chinese hamster V79 cells	10, 20, 40 & 80 µg/mL in DMSO	94%	Positive (40 µg/mL)	Not tested	80 µg/mL cytotoxic	Chen et al. (1981)
Mutation (colony formation)	Mouse lymphoma (L5178Y) cells	−S9: 125, 250, 500, 1 000, 1 500, 1 800, 2 000 µg/mL +S9 250, 500, 1 000, 1 800, 2 000, 2 200 µg/mL in DMSO	96.0% w/w, Batch/lot no.: 9010501, 0.19% w/w MeOOOPS-triester, 0.83% w/w MeOOSPS-triester, 0.15% w/w isomalathion, <0.02% malaoxon	Positive (2 000 µg/mL)	Positive (2 000 µg/mL)	GLP and TG compliant Small but significant increases in small, large and total colonies Positive increases associated with decreases of 89% (−S9) and 64% and 78% (+S9) in relative total growth	Edwards (2001b)
Chromosome damage (chromosomal aberrations)	CHO cells	−S9 25, 50, 76 & 101 µg/mL +S9 303, 352 & 402 µg/mL in DMSO	NS	Negative	Positive (303 µg/mL)	−S9 = 101 µg/mL cytotoxic, positive concentration-related response	Galloway et al. (1987)
DNA damage (SCE)	CHO cells	−S9 4.03, 12.1 & 40.3 µg/mL +S9 121, 298, 351 & 403 µg/mL in DMSO	NS	Positive (40.3 µg/mL)	Positive (298 µg/mL)	Response generally concentration-related	Galloway et al. (1987)

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
DNA damage (alkaline comet assay)	Rat adrenal gland PC12 cells	20 & 40 mg/L in DMSO	95%	Positive (40 mg/mL)	Not tested	Increased intracellular ROS levels and reduced catalase, superoxide dismutase	Lu et al. (2012)
DNA damage (SCE)	CHO cells	0.03, 0.1, 0.3 & 1.0 mmol/L in DMSO	99%	Positive (0.3 mmol/L)	Not tested	Concentration–response relationship	Nishio & Uyeki (1981)
Mutation (colony formation)	Mouse lymphoma (L5178Y) cells	S9 = 6 trials with concentrations 1.25–60 μg/mL +S9 = 2 trials of 5–60 μg/mL; in ethanol	NS	Equivocal	Negative	−S9: 6 trials resulted in 1 inconclusive, 1 positive, 2 equivocal and 2 negative trials Tested to excessive cytotoxic concentrations	NTP study reported in CEBS with accession number 002-02380-0020-0000-7 (2017)
DNA damage (alkaline & neutral comet assay)	Lymphocytes (isolated from male Wistar rats)	1/10 (0.52), 1/4 (1.3) LC_{50} (mg/L) in DMSO	NS	Positive (0.52 mg/mL) for both SSB and DSB	Not tested	Duration of exposure, 2, 4, 8, or 12 hours; 4-hour LC_{50} > 5.2 mg/L, alkaline measures DNA SSB & alkali-labile sites, neutral measures DSB	Ojha & Gupta (2014)
DNA damage (DNA–protein crosslink)	Lymphocytes (isolated from male Wistar rats)	1/10 (0.52), 1/4 (1.3) LC_{50} (mg/L) in DMSO	NS	Positive (0.52 mg/mL)	Not tested	Duration of exposure, 2, 4, 8, or 12 hours; 4-hour LC_{50} > 5.2 mg/L, assay based on binding of SDS to proteins, and lack of binding to DNA	Ojha & Gupta (2014)
DNA damage (alkaline & neutral comet assay)	Lymphocytes (isolated from male Wistar rats)	Alkaline 1/20 (0.26), 1/10 (0.52), 1/8 (0.65), 1/4 (1.3) LC_{50} (mg/L) Neutral 1/4 LC_{50} (1.3 mg/L) in DMSO	NS	Positive alkaline 1/20 LC_{50} (0.26 mg/L) @ 2 hours; neutral 1/4 LC_{50} (1.3 mg/L)	Not tested	Duration of exposure, 2, 4 hours; 4-hour LC_{50} > 5.2 mg/L, alkaline measures DNA SSB, neutral measures DSB, Fpg–Endo enzyme treatment indicates presence of ROS-generated damage, using comet assay	Ojha & Srivastava, (2014)

End-point	Test object	Concentration	Purity	Results −S9	Results +S9	Comments	Reference
DNA damage (UDS)	Primary hepatocytes from male SD rats	0.02, 0.04, 0.08 & 0.12 μL/mL in DMSO 18-hour exposure	94% w/w, Batch/lot no.: AC 6015-136, 0.1 % w/w MeOOOPS-triester, 0.4 % w/w MeOOSPS-triester, 0.2 % w/w isomalathion, 0.1% w/w malaoxon	Not applicable	Negative	GLP study	Pant (1989)
DNA damage (SCE)	Chinese hamster V79 cells	10, 20 & 30 μg/mL in ethanol	> 99%	Negative	Not tested	Selection of concentrations based on effects on cell growth. Strong increase in polyploidy at 20–40 mg/L	Szekely, Goodwin & Delaney (1992)
Chromosome damage (chromosomal aberrations)	CHO cells	−S9 187, 249, 377 +S9 = trial 1: 502, 1 256, 2 512 μg/mL in DMSO; trial 2: 1 998, 2 498, 3 010 μg/mL in DMSO; trial 3: 3 000, 3 250, 3 500 μg/mL in DMSO	94.4%	Negative	Negative	Most trials harvested at 10–12 hours (not current protocol), one of two +S9 trials with delayed harvest weekly positive; overall call negative. Concentrations tested based on range-finding studies	Ivett et al. (1989)
Malaoxon							
DNA damage (SCE)	CHO cells	S9 Trial 1 = 16.7, 50, 167, 500; trial 2 = 50, 124, 168, 251 +S9 = 167, 500, 1670, 5 000 μg/mL in DMSO	94.4%	Positive (50 μg/L)	Positive (1670 μg/L)	NTP study, no second generation metaphase cells to score at concentrations of 251 μg/mL and higher (−S9) and 5 000 μg/mL (+S9)	Ivett et al. (1989)
DNA damage (alkaline comet assay)	Rat PC12 adrenal gland cells	20 & 40 mg/L in DMSO	95%	Positive (20 mg/L)	Not tested	Increased intracellular ROS levels and reduced catalase, superoxide dismutase	Lu et al. (2012)

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
Mutation (colony formation)	Mouse lymphoma (L5178Y) cells	−S9: 2 trials with concentrations of 25–400 µg/mL +S9: 3 trials with concentrations of 50–300 µg/mL in ethanol	94.4%	Positive (100 µg/mL)	Negative	Relative total growth decreased by ~60% at LEC without S9; no growth at highest concentrations in each trial; increase in both large and small colonies, with some preference for small colonies	Myhr & Caspary, (1991)
DNA damage (SCE)	CHO cells	0.03, 0.1, 0.3 & 1.0 mmol/L in DMSO	96%	Positive (0.1 mmol/L)	Not tested	Concentration–response relationship; malaoxon induced higher level of SCEs than malathion	Nishio & Uyeki (1981)

CHO: Chinese hamster ovary; CEBS: Chemical Effects in Biological Systems; DMSO: dimethylsulfoxide; DSB: double strand break; GLP: good laboratory practice; HTC: hepatoma cell; LC_{50}: median lethal concentration; NS: not specified; ROS: reactive oxygen species; SCE: sister chromatid exchange; SDS: sodium dodecyl sulfate; SSB: single strand breaks; S9: $9000 \times g$ supernatant; −S9: without metabolic activation; +S9: with metabolic activation; TG: test guideline; UDS: unscheduled DNA synthesis; NTP: National Toxicology Program (USA)

Genotoxicity tests in human cells (Table 20)

In assays that used human cells to assess DNA damage, malathion induced 8-hydroxy-2'-deoxyguanosine (8-Oxo-dG) adducts in isolated peripheral blood mononuclear cells, damage that was associated with oxidative stress (Ahmed et al., 2011). Supportive of this hypothesis, malathion appears to selectively induce markers of oxidative stress in Tox21/ToxCast high-throughput screening assays (USEPA interactive Chemical Safety for Sustainability Dashboard, 2016). Malathion was positive in the alkaline comet assay using the hepatocellular carcinoma cell line HepG2, albeit at a very high concentration that was associated with lipid peroxidation (Moore, Yedjou & Tchounwou, 2010), but negative using the same assay platform in isolated lymphocytes (Blasiak et al., 1999). These studies were conducted in the absence of metabolic activation. In primary mucosal epithelial cells from human tonsil tissue, malathion induced DNA damage as measured by the alkaline comet assay (Tisch, Faulde & Maier, 2007); studies were conducted in the absence of metabolic activation only. In the absence of metabolic activation, malathion consistently induced sister chromatid exchanges in phytohaemagglutinin (PHA)-stimulated lymphocytes in whole blood cultures (Herath et al., 1989; Garry et al., 1990; Balaji & Sasikala, 1993), in a lymphoid cell line (LAZ-007) (Sobti, Krishan & Pfaffenberger, 1982), and in fetal lung fibroblasts (Nicholas, Vienne & van den Berghe, 1979). Malathion was also active in inducing sister chromatid exchanges in the two studies that evaluated the response in the presence of metabolic activation (Sobti, Krishan & Pfaffenberger, 1982; Garry et al., 1990). Malathion was negative for inducing unscheduled DNA synthesis in WI-38 cells (USEPA, 1977).

In the absence of metabolic activation, malathion consistently induced chromosomal aberrations in PHA-stimulated human lymphocytes cultured as whole blood or after isolation (Walter, Czajkowska & Lipecka, 1980; Herath et al., 1989; Garry et al., 1990; Balaji & Sasikala, 1993; Edwards, 2001a; Lloyd, 2009) and in LAZ-007 cells, a lymphoid cell line (Sobti, Krishan & Pfaffenberger. 1982). In the presence of metabolic activation, some studies reported a positive clastogenic effect (Garry et al. 1990; Lloyd, 2009), while others were negative (Sobti, Krishan & Pfaffenberger. 1982; Edwards 2001a). Malathion was reported to be negative for clastogenicity in the haematopoietic cell line B411-4 (tested in the absence of metabolic activation only) (Huang, 1973). Malathion induced micronuclei in PHA-stimulated lymphocytes cultured both as whole blood (positive by slope analysis) and after isolation (testing was in the absence of metabolic activation only); the compound was more potent in whole blood cultures (Titenko-Holland et al., 1997). Malathion also induced micronuclei in the hepatoma cell line HepaRG, which express liver-like metabolism (Josse et al., 2014), but not in Molt-4 T-lymphocytes in the absence of metabolic activation (Szekely, Goodwin & Delaney, 1992).

Malathion was reported to induce mutations at the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) locus in isolated T-cells (Pluth et al., 1996); a subsequent expanded evaluation concluded that mutations arose preferentially at G:C basepairs (Pluth et al., 1998).

Malaoxon and isomalathion induced DNA damage in isolated lymphocytes in the absence of metabolic activation only, as measured by the alkaline comet assay (Blasiak et al., 1999). Further, a follow-up study concluded that the malaoxon-mediated damage was likely induced by reactive oxygen species (ROS) (Blasiak & Stankowska, 2001). Isomalathion was reported also to induce micronuclei in the hepatoma cell line HepaRG (Josse et al., 2014).

Table 20. In vitro genotoxicity tests in human cells

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
Malathion							
DNA damage (8-Oxo-dG adducts)	Peripheral blood mononuclear cells (isolated)	5, 10, 20, 50 & 100 µmol/L Vehicle unspecified	NS (technical grade)	Positive (20 µmol/L)	Not tested	Treatment for 6, 12, 24 hours. Adducts measured by ELISA, duration- and concentration-dependent response. Malondialdehyde concentrations were also increased. Effect attenuated by *N*-acetylcysteine & curcumin	Ahmed et al. (2011)
Chromosome damage (chromosomal aberrations)	Lymphocytes (male – whole blood, PHA stimulated)	0.2, 0.2, 2 & 20 µg/mL in 1% acetone	NS	Positive (2 µg/mL)	Not tested	Treatment started 0, 24, 48 hours after mitogen stimulation, termination at 72 hours, most active when administered at 0 time, concentration–response relationship, gaps excluded from analysis. Mitotic index reduced by 71% at 2 µg/mL	Balaji & Sasikala (1993)
DNA damage (SCE)	Lymphocytes (male – whole blood, PHA stimulated)	0.2, 0.2, 2 & 20 µg/mL in acetone	NS	Positive (20 µg/mL)	Not tested	Treatment at 0, 24, 48 hours, termination at 72 hours, concentration-dependent response obtained using all 3 protocols but most active when administered at 0 time	Balaji & Sasikala (1993)
DNA damage (alkaline comet assay)	Lymphocytes (isolated)	25, 75 & 200 µmol/L in ethanol	≥ 99.8%	Negative	Not tested	Inadequate publication. One hour incubation, rationale for concentration selection not provided	Blasiak et al. (1999)
Chromosome damage (chromosomal aberrations)	Lymphocytes (male – whole blood, PHA stimulated)	−S9 150, 300 & 450 µg/mL +S9 300, 600 & 900 µg/mL DMSO vehicle	Batch/lot no.: 9010501, 96.0% w/w malathion, 0.19% w/w MeOOOPS-triester, 0.83% w/w MeOOSPS-triester, 0.15% isomalathion, < 0.02% malaoxon	Positive (450 µg/mL)	Negative	GLP- and TG-compliant study 2 donors, whole blood, positive in one donor at maximum concentration, associated with 50% depression in mitotic index	Edwards (2001a)

End-point	Test object	Concentration	Purity	Results		Comments	Reference
				−S9	+S9		
DNA damage (UDS)	Human fetal lung fibroblasts (WI-38)	−S9: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} mol/L; +S9: 10^{-3}, 10^{-4}, 10^{-5} mol/L	Technical grade (America Cyanamid; lot 40216006.300)	Negative	Negative	Solvent not specified	USEPA (1977) summarized in Waters et al. (1980)
Chromosome damage (chromosomal aberrations)	Lymphocytes (M – whole blood, PHA stimulated)	66, 83, 132 & 660 µg/mL in DMSO	NS	Positive	Positive	Exposed cells in G0, concentration-dependent increase	Garry et al. (1990)
DNA damage (SCE)	Lymphocytes (M – whole blood, PHA stimulated)	83, 132 or 660 µg/mL in DMSO	NS	Positive by trend test	Positive by trend test	Exposed cells in G0, positive is based on concentration-dependent increase but does not indicate if any concentration statistically increased over control	Garry et al. (1990)
Chromosome damage (chromosomal aberrations)	Lymphocytes (whole blood, PHA stimulated)	5, 20, 40 & 50 µg/mL in DMSO	> 98%	Positive (20 µg/mL)	Not tested	Treatment for 4 and 24 hours, no positive control, when gaps excluded, active at 20 µg/mL at 24 hour only but not at higher concentrations. Mitotic index at 24 hour not reduced significantly	Herath et al. (1989)
DNA damage (SCE)	Lymphocytes (M – whole blood), PHA stimulated	5, 20 & 50 µg/mL in DMSO	> 98%	Positive (20 µg/mL)	Not tested	Treatment for 24 hours, concentration-related response	Herath et al. (1989)
Chromosome damage (chromosomal aberrations)	Human haematopoietic cell line B411-4	50 & 100 µg/mL in DMSO	95%	Negative	Not tested	Inadequate publication, lacking critical details. Multiple sample times: 6, 12, 24, 50 hours	Huang (1973)
Chromosome damage (micronucleus formation)	HepaRG hepatoma cell line	10, 25 or 50 µmol/L in DMSO	NS	Not applicable	Positive (10 µmol/L)	Absence of clear dose–response over concentration range tested but all 3 concentrations significant	Josse et al. (2014)

End-point	Test object	Concentration	Purity	Results		Comments	Reference
				−S9	+S9		
Chromosome damage (chromosomal aberrations)	Lymphocytes (M – whole blood, PHA stimulated)	−S9: 125, 500 & 600 µg/mL +S9: 600, 900 & 1 000 µg/mL DMSO vehicle	Batch/lot no.: 1050-OSJ-15A, 99.1% w/w malathion, 0.25% w/w MeOOSPS-triester, 0.3% w/w isomalathion, 0.08% w/w malaoxon	Positive (500 µg/mL)	Positive (900 µg/mL)	GLP- and TG-compliant study Malathion spiked to the specification limit with relevant impurities; used pooled blood of 3 male donors Mitotic index reduced by 32% (−S9) and 14% (+S9) at LEC	Lloyd (2009)
DNA damage (alkaline comet assay)	HepG2 hepatocellular carcinoma cell line	6, 12, 18 & 24 mmol/L in DMSO	98.2%	Positive (24 mmol/L)	Not tested	Treatment for 48 hours, cell viability decreased by > 70%, induced malondialdehyde at 6 mmol/L, a measure of lipid peroxidation	Moore, Yedjou & Tchounwou (2010)
DNA damage (SCE)	Fetal lung fibroblasts	2.5, 5, 10, 20 & 40 µg/mL in ethanol	99%	Positive (20 µg/mL)	Not tested	Treatment either 1× at 4 hours or 2× at 4 & 24 hours, termination at 72 hours. Induced concentration-related response	Nicholas, Vienne & van den Berghe (1979)
Mutation (*HPRT* mutation)	T-lymphocytes (isolated)	30, 50, 80, 150, 300, 450 & 600 µg/mL in DMSO	97–99%	Positive (50, 450 & 600 µg/mL but not 80, 150 or 300 µg/mL)	Not tested	Multiple mutants from different cultures aggregated. Specific deletion more common than in control mutants. 6 of 84 (7.1%) *HPRT* mutants arising in in vitro malathion-treated human T-lymphocytes were characterized by specific genomic deletions in a 125-bp region of exon 3	Pluth et al. (1996)
Mutation (*HPRT* mutation)	T-lymphocytes (isolated)	10–650 µg/mL in DMSO	97–99%	Positive	Not tested	Extended evaluation of *HPRT* mutations 24 from control, 77 from 6 in vitro treatments with cells from 4 male donors, mutations induced preferentially at G:C basepairs	Pluth et al. (1998)
DNA damage (SCE)	Lymphoid cells (LAZ-007)	−S9: 0.02, 0.2, 2 & 20 µg/mL +S9: 20 µg/mL in ethanol	NS	Positive (0.2 µg/mL)	Positive (20 µg/mL)	Concentration-induced response	Sobti, Krishan & Pfaffenberger (1982)

End-point	Test object	Concentration	Purity	Results −S9	+S9	Comments	Reference
Chromosome damage (micronucleus formation)	T-lymphocytes (Molt-4)	7.5, 15, 30, 60, 80 & 120 µg/mL in ethanol	> 99%	Negative	Not tested	Higher concentrations cytotoxic	Szekely, Goodwin & Delaney (1992)
DNA damage (alkaline comet assay)	Tonsil specimens	0.05, 0.1, 0.5, 0.75 & 1.0 mmol/L in DMSO	99.2%	Positive	Not tested	Inadequate publication lacking critical information on cell samples used as tonsil specimens taken from 85 patients; publication in German	Tisch, Faulde & Maier (2007)
Chromosome damage (micronucleus formation)	Lymphocytes (whole blood and isolated), PHA-stimulated	5, 20, 50 & 100 µg/mL in DMSO	95%	Positive (by slope analysis whole blood, 75 µg/mL isolated cells)	Not tested	Impurities include malaoxon, Kinetochore-negative micronuclei (malathion mostly induced chromosome breakage). No concentration–response relationship in micronucleus formation in whole blood culture	Titenko-Holland et al. (1997)
Chromosome damage (chromosomal aberrations)	Lymphocytes (isolated, PHA stimulated)	10, 30, 50 & 70 µg/mL in ethanol	99%	Positive (10 µg/mL)	Not tested	Treatment 24 hours before PHA – 96-hour cultures; 4, 18 and 48 hours after PHA, 72 hours cultures. Analysis includes gaps. Active with maximum exposure duration & maximum response at lowest concentration	Walter, Czajkowska & Lipecka (1980)
Malaoxon							
DNA damage (alkaline comet assay)	Lymphocytes (isolated)	25, 75 & 200 µmol/L in ethanol 1 hour exposure	> 98%	Positive (25 µmol/L)	Not tested	Concentration-dependent response, extent of damage reduced by pretreatment with α-tocopherol and treatment with catalase; detected presence of Fpg sites, indicative of ROS	Blasiak & Stankowska (2001)
DNA damage (alkaline comet assay)	Lymphocytes (isolated)	25, 75 & 200 µmol/L in ethanol 1 hour incubation	≥ 99.8%	Positive (75 µmol/L)	Not tested	Concentration-dependent response, more potent than malathion or isomalathion. Damage repaired within 1 hour	Blasiak et al. (1999)
Isomalathion							
DNA damage (alkaline comet assay)	Lymphocytes (isolated)	25, 75 & 200 µmol/L in ethanol 1 hour incubation	≥ 99.8%	Positive (25 µmol/L)	Not tested	Concentration-dependent response, more potent than malathion but less than malaoxon. Damage repaired within 1 hour	Blasiak et al. (1999)

End-point	Test object	Concentration	Purity	Results		Comments	Reference
				−S9	+S9		
Chromosome damage (micronucleus formation)	HepaRG hepatoma cell line	0.05, 5, 10, 25 & 50 μmol/L in DMSO	NS	Not applicable	Positive (25 μmol/L)	Concentration-dependent response. Also induced oxidative stress as assessed by ROS formation	Josse et al. (2014)

DMSO: dimethylsulfoxide; ELISA: enzyme-linked immunosorbent assay; Fpg: formamidopyrimidine-DNA-glycosylase; HepG2:hepatocellular carcinoma; *HPRT*: hypoxanthine-guanine phosphoribosyltransferase locus; LEC: lowest effective concentration; 8-Oxo-dG: 8-hydroxy-2'-deoxyguanosine; NS: not specified; PHA: phytohaemagglutinin; ROS: reactive oxygen species; S9: 9000 × *g* supernatant; −S9: without metabolic activation; +S9: with metabolic activation; SCE: sister chromatid exchange; TG: test guideline; w/w: weight per weight; UDS: unscheduled DNA synthesis

(b)   In vivo studies

Genotoxicity tests in nonmammalian systems (Table 21)

In laboratory studies, malathion or products containing malathion tested positive for the induction of chromosomal aberrations or micronuclei in a number of different species, including plants (*Allium* root tips) (Hoda & Sinha, 1991; Kumar, Khan & Sinha, 1995); Tradescantia early meiotic pollen mother cells (but only under certain conditions; Ma et al., 1983); the erythrocytes of tadpoles of Indian skittering frogs (*Euflictis cyanophlyctis*) (Giri et al., 2012); erythrocytes of several species of fish (*Channa punctatus* (Bloch)) (Kumar et al., 2010); *Oreochromis niloticus* (also head kidney cells, Kandiel et al., 2014); and in the erythrocytes of Japanese quail (*Coturnix japonica*) (Hussain et al., 2015).

In *Drosophila melanogaster*, malathion by feeding and/or injection was reported negative for the induction of chromosome damage and sex-linked recessive lethals in germ cells by Valencia (1981) and Velázquez et al. (1987) or wing-spot mutations by Osaba et al. (1999), but reported positive by feeding for sex-linked recessive lethals and dominant lethals by Hoda & Sinha (1991) and Kumar, Khan & Sinha (1995).

Malathion also induced DNA damage in lymphocytes and cells of the gill and kidney sampled from *Channa punctatus* (Bloch), as measured by the alkaline comet assay (Kumar et al., 2010) and in liver cells of *O. niloticus*, as measured by DNA fragmentation assay (Kandiel et al. 2014). Malathion was reported to not induce DNA damage in *Litopenaeus stylirostris* (shrimp), measured using the alkaline unwinding assay (Galindo Reyes et al., 2002) or 8-Oxo-dG adducts in the DNA of liver cells of seabream (*Sparus aurata*) (Rodríguez-Ariza et al., 1999).

Malaoxon in food induced an increase in reciprocal translocations and sex-linked recessive lethals in *D. melanogaster* but not for sex-linked recessive lethals when administered by injection (Foureman et al., 1994).

Table 21. In vivo genotoxicity tests in nonmammalian systems

End-point	Test object	Concentration or dose	Purity	Results	Comments	Reference
Malathion						
Chromosome damage (micronuclei)	*E. cyanophlyctis* (Indian skittering frog) (erythrocytes)	0.5, 1.0, 2.0 mg/L Treatments at 24, 48, 72 and 96 hours	50% EC commercial formulation	Positive (0.05 mg/L)	The 96-hour LC_{50} for malathion was 3.588 mg/L. Dose-dependent increase	Giri et al. (2012)
Mutation (sex-linked recessive lethals)	*D. melanogaster* (Muller-5)	0.006% in feed		Positive	Inadequate publication due to lack of protocol details	Hoda & Sinha (1991)
Mutation (dominant lethals)	*D. melanogaster* (Oregon R) (germ cells)	0.006% in feed		Positive	Inadequate publication due to lack of protocol details	Hoda & Sinha (1991)
Chromosome damage (Chromosomal aberrations)	*Allium cepa* (onion) (root tips)	50, 100, 200, 400 & 800 ppm 24 hour treatment, water suspension	Not specified	Positive	Mostly clastogenic but some effects on malsegregation	Hoda & Sinha (1991)
Chromosome damage (micronuclei in erythrocytes)	*C. japonica* (Japanese quail, male) (erythrocytes)	20–120 mg/kg bw per day in corn oil Administered via crop tubing daily for 17, 34 and 51 days	95%	Positive (60 mg/kg bw per day)	Increased at all sample times; also frequency of binucleate erythrocytes increased	Hussain et al. (2015)
Chromosome damage (micronuclei in erythrocytes) Chromosome damage (Chromosomal aberrations in head kidney cells) Chromosome damage (DNA fragmentation in liver cells)	*O. niloticus* (fish - Nile tilapia)	Acute: 5 ppm Chronic: 1 ppm Fish maintained in water containing malathion for 96 hours (acute) or 10 days (chronic)	57%	Positive	Study of limited value since 96-hour LC_{50} = 5 ppm	Kandiel et al. (2014)
Mutation (dominant lethal)	*D. melanogaster* (Oregon R) (germ cells)	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 & 5.0 µg/L In feeding solution	50% commercial grade	Positive (2.5 µg/L)	Dose–response relationship	Kumar, Khan & Sinha (1995)

End-point	Test object	Concentration or dose	Purity	Results	Comments	Reference
Mutation (sex-linked recessive lethals)	*D. melanogaster* (Oregon R) (Germ cells)	2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 & 7.5 µg/L In feeding solution	50% commercial grade	Positive (4.0 µg/L)	Dose–response relationship	Kumar, Khan & Sinha (1995)
Chromosomal damage (chromosomal aberrations)	*A. cepa* (onion) (root tips)	40, 42.5, 45, 47.5, 50, 52.5, 55, 57.5. 60 & 62.5, 65 mg/L In feeding solution	50% commercial grade	Positive (50 µg/L)	Dose–response relationship	Kumar, Khan & Sinha (1995)
Chromosome damage (micronuclei in erythrocytes) DNA damage (alkaline comet assay – gill & kidney cells, lymphocytes (isolated))	*Channa punctatus* (Bloch), fish	0.59, 0.74, 1.48 ppm Fish maintained in water containing malathion with sampling on days 0, 1, 3, 7, 15, 22 and 29 Semi-static system, water changed every second day	50% commercial grade	Positive (0.59 ppm 1/10th LC$_{50}$)	LC$_{50}$ 5.93 ppm, dose–response observed	Kumar et al. (2010)
Chromosome damage (micronucleus formation)	*Tradescantia* clones 03 & 4430 (early meiotic pollen mother cells)	a) 5.5–4125 ppm; absorption of malathion /water mixture (± DMSO and/or S9) through the stem b) 0.110–0.435%; spraying a malathion /water mixture onto the plant cuttings in enclosed chambers c) 0.616%; spraying a malathion/water mixture on an open population of plants in the greenhouse d) 0.026–0.561%; absorption of malathion fumes through the leaves and buds Most treatments were administered for 6 hours, followed by a 24-hour recovery time		Positive (~0.255%)	Positive results variable and associated with high levels of toxicity	Ma et al. (1983)

End-point	Test object	Concentration or dose	Purity	Results	Comments	Reference
Wing-spot test	*D. melanogaster* (standard and NORR strains) Somatic cells	0.000 1%, 0.000 25%, 0.000 5%, 0.007 5% standard cross; 0.007 5%, 0.001% NORR cross in 3% Tween 80 plus 3% ethanol In feeding solution	≥ 95%	Negative	Active only at one intermediate dose so response likely not relevant	Osaba et al. (1999)
DNA damage (alkali sensitive adducts or strand breaks)	*Litopenaeus stylirostris* (shrimp) larvae	Unspecified Larvae maintained in water changed daily for 4 days, damage detected by alkaline unwinding assay	Unspecified	Negative	Inadequate publication given absence of critical details; LC_{50} = 34.2 mg/L	Galindo Reyes et al. (2002)
DNA damage (adduct) (8-Oxo-dG)	*Sparus aurata* (seabream) (liver)	6.38 mg/kg bw, IP × 1 sampled 7 days later	NS	Negative	Publication lacks specific details on dose selection and single dose only tested. 8-OH-dG measured by HPLC-EC	Rodríguez-Ariza et al. (1999)
Mutation (sex-linked recessive lethals)	*D. melanogaster* (Canton-S) (Germ cells)	0.25 & 0.5 ppm In feeding solution	Technical grade from America Cyanamid	Negative	Solvent not specified	Valencia (1981)
Chromosome damage (sex chromosome loss and non-disjunction)	*D. melanogaster* (males with rearranged Y chromosome) (germ cells)	0.5 ppm In feeding solution	Technical grade from America Cyanamid	Negative	Solvent not specified	Valencia (1981)
Mutation (sex-linked recessive lethals)	*D. melanogaster* (MRA) (Germ cells)	50 ppm adult feeding 10, 25 ppm adult injection 100 ppm larval feeding	50% dissolved in DMSO then diluted in 5% sucrose to give a final DMSO concentration of 0.1%	Negative		Velázquez et al. (1987)
Chromosome damage (sex chromosome loss and non-disjunction)	*D. melanogaster* (Ring-X) (germ cells)	5 & 10 ppm adult feeding 5 ppm adult injection 7, 10 & 20 ppm larval feeding	50% dissolved in DMSO then diluted in 5% sucrose to give a final DMSO concentration of 0.1%	Negative		Velázquez et al. (1987)

End-point	Test object	Concentration or dose	Purity	Results	Comments	Reference
Malaoxon						
Chromosome damage (reciprocal translocation)	*D. melanogaster* (Canton-S) (germ cells)	5 ppm in water	94.4%	Positive		Foureman et al. (1994)
Mutation (sex-linked recessive lethals)	*D. melanogaster* (Canton-S) (germ cells)	5 ppm feeding	94.4%	Positive (feed)		Foureman et al. (1994)
		2 ppm injection, in water		Negative (injection)		

bw: body weight; 8-Oxo-dG: 8-hydroxy-2′-deoxyguanosine; DMSO: dimethyl sulfoxide; HPLC-EC: high pressure liquid chromatography-electrochemical-electrochemical detection; IP: intraperitoneal; LC_{50}: mean lethal concentration; LD_{50}: median lethal dose; LED: lowest effective dose; NS: not specified; ppm: parts per milion

Genotoxicity tests in mammalian systems (Table 22)

Malathion was positive for DNA damage measured using the alkaline comet assay in blood leukocytes of rats treated by intraperitoneal injection for 5 days (Moore, Patlolla & Tchounwou, 2011); in cells of the liver, brain, kidney and spleen of rats treated orally once or once a day for 60 days (Ojha et al., 2013); and in blood leukocytes and brain cells of rats treated once only or once a day for 28 days via intraperitoneal injection (Réus et al. 2008). Malathion was negative for DNA damage in a rat hepatocyte unscheduled DNA synthesis assay when administered by gavage (Meerts, 2003). Malathion was reported to induce sister chromatid exchanges in bone marrow cells of mice treated acutely by intraperitoneal injection (Giri et al., 2002) or for 24 weeks when fed a diet of malathion formulation–treated feed (Amer et al., 2002). Giri et al. (2002) also reported an increase in sperm head shape abnormalities in mice treated via an intraperitoneal injection over 5 days and sampled at 35 days.

A number of in vivo rodent studies report malathion and malathion formulations as clastogenic. Increased chromosome damage has been reported in bone marrow cells of various strains of gavage-administered mice (Giri et al., 2002) or mice chronically administered for 7 days (Kumar, Khan & Sinha, 1995), 10 days (Hoda & Sinha, 1991), by intraperitoneal injection (Dulout, Pastori & Olivero, 1983; Giri et al., 2002) or by skin painting (Salvadori et al., 1988). Positive clastogenic results are reported also for primary spermatocytes of mice treated dermally (Salvadori et al. 1988), in bone marrow cells of rats treated acutely via intraperitoneal injection (Moore, Patlolla & Tchounwou., 2011), in spleen cells of mice treated once via an intraperitoneal injection (Amer et al., 1996) as well as in Syrian hamsters treated with a formulation via intraperitoneal injection (Dzwonkowska & Hübner, 1986). Malathion has also been reported positive for clastogenicity in bone marrow cells of mice treated via intraperitoneal injection for 35 days (Abraham et al., 1997), in primary spermatocytes of mice maintained on treated water for 50 and 100 days (Bulsiewicz et al., 1976) and in bone marrow cells, spleen cells and spermatocytes of mice fed for 6 and 12 weeks with a malathion formulation–treated grain that had been stored for 24 weeks (Amer et al. 2002). Related to these positive effects with chromosomal aberrations, Giri, Giri & Sharma (2011) reported an increase in the frequency of mouse bone marrow micronucleated polychromatic erythrocytes (MN-PCE) when malathion was administered orally or by intraperitoneal injection acutely, as did Dulout et al. (1982) for mice treated acutely via intraperitoneal injection or by skin painting. Abraham et al. (1997) reported positive findings for the induction of bone marrow MN-PCE in mice treated via intraperitoneal injection for 35 days and sampled weekly. Rats treated once a day for 28 days by intraperitoneal injection exhibited an increased frequency of micronucleated erythrocytes but not in MN-PCE following a single injection (Réus et al., 2008). Because the spleen of rats (as opposed to that of mice) efficiently removes micronuclei from erythrocytes, these findings are suspect.

Other investigators reported negative findings for the induction of chromosomal aberrations by malathion or malathion-containing products in bone marrow cells and spermatogonia of mice treated via intraperitoneal injection (Degraeve & Moutschen, 1984); in bone marrow cells, spermatogonia and primary spermatocytes of mice maintained on treated drinking water for 5 days per week for 7 weeks (Degraeve, Chollet & Moutschen, 1984a), in primary spermatocytes of mice sampled 10–15 days after a single intraperitoneal injection (Degraeve, Chollet & Moutschen, 1984b), in bone marrow cells of mice dosed by intraperitoneal injection or gavage (Kurinniy 1975; NTP, 2016) and in bone marrow cells of rats treated by gavage (Gudi, 1990). Malathion was also reported negative in studies that evaluated the induction of micronuclei in bone marrow erythrocytes of mice treated acutely by intraperitoneal injection with malathion (Navarro, 1995).

Malathion was negative in the mouse dominant lethal test when administered once orally by gavage (USEPA 1977), by a single intraperitoneal injection (Degraeve & Moutschen, 1984), or in drinking water for seven weeks (Degraeve, Chollet & Moutschen, 1984a).

Malaoxon was reported as negative for the induction of chromosomal aberrations and sister chromatid exchanges in the bone marrow cells of male mice following a single intraperitoneal injection (NTP, 2016).

Table 22. In vivo genotoxicity tests in mammalian systems

End-point	Test object	Dose	Purity	Result	Comments	Reference
Malathion						
Chromosomal damage (chromosomal aberrations)	Male Swiss albino mice (bone marrow)	$1/15^{th}$ LD$_{50}$, IP daily for 35 days Vehicle not specified Terminated at weekly intervals during treatment and during a 35 day recovery period	NS	Positive	Inadequate publication. Significant increase in frequency of aberrant cells (no *P* value calculated); increase directly proportional to treatment duration; level returned to control value within one week after end of treatment	Abraham et al. (1997)
Chromosome damage (chromosomal aberrations)	Female Syrian hamster (bone marrow)	240, 480, 1200 & 2400 mg/kg bw, IP × 1 Vehicle not specified Sampled at 24 hours	Sadofos 30 (30%)	Positive at lowest dose only	Limited study since positive at lowest dose tested only, gaps excluded. LD$_{50}$ = 2400 mg/kg bw	Dzwonkowska & Hübner (1986)
Chromosome damage (chromosomal aberrations)	Mice, unspecified strain (spleen cells)	30 mg/kg bw in DMSO, IP × 1 Sampled at 6, 24, 48 hours	100% (synthesized)	Positive (excluding gaps) at all sampling times	Inadequate publication due to lack of detail on sample processing and scoring criteria. Single dose represented 1/8-1/10 LD$_{50}$	Amer et al. (1996)
Chromosome damage (chromosomal aberrations)	BALB/c mouse (bone marrow)	115, 230 & 460 mg/kg bw in corn oil, IP × 1 Terminated at 6, 12 & 24 hours	95.5%	Positive (excluding gaps) based on dose–response relationship	Data analysis limited due to lack of pairwise comparisons	Dulout, Pastori & Olivero (1983)
Chromosome damage (chromosomal aberrations)	Male CFW mice (primary spermatocytes)	0.3% solution of Sadofos-30 containing ~30% malathion administered in water Gavage & oral in water for 50 or 100 days	NS	Positive	Inadequate publication. Gaps included in the analysis and the increase was in events related to chromosome number (polyploidy, univalents)	Bulsiewicz et al. (1976)
Mutation (dominant lethal)	Male ICR/SIM mice (testis)	1 250, 2 500 & 5 000 mg/kg bw in corn oil, feeding	Technical grade from America Cyanamid; lot 40216006.300	Negative	Limited study. Mice maintained on diet for 7 weeks, with the amount ingested not specified	USEPA (1977) summarized in Waters et al. (1980)
Chromosome damage (chromosomal aberrations)	Male Q strain mice (bone marrow, spermatogonia)	300 mg/kg bw, IP × 1 Solvent not specified Sampled 12, 24, 36 hours	> 99%	Negative	Inadequate publication with missing critical protocol and data analysis information	Degraeve & Moutschen (1984)

End-point	Test object	Dose	Purity	Result	Comments	Reference
Mutation (dominant lethal)	Male Q strain mice (testis)	300 mg/kg bw, IP × 1 Solvent not specified	> 99%	Negative	Inadequate study given testing of single dose although stated to be a maximum dose, no information provided. No increase in pre- or postimplantation fetal lethality	Degraeve & Moutschen (1984)
Chromosome damage (chromosomal aberrations)	Male Q strain mice (bone marrow, spermatogonia, primary spermatocytes)	8 ppm in drinking water 5 days per week for 7 weeks	99%	Negative	Inadequate study. No positive control	Degraeve, Chollet & Moutschen (1984a)
Mutation (dominant lethal)	Male Q strain mice (testis)	8 ppm in drinking water 5 days per week for 7 weeks	99%	Negative	Inadequate study. No positive control	Degraeve, Chollet & Moutschen (1984a)
Chromosome damage (chromosomal aberrations)	Male Q strain mice (primary spermatocytes)	300 mg/kg bw, IP × 1 Solvent unspecified Sampled at 10–11, 12–13, 14–15 days	99%	Negative	Limited study given use of single dose although stated to be the maximum dose possible and the lack of detail	Degraeve, Chollet & Moutschen (1984b)
Chromosome damage (chromosomal aberrations)	Strain 615 mice (sex unspecified) (bone marrow)	0.8, 0.4, 0.2, 0.1 × LD_{50}, IP once per day for 4 days	99%	Negative	In Chinese, English abstract	Ni et al. (1993)
Chromosome damage (chromosomal aberrations)	Male Swiss (Rockland) mice (bone marrow)	120, 240 & 480 mg/kg bw in corn oil, IP × 1, dermal × 1 Sampled at 48 hours	99.5%	Positive (120 mg/kg bw)	Questionable study given the results. For dermal, applied to abdomen (single housed). More active by dermal than IP. No dose–response	Dulout et al. (1982)
Chromosome damage (chromosomal aberrations)	Male & female Swiss albino mice (bone marrow)	234, 468 & 701 μL/kg bw in corn oil, IP × 2 Terminated 24 hours after 2nd treatment	Technical grade, purity 1 103 g/L	Negative	Inadequate study since positive control induced a significant increase in MN-NCE at 48 hours; magnitude only slightly lower than that induced in PCE. Doses were 25, 50, 75% of the LD_{50}	Navarro (1995)
Chromosome damage (chromosomal aberrations)	Male & female Swiss albino mice (bone marrow)	0.2 μg/kg bw per day for 10 days, gavage Solvent not specified. Terminated on day 11	NS	Positive	Inadequate publication given lack of information on scoring criteria. Sexes mixed, effect reduced by concurrent treatment with vitamin C	Hoda & Sinha (1991)

End-point	Test object	Dose	Purity	Result	Comments	Reference
Chromosome damage (chromosomal aberrations)	Male & female Swiss albino mice (bone marrow)	2.5, 5 & 10 mg/kg bw × 1, 2.0 mg/kg bw × 5, IP Distilled water vehicle Sampled at 24- and 48-hour intervals	NS	Positive (2.5 mg/kg bw single treatment, 2.0 mg/kg bw per day repeated treatment)	Inadequate publication, sexes mixed without providing details. 10 mg/kg bw maximum sublethal dose. Appropriately, gaps excluded from analysis	Giri et al. (2002)
Chromosome damage (chromosomal aberrations)	Male & female Swiss albino mice (bone marrow)	5 mg/kg bw × 1, 2.0 mg/kg bw × 5, gavage Distilled water vehicle Sampled at 24- and 48-hour intervals	NS	Positive	Inadequate publication, sexes mixed. Appropriately, gaps excluded from analysis	Giri et al. (2002)
Chromosome damage (chromosomal aberrations)	Male Swiss albino mice (bone marrow)	1/15th LD_{50}, IP daily for 35 days Solvent not specified Terminated at weekly intervals during treatment and during a 35-day recovery period	NS	Positive	Inadequate publication. Statistically significant increase in frequency of MN formation (no P value calculated); increase directly proportional to treatment duration; level returned to control value within 1 week of treatment. Does not indicate cell type scored but cites paper that scored PCE	Abraham et al. (1997)
Mutation (sperm head abnormalities)	Male Swiss albino mice (sperm)	2.5, 5 & 10 mg/kg bw, IP × 5 at 24-hours intervals Distilled water vehicle Sampled at 35 days	NS	Positive (2.5 mg/kg bw)		Giri et al. (2002)
DNA damage (SCE)	Male & female Swiss albino mice (bone marrow)	2.5, 5 & 10 mg/kg bw, IP × 1 Distilled water vehicle Sampled at 24 hours	NS	Positive (2.5 mg/kg bw)	Inadequate publication. Sexes mixed with no details	Giri et al. (2002)
Chromosome damage (micronuclei)	Male & female Swiss albino mice (bone marrow)	2.5, 5, 10 mg/kg bw × 1, 5.0 mg/kg bw × 2, IP Distilled water vehicle Sampled after 24 or 48 hours	95%	Positive	Inadequate publication. Sexes mixed with no details. Dose–response evident	Giri, Giri & Sharma (2011)

End-point	Test object	Dose	Purity	Result	Comments	Reference
Chromosome damage (micronuclei)	Male & female Swiss albino mice (bone marrow)	5 mg/kg bw in distilled water, gavage × 1 or × 2 Sampled at 24 hours	95%	Positive	Inadequate study: used IP solvent control and sexes mixed with no details	Giri, Giri & Sharma (2011)
Chromosome damage (chromosomal aberrations) (bone marrow)	Swiss albino mice, sex unspecified	0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 & 6.0 mg/kg bw per day for 7 days, gavage Terminated at 24 hours	Technical grade formulation of 50%	Positive (1.5 mg/kg bw)	Inadequate publication given lack of information on scoring criteria and gaps included in the analysis. Dose-dependent response	Kumar, Khan & Sinha (1995)
Chromosome damage (chromosomal aberrations)	Male Swiss Webster mice (bone marrow, spermatocytes)	Single treatment: 500, 1 000 & 1 500 mg/kg bw, dermal Multiple treatments: 25, 500 & 1000 mg/kg bw per day for 10 days, dermal Corn oil vehicle Terminated 24 hours after last treatment	Commercial malathion (Malatol 100 CE, lot no. 4263-01; Cyanamid Química do Brasil Ltda.)	Positive (multiple treatments = bone marrow, 250 mg/kg bw; primary spermatocytes, 500 mg/kg bw)	Gaps excluded from analysis, highest response at lowest dose. Single-dose administration gave negative results. Also induced increase in univalents in primary spermatocytes	Salvadori et al. (1988)
Chromosome damage (chromosomal aberrations)	Male white Swiss mice (bone marrow, spermatocytes, spleen cells)	8.36, 25.08 & 41.80 mg/kg in wheat grain for 6 or 12 weeks, with treated wheat grain stored for 4, 12 or 24 weeks	57% (Keminof, Denmark)	Positive	Inadequate study. Gaps excluded in bone marrow analysis; unclear how aberrations in spermatocytes were scored, lack of information on spleen cell cultures. Positive results also obtained with treated grain stored for 24 weeks; negative results with mice fed with grain stored for 4 weeks, no dose–response	Amer et al. (2002)
DNA damage (SCE)	Male white Swiss mice (spleen cells)	8.36, 25.08 & 41.80 mg/kg in wheat grain for 6 or 12 weeks with treated grain stored for 4, 12 or 24 weeks	57% (Keminof, Denmark)	Positive	Negative results with mice fed with grain stored for 4 weeks, no dose–response	Amer et al. (2002)

End-point	Test object	Dose	Purity	Result	Comments	Reference
Chromosome damage (chromosomal aberrations)	Male & female SD rats (bone marrow)	0.4 (500), 0.8 (1 000), 1.6 (2 000) mL(mg)/kg bw in corn oil, gavage Terminated at 12, 24 & 48 hours	Batch/lot no.: AC 6015-136, 94.0 % w/w malathion, 0.1 % w/w MeOOOPS-triester, 0.4 % w/w MeOOSPS-triester, 0.2 % w/w isomalathion. 0.1% w/w malaoxon	Negative	GLP- and TG-compliant study. Frequency of chromosomal aberrations in control and treated animals unreasonable low: 5 chromatid-type aberrations in 5 950 cells of control and treated animals suggesting scoring criteria issues and/or number of cells scored underpowered	Gudi (1990)
Chromosome damage (chromosomal aberrations)	Male SD rats (bone marrow)	2.5, 5, 10 & 20 mg/kg bw in DMSO, IP × 5 at 24-hour intervals	98.2%	Positive (5 mg/kg bw per day)	Inadequate publication, no information on types of chromosomal aberrations detected or if gaps included or excluded from analysis	Moore, Patlolla & Tchounwou (2011)
DNA damage (alkaline comet assay)	Male SD rats (leukocytes)	2.5, 5, 10 & 20 mg/kg bw in DMSO IP × 5 at 24-hour intervals	98.2%	Positive (2.5 mg/kg bw per day)	Not clear what cell type scored since text says leukocytes and lymphocytes in different sections	Moore, Patlolla & Tchounwou (2011)
DNA damage (alkaline comet assay)	Male Wistar rats (liver, brain, kidney, spleen cells)	687.5 mg/kg bw, gavage Terminated at 24, 48, 72 hours (acute) 23 mg/kg bw per day for 60 days, gavage Terminated at 24 hours	NS	Positive	Dose = 1/2 reported LD_{50} of 1375 mg/kg bw, all tissues affected, for acute, greatest increase in damage at 24 hours, more damage in chronic	Ojha et al. (2013)
DNA damage (alkaline comet assay)	Male Wistar rats (hippocampus cells & leukocytes)	25, 50, 100, 150 mg/kg bw in 0.9% NaCl IP injection × 1 day (acute) or × 28 days (chronic)	NS	Positive (at and above 50 mg/kg bw per day for 28 days)	150 mg/kg bw = 1/9th the LD_{50}	Réus et al. (2008)

End-point	Test object	Dose	Purity	Result	Comments	Reference
Chromosome damage (micronuclei)	Male Wistar rats (erythrocytes)	25, 50, 100, 150 mg/kg bw in 0.9% NaCl IP injection × 1 day (acute) or × 28 days (chronic)	NS	Positive (150 mg/kg bw per day for 28 days)	Questionable chronic study given screening of MN from blood by rat spleen. 150 mg/kg bw = 1/9 the LD_{50}; acute - scored MN in PCE; chronic, scored MN in total erythrocytes	Réus et al. (2008)
DNA damage (UDS)	Male Wistar rats (hepatocytes)	500, 1 000 & 1 500 mg/kg bw in corn oil	96%	Negative	GLP- and TG-compliant study	Meerts (2003)
Malaoxon						
Chromosome damage (chromosomal aberrations)	Male B6C3F1 mice	7.5, 15, 30 mg/kg bw, IP × 1 Terminated at 17 hours 5, 10 & 20 mg/kg bw, IP × 1 Terminated at 24 hours Phosphate-buffered saline vehicle	> 95%	Negative	Potential solubility issues given low solubility of compound in water	NTP (2016)
DNA damage (SCE)	Male B6C3F1 mice	7.5, 15 & 30 mg/kg bw in phosphate-buffered saline	> 95%	Negative	Potential solubility issues given low solubility of compound in water	NTP (2016)

bw: body weight; CEBS: Chemical Effects in Biological Systems; DMSO: dimethyl sulfoxide; GLP: good laboratory practice; IP: intraperitoneal; LD_{50}: median lethal dose; MN: micronuclei; MN-NCE: micronucleated normochromatic erythrocyte; NS: not specified; PCE: polychromatic erythrocyte; NTP: United States National Toxicology Program; SCE: sister chromatid exchange; TG: test guideline; UDS: unscheduled DNA synthesis

(c) *Observations in humans*

Compared to a control population, 14 patients treated for acute intoxication with a malathion-based product exhibited increased levels of chromosome damage in mitogen-stimulated lymphocytes cultured from their blood shortly after admittance to the hospital (van Bao et al., 1974).

In workers exposed selectively to malathion in the California Mediterranean Fruit Fly Eradication Program, the frequency of erythrocyte glycophorin A mutations was not increased significantly (the number of participants evaluated was very low) and neither was there a significantly increased frequency of micronuclei in lymphocytes isolated from blood and mitogen-stimulated to proliferate in vitro (Titenko-Holland et al., 1997; Windham et al., 1998). Pluth et al. (1996) reported a spectrum of *HPRT* mutations on T-lymphocytes from a single worker exposed to malathion and other pesticides similar to that obtained when T-lymphocytes were exposed to malathion in vitro.

Workers exposed to a combination of malathion and chlorpyrifos exhibited an increased frequency of micronuclei in isolated lymphocytes (Omari, 2011).

Workers exposed to multiple pesticides, including malathion, have been reported as having increased levels of DNA damage in unstimulated leukocytes or isolated lymphocytes compared to controls, as measured by the alkaline comet assay (Garaj-Vrhovac & Zeljezic, 2000, 2001; Singh et al., 2011; Benedetti et al., 2013; Varona-Uribe et al., 2016) and by the increased frequencies of sister chromatid exchanges in mitogen-stimulated lymphocytes (Rupa et al., 1988, 1991; De Ferrari et al., 1991; Garaj-Vrhovac & Zeljezic, 2001, 2002).

Exposed workers were also reported as exhibiting increased frequencies of chromosomal aberrations (Yoder, Watson & Benson, 1973; Páldy et al., 1987; Rupa et al., 1988, 1989; De Ferrari et al., 1991; Garaj-Vrhovac & Zeljezic, 2001, 2002) and micronuclei (Garaj-Vrhovac & Zeljezic 2001, 2002; Benedetti et al., 2013) in mitogen-stimulated blood lymphocytes. In contrast, Lucero et al. (2000) reported a lack of significant increase in the frequency of micronuclei in buccal epithelial cells and mitogen-stimulated lymphocytes sampled from exposed workers, while Davies et al. (1998) reported the same negative finding for the frequency of micronuclei in mitogen-stimulated blood lymphocytes in a population of British Columbia female seasonal farmworkers.

Pluth et al. (1996) reported a spectrum of *HPRT* mutations in T-lymphocytes from a single worker exposed to malathion and other pesticides similar to that obtained when T-lymphocytes from unexposed individuals were exposed to malathion in vitro.

The results of observations in humans are summarized in Table 23.

Table 23. Genotoxicity observations in humans exposed to malathion

End-point	Tissue or cell type	Exposure conditions	Response/significance	Comments	Reference
Chromosome damage (chromosomal aberrations)	Lymphocytes (whole blood, PHA stimulated)	60 workers actively engaged in the processing unit and in direct contact with malathion. Participants were classified depending on the period of exposure (0–5, 60–10, 11–15, 15–20, > 20 years; number of participants = 7, 8, 25, 12 and 8, respectively)	Negative	20 on-site control, 4/group. Inadequate publication lacking critical details. Negative if gaps excluded. Exposure not likely to have been limited to malathion	Singaravelu, Mahalingam & Muthu (1998)
Chromosome damage (micronuclei)	Lymphocytes (isolated, PHA stimulated, cytochalasin B)	38 (29 male, 9 female) malathion-exposed workers involved in the Mediterranean Fruit Fly Eradication Program, California	Negative	P values after 6 months of exposure vs control group (n = 16, 9 male, 7 female), malathion diacid levels in urine ranged from not detected (< 5 ppb) to 2 200 ppb, kinetochore status did not differ. Exposure to multiple agents	Titenko-Holland et al. (1997)
Chromosome damage (chromosomal aberrations)	Lymphocytes (whole blood, PHA stimulated)	14 patients with acute intoxication with a malathion-based formulation: blood analyses immediately (3–6 days), and 1 and 6 months after intoxication	Positive ($P < 0.001$)	P values for intoxicated group vs control group (n = 15). Exposure to multiple agents	van Bao et al. (1974)
Mutation (glycophorin A assay)	Erythrocytes	1992: 9 male and female workers; 1993: 10 male and female workers in the California Mediterranean Fruit Fly Eradication Program	Negative	Very limited number/group: 1 in 1992, 4 in 1993; some workers also use diazinon	Windham et al. (1998)
Chromosome damage (micronuclei)	Lymphocytes (isolated, PHA stimulated, cytochalasin B)	Sept 1993: 24 male and female workers; Dec 1993: 14 male and female workers in the California Mediterranean Fruit Fly Eradication Program; some workers also used diazinon	Negative	Sept 1993: 10 male and female controls; Dec 1993: 6 male and female controls; malathion diacid levels in urine ranged from not detected (< 5 ppb) to 2 200 ppb, expanded analysis from Titenko-Holland et al. (1997)	Windham et al. (1998)
Chromosome damage (micronuclei)	Lymphocytes (isolated, PHA stimulated, cytochalasin B)	23 healthy Jordanian non-smoking workers with varied durations of exposure (3–30 years). Malathion was used together with chlorpyrifos	Positive ($P < 0.01$ at 8 months of exposure; $P < 0.05$ after 8 months of no exposure)	P values for exposed group after 8 months of exposure vs control group (n = 22), also sampled 8 months after no exposure; lower levels of MN but still elevated. Exposure to multiple agents	Omari (2011)

End-point	Tissue or cell type	Exposure conditions	Response/significance	Comments	Reference
DNA damage (alkaline comet assay)	Leukocytes (whole blood)	81 soybean workers (65 male, 16 female) exposed to 6 herbicides, 14 insecticides (including malathion) and 5 fungicides throughout the growing season	Positive ($P < 0.001$ for males, $P < 0.05$ for females)	Compared to 46 controls (19 male, 27 female), no correlation for age and exposure time. Exposure to multiple agents	Benedetti et al. (2013)
Chromosome damage (micronuclei)	Epithelial cells (buccal)	81 soybean workers (65 male, 16 female) exposed to 6 herbicides, 14 insecticides (including malathion) and 5 fungicides throughout the growing season	Positive ($P < 0.001$ for males, $P < 0.05$ for females)	Compared to 46 controls (19 male, 27 female), no correlation for age and exposure time. Exposure to multiple agents	Benedetti et al. (2013)
Chromosome damage (micronuclei)	Lymphocytes (isolated, PHA stimulated, cytochalasin B)	18 British Columbia seasonal farmworkers (female) exposed to the herbicides simazine, paraquat, napropamide and glyphosate, the fungicides captan and triforine and the insecticides diazinon, malathion, carbofuran and endosulfan	Negative	Compared to 21 age-matched female controls; trend for increased response with increasing work duration. Exposure to multiple agents	Davies et al. (1998)
Chromosome damage (chromosomal aberrations)	Lymphocytes (isolated, PHA stimulated)	(A) 32 healthy individuals exposed to pesticides while working in the flower industry; (B) 32 individuals exposed to pesticides while working in the flower industry and hospitalized for bladder cancer but not yet treated; (C) 31 matched controls. Exposure included 18 nitro-organic herbicides and fungicides, 9 nitro-organic fungicides, 12 organophosphate and organothiophosphate insecticides, 4 hydrocarbon-derivative herbicides and 5 inorganic fungicides and insecticides	Positive ($P < 0.01$ for A vs C, $P < 0.05$ for B vs C)	Response not confounded by age or smoking habit. Observed also increased frequencies of aneuploid and polyploid cells. Exposure to multiple agents	De Ferrari et al. (1991)
DNA damage (SCE)	Lymphocytes (isolated, PHA stimulated)	(A) 28 healthy individuals exposed to pesticides while working in the flower industry; (B) 14 individuals exposed to pesticides while working in the flower industry and hospitalized for bladder cancer but not yet treated; and (C) 15 matched controls. Exposure included 18 nitro-organic herbicides and fungicides, 9 nitro-organic fungicides, 12 organophosphate and organothiophosphate insecticides, 4 hydrocarbon-derivative herbicides and 5 inorganic fungicides and insecticides	Positive ($P < 0.01$ for A vs C, $P < 0.001$ for B vs C)	Response not confounded by age or smoking habit. Exposure to multiple agents	De Ferrari et al. (1991)

End-point	Tissue or cell type	Exposure conditions	Response/significance	Comments	Reference
DNA damage (alkaline comet assay)	Leukocytes (whole blood)	10 workers (7 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid and malathion)	Positive ($P < 0.001$)	P values for exposed group after 8 months of exposure vs control group ($n = 10$, 7 male, 3 female); values reduced after 8 months of non-exposure. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2000)
Chromosome damage (chromosomal aberrations)	Lymphocytes (whole blood, PHA stimulated)	20 workers (17 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.001$)	P values for exposed group after 8 months of exposure vs 20 controls (12 male, 8 female); gaps excluded, values reduced but still elevated vs controls after 8 months on non-exposure. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2001)
DNA damage (alkaline comet assay)	Leukocytes (whole blood)	20 workers (17 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.001$)	P values for exposed group after 8 months of exposure vs 20 controls (12 male, 8 female); values reduced but still elevated vs controls after 8 months on non-exposure. Expanded from the Garaj-Vrhovac & Zeljezic, 2000 study. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2001)
Chromosome damage (micronuclei)	Lymphocytes (whole blood, PHA stimulated, cytochalasin B)	20 workers (17 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.05$)	P values for exposed group after 8 months of exposure vs 20 controls (12 male, 8 female), values reduced but still elevated vs controls after 8 months on non-exposure. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2001)
DNA damage (SCE)	Lymphocytes (whole blood, PHA stimulated)	20 workers (17 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.001$)	P values for exposed group after 8 months of exposure vs 20 controls (12 male, 8 female); values reduced but still elevated vs controls after 8 months on non-exposure. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2001)
Chromosomal aberrations	Lymphocytes (isolated, PHA stimulated)	10 workers (7 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.05$)	P value for exposed workers vs 20 controls (12 male, 8 female). Exposed population from Garaj-Vrhovac & Zeljezic (2000) study, control from 2001 study. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2002)

End-point	Tissue or cell type	Exposure conditions	Response/significance	Comments	Reference
DNA damage (alkaline comet assay)	Leukocytes	10 workers (7 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.01$)	P value for exposed workers vs 20 controls (12 male, 8 female). Exposed population from Garaj-Vrhovac & Zeljezic (2000) study, control from 2001 study. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2002)
Chromosome damage (micronuclei)	Lymphocytes (isolated, PHA stimulated, cytochalasin B)	10 workers (7 male, 3 female) in pesticide production simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.05$)	P value for exposed workers vs 20 controls (12 male, 8 female). Exposed population from Garaj-Vrhovac & Zeljezic (2000) study, control from 2001 study. Exposure to multiple agents	Garaj-Vrhovac & Zeljezic (2002)
Chromosome damage (micronuclei)	Lymphocytes (isolated, PHA stimulated, cytochalasin B)	64 greenhouse workers (male) exposed to 16 pesticides including malathion (usage 12.5%), 1 bactericide and 9 fungicides	Negative	P value for exposed workers vs 50 controls (male) matched for smoking, mean age of control 6 years greater. Exposure to multiple agents	Lucero et al. (2000)
Chromosome damage (micronuclei)	Epithelial cells (buccal)	59 greenhouse workers (male) exposed to 16 pesticides including malathion (usage 12.5%),1 bactericide and 9 fungicides	Negative	P value for exposed workers vs 49 controls (male) matched for smoking, mean age of control 6 years greater. Exposure to multiple agents	Lucero et al. (2000)
Chromosome damage (chromosomal aberrations)	Lymphocytes (isolated, PHA stimulated)	80 workers (male) exposed to ~80 different kinds of formulations from groups of organic phosphates, dithiocarbamates, nitro compounds, triazines, urea compounds, phthalimides, organochlorines, phenoxyacetic acids, pyrethroids, carbamates, heterocyclic compounds and sulfur- and copper-containing chemicals	Positive ($P < 0.001$)	Compared to 24 controls (male); increasing damage generally with increasing duration of exposure; blind scoring. Exposure to multiple agents	Páldy et al. (1987)
Chromosome damage (chromosomal aberrations	Lymphocytes (isolated, PHA stimulated)	25 vegetable-garden workers (male), smokers and alcohol consumers, exposed to seven pesticides including malathion	Positive ($P < 0.05$)	P value for exposed workers, independent of years worked, vs control I (20 healthy male non-smokers and non-alcohol consumers) or control II (10 healthy male smokers and alcohol consumers); analysis based on all aberrations, including gaps, response independent of years worked. Exposure to multiple agents	Rupa et al. (1988)

End-point	Tissue or cell type	Exposure conditions	Response/significance	Comments	Reference
DNA damage (SCE)	Lymphocytes (whole blood, PHA stimulated)	25 vegetable-garden workers (male), smokers and alcohol consumers, exposed to seven pesticides including malathion	Positive ($P < 0.05$)	P value for exposed workers, independent of years worked, vs control I (20 healthy male non-smokers and non-alcohol consumers) or control II (10 healthy male smokers and alcohol consumers); response independent of years worked. Exposure to multiple agents	Rupa et al. (1988)
Chromosome damage (chromosomal aberrations)	Lymphocytes (whole blood, PHA stimulated)	50 smoking cotton-field workers (male; based on Rupa et al., 1991) exposed to 11 pesticides including malathion (50% purity)	Positive ($P < 0.05$)	Frequency independent of years worked, total number of aberrations compared to 20 non-smoking male controls and 27 smoking male controls. Analysis excluded gaps. Exposure to multiple agents	Rupa, Reddy & Reddi (1989)
DNA damage (SCE)	Lymphocytes (whole blood, PHA stimulated)	61 non-smoking, cotton-field workers (male) regularly exposed to 11 pesticides including malathion	Positive ($P < 0.05$)	P value for pesticide applicators vs 45 controls (male), increasing frequency with exposure duration. Exposure to multiple agents	Rupa et al. (1991)
DNA damage (alkaline comet assay)	Lymphocytes (isolated)	70 male and female workers spraying pesticides for community health programmes in Delhi, India, exposed to pirimiphos-methyl, chlorpyrifos, temephos and malathion	Positive ($P < 0.001$)	P value vs 70 matched controls; exposure to multiple agents	Singh et al. (2011)
DNA damage (alkaline comet assay)	Leukocytes	223 rice field workers (98% male) in Colombia	Positive (95% CI: 2.34–21.60)	Cross-sectional study, 31 pesticides were quantified in blood, serum and urine. Maximum-likelihood factor analysis identified 8 different mixtures. Robust regressions were used to explore associations between the factors identified and the comet assay. The mixture of pirimiphos-methyl, malathion, bromophosmethyl and bromophosethyl (but not malathion alone) was associated with increased tail length	Varona-Uribe et al. (2016)

End-point	Tissue or cell type	Exposure conditions	Response/significance	Comments	Reference
Chromosome damage (chromosomal aberrations)	Lymphocytes (whole blood, PHA stimulated)	16 workers (male) exposed to 17 pesticides including malathion, sampled at midwinter ebb in spraying operations and again during the peak spraying	Positive (no P value)	No statistics provided vs 16 male controls but a 5-fold increase in damage between sampling times and 3.5-fold greater than concurrent control samples; slides scored blind. Exposure to multiple agents	Yoder, Watson & Benson (1973)
DNA damage (SCE)	Lymphocytes (whole blood, PHA stimulated)	20 workers (17 male, 3 female) working in pesticide production simultaneously exposed to 5 pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	Positive ($P < 0.001$)	P value by Mann–Whitney test for exposed group vs control group ($n = 20$, 12 male, 8 female), increase was present at the beginning of 8-month exposure, at the end of 8-month exposure and 8 months later. Also, significant increase in high frequency cells (> 95 percentile of pooled distribution). Exposure to multiple agents	Zeljezic & Garaj-Vrhovac (2002)

MN: micronuclei; PHA: phytohaemagglutinin; ppb: parts per billion; SCE: sister chromatid exchange.

2.5 Reproductive and developmental toxicity

(a) Single-generation and multigeneration studies

In a two-generation reproductive toxicity study by Schroeder (1990), malathion (purity 94%) was admixed in the diet at 0, 550, 1700 or 1800 ppm and fed ad libitum to two parental generations of Sprague Dawley–derived COBS®CD® rats (25/sex per dose) and their offspring through premating, mating, gestation and lactation. Observations for mortalities and clinical signs were made daily, with more detailed clinical examinations performed on each rat weekly. Body weight and feed consumption were recorded weekly. Pups were examined, weighed, counted and sexed on postnatal days 0, 4, 7, 14 and 21. Standard reproduction, offspring and litter parameters were recorded or calculated. At scheduled termination, the rats were necropsied and their organs weighed and tissues histopathologically examined.

The doses achieved during premating, gestation and lactation are summarized in Table 24. There were no treatment-related mortalities, clinical signs, effects on body weight or feed consumption, macroscopic or microscopic findings in parental rats. There were no effects on reproduction parameters or reproductive tissues. In F_{1A} litters, pup weights were 14% lower ($P < 0.05$) than the control on day 21 of lactation at 5000 and 7500 ppm. In F_{1B} litters, pup weights were 10.7% lower than the control on day 21 of lactation. In F_{2A} litters, pup weights were 10% lower than the control ($P < 0.05$) at 7500 ppm. In F_{2B} litters, pup weights were significantly lower ($P < 0.05$) than the control on day 7, 14 and 21 of lactation (-15.2%, -17.0% and -19.8%, respectively) at 5000 ppm and on day 21 of lactation (-13.7%; $P < 0.05$) at 7500 ppm.

The NOAEL for both reproductive toxicity and parental toxicity was 7500 ppm (equal to 595 mg/kg bw per day in males and 655 mg/kg bw per day in females), the highest tested dose. The NOAEL for offspring toxicity was 1700 ppm (equal to 130 mg/kg bw per day in males and 152 mg/kg bw per day in females) for reduced pup weights at 5000 ppm (equal to 393 mg/kg bw per day in males and 438 mg/kg bw per day in females).

Table 24. Achieved doses of malathion in rats

Phase	Achieved dose in mg/kg bw per day							
	Males				Females			
	550 ppm	1 700 ppm	5 000 ppm	7 500 ppm	550 ppm	1 700 ppm	5 000 ppm	7 500 ppm
Premating								
F_0	42.71	131.79	393.54	594.98	49.91	151.89	438.09	655.05
F_1	42.61	129.97	394.41	628.04	51.23	154.25	464.71	751.84
Gestation								
$F_0 - F_{1A}$	–	–	–	–	47.35	143.44	420.16	629.91
$F_0 - F_{1B}$	–	–	–	–	44.08	135.70	393.67	599.78
$F_1 - F_{1A}$	–	–	–	–	45.68	139.00	418.57	685.40
$F_1 - F_{1B}$	–	–	–	–	39.58	124.12	383.84	582.20

ppm: parts per million; F_0: parental generation; F_{1A}: first generation –first litter; F_{1B}: first generation –second litter

Results expressed as the mean achieved dose in mg/kg bw per day

Source: Schroeder (1990)

Uzun et al. (2009) administered malathion (purity not specified) in corn oil by gavage to groups of six sexually mature male Wistar rats for 4 weeks at 0 or 27 mg/kg bw per day in the

presence or absence of 200 mg/kg bw per day vitamin C and vitamin E. The rats were terminated after 4 weeks and their reproductive tissues dissected to analyse testicular sperm counts, epididymal sperm motility and epididymal sperm morphology. The reproductive organs were not weighed or histopathologically examined. Blood was also sampled at termination to analyse luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone. Sperm counts and sperm motility were significantly lower than the control ($P < 0.05$) in malathion-treated rats (−22% and −36% respectively), with vitamin co-treatment having a marginal effect on these parameters (−13% and −26% lower than the control, respectively). Abnormal sperm morphology was significantly increased ($P < 0.05$) in malathion-treated rats (2.72% versus 1.71% in the control), with vitamin co-treatment also having a marginal effect on these parameters (1.97% versus 1.71% in the control). Significant reductions ($P < 0.05$) in FSH (−17%), LH (−32%) and testosterone (−27%) occurred in malathion-treated rats, while vitamin co-treatment had no effect. The authors observed "fewer spermatogenic cells in some of the seminiferous tubules [in malathion-treated rats], and necrosis in some seminiferous tubules and oedema in interstitial tissue". Pictures of representative histopathological changes were shown, but there was no quantitative evaluation of these microscopic changes. This study is considered to have limited value for risk assessment purposes because of the small group size, the use of only one dose of malathion, the absence of organ weight data and the absence of histopathological examination of reproductive tissues.

Geng et al. (2015) administered malathion (purity 95%) in corn oil by gavage at 0, 33.75, 54 or 108 mg/kg bw per day for 60 days to groups of 10 male Wistar rats (age unspecified; 80–100 g bw). Body weights were recorded weekly while feed consumption was not recorded. Following termination, the testes were weighed. Sperm counts, motility and morphology were recorded. Blood samples were collected at termination to analyse serum LH, FSH and testosterone concentrations. Acetylcholinesterase activity was not analysed. The following testicular enzymes were analysed in homogenized left testis samples: acid phosphatase (ACP), lactate dehydrogenase, succinate dehydrogenase (SDH) and GGT. Histopathology was performed on the right testis. The seminal vesicle, epididymis or prostate were not microscopically examined. Apoptosis was analysed in testes by terminal deoxynucleotidyl transferase nick end labelling (TUNEL) assay. Bax and Bcl-2 protein expression was analysed in testes using immunohistochemistry.

There were no deaths and no clinical signs. Terminal body weight and overall body-weight gain was significantly lower ($P < 0.05$) than the controls at the highest dose (−17% and −24%, respectively). Effects on the testes, sperm characteristics, hormone levels and testicular enzymes are summarized in Table 25. The authors stated that the testes appeared small and mauve in colour at 54 and 108 mg/kg bw per day. While absolute testis weight was approximately 20% lower than the control ($P < 0.01$) at 54 and 108 mg/kg bw per day, relative testis weight was consistent across all groups. Sperm counts were significantly reduced ($P < 0.01$) at the highest dose while sperm motility was decreased and dismorphology rates were increased at 54 and 108 mg/kg bw per day. Serum LH was reduced at 54 and 108 mg/kg bw per day, while serum FSH and testosterone were reduced at 108 mg/kg bw per day. Changes in testicular enzymes include reduced acid phosphatase (108 mg/kg bw per day) and GGT (54 and 108 mg/kg bw per day) and increased lactate dehydrogenase (108 mg/kg bw per day). There was no treatment-related change in succinate dehydrogenase. The results of the histopathological examination of the testes were not quantified; the authors stated that at 54 and 108 mg/kg bw per day "severe alterations in the seminiferous tubules including the loss, derangement and sloughing of the spermatogenic cells, vacuolization in Sertoli cell cytoplasm and destruction of Sertoli cell cytoskeleton" occurred. Graphically presented data showed a significant dose-related increase ($P < 0.01$) in apoptosis in spermatogenic cells at every dose of malathion. Bax protein levels were significantly lower than the control ($P < 0.05$) at 54 and 108 mg/kg bw per day, while at these same doses, Bcl-2 proteins levels were significantly increased ($P < 0.01$).

A number of factors confound the interpretation of these observations, including uncertainty of the age of the rats and the possibility that the effects were secondary to the reduction in body-weight gain. In addition, there were limited methodological details surrounding the histopathological examination of the testes including how slides were evaluated and graded.

Table 25. Effect of malathion on testicular parameters in rats

Parameter	Measure per dose			
	0 mg/kg bw per day	33.75 mg/kg bw per day	54 mg/kg bw per day	108 mg/kg bw per day
Terminal body weight (g)	402.10	380.40	345.5	334.9* (−17%)
Testes weight (g)	4.26	3.80	3.43** (+19%)	3.36** (+21%)
Relative testes weight (%)	0.011	0.010	0.010	0.010
Sperm counts (10^9)	9.26	9.19	7.36	5.54** (−40%)
Sperm motility (%)	78.50	73.70	41.60** (−47%)	36.10** (−54%)
Dismorphology rates (%)	0.24	0.37	0.57** (+140%)	0.63** (+160%)
LH (mIU/mL)	1.08	0.73	0.52** (−52%)	0.49** (−55%)
FSH (mIU/mL)	2.05	1.79	1.53	1.25** (−39%)
Testosterone (nmol/L)	3.35	2.94	2.67	1.91* (−43%)
ACP (U/g protein)	1.46	1.31	1.20	1.13** (−23%)
LDH (U/g protein)	0.19	0.24	0.25	0.31** (+63%)
SDH (U/mg protein)	96.81	100.21	84.42	87.36
GGT (U/g protein)	1.22	1.19	0.95** (−22%)	0.82** (−33%)

ACP: acid phosphatase; FSH: follicle-stimulating hormone; GGT: gamma-glutamyltransferase; IU: International Unit; LDH: lactate dehydrogenase; LH: luteinizing hormone; SDH: succinate dehydrogenase; U: enzyme unit; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the concurrent control in parentheses.

Source: Schroeder (1990)

In a uterotrophic assay by Barnett Jr (2011b), groups of eight ovariectomized female Crl:CD[SD] rats were administered by gavage malathion (purity 96.0%) in corn oil at 0, 100, 300 or 1000 mg/kg bw per day for three days. A positive control group of eight rats was administered 5 µg/kg bw per day 17α-ethynyl estradiol (purity 99%) as two subcutaneous injections of 2.5 µg/kg bw for three days. Estrous cycling was examined for five days prior to commencing dosing. The rats were observed twice daily for mortality and clinical signs. Body weight and feed consumption were recorded daily. Vaginal patency was recorded twice on the day of termination. The rats were terminated 24 hours after the final dose, and blood and brain acetylcholinesterase activity analysed. Each rat was necropsied, and the uterus, liver and brain weights recorded. There were no deaths or treatment-related clinical signs. At 1000 mg/kg bw per day, there was a significant loss of body weight on day 2 –3 (−0.8 g; $P < 0.05$). The positive control group also had a significant loss of body weight on day 2–3 (−2.7 g; $P < 0.01$). Overall body-weight gain from day 1–4 was −14%, −3.2% and −35.5% lower than the control at 100, 300 and 1000 mg/kg bw per day, respectively. At 1000 mg/kg bw per day, daily feed consumption was significantly lower than the control on days 2, 3 and 4 (−10%, −8% and −6%, respectively; $P < 0.01$ or 0.05). Vaginal patency was confirmed at termination. There were no treatment-related macroscopic findings. Mean absolute liver weight was increased at 1000 mg/kg bw per day (+24%; $P < 0.01$), while relative liver weight was increased at 300 (+10%, $P < 0.05$) and 1000 mg/kg bw per day (+25%, $P < 0.01$). The absolute and relative weights of the wet and blotted uteri in malathion-dosed rats were comparable to the vehicle control group values. Toxicologically and statistically significant inhibition of erythrocyte acetylcholinesterase occurred at every dose of malathion (−20.2%, −38.3% and −68.7% at 100, 300 and 1000 mg/kg bw per day, respectively; $P < 0.01$), while brain acetylcholinesterase activity was significantly lower ($P < 0.01$) than the control at 1000 mg/kg bw per day (−15.7%). On the basis of these findings, malathion was not positive for estrogenic activity in the uterotrophic bioassay at doses up to the limit dose of 1000 mg/kg bw per day.

A Hershberger assay was conducted by Barnett Jr (2011c) to determine the effect of malathion on the weights of the following androgen-dependent tissues in castrate-peripubertal male rats: ventral prostate, seminal vesicle plus fluids and coagulating glands, levator ani-bulbocavernosus muscle, paired Cowper's glands and the glans penis. In Phase 1 of the study, to evaluate the potential androgenic activity of malathion, malathion (purity 96.0%) in corn oil was administered by gavage at 0, 100, 300 or 1000 mg/kg bw per day for 10 days to groups of eight male Crl:CD[SD] rats. A positive control group of eight rats was administered 0.4 mg/kg bw per day testosterone propionate subcutaneously for 10 days.

In Phase 2 of the study, which evaluated the potential antiandrogenic activity of malathion, groups of eight male rats were administered by gavage with malathion in corn oil at 0, 100, 300 or 1000 mg/kg bw per day for 10 days. A positive control group of eight rats was administered by gavage 3 mg/kg bw per day flutamide in corn oil for 10 days. All groups received a subcutaneous dose of 0.4 mg/kg bw per day of testosterone propionate for 10 days to enable the detection of potential antiandrogenic activity. Observations for mortality and clinical signs were made daily. Body weight and feed consumption were recorded daily. Preputial separation was evaluated prior to commencing dosing. Following termination, the rats were necropsied, and blood and brain acetylcholinesterase activity analysed (Phase 1 only) and selected organs weighed.

Phase 1: There were no treatment-related deaths or clinical signs. There were no significant intergroup differences in absolute body weight, while body-weight gain was significantly lower than the control at 1000 mg/kg bw per day from days 3–4 (−89%, $P < 0.01$) and 4–5 (−59%, $P < 0.05$); overall body-weight gain (days 1–11) was 15% lower than the control but this difference was not statistically significant. Feed consumption was significantly lower than the control at 1000 mg/kg bw per day at days 3–4 (−9%, $P < 0.05$), 4–5 (−10%, $P < 0.05$) and 7–8 (−19%, $P < 0.01$); overall feed consumption was 9.4% lower ($P < 0.05$) than the control. There were no treatment-related macroscopic findings. In the positive control, there were significant increases ($P < 0.01$) in the weight of the levator ani-bulbocavernosus muscle, seminal vesicles with coagulating glands and fluid, Cowper's glands, ventral prostate and glans penis. In malathion-dosed rats, no change in androgen-dependent organ weights occurred. Absolute liver weight increased by 29% ($P < 0.01$) at 1000 mg/kg bw per day, while relative liver weight increased by 9% ($P < 0.01$) and 32% ($P < 0.01$) at 300 and 1000 mg/kg bw per day, respectively. Similarly, absolute paired kidney weight increased by 18% ($P < 0.05$) at 1000 mg/kg bw per day, while relative kidney was increased by 7% ($P < 0.05$) and 21% ($P < 0.01$) at 300 and 1000 mg/kg bw per day, respectively. Erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$) than the control at every dose of malathion (−21.6%, −61.2% and −88.1% at 100, 300 and 1000 mg/kg bw per day, respectively). Brain acetylcholinesterase activity was significantly lower than the control at 1000 mg/kg bw per day (−39.1%, $P < 0.01$).

Phase 2: There were no treatment-related deaths or clinical signs. There were no significant intergroup differences in absolute body weight, while overall body-weight gain was 14% lower than the control ($P < 0.01$), at 1000 mg/kg bw per day. Feed consumption was comparable across all groups. There were no treatment-related macroscopic findings. In malathion-dosed rats, no change in androgen-dependent organ weights occurred. At 1000 mg/kg bw per day, absolute and relative liver weights increased (+21% and +27%, respectively; $P < 0.05$), while relative paired kidney weight also increased (+13%, $P < 0.01$).

Based on these findings, malathion did not show an androgenic or antiandrogenic response in male rats.

As part of a screen for endocrine disruption potential by the USEPA, Palmer (2011a) examined the effect of malathion on the reproduction of the fathead minnow (*Pimephales promelas*). Groups of 24 fish were exposed to malathion (purity 96.0%) for 21 days at concentrations of 0, 0.10. 0.32 or 1.00 mg/L (analytical concentrations of 0, 0.08, 0.25 and 0.82 mg/L, respectively). The fish were observed daily for survival, adverse signs, fecundity (egg production) and fertility

(determination of fertile versus non-fertile eggs). At termination, the fish were weighed and their length recorded. Secondary sex characteristics were recorded (pigmentation patterns, tubercles, fatpads and ovipositors). Blood was collected to analyse serum vitellogenin. Each fish was macroscopically examined and gonadal sex determined. Their gonads were weighed and histopathologically examined.

There was no effect on survival. At the highest concentration, eight fish showed various signs of toxicity including bruising on the body, loss of colour or no colour in the tail, erratic swimming behaviour and/or lethargy. There was no treatment-related effect on fish growth, fecundity or fertility. At the highest dose in males, mean fatpad and tubercle score were significantly lower ($P < 0.05$) than the control (2.3 versus 4.0, and 11.4 versus 23.8, respectively). There was no effect on the gonadosomatic index in males, while a slight increase occurred in females (1.4 versus 11.9 in the control). There were no treatment-related effects on vitellogenin. Microscopic examination detected an increased presence of diploid spermatogonia with a decreased presence of spermatocysts containing spermatocytes (primary and secondary) and spermatids in the testis germinal epithelium in males at the highest concentration. In females at this same concentration, there was a slight though significant increase ($P < 0.05$) in atresia in the ovaries.

In an amphibian metamorphosis assay, African Clawed Frog tadpoles (*Xenopus laevis*) were exposed to malathion (purity 96.0%) for 21 days at concentrations of 0, 0.04, 0.13 and 0.40 mg/L (analytical concentrations of 0, 0.03, 0.11 and 0.32 mg/L. There were no treatment-related effects on tadpole growth or development (Palmer 2011b, c).

Wagner (2011) examined the potential of malathion (purity 96%) to affect the steroidogenic pathway in the H295R human adrenocarcinoma cell line. Malathion in dimethyl sulfoxide (DMSO) was tested at concentrations of 0.0001, 0.001, 0.01, 0.1, 1, 10, and 100 µmol/L (3 replicates per plate) in three independent assays. Testosterone and estradiol were analysed by HPLC/MS-MS. Following the 48-hour incubation, precipitation was noted at 100 µmol/L in two assays, while cytotoxicity did not exceed 20% at any concentration. A statistically significant reduction ($P < 0.05$; −19%) in testosterone concentration occurred at a malathion concentration of 100 µmol/L) in one assay but at no other concentrations or in the other independent assays; precipitation was observed at 100 µmol/L malathion in those other two assays. Similarly, estradiol was significantly increased ($P < 0.05$; +12%) at 10 µmol/L in one assay but at no other concentrations or in the other independent assays. In this same assay, precipitation occurred at the highest dose of 100 µmol/L. In the only assay where precipitation did not occur, estradiol did not significantly increase at 10 or 100 µmol/L (+14 and +13%, respectively; $P > 0.05$). Based on these results the authors concluded that malathion caused an equivocal response in the steroidogenesis assay. However, as these changes were both small and inconsistent across the three assays, they are unlikely to represent a treatment-related effect.

Wilga (2011) evaluated the potential of malathion (purity 96%) in DMSO to inhibit aromatase activity using human CYP19 and P450 reductase recombinant microsomes at concentrations from 10^{-10} to 10^{-3} mol/L. Three independent assays were performed, with each concentration tested in triplicate. The positive control substance, 4-hydroxyandrostenedione (purity 99.6%) was included in each assay at concentrations from 10^{-10} to 10^{-5} mol/L. Over the three assays, malathion decreased aromatase activity at 10^{-4} to 10^{-3} mol/L. As slight precipitation occurred at 10^{-3} mol/L in the first assay, the highest concentration in subsequent assays was reduced to $10^{-3.5}$ mol/L. At this concentration, malathion inhibited aromatase activity by 64.6% of control activity. Using the USEPA's interpretation procedure for aromatase inhibition, malathion was classified as equivocal; however, the authors concluded that malathion was not an inhibitor of aromatase activity.

Willoughby (2011a) evaluated the potential of malathion (purity 96%) in DMSO to act as an agonist of human estrogen receptor alpha (hERα) in a transcriptional activation assay using the hERα-HeLa-9903 cell line at concentrations of 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} mol/L. Each concentration was tested in replicates of six wells per plate at 24-hour-long exposures. In addition, two replicates per plate tested the positive control hERα antagonist, ICI 182,780. In a preliminary assessment of cytotoxicity and precipitation, cytotoxicity occurred at 10^{-4} mol/L. In two valid independent assays, malathion did not result in an increase in luciferase activity at any of the viable concentrations tested. On this basis the author concluded that malathion is not an agonist of hERα.

Willoughby (2011b) evaluated the potential of malathion (purity 96%) to act as an agonist of human androgen receptors in rat ventral prostate tissue homogenate at concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} mol/L. Two positive controls were included in each of the three binding assays in replicates of 3: a positive control, R1881 (purity 98%) (10^{-11} to 10^{-6} mol/L), and a weak positive control, dexamethasone (purity 99%) (10^{-10} to 10^{-3} mol/L). A solvent (DMSO) control (6 replicates) was also included in each assay. In the first assay, the mean specific binding of 1 nmol/L [^{3}H]-R1881 was 51.6% at 10^{-3} mol/L malathion; dexamethasone had a logIC$_{50}$ of −4.3 mol/L while the logIC$_{50}$ of R1881 was −9.9 mol/L. In the second assay, the mean specific binding of 1 nmol/L [^{3}H]-R1881 was 50.4% at 10^{-3} mol/L malathion; dexamethasone had a logIC$_{50}$ of −4.4 mol/L, while the logIC$_{50}$ of R1881 was −9.0 mol/L. In the third assay, the mean specific binding of 1 nmol/L [^{3}H]-R1881 was greater than 75% at every soluble concentration of malathion; dexamethasone had a LogIC$_{50}$ of −4.6 mol/L while the logIC$_{50}$ of R1881 was −9.0 mol/L. The authors classified malathion as equivocal in the first two assays and a non-binder in the third. On this basis malathion was classified as equivocal for binding to the androgen receptor.

Barnett Jr (2012d) undertook a pubertal development and thyroid function study to determine the potential of malathion to interact with the endocrine system. In the main study, groups of 20 peripubertal Crl:CD[SD] rats per sex were administered malathion (purity 95.8%) in corn oil by gavage at 0, 250, 500 or 1000 mg/kg bw per day from postnatal days 23–54 (males) and 22–43 (females). The highest dose was reduced to 750 mg/kg bw per day due to overt toxicity in females. Additional groups of eight rats per sex were dosed similarly to analyse acetylcholinesterase activity. The rats were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded daily. Once vaginal patency was observed, daily vaginal smears were taken to determine the stage of the estrous cycle. Thyroid hormones (thyroxine, thyroid stimulating hormone and testosterone) and clinical chemistry parameters were analysed in blood samples collected at termination. Erythrocyte and brain acetylcholinesterase activities were analysed in the additional groups of eight rats following termination. All rats were necropsied, selected organs weighed and tissues histopathologically examined.

Treatment-related deaths occurred at 500 and 750 mg/kg bw per day in the males in the main study (2 and 14 rats, respectively), with an additional high-dose male terminated in extremis. At 500 mg/kg bw per day, the deaths occurred on postnatal days 45 and 50, while at 750 mg/kg bw per day, the deaths occurred between postnatal days 25 and 53. Clinical signs observed prior to death included urine-stained abdominal fur, slight-to-severe excess salivation, prostrate behaviour, tremors, lacrimation, decreased motor activity, ataxia, loss of righting reflex, chromodacryorrhea, slight dehydration, ungroomed coat, dyspnoea, coldness to touch and hunched posture. Similar clinical signs were observed in survivors at these same doses. Among the females, treatment-related deaths occurred at the highest dose (two rats, which died on postnatal days 25 and 41), with clinical signs consistent with those observed in males. In both sexes, there was no treatment-related effect on body weight or feed consumption, sexual maturation (preputial separation or vaginal patency) or estrous cycling.

The results of the analysis of clinical chemistry parameters and hormone levels are summarized in Table 26. In the male rats, testosterone was decreased at every dose; in the absence of other antiandrogenic findings, the toxicological relevance of this decrease is unclear. In addition,

thyroxine was reduced at every dose but did not follow a dose–response relationship. There was no treatment-related effect on TSH levels. A number of significant changes in clinical chemistry parameters occurred, although some of these did not follow a dose–response relationship or were inconsistent in males and females; the most consistent findings were increased cholesterol and decreased alkaline phosphatase, triglycerides, albumin, globulin, albumin to globulin ratio, calcium and potassium.

Table 26. Effect of malathion on clinical chemistry parameters, hormone levels and organ weights in prepubertal rats

	Measure and per cent change per dose of malathion							
	Males				**Females**			
Parameter	**0 mg/kg bw per day**	**250 mg/kg bw per day**	**500 mg/kg bw per day**	**750/1000 mg/kg bw per day**	**0 mg/kg bw per day**	**250 mg/kg bw per day**	**500 mg/kg bw per day**	**750/1000 mg/kg bw per day**
Testosterone (ng/mL)	2.15	0.86** (−60%)	0.48** (−78%)	0.21** (−90%)	–	–	–	–
T4 (µg/dL)	5.12	7.24** (+41%)	6.92** (+35%)	6.76** (+32%)	4.11	4.89*	4.75	5.12**
TSH (ng/mL)	2.78	4.01	3.41	3.26	1.89	2.26	2.49	2.14
AST (IU/L)	212.1	195.4	154.0**	143.1**	216.0	234.1	213.4	210.4
ALP (IU/L)	514.1	524.6	415.2*	330.0**	402.5	397.6	388.8	297.5**
Total bilirubin (mg/dL)	0.159	0.133**	0.111**	0.101**	0.154	0.128**	0.107**	0.113
Cholesterol (mg/dL)	90.6	95.7	101.1*	108.3**	92.4	103.2*	104.4*	109.0**
Triglycerides (mg/dL)	465.0	312.1**	164.8**	150.0**	157.3	141.6	93.8**	74.5**
Albumin (g/dL)	3.39	3.20**	3.18**	3.21*	3.38	3.26*	3.12**	3.10**
Globulin (g/dL)	2.28	2.47**	2.67**	2.66**	2.16	2.41	2.27*	2.40**
Albumin : globulin ratio	1.49	1.30**	1.20**	1.21**	1.57	1.45**	1.38**	1.29**
Glucose (mg/dL)	124.6	123.1	146.7**	174.0**	145.1	132.6**	135.8*	142.6
BUN (mg/dL)	15.1	14.3	13.4	13.3	17.5	15.5**	14.5**	13.4**
Creatinine (mg/dL)	0.26	0.26	0.27	0.25	0.25	0.23	0.21**	0.20**
Calcium (mg/dL)	10.50	10.18**	10.15**	10.18**	10.82	10.75	10.53*	10.41**
Potassium (mmol/L)	6.65	6.33	6.16**	6.05**	7.27	7.10	6.53**	6.16**
Chloride (mmol/L)	93.8	94.8	95.6*	97.0*	97.7	96.5	96.6	95.6
Absolute liver weight (g)	15.44	17.00 (+10%)	18.72** (+21%)	18.64* (+21%)	8.82	9.31 (+6%)	9.37 (+6%)	10.24** (+16%)
Relative liver weight (%)	4.70	5.18** (+10%)	5.75** (+22%)	5.91** (+26%)	5.04	5.22 (+4%)	5.34** (+6%)	5.76** (+14%)
Absolute kidney weight (g)	2.49	2.63	2.79** (+12%)	2.74	1.64	1.70	1.76* (+7%)	1.84** (+12%)
Relative kidney weight (%)	0.76	0.81** (+7%)	0.86** (+13%)	0.88** (+16%)	0.94	0.95	1.01** (+7%)	1.04** (+11%)

ALP: alkaline phosphatase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; IU: International Unit; T4: thyroxine; TSH: thyroid stimulating hormone; *: $P < 0.01$; **: $P < 0.001$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Significant increases ($P < 0.01$ or 0.05) in absolute and/or relative liver and kidney weights occurred at and above 250 mg/kg bw per day in males and 500 mg/kg bw per day in females (Table 26). None of these increases were accompanied by any microscopic changes, and the majority were of a magnitude that would not be considered toxicologically significant. In males, there was significant decrease ($P < 0.01$) in the absolute weight of the levator ani-bulbocavernosus complex at 500 and 750/1000 mg/kg bw per day (−14% and −17%, respectively). These decreases were not considered treatment related as there was no dose–response relationship, there were no corroborating effects on other androgen-responsive organs, and the mean values were within the historical control range. There were no treatment-related histopathological findings in males. In females, there was a significant ($P < 0.05$) increase in the number of primary follicles in both left and right ovaries combined at 750/1000 mg/kg bw per day (28 primary follicles versus 20 in the control); however, this difference was not considered treatment-related as no increase occurred in the left and right ovary separately.

Cholinesterase study: Deaths and clinical signs occurred at the highest dose (six males and four females were found dead on postnatal days 24–49 and 23–25, respectively). The clinical signs were consistent with those that occurred during the main study. There were no effects on body weight, feed consumption or the occurrence of macroscopic abnormalities. Toxicologically and statistically significant ($P < 0.01$) inhibition of erythrocyte acetylcholinesterase occurred at 250 and 500 mg/kg bw per day in males (−79.2% and −87.7%, respectively; apart from the dead rats, no data were available at the highest dose) and at every dose in females (−74.1%, −89.1% and −97.3% at 250, 500 and 750/1000 mg/kg bw per day, respectively). Brain acetylcholinesterase activity was significant lower ($P < 0.01$) than the control in males at 250 and 500 (−17.4% and −34.6%, respectively; apart from the dead rats, no data were available at the highest dose) and in females at every dose (−8.9%, −23.6% and −42.1% at 250, 500 and 750/1000 mg/kg bw per day, respectively).

Based on these findings, malathion did not cause any antiandrogenic, estrogenic or antiestrogenic effects in rats or any effect on pubertal development or thyroid function up to 750 mg/kg bw per day.

In a published in vitro study by Kjeldsen, Ghisari & Bonefeld-Jørgensen (2013), malathion (purity > 93%) and a number of other pesticides were screened for their potential to interact with the human estrogen or androgen receptors or to interfere with aromatase activity. The estrogen receptor transactivation assay was conducted in human breast carcinoma MVLN cells, the androgen receptor transactivation assay was conducted in hamster ovary CHO-K1 cells and the aromatase activity assay was conducted in human choriocarcinoma JEG-3 cells. Malathion weakly induced estrogen receptor transactivation at a concentration of 1×10^{-5} mol/L (+113% relative to the solvent control). In the presence of 25 pmol/L estradiol, the same concentration of malathion had no effect on estrogen receptor transactivation. In contrast, the positive control (estradiol) induced estrogen receptor transactivation at a concentration of 6.3×10^{-12} mol/L (+248% relative to the solvent control). The conclusion reached was that malathion had no effect on androgen receptor transactivation or on aromatase activity.

(b)   Developmental toxicity

Rats

In a pilot study by Lochry (1988), groups of eight pregnant Crl:CD[SD]BR rats were administered malathion (unspecified purity) in corn oil by gavage at doses of 0, 300, 600, 800 or 1000 mg/kg bw per day from gestation days 6–15. The dams were observed daily for clinical signs, with body weight recorded throughout this period; feed consumption was not reported, and there was no statistical analysis of body weight data. On gestation day 20, the surviving dams were terminated and necropsied; the following parameters were recorded: corpora lutea counts, total resorptions, number of implantations, live fetuses, dead fetuses and pup sex ratio. Fetuses were examined for external, visceral and skeletal abnormalities. At the highest dose, three dams died on gestation days 11 or 12,

with clinical signs (salivation, urine-stained abdomen and chromorrhinorrhea) and body-weight loss (6–26 g in two dams) prior to death. In survivors, clinical signs were observed at and above 600 mg/kg bw per day: salivation (one, four and eight dams at 600, 800 and 1000 mg/kg bw per day, respectively) and urine-stained abdominal fur (four dams at 1000 mg/kg bw per day). Mean body weight was generally lower than the control at and above 600 mg/kg bw per day (up to 5% lower than the control at the highest dose), with body-weight gain reduced over the first few days of dosing at 800 and 1000 mg/kg bw per day (body-weight gain was −6 g at the highest dose versus +9.1 g in the control) from days 6–9. There were no treatment-related macroscopic findings or effects on litter parameters or incidence of external, visceral or skeletal abnormalities in fetuses.

In the main developmental toxicity study by Lochry (1989), groups of 25 pregnant Crl:CD[SD]BR rats were administered malathion (purity 94%) in corn oil by gavage at doses of 0, 200, 400 or 800 mg/kg bw per day from gestation days 6–15. Dams were observed daily for clinical signs, with body weight and feed consumption recorded throughout this period and from gestation days 16–20. On gestation day 20, the surviving dams were terminated and necropsied, and the following parameters recorded: gravid uterine weight, corpora lutea counts, total resorptions, number of implantations, live fetuses, dead fetuses and pup sex ratio. Fetuses were examined for external, visceral and skeletal abnormalities. There were no treatment-related deaths. Treatment-related clinical signs were confined to the highest dose and included urine staining of the abdomen in five dams and chromodacryorrhea and chromorrhinorrhea in one dam. Body-weight gain was significantly lower ($P < 0.01$ or 0.05) than the control at the highest dose over days 6–9 (+11 versus +14.8 g) and 6–12 (+27.3 versus +33.8 g), with a compensatory increase observed from days 16–20 (+73.4 versus +62.0 g). Feed consumption during the dosing period was also significantly lower ($P < 0.01$) than the control (66.8 versus 70.7 g). There were no treatment-related macroscopic findings or effects on litter parameters or on the incidence of external, visceral or skeletal abnormalities in fetuses. The NOAEL for maternal toxicity was 400 mg/kg bw per day for clinical signs and reduced body-weight gain and feed consumption at 800 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 800 mg/kg bw per day, the highest tested dose.

Rabbits

In a range-finding study, Siglin, Voss & Becci (1985) administered malathion (purity 92.4%) in corn oil by gavage to groups of five pregnant New Zealand White rabbits at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw per day from gestation days 6–18. The dams were observed daily for clinical signs, and body weight was recorded on gestation days 0, 6, 12, 15, 18, 24 and 29. Feed consumption was not recorded. On gestation day 29, the surviving dams were terminated and necropsied, and the following parameters recorded: gravid uterine weight; ovary weight; corpora lutea counts; number of implantations, resorptions, live fetuses and dead fetuses; and pup sex ratio. The fetuses were examined for external, visceral and skeletal abnormalities. Maternal deaths occurred at 200 and 400 mg/kg bw per day (four and two dams, respectively) between gestation days 7 and 17, with clinical signs consisting of decreased activity, tremors and salivation also at these doses in the majority of the rabbits. There was no effect on body weight or body-weight gain, on litter parameters or on the incidence of external, visceral or skeletal abnormalities in fetuses and there were no treatment-related macroscopic findings.

In the main study by the same authors (Siglin, Voss & Becci, 1985), groups of 20 pregnant New Zealand White rabbits were administered malathion (purity 92.4%) in corn oil by gavage at doses of 0, 25, 50 or 100 mg/kg bw per day from gestation days 6–18. The dams were observed daily for clinical signs. Body weight was recorded on gestation days 0, 6, 12, 15, 18, 24 and 29. Feed consumption was not recorded. On gestation day 29, the surviving dams were terminated and necropsied, and the following parameters recorded: gravid uterine weight; ovary weight; corpora lutea counts; number of implantations, resorptions, live fetuses and dead fetuses; and pup sex ratio. The fetuses were examined for external, visceral and skeletal abnormalities.

There were no treatment-related deaths or clinical signs. There was no significant difference in mean body weight between treated and control dams. At 50 and 100 mg/kg bw per day, mean

body-weight gain was significantly lower ($P < 0.05$) than the control over gestation days 6–18 (−0.03 g versus 0.19 g in the control). One control dam, one mid-dose dam and two high-dose dams aborted their litters on gestation days 21–26. At 50 and 100 mg/kg bw per day, the number of resorption sites and the percentage of resorptions was increased but not significantly or dose-relatedly (Table 27). Further, an expert review of the resorption data (Robinson, 2002) concluded that these slightly higher resorption values were within the historical control range (0–43%) and the apparent increase was not corroborated by the live litter size or the number of late resorptions, which were unaffected by treatment. This would not be expected if the compound induced an increased resorption rate. Based on these considerations, the expert review concluded that there was no evidence of a toxicologically significant embryo-lethal effect (Robinson, 2002). There were no treatment-related macroscopic findings in the dams or external, visceral or skeletal abnormalities in fetuses. The NOAEL for maternal toxicity was 25 mg/kg bw per day for reduced body-weight gain at 50 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest tested dose. These findings indicate that malathion is not teratogenic.

Table 27. Effect of malathion on litter resorptions in rabbits

| Parameter | No. or % measure per dose | | | |
	0 mg/kg bw per day	25 mg/kg bw per day	50 mg/kg bw per day	100 mg/kg bw per day
Resorption sites	0.9 ± 1.2	0.7 ± 1.1	2.3 ± 2.8	2.0 ± 2.7
Per cent resorptions (%)	15.6 ± 26.9	12.3 ± 20.7	29.2 ± 34.2	28.4 ± 34.9
Postimplantation loss (%)	$24.7 + 31.2$	$14.9 + 21.4$	$29.2 + 34.2$	$30.0 + 34.4$
Total live fetuses (no.)	5.6 ± 3.0	7.8 ± 3.2	5.8 ± 3.6	6.0 ± 3.3

Results expressed as the mean ± 1 standard deviation.

Source: Siglin, Voss & Becci (1985)

2.6   Special studies

(a)   Neurotoxicity

Fletcher (1989) examined the potential of malathion to cause delayed peripheral neuropathy in White Leghorn hens. In the acute toxicity phase of the study, the LD_{50} was estimated to be 775 mg/kg bw (range: 610–984 mg/kg bw). In the atropine sulfate sufficiency phase of the study, efficacious doses of atropine were determined to be 1.3 times the LD_{50} on day 1 and 1.1 times the LD_{50} on day 21. In the main phase of the study, a group of 60 hens received a single intramuscular injection of atropine sulfate (10 mg/kg bw), followed one hour later by a single gavage dose of malathion (purity 93.6%) in water at 1.3 times the LD_{50}. Atropine (30 mg/kg bw) was administered at 0.5, 1, 3 and 50 hours after dosing. Twenty-one days after dosing, a second dose of malathion was given (1.1 times the LD_{50}) following this same procedure. Vehicle (water) and positive control (500 mg/kg bw tri-o-tolyl phosphate) groups comprised 15 hens.

Thirty-nine hens died within 15 days of the first dose of malathion, with seven of the remaining hens dying within 7 days of the second dose. One control hen died on day 33 and all positive control hens were terminated on day 16 in a moribund condition. Clinical signs were observed in malathion-treated hens within approximately one hour of dosing and included general weakness, ataxia, inability to stand, sitting on haunches, diarrhoea, paralysis of legs and wings, and pale comb. Similar signs occurred after the second dose of malathion and resolved 5 or 6 days after dosing. No clinical signs were observed in negative controls. The positive controls showed signs of moderate to severe ataxia, inability to stand, paralysis of legs and wings, general weaknesses and

sitting on haunches by day 10. Body-weight gain over days 1–21 was significantly lower ($P < 0.05$) than the control in malathion-treated hens (−234 versus −72 g, respectively). Reduced feed consumption occurred for approximately week after the first dose of malathion (5–65 g/day versus 73–112 g/day in the vehicle control). Feed consumption was also lower in the positive control group (41–86 g/day). Necropsy and histopathology revealed no treatment-related effects on nervous tissue. In the positive control group, neural lesions were observed histopathologically (axonal degeneration and demyelination, hyperplasia of Schwann cells, increased glial cell proliferation). There was no evidence that malathion caused delayed peripheral neuropathy.

In an acute neurotoxicity study by Lamb (1994a), groups of 27 Crl:CD®BR rats of each sex received a single gavage dose of malathion (purity 96.2%) in corn oil at 0, 500, 1000 or 2000 mg/kg bw. These doses were based on the range-finding study by Nemec (2000), where the time to peak effect was estimated to be 15 minutes. In each group, seven rats per sex were allocated to the neuropathology evaluation and 20 to the analysis of cholinesterase. Observations were made daily for deaths and clinical signs. Body weights were recorded on day 1 and weekly thereafter. Feed consumption was not recorded. A functional observational battery and motor activity assessment were performed at 15 minutes and 7 and 14 days after dosing in 12 rats per sex per group. Plasma, erythrocyte and brain cholinesterase activities were analysed in samples collected from five rats per sex per dose pretreatment and at 15 minutes, 7 days and 15 days (termination) after dosing. The remaining rats were terminated on day 15, necropsied and the nervous system examined histopathologically.

One high-dose male was terminated in a moribund condition seven days after dosing. Salivation occurred within 15 minutes of dosing in every treated group (two males in each group; one, one and four females at 500, 1000 and 2000 mg/kg bw, respectively). Additional clinical signs at the highest dose included yellow material around the uro/anogenital region (six males and nine females), red material around the nose and mouth (two males and seven females) and decreased defecation (three males) or small faeces (13 females). There was no treatment-related effect on body weight. Salivation was observed at 15 minutes after dosing (males: 0, 0, 1 and 4 of 12 rats at 0, 500, 1000 and 2000 mg/kg bw per day, respectively; females: 0, 0, 1 and 2 of 12 rats at 0, 500, 1000 and 2000 mg/kg bw per day). No other treatment-related observations were made during the functional observational battery. In high-dose males, a significantly lower ambulatory activity ($P < 0.05$) was observed 15 minutes after dosing (321 versus 422 counts in the control during subsession 1; total counts were 653 versus 940, respectively). There was no treatment-related effect on plasma cholinesterase or brain acetylcholinesterase activity in either sex. At the highest dose, erythrocyte acetylcholinesterase activity was significantly lower than the control only in females 7 days after dosing (−39%, $P < 0.05$); erythrocyte acetylcholinesterase was 40% lower than the control in males but the difference was not statistically significant. There was no treatment-related effect on brain weights or macroscopic or microscopic findings in nervous tissue.

The NOAEL was 1000 mg/kg bw for reduced erythrocyte acetylcholinesterase activity in females and reduced ambulatory activity in males at 2000 mg/kg bw.

In a 13-week neurotoxicity study by Lamb (1994b), groups of 25 Crl:CD BR rats of each sex were fed diets containing 0, 50, 5000 or 20 000 ppm malathion (purity 96.4%) ad libitum. The achieved doses were 0, 4, 352 and 1486 mg/kg bw per day in males and 0, 4, 395 and 1575 mg/kg bw per day in females, respectively. Observations were made daily for deaths and clinical signs, with body weight and feed consumption recorded weekly. A functional observational battery and motor activity assessment (10 rats/sex per dose) were performed pretreatment and during weeks 4, 8 and 13. Plasma, erythrocyte and brain cholinesterase activities were analysed in samples collected from five rats per sex per dose and during weeks 3, 7 and 13. Following scheduled termination on day 91, all the rats were necropsied and brain weights recorded. Nervous tissue was examined histopathologically.

There were no deaths. The only treatment-related clinical sign was the presence of yellow or orange material around the ano/uro-genital region and on the tail of high-dose rats (nine males and eight females). At 20 000 ppm, mean body weight was 9–20% ($P < 0.01$ or 0.05) and 9–13% ($P < 0.01$) lower than the control in males and females, respectively, throughout the exposure period. Also at 20 000 ppm, body-weight gain was significantly lower ($P < 0.01$) than the control in males during the first week of exposure (−79%) and in females during weeks 0–1 (−57%), 4–5 (−41%), 10–11 (−71%). At the highest dose, cumulative body-weight gain to week 12 was significantly lower ($P < 0.05$) than the control in males (−15%), while cumulative body-weight gain to week 13 was significantly lower ($P < 0.01$) than the control in females (−24%). Mean feed consumption was also reduced at the highest dose in males during weeks 0–1 (−19%, $P < 0.01$), 6–7 (−10%, $P < 0.05$) and in females throughout most of the exposure period (−9 to −20%, $P < 0.01$ or 0.05). There were no treatment-related functional observational battery findings or effects on locomotor activity.

The effect of malathion on cholinesterase activity is summarized in Table 28. Significant inhibition ($P < 0.01$ or 0.05) of plasma cholinesterase activity occurred at 5000 and 20 000 ppm in males during week 3 but only at 20 000 ppm in females or at other sampling points. Toxicologically and statistically significant inhibition of erythrocyte cholinesterase activity occurred at 5000 and 20 000 ppm in both sexes, and at every sampling point. Acetylcholinesterase activity in different brain regions was significantly lower than in the control at 20 000 ppm more consistently in females than males. There was no treatment-related effect on brain weights or macroscopic or microscopic findings in nervous tissue.

The NOAEL was 5000 ppm (equal to 352 mg/kg bw per day in males and 395 in females) based on the inhibition of brain acetylcholinesterase activity, clinical signs and reduced body weight at 20 000 ppm (equal to 1486 mg/kg bw per day in males and 1575 mg/kg bw per day in females).

Table 28. Effect of malathion on cholinesterase in rats over 13 weeks of dietary exposure

Parameter	Cholinesterase activity per dietary concentration			
	0 ppm	50 ppm	5 000 ppm	20 000 ppm
Plasma ChE (IU/mL)				
Males				
Week 3	0.36	0.320	0.292* (−18%)	0.170* (−53%)
Week 7	0.309	0.309	0.248	0.142** (−54%)
Week 13	0.303	0.277	0.266	0.150** (−51%)
Females				
Week 3	0.933	1.176	0.92	0.42** (−55%)
Week 7	1.777	1.325	1.250 (−30%)	0.430** (−76%)
Week 13	1.517	2.257	1.291	0.453* (−70%)
Erythrocyte AChE (IU/mL)				
Males				
Week 3	0.88	0.90	0.43** (−51%)	0.41** (−53%)
Week 7	0.80	0.79	0.31** (−61%)	0.26** (−68%)
Week 13	1.06	1.04	0.54** (−49%)	0.39** (−63%)
Females				
Week 3	0.99	1.04	0.49** (−51%)	0.36** (−64%)
Week 7	0.90	0.87	0.42** (−53%)	0.39** (−57%)
Week 13	1.22	1.23	0.62** (−49%)	0.39** (−68%)
Brain hippocampus (IU/g)				

Parameter	Cholinesterase activity per dietary concentration			
	0 ppm	50 ppm	5 000 ppm	20 000 ppm
Males				
Week 3	3.33	3.19	2.96	2.70
Week 7	5.88	5.71	5.99	4.8
Week 13	5.61	4.99	5.50	6.15
Females				
Week 3	3.26	3.16	3.33	2.36** (−44%)
Week 7	6.34	6.32	5.54	3.94** (−38%)
Week 13	5.02	5.70	4.56	2.68** (−47%)
Brain olfactory (IU/g)				
Males				
Week 3	5.46	6.67	6.39	4.77
Week 7	12.98	13.18	12.32	11.51
Week 13	12.67	13.62	13.26	8.30* (−34%)
Females				
Week 3	7.23	6.56	6.62	5.00* (−31%)
Week 7	12.45	15.50	12.60	9.09* (−27%)
Week 13	16.70	12.46	11.78	8.27** (−50%)
Mid brain (IU/g)				
Males				
Week 3	5.24	5.19	5.07	4.34
Week 7	7.27	7.27	6.37	6.08
Week 13	9.69	9.51	9.02	7.4** (−24%)
Females				
Week 3	4.68	5.20	4.76	3.84
Week 7	7.67	6.03	6.66	4.99** (−35%)
Week 13	8.84	9.86	8.86	5.30** (−40%)
Brainstem (IU/g)				
Males				
Week 3	4.40	4.40	4.89	3.67
Week 7	7.28	7.79	6.72	6.47
Week 13	8.56	8.09	8.03	7.06** (−18%)
Females				
Week 3	4.35	4.78	4.15	3.38
Week 7	7.13	8.01	6.92	5.95
Week 13	8.71	9.10	7.34	5.61** (−36%)
Brain cerebellum (IU/g)				
Males				
Week 3	2.62	2.33	2.28	2.50
Week 7	3.04	3.16	2.91	2.96
Week 13	3.80	3.48	3.39	3.32

| | Cholinesterase activity per dietary concentration | | | |
Parameter	0 ppm	50 ppm	5 000 ppm	20 000 ppm
Females				
Week 3	2.50	2.65	2.49	2.0* (−20%)
Week 7	3.09	2.85	2.99	2.60
Week 13	3.67	3.86	3.68	2.50** (−32%)
Brain cortex (IU/g)				
Males				
Week 3	5.15	5.18	5.21	4.57
Week 7	8.32	8.78	7.65	6.12** (−26%)
Week 13	10.02	10.08	10.74	7.72
Females				
Week 3	5.85	5.47	4.81** (−18%)	4.00** (−32%)
Week 7	9.13	7.75	7.28	5.46**
Week 13	10.47	10.29	9.20	4.92** (−53%)

AChE: acetylcholinesterase; ChE: cholinesterase; IU: International Unit; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Source: Lamb (1994b)

In a range-finding study by Fulcher (2002a) designed to establish appropriate doses for both a developmental neurotoxicity study and a cholinesterase study, groups of 15 pregnant Crl:CD BR rats were administered malathion (purity 96%) in corn oil by gavage from gestation day 6–20 or day 10 of lactation. Pups (3/sex per group from 10 litters) were dosed from postnatal days 11–21. In the initial phase of the study, doses of 0, 7.5, 750 or 1000/1250 mg/kg bw per day were administered to the dams and doses of 0, 7.5, 200 or 450 mg/kg bw per day were administered to the pups. Due to overt toxicity, these doses were reduced to 0, 7.5, 35, 75 and 150 mg/kg bw per day in Phase 2 of the study. Observations for mortality and clinical signs, body weight and feed consumption were recorded regularly throughout the study. Standard litter and offspring parameters were recorded. Plasma, erythrocyte and brain cholinesterase activities were analysed in samples collected on gestation day 20 in dams and postnatal day 21 in pups. A limited range of clinical chemistry parameters were analysed in both dams and pups (sodium, potassium, bicarbonate, chloride and calcium). Following scheduled termination (gestation day 20 for dams and postnatal day 21 for pups), the rats were necropsied and any macroscopic abnormalities processed for histopathological examination.

In Phase 2 of the study, there were no deaths. The dams salivated at and above 35 mg/kg bw per day, while no clinical signs occurred in pups. There was no treatment-related effect on body weight or feed consumption. Litter, offspring and clinical chemistry parameters were unremarkable. There were no treatment-related macroscopic findings. Erythrocyte acetylcholinesterase activity was significantly lower than the control in the dams at 75 (−33%, $P < 0.05$) and 150 mg/kg bw per day (−59%, $P < 0.01$), while plasma cholinesterase and brain acetylcholinesterase were unaffected by treatment. In dams terminated on gestation day 20, plasma cholinesterase was significantly lower than the control ($P < 0.01$ or 0.05) at and above 35 mg/kg bw per day (−9% to −11%), whereas there was no effect on erythrocyte or brain acetylcholinesterase activities. In pups terminated on postnatal day 21, erythrocyte acetylcholinesterase activity was significantly lower than the control at every dose (−19%, −30%, −42% and −58% in males and −12%, −25%, −41% and −65% in females at 7.5, 35, 75 and 150 mg/kg bw per day, respectively), whereas brain acetylcholinesterase activity was significantly lower than the control at 75 (−9% in males and −7% in females, $P < 0.01$) and 150 mg/kg bw per day (−18% in males and −19% in females, $P < 0.01$). There were no significant intergroup differences in plasma cholinesterase activity in male pups while in the female pups plasma

cholinesterase was 16 ($P < 0.01$) and 26% ($P < 0.01$) lower than the control at 75 and 150 mg/kg bw per day, respectively.

In a developmental neurotoxicity study that was not guideline compliant, groups of at least 21 pregnant Crl:CD BR rats were administered malathion (purity 96%) in corn oil by gavage at doses of 0, 5, 50 or 150 mg/kg bw per day from gestation day 6 to day 10 of lactation. Offspring (seven per litter) were also given comparable doses of malathion by gavage from postnatal day 11–21. The doses were based on the results of the range-finding study by Fulcher (2002a) and the cholinesterase study by Fulcher (2001). Observations for mortalities and clinical signs were made daily, with body weight and feed consumption recorded regularly. The behaviour of 10 dams per group was assessed on gestation days 12 and 18 and postnatal days 4 and 10. The following reproduction parameters were determined: gestation index; gestation length; postimplantation and survival index; live birth index; viability index; and lactation index. The following offspring parameters were determined: number of live and dead offspring; individual body weights; sex; clinical signs and the time of vaginal opening and balanopreputial separation. On postnatal day 4, the litters were culled to eight pups and each litter assigned to an assessment of behaviour (postnatal days 4, 11, 21, 35, 45 and 60 in 10 pups/sex per group), motor activity (postnatal days 13, 17 and 22 in one pup/sex per litter), auditory startle response habituation and auditory startle pre-pulse inhibition (postnatal days 23 and 60 in one pup/sex per litter), and learning and memory (postnatal days 23 or 24, and 61 or 62 in one pup/sex per litter). The dams were terminated after weaning on postnatal day 20 or 21 and necropsied; the weights of the brain, pituitary and sex organs were recorded. The offspring were terminated on postnatal day 11 (10 offspring/sex per group), 21 (10 offspring/sex per group) or 65 (all remaining offspring), necropsied, brain weight and brain morphometry recorded and nervous tissue histopathologically examined.

There were no treatment-related deaths. In the dams, there was an increase in post-dosing salivation at the highest dose (5, 4, 3 and 20 dams at 0, 5, 50 and 150 mg/kg bw per day) but there was no effect on body weight, feed consumption or reproduction parameters or on treatment-related behavioural abnormalities, macroscopic findings or effect on brain weight. There was no treatment-related effect on litter parameters and no effect on survival or body weight in offspring. At 150 mg/kg bw per day, four offspring from the same litter exhibited clinical signs after 7–9 days of exposure to malathion (postnatal day 17–19), including whole-body tremors, underactivity, prostrate posture, partially closed eyelids and abnormal gait. Abnormalities observed in offspring at 150 mg/kg bw per day during the functional observational battery included failure to show an immediate surface righting reflex (5 females versus 1 female in the control on postnatal day 11). In offspring there was no effect on brain weight or the incidence of neuropathological findings.

The NOAEL for both maternal toxicity and offspring toxicity was 50 mg/kg bw per day for clinical signs at 150 mg/kg bw per day (Fulcher, 2002b; Reiss, 2004).

Myers (2003, 2004) examined the potential of malathion to affect the thickness of the corpus callosum in rat pups. Dams were administered malathion (unspecific purity and vehicle) by gavage from gestation days 6–10 followed by direct dosing of offspring (20/sex per dose) from postnatal days 11–21 at 0, 5, 50 or 150 mg/kg bw per day. Ten offspring per sex per dose were terminated on postnatal day 21 while the remainder were observed undosed until postnatal day 65. Histopathologic examination of standard sections of brain tissue showed no treatment-related effect on the thickness of the corpus callosum.

(b)   Cholinesterase inhibition

Rodriguez et al. (1997) compared the potential of enantiomers of malaoxon to inhibit different cholinesterases, including erythrocyte and human plasma cholinesterase. Rat and electric eel erythrocyte acetylcholinesterase were the most sensitive to malaoxon, with bovine and human

erythrocyte acetylcholinesterase less sensitive; inhibitory potency varied approximately 100-fold (Table 29). In all experiments, *R*-malaoxon was more potent than *S*-malaoxon.

Table 29. Interspecies comparison of cholinesterase inhibition by enantiomers of malaoxon

Species/cholinesterase	*R*-malaoxon	*S*-malaoxon	R : S ratio	Racemic malaoxon
Rat erythrocyte AChE	1.52×10^5	3.00×10^4	5	1.01×10^5
Sheep red blood cell AChE	1.96×10^5	8.70×10^3	22.5	1.54×10^5
Electric eel erythrocyte AChE	9.39×10^5	5.79×10^4	16.2	4.74×10^5
Human plasma ChE	1.46×10^4	4.24×10^3	3.4	7.78×10^3
Human erythrocyte AChE	1.27×10^5	1.09×10^4	11.6	6.5×10^4

AChE: acetylcholinesterase; ChE: cholinesterase

Results are expressed as the mean bimolecular reaction constants (ki; mol/L per minute).

Source: Rodriguez et al. (1997)

In non-guideline studies (Fulcher, 2001, 2003) designed to examine the effect of malathion on cholinesterase activity, groups of nine pregnant Crl:CD BR rats were administered malathion (purity 96%) in corn oil by gavage at 0, 5, 50 or 150 mg/kg bw per day from gestation days 6–20. Three hours after dosing on gestation day 20, eight dams were terminated and plasma, erythrocyte and brain cholinesterase activities of the dams and fetuses analysed. Additional groups of 10 pregnant rats were administered malathion by gavage at the same doses from gestation day 6 to postnatal day 10. Offspring in eight litters per group were then administered comparable doses of malathion from postnatal days 11– 21. Selected offspring were terminated on postnatal days 4, 21 and 60, and plasma, erythrocyte and brain cholinesterase activities analysed. In an additional part of the study, pups from eight control dams received a single gavage dose of malathion on postnatal day 11 at 0, 5, 50, 150 or 450 mg/kg bw and were terminated 2 hours later to analyse plasma, erythrocyte and brain cholinesterase activity. Additional groups of eight male and eight female young adult rats were administered a single gavage dose of malathion at 0, 5, 50, 150 or 450 mg/kg bw and terminated 2 hours later to analyse plasma, erythrocyte and brain cholinesterase activities. Alternatively, groups of eight young adult rats received 11 consecutive daily doses of malathion at 0, 5, 50 or 150 mg/kg bw per day and were terminated 2 hours after the final dose to analyse plasma, erythrocyte and brain cholinesterase activities. Throughout all phases of the study, mortality, clinical signs, body weight, litter and offspring parameters were recorded. Following termination, the rats were necropsied and brain weights recorded.

There were no treatment-related deaths or clinical signs in the dams. Tremors were observed in the offspring (5 of 16) that received a single dose of 450 mg/kg bw per day, with one rat terminated in a moribund condition 1 hour after dosing. Body weight, litter and fetal parameters were unaffected by treatment. There were no treatment-related macroscopic findings or effect on brain weight.

In the dams exposed to malathion from gestation days 6–20 and terminated on gestation day 20, erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$) than the control at 50 and 150 mg/kg bw per day (−19% and −51%, respectively); there was no effect on plasma or brain cholinesterase activities. In fetuses from these same dams, plasma and erythrocyte cholinesterase activities were significantly lower ($P < 0.01$ or 0.05) than the control (plasma: −14% and −15%, respectively; erythrocytes: −11% and −19%, respectively), while there was no change in brain acetylcholinesterase activity. In pups from these same-treated dams terminated on postnatal day 4, plasma, erythrocyte and brain cholinesterase activities were unaffected by treatment.

In offspring administered a single dose of malathion on postnatal day 11, plasma cholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control in both sexes at and above 50 mg/kg bw per day (−19%, −36% and −54% in males and −16%, −35% and −52% in females at 50, 150 and 450 mg/kg bw, respectively). Erythrocyte acetylcholinesterase was significantly lower

than the control ($P < 0.01$ or 0.05) at every dose in males and at and above 50 mg/kg bw in females, (−16%, −25%, −55% and −72% in males and −7%, −23%, −48% and −61% in females at 5, 50, 150 and 450 mg/kg bw, respectively). Brain acetylcholinesterase was significantly lower ($P < 0.01$) than the control at 150 and 450 mg/kg bw in both sexes (−44% and −84% in males and −48% and −81% in females, respectively).

In young adult rats administered a single dose of malathion, plasma and erythrocyte cholinesterase activities were significantly lower ($P < 0.001$ or 0.01) than the control in males (−24% and −25%, respectively), but there was no effect on brain acetylcholinesterase activity. In high-dose females, statistically but not toxicologically significant inhibition of erythrocyte acetylcholinesterase occurred (−17%, $P < 0.001$), but there was no effect on plasma or brain cholinesterase activities.

In offspring terminated on postnatal day 21 after 11 consecutive daily doses, plasma cholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control in both sexes at 50 and 150 mg/kg bw per day (−19% and −24% in males and −19% and −32% in females, respectively). Erythrocyte acetylcholinesterase activity was significantly lower than the control in males at every dose (−17%, −39% and −67% at 5, 50 and 150 mg/kg bw per day, respectively), while in females significant inhibition occurred at 50 and 150 mg/kg bw per day (−34% and −68%, respectively). Brain acetylcholinesterase activity was significantly lower than ($P < 0.01$) the control only at the highest dose (−16% in both sexes).

In young adults administered 11 consecutive doses of malathion, plasma cholinesterase was significantly lower ($P < 0.05$) than the control only in males at 50 and 150 mg/kg bw per day (−11% and −13% respectively). Erythrocyte acetylcholinesterase was significantly lower ($P < 0.01$) than the control at 50 and 150 mg/kg bw per day in both sexes (−20% and −43% in males, and −20% and −48% in females, respectively). There was no effect on brain acetylcholinesterase in either sex.

In offspring terminated on postnatal day 60, there was no treatment-related effect on plasma, erythrocyte or brain cholinesterase activities.

Barnett Jr (2006a) undertook a range-finding study in Crl:CD[SD] juvenile rats to determine the effect of malathion or malaoxon on acetylcholinesterase activity. Groups of five rat pups per sex were administered malathion (purity 96%) in corn oil by gavage from postnatal days 11–21 at doses of 0, 5, 15 or 50 mg/kg bw per day or malaoxon (purity 97.7%) at doses of 0, 0.05, 0.1 or 1 mg/kg bw per day. The rats were observed daily for mortality and clinical signs. Body weights were recorded daily during the dosing period. The rats were terminated on postnatal day 21 and blood and brain acetylcholinesterase activity analysed. There were no treatment-related deaths, clinical signs, effects on body weight or macroscopic abnormalities. Brain acetylcholinesterase activity was not affected by treatment. Toxicologically significant inhibition of erythrocyte acetylcholinesterase activity occurred in females at 50 mg/kg bw per day malathion (−28.2%) and 1 mg/kg bw per day malaoxon (−30.4% in males and −29.4% in females).

Barnett Jr (2006b) determined the time to peak cholinesterase inhibition in young preweanling Crl:CD[SD] rats (20/sex per group) following repeated gavage dosing with 0 or 150 mg/kg bw per day malathion (purity 96%) or 4 mg/kg bw per day malaoxon (purity not specified) in corn oil from postnatal days 11–21. Five malathion-treated rats per sex were terminated at 1, 2, 3 and 4 hours after dosing while five malaoxon-treated rats per sex, were terminated at 30, 60, 90 and 120 minutes after dosing to analyse erythrocyte and brain acetylcholinesterase activities. There were no deaths. Clinical signs including whole-body tremors, reduced motor activity, prostrate positioning, soft or liquid faeces, impaired righting reflex and dehydration, were observed only at 150 mg/kg bw per day malathion in both sexes from postnatal days 13–18. In malathion-treated rats, toxicologically significant inhibition of erythrocyte acetylcholinesterase activity occurred at all time points in both sexes (−20.7% to −50.7% in males and −38.6% to −62.2% in females), with the peak effect 2 hours after dosing. Brain acetylcholinesterase activity was inhibited at every time point (−11.2% to −14.0% in males and −9.5% to −21.4% in females), with the peak effect 2 hours after dosing. In malaoxon-

treated rats, inhibition of erythrocyte activity occurred at most time points (−41.0% to −62.2% in males and −50.3% to −59.4% in females), with the peak effect 90 minutes after dosing in males and 2 hours after dosing in females. Inhibition of brain acetylcholinesterase activity occurred at most time points in males (−4% to −15.7%) with the time of peak effect 30 minutes after dosing. In females, brain acetylcholinesterase activity was inhibited at 30 and 60 minutes after dosing (−8.3% and −2.2%, respectively).

In the second part of this study, the inhibition of cholinesterase activity was determined following a single gavage dose of 0, 50, 150 or 450 mg/kg bw per day malathion on postnatal day 21 (6–10 rats per sex per group). Rats were terminated 2 hours after dosing to analyse erythrocyte and brain acetylcholinesterase activities. There were no treatment-related deaths. Slight whole-body tremors, miosis and urine-stained abdominal fur were observed in one high-dose female. Erythrocyte acetylcholinesterase activity was inhibited in a dose-related manner in males (−19.6%, −27.1% and −32.5%, respectively), while in females the level of inhibition at 150 mg/kg bw was higher than that at 450 mg/kg bw (−13.4%, −35.5% and −22.8%, respectively). Only slight inhibition of brain acetylcholinesterase occurred in high-dose males (−14.9%) and females (−5.7%).

Barnett Jr (2006c) undertook a further study to determine the effect of malathion or malaoxon on acetylcholinesterase activity in Crl:CD[SD] juvenile rats. Groups of five pups per sex were administered malathion (purity 96%) in corn oil by gavage from postnatal days 11–21 at doses of 0, 5, 25, 50 or 150 mg/kg bw per day or malaoxon (purity 97.7%) at doses of 0, 0.1, 1, 2.5 or 4 mg/kg bw per day. The pups were observed daily for mortality and clinical signs. Body weights were recorded daily during the dosing period. The rats were terminated on postnatal day 21, and blood and brain acetylcholinesterase activity analysed. There were no treatment-related deaths. At 150 mg/kg bw per day malathion, clinical signs were observed in both sexes: these included tremors, decreased motor activity, impaired righting reflex, splayed forelimbs and pale extremities. No clinical signs occurred in pups exposed to malaoxon. There was no effect on body weight. Erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at and above 25 mg/kg bw per day malathion in both sexes; however, toxicologically significant inhibition occurred only at 50 and 150 mg/kg bw per day (−15.1%, −34.1% and −54.1 % in males and −17.6%, −30.1% and −51.7% in females at 25, 50 and 150 mg/kg bw per day, respectively). Erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at and above 1 mg/kg bw per day malaoxon in both sexes; however, toxicologically significant inhibition occurred only at 2.5 and 4 mg/kg bw per day (−14.1%, −45.8% and −51.1 % in males and −13.5%, −34.7% and −45.3% in females at 1, 2.5 and 4 mg/kg bw per day, respectively). Brain acetylcholinesterase activity was significantly lower than the control only at 150 mg/kg bw per day malathion (−14.5% and −16.8% in males and females, respectively) and was unaffected by malaoxon. The NOAEL for erythrocyte cholinesterase inhibition was 25 mg/kg bw per day for malathion and 1 mg/kg bw per day for malaoxon.

Pratt (2006) compared the acute oral toxicity and inhibition of acetylcholinesterase activity of malathion and desmethyl malathion in female CD rats. In a pilot experiment, groups of five female rats were administered a single gavage dose of malathion (purity 96.8%) in DMSO at doses of 1500 or 2000 mg/kg bw. Blood was sampled prior to dosing and at 2 and 24 hours after dosing to analyse erythrocyte acetylcholinesterase activity. At 2000 mg/kg bw malathion, all the rats exhibited salivation immediately after dosing and piloerection approximately 4 hours after dosing; two rats died. Acetylycholinesterase activity was inhibited by 10–71%. At 1500 mg/kg bw malathion, there were no deaths. Salivation was observed immediately after dosing in all rats, with piloerection from 30 minutes after dosing. Acetylcholinesterase activity was inhibited by 42–59% at 2 hours after dosing and approximately 50% at 24 hours after dosing.

In the main study, groups of 10 females rats were administered a single dose of malathion (purity 96.8%) or desmethyl malathion (purity 90–92.7%) by gavage at 1500 mg/kg bw in DMSO. A control group of 10 rats were dosed with the vehicle alone. Deaths and clinical signs were recorded

daily until termination on day 14. Body weight was recorded prior to dosing and at termination. Blood was sampled prior to dosing and at 2 and 24 hours after dosing to analyse erythrocyte acetylcholinesterase activity. There were no treatment-related deaths. The rats dosed with malathion displayed salivation and piloerection from approximately 30 minutes after dosing. Salivation ceased by one hour after dosing while piloerection ceased by day 2. No clinical signs were observed in rats dosed with desmethyl malathion. The rats were terminated on day 14 and necropsied. There was no effect on body weight and no treatment-related macroscopic findings. Both malathion and desmethyl malathion caused a signification reduction in acetylcholinesterase activity, with the magnitude and duration of the reduction higher with malathion (Table 30).

Table 30. Effect of malathion or desmethyl malathion on erythrocyte acetylcholinesterase activity in female rats

Time	Erythrocyte AChE activity		
	Control	1 500 mg/kg bw malathion	1 500 mg/kg bw desmethyl malathion
Pre-dose	2 330 ± 197	2 285 ± 192	2 320 ± 203
2 hours	2 365 ± 228	1 205 ± 80 (−49%)**	1 585 ± 78 (−33%)*
24 hours	2 248 ± 198	1 770 ± 99 (−21%)**	2 023 ± 116 (−10%)*

AChE: acetylcholinesterase; bw: body weight; U: enzyme unit; SD: standard deviation; *: $P < 0.01$ compared with the control; **: $P < 0.001$ compared with desmethyl malathion

Results expressed as the mean activity (U/L) ± 1 SD, with the % decrease (−) relative to the control in parentheses.

Source: Pratt (2006)

Using data from 2-year rat chronic toxicity and carcinogenicity studies on malathion (Daly, 1996a) and malaoxon (Daly, 1996b), Reiss (2006a) undertook BMD modelling on erythrocyte and brain acetylcholinesterase inhibition. Data from 90-day, 180-day, 1-year and 2-year sampling times were analysed while feed consumption data were reanalysed to obtain more accurate dose estimates. Two BMD models were used based on USEPA methodology: a basic model consisting of an exponential declining curve and an expanded model incorporating saturable metabolism. The data from the four sampling points were meta-analysed jointly. The results are summarized in Table 31. The authors concluded that the use of a BMD_{20} for erythrocyte acetylcholinesterase activity would be protective for the inhibition of brain acetylcholinesterase activity at the 10% level by at least a factor of 2 for malathion and 20 for malaoxon. The authors also proposed toxicity adjustment factors (TAFs) for malaoxon from 37–38 in males and 65–69 in females for the inhibition of erythrocyte acetylcholinesterase activity.

Table 31. BMDs for erythrocyte and brain acetylcholinesterase activities from 2-year rat chronic toxicity and carcinogenicity studies on malathion and malaoxon

Compound	Sex	Brain AChE BMD_{10}	Erythrocyte AChE	
			Inhibition level (%)	BMD
Malathion	Male	231.1	10	42.8
			15	67.5
			20	95.2
	Female	337.7	10	46.2
			15	73.3
			20	104.0
	Male	52.0	10	1.1

Compound	Sex	Brain AChE BMD$_{10}$	Erythrocyte AChE	
			Inhibition level (%)	BMD
Malaoxon				
			15	1.8
			20	2.6
	Female	74.7	10	0.71
			15	1.1
			20	1.5

AChE: acetylcholinesterase; BMD: benchmark dose; BMD$_{10}$: estimated benchmark dose for a 10% inhibition; F: female; M: male

Source: Reiss (2006a)

Reiss (2006b) undertook a similar analysis of erythrocyte and brain acetylcholinesterase activity after repeated oral dosing of rat pups with malathion or malaoxon from postnatal days 11–21. The results are summarized in Table 32. The authors concluded that the use of a BMD$_{20}$ for erythrocyte acetylcholinesterase activity would be protective for the inhibition of brain acetylcholinesterase activity at the 10% level by at least a factor of 2.8 for malathion and 3.6 for malaoxon. The authors also proposed TAFs of 30 in males and 26–28 in females for the inhibition of erythrocyte acetylcholinesterase activity by malaoxon.

Table 32. BMDs for erythrocyte and brain AChE activities in rat pups

Compound	Sex	Brain AChE BMD$_{10}$	Erythrocyte AChE	
			Inhibition level (%)	BMD
Malathion	Male	91.2[a]	10	12.7
			15	20.1
			20	28.1
	Female	85.7[a]	10	13.6
			15	21.5
			20	30.3
Malaoxon	Male	> 4[b]	10	0.43
			15	0.67
			20	0.93
	Female	> 4[b]	10	0.53
			15	0.82
			20	1.1

AChE: acetylcholinesterase; BMD: benchmark dose; BMD$_{10}$: estimated benchmark dose for a 10% inhibition; bw: body weight

[a] Brain AChE data were insufficient to estimate a BMD: no inhibition occurred at 50 mg/kg bw per day while inhibition greater than 10% occurred at the next highest dose of 150 mg/kg bw per day.

[b] Brain AChE data were insufficient to estimate a BMD because inhibition was less than 10% at the highest dose of 4 mg/kg bw per day.

Source: Reiss (2006b)

Stannard (2006a) examined the time to peak effect on erythrocyte and brain acetylcholinesterase activities of a single gavage dose of malathion in young preweanling Crl:CD®(SD) IGS BR rats. In the first experiment, four groups of rats (6/sex) were administered a

single gavage dose of 150 mg/kg bw malathion (purity 96%) in corn oil on postnatal day 11 and terminated at 10, 20, 30 or 40 minutes after dosing. In a second experiment, which incorporated a control group of five rats per sex, four groups of rats (6/sex) were administered a single gavage dose of 150 mg/kg bw malathion on postnatal day 11 and terminated at 30, 50, 60 or 80 minutes after dosing. In a third experiment, three groups of rats (5/sex) were administered a single gavage dose of 150 mg/kg bw malathion and terminated at 60, 90 or 120 minutes after dosing. Assessment of mean erythrocyte and brain acetylcholinesterase activity over the three experiments indicated that the time to peak effect was approximately 50–60 minutes after dosing. However, due to a number of limitations the authors concluded that the study objective had not been met. In a repeat study (Stannard, 2006b), six groups of preweaning Crl:CD®(SD) IGS BR rats (8/sex) were administered a single gavage dose of 150 mg/kg bw malathion (purity 96%) in corn oil on postnatal day 11 and one group terminated at 30, 60, 90, 120, 240 or 360 minutes after dosing. A concurrent control group comprised 16 rats per sex. Blood and brain acetylcholinesterase activity were analysed. The level of inhibition of erythrocyte acetylcholinesterase activity was 72–57% after 30–60 minutes and 36–37% for brain acetylcholinesterase at 60 minutes. The time to peak effect on erythrocyte and brain acetylcholinesterase activities was 60 minutes.

A range-finding study by Stannard (2006c) assessed the effects of a single dose of malaoxon (purity 97.7%) in corn oil on acetylcholinesterase activity administered on postnatal day 11 to juvenile Crl:CD®(SD) IGS BR rats (up to 6/sex per dose). Three experiments collectively tested doses ranging from 0.1 to 30 mg/kg bw. Successive reductions in the dose were made across the three experiments as overt signs of toxicity (deaths and clinical signs within 30 minutes) and large reductions in erythrocyte and brain acetylcholinesterase occurred at 20 and 30 mg/kg bw. Toxicologically significant inhibition of erythrocyte acetylcholinesterase activity (i.e. > 20% inhibition) occurred at and above 0.5 mg/kg bw, while brain acetylcholinesterase activity inhibition occurred at and above 5 mg/kg bw. In the main study by Stannard (2006d), the time to peak effect on erythrocyte and brain acetylcholinesterase activities was examined following a single gavage dose of 7 mg/kg bw malaoxon (purity 97.7%) in corn oil to juvenile Crl:CD®(SD) IGS BR rats on postnatal day 11 (six groups of eight rats/sex). One group of rats per sex was terminated at 20, 40, 60, 90, 120 and 240 minutes after dosing, and erythrocyte and brain acetylcholinesterase activities analysed in all the rats, including a control group of 16 rats per sex. There were no mortalities or treatment-related clinical signs. The time to peak effect was determined to be 20 minutes, with the maximum level of inhibition of erythrocyte acetylcholinesterase activity at 48% in males and 49% in females, while brain acetylcholinesterase activity was inhibited by 46% in both sexes.

The effect of a single dose of malathion or malaoxon on acetylcholinesterase activity in juvenile Crl:CD®(SD) IGS BR rats was examined by Stannard (2006e). Both compounds were administered in corn oil by gavage on postnatal day 11 under the following conditions: groups of 12 rats per sex received malathion (purity 96%) at 0, 5, 15, 40 or 60 mg/kg bw; groups of 12 males received malaoxon (purity 97.7%) at 0, 0.5, 1.0, 2.0 or 3.5 mg/kg bw; groups of 12 females received malaoxon (purity 97.7%) at 0, 0.1, 0.25, 0.75 or 2.0 mg/kg bw. The rats that received malaoxon were terminated after 20 minutes and those that received malathion were terminated after 60 minutes. Blood and brain acetylcholinesterase activity was analysed. In malathion-dosed rats, there were no deaths or clinical signs. Toxicologically significant inhibition of erythrocyte and brain acetylcholinesterase activity occurred in both sexes at 40 and 60 mg/kg bw (Table 33). In malaoxon-dosed rats, there were no deaths or clinical signs. Toxicologically significant inhibition of erythrocyte and brain acetylcholinesterase activity occurred in males at 2.0 and 3.5 mg/kg bw, and in females at 0.75 and 2.0 mg/kg bw (Table 33).

Table 33. Inhibition of acetylcholinesterase in juvenile rats following an acute oral dose of malathion or malaoxon

Treatment	AChE activity per treatment per dose			
	Males		Females	
	Erythrocyte AChE (U/L)	Brain AChE (U/L)	Erythrocyte AChE (U/L)	Brain AChE (U/L)
Malathion				
0 mg/kg bw	2 250	4 529	2 252	4 500
5 mg/kg bw	2 102 (6.6%)	4 050 (−10.6%)	2 084 (−7.5%)	3 917 (−13.0%)
15 mg/kg bw	1 979 (−12.0%)	3 854 (−14.9%)	1 994 (−11.5%)	3 779 (−16.0%)
40 mg/kg bw	1 306 (−41.9%)	3 629 (−19.9%)	1 429 (−36.5%)	3 300 (−26.7%)
60 mg/kg bw	973 (−56.8%)	3 075 (−32.1%)	783 (−65.2%)	2 725 (−39.4%)
Malaoxon				
0 mg/kg bw	2 225	4 313	2 205	4 338
0.1 mg/kg bw	–	–	2 115 (−4.1%)	4 242 (−3.3%)
0.24 mg/kg bw	–	–	1 914 (−13.2%)	4 029 (−8.2%)
0.5 mg/kg bw	2 148 (−3.5%)	4 242 (−1.6%)	–	–
0.75 mg/kg bw	–	–	1 394 (−36.8%)	3 638 (−17.1%)
1.0 mg/kg bw	2 007 (−9.8%)	4 133 (−4.2%)	–	–
2.0 mg/kg bw	1 429 (−35.8%)	3 850 (−10.7%)	865 (−60.8%)	3 054 (−30.4%)
3.5 mg/kg bw	1 046 (−53.0%)	3 408 (−21.0%)	–	–

AChE: acetylcholinesterase; bw: body weight; U: enzyme unit

Results expressed as the mean, with the % decrease (−) relative to the control in parentheses.

Source: Stannard (2006e)

Barnett Jr (2007) performed an acute range-finding study in juvenile Crl:CD[SD] rats to determine the effect of malaoxon on acetylcholinesterase activity. Groups of five rat pups per sex were administered malaoxon (purity 97.7%) in corn oil by gavage on postnatal day 11 at doses of 0, 3.5, 7 or 10 mg/kg bw. Blood and brain samples were collected approximately 20 minutes after dosing to analyse acetylcholinesterase activity. No deaths or clinical signs were observed. There was a dose-related reduction in erythrocyte acetylcholinesterase activity, which was toxicologically significant at 7 and 10 mg/kg bw (−18.1%, −33.0% and −42.8% in males and −19.5%, −35.7% and −51.3% in females at 3.5, 7 and 10 mg/kg bw). In both sexes, toxicologically significant inhibition of brain acetylcholinesterase occurred only at 10 mg/kg bw (−27%).

Barnett Jr (2008a) examined the time to peak effect on cholinesterase activity of an acute oral dose of malathion or malaoxon in juvenile Crl:CD[SD] rats. Pups were administered malathion (purity 96.0%) in corn oil by gavage on postnatal day 11 at a dose of 0 (32/sex) or 150 mg/kg bw (56/sex) or to malaoxon (purity 97.7%) at 10 mg/kg bw (56/sex). Eight pups per sex were terminated at 20, 40, 60, 80, 100, 120 and 150 minutes to analyse erythrocyte and brain acetylcholinesterase activities. Five male pups and nine female pups administered malathion displayed whole-body tremors at 18–62 minutes after dosing. One female pup dosed with malaoxon displayed whole-body tremors 36 minutes after dosing. In malathion-treated rats, the inhibition of erythrocyte acetylcholinesterase activity ranged from −24.2% to −44.3% in males and −30.0% to −53.6% in females, with the time to peak effect of 40–60 minutes. The inhibition of brain acetylcholinesterase activity ranged from −20.0% to −36.4% in males and −25.2% to −55.5% in females, with the time to peak effect of 60–80 minutes. In malaoxon-treated rats, the inhibition of erythrocyte

acetylcholinesterase activity ranged from −24.2% to −44.3% in males and −30.0% to −53.6% in females, with the time to peak effect of 40–60 minutes. The inhibition of brain acetylcholinesterase activity ranged from −18.6% to −30.9% in males and −5.9% to −32.7% in females, with the time to peak effect of 60–80 minutes.

Barnett Jr (2008b) examined the time to peak effect on cholinesterase activity in juvenile Crl:CD[SD] rats following an acute oral dose of malaoxon. In a range-finding experiment, rat pups received a single gavage dose of 12.5 (five/sex) or 15 mg/kg bw (15/sex) malaoxon (purity 96.0%) in corn oil on postnatal day 11 and were observed for up to 4 hours after dosing. In the main experiment, groups of 40 pups per sex were dosed with 12.5 mg/kg bw malaoxon on postnatal day 11. A vehicle control group comprised eight pups per sex. At 10, 30, 60, 90 and 240 minutes after dosing, four pups per sex were terminated and their blood and brain acetylcholinesterase activity analysed. In the range-finding experiment, deaths occurred in males (one and five pups at 12.5 and 15 mg/kg bw, respectively) and females (three pups at 15 mg/kg bw). Decreased motor activity was observed in one male at 12.5 mg/kg bw, while decreased motor activity, salivation and loss of righting reflex were observed in two males and three females at 15 mg/kg bw. In the main experiment, there were no deaths, while five males and seven females had slight or moderate tremors 23–87 minutes after dosing. One other male had intermittent whole-body twitches at 30 minutes after dosing. Erythrocyte acetylcholinesterase was inhibited at every time point, with maximum inhibition 60 minutes after dosing in males (−78.4% compared to the control) and 30–90 minutes in females (−75.8% to −79.1% compared to the control). Brain acetylcholinesterase activity was inhibited in both sexes, reaching maximum inhibition at 60 minutes in males (−54.6% compared to the control) and 90 minutes in females (−49.8% compared to the control).

Barnett Jr (2008c) examined the time to peak effect of malathion on cholinesterase activity in juvenile Crl:CD[SD] rats. Rat pups were administered a single gavage dose of malathion (purity 96.0%) in corn oil at 0 (8 pups/sex) or 150 mg/kg bw (40 pups/sex) on postnatal day 11. At 30, 60, 80, 100 and 150 minutes after dosing, eight pups per sex were terminated and blood and brain acetylcholinesterase activity analysed. All pups survived to scheduled termination. Clinical signs were observed 16–143 minutes after dosing and included whole-body tremors (10 males, 6 females), whole-body and head tremors (five males, five females), whole-body tremors and body jerks, (1 male), body jerks (2 females), head tremors (1 male, 1 female) and whole-body and head tremors, and body jerks (1 female). Erythrocyte acetylcholinesterase was inhibited in both sexes at every time point (−47.2% to −75.8% in males and −55.3% to −78.0% in females) reaching a maximum at 60 minutes after dosing. Brain acetylcholinesterase activity was inhibited in both sexes at every time point (−29.3% to −69.6% in males and −36.4% to −68.7% in females), reaching a maximum at 60 minutes after dosing.

Barnett Jr (2008d) compared the effect of malathion and malaoxon on erythrocyte and brain acetylcholinesterase activities at the time of peak effect in juvenile Crl:CD[SD] rats. On postnatal day 11, groups of 12 pups per sex were administered a single gavage dose of malathion (purity 96.0%) in corn oil at 0, 10, 25, 50, 100 or 150 mg/kg bw. Separate groups of 12 pups per sex received malaoxon (purity 97.7%) at 0, 1.0, 3.5, 7.0, 10.0 or 12.5 mg/kg bw. At 60 minutes after dosing, the pups were terminated and blood and brain acetylcholinesterase activity analysed. In malathion-dosed rats, there were no treatment-related deaths. Slight or moderate whole-body tremors were observed at 100 (five males, one female) and 150 mg/kg bw (five males, seven females), while body jerks were observed at 150 mg/kg bw (3 females) at 41–57 minutes after dosing. Toxicologically relevant and statistically significant inhibition of erythrocyte acetylcholinesterase occurred at and above 50 mg/kg bw in both sexes, while brain acetylcholinesterase activity was significantly lower than the control at 100 and 150 mg/kg bw (Table 34). In malaoxon-dosed pups, slight whole-body tremors were observed between 33 and 57 minutes after dosing (one male and three females at 10 mg/kg bw; two males at 12.5 mg/kg bw). Toxicologically and statistically significant inhibition of erythrocyte

acetylcholinesterase activity occurred at and above 3.5 mg/kg bw, while brain acetylcholinesterase was significantly lower than the control at and above 7.0 mg/kg bw in males and at 10.0 and 12.5 mg/kg bw in females (Table 25).

Table 34. Inhibition of erythrocyte and brain acetylcholinesterase in rat pups 60 minutes after a single dose of malathion or malaoxon

Treatment	AChE activity per treatment per dose			
	Males		Females	
	Erythrocyte (U/mL)	Brain (U/g)	Erythrocyte (U/mL)	Brain (U/g)
Malathion				
0 mg/kg bw	2.04	5.11	2.173	4.98
10 mg/kg bw	1.95 (−4.7%)	5.20	1.944 (−10.5%)	5.11
25 mg/kg bw	1.75 (−14.5%)	5.11 (−0.1%)	1.780* (−18.1%)	4.86 (−2.4%)
50 mg/kg bw	1.25** (−39.0%)	4.38 (−14.3%)	1.550** (−28.7%)	4.66 (−6.6%)
100 mg/kg bw	1.04** (−49.0%)	3.33** (−34.9%)	0.801** (−63.1%)	2.89* (−42.0%)
150 mg/kg bw	0.67** (−67.3%)	2.82** (−44.8%)	0.483** (−77.8%)	1.76** (−64.7%)
Malaoxon				
0 mg/kg bw	2.32	5.14	2.127	5.14
1 mg/kg bw	1.89 (−18.4%)	5.27	1.77 (−16.8%)	5.07 (−1.5%)
3.5 mg/kg bw	1.13** (−51.3%)	4.65 (−9.6%)	1.20** (−43.8%)	4.65 (−9.5%)
7 mg/kg bw	0.63** (−72.7%)	3.82** (−25.8%)	0.73** (−65.5%)	4.31 (−16.2%)
10 mg/kg bw	0.55** (−76.3%)	2.84** (−44.8%)	0.55** (−74.0%)	2.66** (−48.3%
12.5 mg/kg bw	1.05** (−54.7%)	3.95 (−23.2%)	0.49** (−76.9%)	2.50** (−51.3%)

AChE: acetylcholinesterase; bw: body weight; U: enzyme unit; *: $P < 0.01$; **: $P < 0.001$

Results expressed as the mean, with the % decrease (−) relative to the control in parentheses.

Source: Barnett Jr (2008d)

An acute range-finding study was conducted by Barnett Jr (2008e) in adult Crl:CD[SD] rats to determine the time to peak effect of erythrocyte acetylcholinesterase activity inhibition by malathion (purity 96%), desmethyl-malathion sodium (45.9% purity as the free acid), MMCA (purity 92.2%) or MDCA (purity 98.8%). The vehicle was DMSO. Rats were administered a single gavage dose of malathion at 0, 1000, 1500 or 2000 mg/kg bw (10/sex per dose) or a single gavage dose of MMCA (10/sex per dose), MDCA (10/sex per dose) or desmethyl-malathion sodium (5/sex per dose) at 1500 mg/kg bw. The rats were observed for mortality and clinical signs following dosing. Blood was sampled (five rats/sex per dose) prior to dosing and at 2 and 8 hours after dosing to analyse erythrocyte acetylcholinesterase activity. The rats (five rats/sex per dose) were terminated after 2 or 8 hours and their brains analysed for acetylcholinesterase activity.

There were no deaths. Clinical signs were confined to malathion-treated rats (1500 and 2000 mg/kg bw) and included red perioral substance; urine-stained abdominal fur; excess salivation; prostrate posture; decreased motor activity, fasciculations on the head, whole body and/or both hindpaws, no pupillary response; hunched posture; gasping; rales; ataxia; lacrimation; miosis; whole-body twitches, slight whole-body tremors; and bradypnea. The results of erythrocyte and brain acetylcholinesterase activity are summarized in Table 35. At every dose of malathion, erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05; 23–69%) than the control in both sexes. At 1500 and 2000 mg/kg bw, brain acetylcholinesterase activity was significantly lower ($P < 0.01$) than the control in females 8 hours after dosing. Toxicologically significant (i.e. > 20%) inhibition of brain acetylcholinesterase activity occurred at 2000 mg/kg bw in both sexes 2 hours after

dosing and in males at 8 hours after dosing. Of the metabolites, only MMCA caused a significant reduction in erythrocyte acetylcholinesterase activity 8 hours after a dose of 1500 mg/kg bw. All remaining cholinesterase levels in the three metabolites were comparable with the controls.

Table 35. Effect of a single dose of malathion or malathion metabolites on acetylcholinesterase activity

| | AChE activity per treatment per dose | | | |
| | Erythrocyte AChE (U/mL) | | Brain AChE (U/g) | |
Treatment	Males	Females	Males	Females
Malathion				
Control – 2 hours	1.03	1.24	14.68	15.05
1 000 mg/kg bw – 2 hours	0.74* (−28%)	0.96* (−23%)	15.30 (+4%)	14.45 (−4%)
1 500 mg/kg bw – 2 hours	0.59** (−43%)	0.70** (−44%)	13.98 (−5%)	11.14 (−26%)
2 000 mg/kg bw – 2 hours	0.53** (−49%)	0.38** (−69%)	9.98 (−32%)	5.29 (−65%)
Control – 8 hours	1.32	1.27	15.77	15.10
1 000 mg/kg bw – 8 hours	0.85** (−36%)	0.64** (−50%)	14.43 (−8.5%)	12.05 (−20%)
1 500 mg/kg bw – 8 hours	0.60** (−55%)	0.49** (−61%)	12.17 (−23%)	8.25** (−45%)
2 000 mg/kg bw – 8 hours	0.45** (−66%)	0.56** (−56%)	8.71 (−45%)	8.03** (−47%)
Desmethyl-malathion sodium				
1 500 mg/kg bw – 2 hours	Not analysed	Not analysed	Not analysed	Not analysed
1 500 mg/kg bw – 8 hours	1.24 (−20%)	1.30 (+5%)	15.50 (−2%)	15.12 (0%)
MMCA				
1 500 mg/kg bw – 2 hours	0.99 (−4%)	1.11 (−10%)	14.75 (0%)	15.20 (+1%)
1 500 mg/kg bw – 8 hours	0.94** (−29%)	1.15 (−9%)	14.23 (−10%)	14.98 (−1%)
MDCA				
1 500 mg/kg bw – 2 hours	0.99 (−4%)	1.10 (−11%)	14.19 (−3%)	14.37 (−5%)
1 500 mg/kg bw – 8 hours	1.17 (−12%)	1.23 (−3%)	14.67 (−7%)	14.74 (−2%)

AChE: acetylcholinesterase; bw: body weight; MDCA: malathion dicarboxylkic acid; MMCA: malathion monocarboxylic acid; U; enzyme unit; *: $P < 0.01$; **: $P < 0.001$

Results expressed as the mean, with the % increase (+) or decrease (−) relative to the control in parentheses.

Source: Barnett (2008)

Reiss & Edwards (2008) conducted BMD modelling of brain and erythrocyte acetylcholinesterase inhibition based on the studies of Fulcher (2001) and Barnett Jr. (2008). BMDs for malathion were estimated for 10% and 20% response levels for brain acetylcholinesterase cholinesterase inhibition and at 10%, 20% and 30% response levels for erythrocyte acetylcholinesterase inhibition. Adequate dose–response data are not available for desmethyl-malathion sodium, MMCA or MDCA. For brain acetylcholinesterase activity, the BMD_{10} of malathion was estimated to be 1078 mg/kg bw in males and 739 mg/kg bw in females, while the BMD_{20} was 1433 mg/kg bw and 1024 mg/kg bw, respectively. For the inhibition of erythrocyte acetylcholinesterase activity, the BMD_{10} was estimated to be 181 mg/kg bw in males and 183 mg/kg bw in females; the BMD_{20} was 390 mg/kg bw and 388 mg/kg bw, respectively; the BMD_{30} was 638 mg/kg bw and 620 mg/kg bw, respectively.

Reiss (2008) undertook BMD modelling of erythrocyte and brain acetylcholinesterase data from an acute oral cholinesterase study with malathion and malaoxon in juvenile rats (Barnett Jr, 2008d) to estimate an acute TAF for malaoxon. BMDs were estimated using USEPA methodologies developed for the Organophosphate Cumulative Risk Assessment, including a simple model that represents an exponential decline in cholinesterase levels with dose and an expanded model that includes saturable metabolism and allows for a minimal response at low doses before beginning an exponential decline. For erythrocyte acetylcholinesterase inhibition, the simple model provided the best fits to the dose–response data. For brain acetylcholinesterase, the expanded model provided the best fit for the malathion data and the simple model provided the best fit for the malaoxon data. The BMDs and TAFs estimated for acetylcholinesterase inhibition are summarized in Table 36.

Table 36. BMDs and TAFs for AChE inhibition in juvenile rats

Compound	Sex	BMD_{10} (mg/kg bw)	BMD_{15} (mg/kg bw)	BMD_{20} (mg/kg bw)
Erythrocyte AChE				
Malathion	Male	10.8	16.0	23.6
	Female	12.3	19.0	26.1
Malaoxon	Male	0.50	0.77	1.1
	Female	0.69	1.1	1.5
TAF	Male	21.6	21.9	21.5
	Female	17.8	17.3	17.4
Brain AChE				
Malathion	Male	41.6	–	–
	Female	39.6	–	–
Malaoxon	Male	2.8	–	–
	Female	3.6	–	–
TAF	Male	14.8	–	–
	Female	11.0	–	–

AChE: acetylcholinesterase; BMD: benchmark dose; BMD_{10}: estimated benchmark dose for 10% inhibition, BMD_{20}: estimated benchmark dose for 20% inhibition; BMD_{30}: estimated benchmark dose for a 30% inhibition; bw: body weight; TAF: toxicity adjustment factor

Source: Reiss (2008)

Barnett Jr (2014) examined the time to peak effect on cholinesterase activity of an acute gavage dose of 200 mg/kg bw MDCA (purity 98.8%) in groups of 32 adult Crl:CD[SD] rats per sex. Control groups of 16 rats per sex were dosed with the vehicle (DMSO). The rats were observed for one hour after dosing and prior to scheduled termination (eight rats per sex per group at 2, 4, 8 and 12 hours after dosing). Blood and brain samples were analysed for acetylcholinesterase activity after termination. There were no deaths or treatment-related clinical signs. There was no treatment-related effect on either erythrocyte or brain acetylcholinesterase activity.

(c)   Immunotoxicity

Groups of 15 female Crl:CD-1[ICR] mice were exposed to malathion (purity 96.0%) at dietary concentrations of 0, 50, 100, 700 or 7000 ppm ad libitum for 6 weeks (equivalent to 0, 8.9, 17.6, 126.8 and 1215.8 mg/kg bw per day, respectively). An additional group of 15 mice served as the positive control. Four days prior to termination, all the mice were administered an intravenous dose of sheep red blood cells. Positive controls also received 50 mg/kg per day cyclophosphamide for four days prior to sacrifice (10 mg/mL). Mortality and clinical signs were observed throughout the

exposure period, and body weight and feed consumption recorded. acetylcholinesterase activity was analysed in blood and brain samples collected at termination. All the mice were necropsied and the spleen and thymus weighed. The spleen was retained for immunological evaluation. There no deaths, treatment-related clinical signs or effects on body weight or feed consumption. There was no treatment-related macroscopic findings or effects on spleen or thymus weights. In contrast, absolute and relative thymus and spleen weights were significantly reduced ($P < 0.01$) in the positive control. Statistically significant and toxicologically relevant inhibition of erythrocyte acetylcholinesterase occurred at 700 and 7000 ppm (-51.2% and -87.1%, respectively; $P < 0.01$). There was no effect on brain acetylcholinesterase activity. Immunological evaluation revealed no change in spleen cell numbers or spleen immunoglobulin-M response to sheep red blood cells.

The NOAEL for immunotoxicity was 7000 ppm (equal to 1215.8 mg/kg bw per day), the highest tested dose (Barnett Jr, 2011d).

(d)   In silico toxicity predictions

The theoretical toxicity of malathion and a storage impurity, 2-mercaptosuccinic acid diethyl ester, were estimated by Clerkin (2015) using quantitative structure–activity relationships (QSAR) contained within the Organisation for Economic Co-operation and Development (OECD)–developed QSAR Application Toolbox (Version 3.2; 2013). Alerts for the storage impurity were generally comparable with malathion (Table 37). Malathion is a Cramer Class III compound while the storage impurity is in Cramer Class I. There were no structural alerts for DNA binding using the OECD QSAR Application Toolbox.

The storage impurity had a low-level alert for 'keratinocyte gene expression' that was not present for malathion. However, as a guinea-pig maximization test had previously demonstrated positive results with malathion, this alert for the storage impurity is of limited toxicological concern. The estimated oral and dermal LD_{50} values for malathion using read-across were 4140 and 2260 mg/kg bw, respectively, while those for the storage impurity were 3870 and 3040 mg/kg bw, respectively. The general pattern of results suggests that the impurity is of no greater toxicity than malathion.

Table 37. In silico toxicity predictions for malathion and a storage impurity

End-point	Malathion	2-Mercaptosuccinic acid diethyl ester
DNA binding by OASIS v 1.2	SN2 SN2 >> DNA alkylation SN2 >> DNA alkylation >> Alkylphosphates, Alkylthiophosphates and Alkylphosphonates	Radical Radical >> Generation of reactive oxygen species Radical >> Generation of reactive oxygen species >> Thiols
DNA binding by OECD	No alert found	No alert found
DPRA cysteine peptide depletion	Not possible to classify according to these rules	Not possible to classify according to these rules
DPRA lysine peptide depletion	Not possible to classify according to these rules	Not possible to classify according to these rules
Estrogen receptor binding	Non-binder, non-cyclic structure	Non-binder, non-cyclic structure
Protein binding potency	Not possible to classify according to these rules (GSH)	Not possible to classify according to these rules (GSH)
Toxic hazard classification by Cramer (original)	High (Class III)	Low (Class I)
Bioaccumulation – metabolism half-lives	Very fast	Very fast

End-point	Malathion	2-Mercaptosuccinic acid diethyl ester
Carcinogenicity (genotox and non-genotox) alerts by ISS	No alert found	No alert found
DNA alerts for Ames, MN and CA by OASIS v1.2	SN2 SN2 >> DNA alkylation SN2 >> DNA alkylation >> Alkylphosphates, Alkylthiophosphates and Alkylphosphonates	No alert found
Eye irritation/corrosion Exclusions rules by BfR	No alert found Solubility < 0.01 g/kg	No alert found Solubility < 0.01 g/kg
Eye irritation/corrosion inclusion rules by BfR	Inclusion rules not met	Inclusion rules not met
In vitro mutagenicity (Ames test) alerts by ISS	No alert found	No alert found
In vivo mutagenicity (micronucleus) alerts by ISS	H-acceptor-path3-H-acceptor	H-acceptor-path3-H-acceptor
Keratinocyte gene expression	Not possible to classify according to these rules	Low gene expression Low gene expression >> Thiols
Protein binding alerts for skin sensitization by OASIS v1.2	SN2 SN2 >> Nucleophilic substitution at sp3 carbon atom SN2 >> Nucleophilic substitution at sp3 carbon atom >> (Thio)Phosphates	SN2 SN2 >> Interchange reaction with sulfur-containing compounds SN2 >> Interchange reaction with sulfur-containing compounds >> Thiols and disulfide compounds
rtER Expert System ver.1 – USEPA	No alert found	No alert found
Skin irritation/corrosion exclusion rules by BfR	No alert found Solubility < 0.01 g/kg	No alert found Solubility < 0.01 g/kg
Skin irritation/corrosion inclusion rules by BfR	Inclusion rules not met	Inclusion rules not met
Repeated dose (HESS)	Not categorized	Not categorized

BfR: German Bundesinstitut für Risikobewertung; CA: chromosomal aberrations: DPRA: direct peptide reactivity assay; GSH: glutathione; HESS: Hazard Evaluation Support System; ISS: Istituto Superiore di Sanità; OASIS: Organization for the Advancement of Structured Information Standards; OECD: Organisation for Economic Co-operation and Development; MN, micronuclei; ROS; reactive oxygen species; rtER: rainbow trout estrogen receptor; SN2, bimolecular nucleophilic substitution; USEPA: United States Environmental Protection Agency
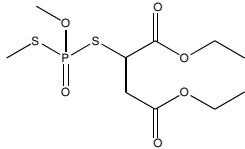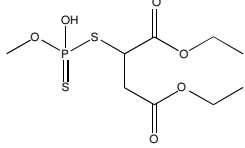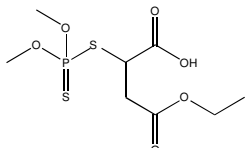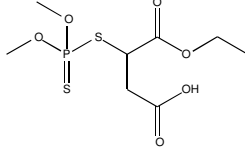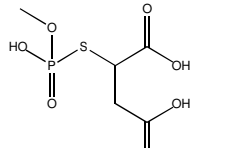
Source: Clerkin (2015)
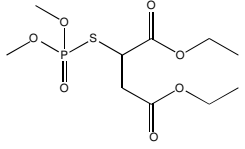
(e) Intestinal microbiota effects

An extensive literature search indicated that malathion had no potential adverse effects on the intestinal microbiota. Based on the mode of action, malathion would most likely not affect the intestinal microbiota. In addition, no studies were found on the ability of intestinal microbiota to metabolize malathion.

(f)   *Studies on metabolites and impurities*

Toxicity tests were conducted on malathion metabolites, the details of which are in Table 38.

Table 38. Malathion metabolites or impurities

Common name	Chemical name (CAS)	Structure	Description
Malaoxon	Butanedioic acid, 2-[(dimethoxyphosphinyl)thio]-1,4-diethyl ester		Rat metabolite Crop metabolite (apple, cotton, wheat, alfalfa, lettuce)
Isomalathion	Butanedioic acid, 2-[[methoxy(methylthio)phosphinyl]thio]-1,4-diethyl ester		Crop metabolite (alfalfa)
Desmethyl malathion	Butanedioic acid, 2-[(mercaptomethoxyphosphinyl)thio]-1,4-diethyl ester		Rat metabolite Crop metabolite (apple, cotton, wheat, alfalfa, lettuce) Simulated processing metabolite
Malathion monocarboxylic acid (MMCA)	Butanedioic acid, 2-[(dimethoxyphosphinothioyl)thio]-monoethyl ester		Rat metabolite Crop metabolite (apple, cotton, wheat, alfalfa, lettuce) Livestock metabolite (lactating goat, laying hen)
Malathion dicarboxylic acid (MDCA)	Butanedioic acid, 2-[(dimethoxyphosphinothioyl)thio]-monoethyl ester		Rat metabolite Crop metabolite (apple, cotton, wheat, alfalfa, lettuce) Livestock metabolite (lactating goat, laying hen)
Desmethyl-malaoxon dicarboxylic acid	Butanedioic acid, 2-[(methoxyphosphinyl)thio]		Processing metabolite – generated by the high-temperature hydrolysis of MDCA

CAS: Chemical Abstracts Service

Acute toxicity

The results of acute oral toxicity tests on malathion metabolites in rats are summarized in Table 39.

Table 39. Results of studies of the acute toxicity of malathion metabolites and impurities

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD$_{50}$ (mg/kg bw) or LC$_{50}$ (mg/L)	Reference
Desmethyl malathion, sodium salt							
Rat	CD	F	Oral	92.7	DMSO	>2 000[a]	Pratt (2005)
Desmethyl-malathion monocarboxylic acid, potassium salt							
Rat	Crl:WI(Han)	F	Oral	77.6	Water	>2 000	Leoni (2012)
MMCA							
Rat	CD	F	Oral	92.2	DMSO	>2 000[b]	Sanders (2008a)
MDCA							
Rat	CD	F	Oral	98.8	DMSO	>2 000	Sanders (2008b)
Malaoxon							
Rat	SD derived, albino	F	Oral	97.7	Distilled water	50	Lowe (2011b)
Desmethyl-malaoxon dicarboxylic acid, trisodium salt							
Rat	WISTAR rats Crl:WI(Han)	F	Oral	23.9	Nil	>2 000	Allingham (2015)

bw: body weight; F: female; LC$_{50}$: median lethal concentration; LD$_{50}$: median lethal dose; DMSO: dimethyl sulfoxide; MDCA: malathion dicarboxylic acid; MMCA: malathion monocarboxylic acid

Short-term studies of toxicity

In a 14-day range-finding study, Daly (1995) exposed Fischer 344 (CDF (F-344)/Crl BR) rats (5/sex per group) to malaoxon (purity 96%) at dietary concentrations of 0, 10, 25, 100, 2500 or 3500 ppm. The doses achieved were 0, 1.1, 3, 12.1, 293 and 387 mg/kg bw per day in males and 0, 1.1, 3.1, 12.5, 281.6 and 294.7 mg/kg bw per day in females, respectively. Mortalities, clinical signs, body weight and feed consumption were recorded. Haematology and clinical chemistry parameters, including plasma and erythrocyte cholinesterase activities, were analysed in blood collected during week 1 and/or at termination. Following termination, the rats were necropsied and their brain acetylcholinesterase activity analysed. No histopathology was performed. At 3500 ppm, one female was found dead on day 10 and another on day 12, with the remaining three rats terminated in a moribund condition on day 12. Clinical signs consisting of decreased faecal volume, hunched posture, tremors, pale appearance, anogenital staining, lethargy and emaciation were observed at and above 100 ppm in females and 2500 ppm in males. Body-weight gain was 30% lower than the control in high-dose males over the 2 weeks and 16.5% and 49% lower than the control in females at 2500 and 3500 ppm, respectively. Feed consumption was significantly lower ($P < 0.01$) than the control during the first week of exposure at 2500 and 3500 ppm (−12% and −21%, respectively, in males; −18% and −39%, respectively, in females). There was no treatment-related effect on haematological parameters and the majority of clinical chemistry parameters. During week 1, plasma cholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control at 100, 2500 and 3500 ppm (males: −10%, −85% and −82%, respectively; females: −26%, −89% and −92%, respectively). At termination, plasma cholinesterase activity was significantly lower ($P < 0.01$) than the control at 2500 and 3500 ppm in males (males: −88% and −91%, respectively) and at 3500 ppm in females (−93%). During week 1, erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$ or 0.05) than the control in males at 100, 2500 and 3500 ppm (−11%, −18% and −22%, respectively) and in females at the highest dose (−16%). At termination, erythrocyte acetylcholinesterase activity was significantly lower ($P < 0.01$) than the control only in males at 2500 and 3500 ppm (−24% and −17%,

respectively). At termination, brain acetylcholinesterase activity was significantly lower than the control ($P < 0.01$) in males at 2500 and 3500 ppm (−37% and −75%, respectively) and in females only at 3500 ppm (−67%). There were no treatment-related macroscopic findings.

Long-term studies of toxicity

In a pre-GLP study (NCI, 1979b), groups of 50 B6C3F1 mice and 50 F344 rats per sex were fed diets containing malaoxon (purity > 95%) at concentrations of 0, 500 or 1000 ppm for 103 weeks (estimated to be equal to 75 and 150 mg/kg bw per day, respectively, in mice and 25 and 50 mg/kg bw per day, respectively, in rats). Following an additional 2-week observation period, the rats were terminated at 103–105 weeks.

In male mice, there was a significant ($P = 0.028$) dose-related reduction in survival (90%, 84% and 74% at 0, 500 and 1000 ppm, respectively). In the second year of the study, a treatment-related increase in clinical signs consisted of alopecia, pale mucous membranes, abdominal distension and hunched posture. Graphically presented data showed high-dose females as having lower body weights than the control. There were no treatment-related neoplastic or non-neoplastic lesions.

In the rats, there was a small reduction in survival in high-dose males but this was not statistically significant (80%, 82% and 64% at 0, 500 and 1000 ppm, respectively). There were no treatment-related clinical signs or effects on body weight. In females, the combined incidence of C-cell adenomas and carcinomas of the thyroid was increased (0%, 2% and 11% at 0, 500 and 1000 ppm, respectively), although this was comparable to the historical control mean of 7%. Statistical analysis using the Cochran–Armitage test indicated that this increase was significant ($P = 0.009$), with pairwise comparisons using Fisher's exact test indicating a significant increase at the highest dose ($P = 0.024$). The incidence of gastric ulcers, commonly observed in the forestomach, was higher in treated rats (4%, 12% and 15% in males and 0%, 2% and 6% in females at 0, 500 or 1000 ppm, respectively).

The authors concluded that malaoxon was not carcinogenic in rats or mice. The slides from this study were re-examined by Rueber (1985) who concluded that there was an increase in neoplasms at all sites and on this basis malathion was carcinogenic. However, a subsequent re-evaluation of this study by Huff et al. (1985) confirmed the original conclusions of the study author and also concluded that there was equivocal evidence that malaoxon had increased the incidence of C-cell neoplasms of the thyroid.

Groups of 85 Fischer 344 (CDF (F-344)/CrlBR) rats per sex were exposed to malaoxon (purity 96.4%) admixed in the diet at concentrations of 0, 20, 1000 or 2000 ppm (equal to 0, 1, 57 and 110 mg/kg bw per day in males and 0, 1, 68 and 140 mg/kg bw per day in females). Groups of 55 rats per sex were retained for 24 months, while 10 rats per sex per group were terminated at 3, 6 and 12 months. Mortalities and clinical signs were recorded daily and body weight and feed consumption throughout the study. Ophthalmology was performed pretreatment and at 12 months and termination. Plasma, erythrocyte and brain cholinesterase activities were analysed in all rats. Clinical chemistry and haematology parameters were analysed in the blood from all the rats terminated at 6 and 12 months and in the 10 rats per sex per group that were retained at 18 months and termination. Urine was collected at 6, 12 and 18 months and at termination to analyse urinary parameters. All surviving rats were terminated at 24 months. All the rats were necropsied and selected organs weighed. Histopathological examinations were performed on controls and high-dose groups at 12 and 24 months and on rats that died or were terminated during the study. Selected tissues from animals at the intermediate and low doses were also examined.

There was a dose-related increase in mortality that was statistically significant ($P < 0.05$) in females at 1000 and 2000 ppm and in males at 2000 ppm at 24 months (Table 33). The incidence of early deaths was also significantly increased ($P < 0.01$ or 0.05) at the highest dose. Additional statistical analysis was undertaken by Nicolich (1998a) using the Thomas, Breslow and Garth

Analyses, which confirmed the effect on survivorship. The only treatment-related clinical sign was yellow anogenital staining in high-dose females throughout the study and in high-dose males from week 81. At the highest dose, absolute body weight was significantly lower ($P < 0.01$ or 0.05) than the control (−1.4% to −7.1% in males and −4.0% to −8.8% in females). Cumulative body-weight gain (from week 0) was significantly lower than the control ($P < 0.01$ or 0.05) in high-dose males during the first year of dosing (−31% to week 1 and then approximately −5% for the remainder of the first year) and in high-dose females throughout the study (from −49% to week one and approximately 10% thereafter). Cumulative body weight was also significantly lower ($P < 0.01$ and 0.05) than the control in females at 1000 ppm during the early part of the study (up to 13% lower than the control). At the highest dose, feed consumption was generally higher than the control throughout most of the study.

There were no treatment-related ophthalmological findings or effects on haematology, urine analysis parameters and the majority of clinical chemistry parameters. Results of the analysis of cholinesterase activity are summarized in Table 40. Plasma cholinesterase activity and erythrocyte acetylcholinesterase activity were significantly lower ($P < 0.01$ or 0.05) than the control at 1000 and 2000 ppm at every sampling point. At 6 months only, erythrocyte acetylcholinesterase activity was significantly lower than ($P < 0.01$) the control at 20 ppm in both sexes, but as the level of inhibition was at 20%, this finding was not considered toxicologically significant. Brain acetylcholinesterase activity was significantly lower than the control ($P < 0.01$ or 0.01) at 1000 and 2000 ppm. There were no other effects on clinical chemistry parameters.

Table 40. Summary of findings in male and female rats exposed to malaoxon for up to 2 years

Parameter	No. and per cent change compared to control per dietary concentration			
	0 ppm	20 ppm	1 000 ppm	2 000 ppm
Survival (%)				
Males				
12 months	100	98	98	100
18 months	100	91	96	91
24 months	71	65	58	47*
Females				
12 months	100	98	97	97
18 months	100	93	87	78
24 months	87	76	56*	51*
Early deaths (absolute number)				
Males	16	19	23	29*
Females	7	13	24**	27**
Plasma ChE activity (IU/mL)				
Males				
3 months	0.53	0.53	0.13** (−75%)	0.09** (−83%)
6 months	0.62	0.59	0.12** (−81%)	0.07** (−89%)
12 months	0.74	0.79	0.19* (−74%)	0.09** (−88%)
24 months	1.60	1.62	0.36** (−78%)	0.15** (−91%)
Females				
3 months	2.56	2.58	0.36* (−86%)	0.14** (−95%)
6 months	3.20	3.00	0.42** (−87%)	0.14** (−96%)
12 months	3.42	3.36	0.61* (−82%)	0.20** (−94%)

Parameter	No. and per cent change compared to control per dietary concentration			
	0 ppm	20 ppm	1 000 ppm	2 000 ppm
24 months	3.12	3.65	0.53* (−83%)	0.32 (−90%)
Erythrocyte AChE activity (IU/mL)				
Males				
3 months	1.06	0.93	0.40** (−62%)	0.45** (−58%)
6 months	1.15	0.91** (−21%)	0.39** (−66%)	0.43% (−63%)
12 months	1.25	1.08	0.49** (−61%)	0.44** (−65%)
24 months	1.25	1.12	0.68** (−46%)	0.70** (−44%)
Females				
3 months	1.25	1.00	0.47** (−62%)	0.53** (−58%)
6 months	1.29	1.04** (−19%)	0.56** (−57%)	0.52** (−60%)
12 months	1.43	1.18	0.58** (−59%)	0.49** (−66%)
24 months	1.32	1.10	0.72** (−45%)	0.71** (−46%)
Brain AChE activity (IU/g)				
Males				
3 months	10.45	10.25	9.51	8.53** (−18%)
6 months	10.22	10.49	10.03	9.05** (−11%)
12 months	11.45	11.24	10.58	9.46** (−17%)
24 months	10.73	10.61	7.52** (−30%)	2.82** (−74%)
Females				
3 months	10.57	10.38	9.34** (−12%)	2.30** (−78%)
6 months	10.29	10.43	9.64** (−6%)	3.97** (−61%)
12 months	11.27	11.27	10.67* (−5%)	4.26** (−62%)
24 months	10.77	10.63	9.26	4.40** (−62%)

AChE: acetylcholinesterase; BMD: benchmark dose; ChE: cholinesterase; ppm: parts per million; IU: International Unit; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the mean, with the % decrease (−) relative to the control in parentheses.

Source: Daly (1996b)

The only treatment-related macroscopic abnormality was emaciation, which occurred at the highest dose in males (9.4% versus 3.5% in the control) and at 1000 and 2000 ppm in females (14% and 15%, respectively, versus 0% in the control). In rats terminated after 12 months, increased absolute and relative liver weights (+22.4% and +14.8%, respectively; $P < 0.01$ or 0.05) and absolute kidney weights (+10.2%, $P < 0.05$) occurred in high-dose males. In rats terminated after 24 months, absolute and relative adrenals weights were increased in high-dose males (+12.6% and +33.3%, respectively; $P < 0.05$) and absolute and relative spleen weights were decreased in high-dose females (−50.5% and −45.6%, respectively; $P < 0.01$). None of these variations in organ weights were accompanied with any microscopic abnormalities or evidence of organ dysfunction.

Histopathological examination revealed a number of non-neoplastic findings at 1000 and 2000 ppm (Table 41). In the stomach, mineral deposits (minimal to moderate) were detected in the muscularis at the highest dose. In the nasal lumen, the presence of foreign material (minimal to severe) and inflammatory cell debris was increased (minimal to moderately severe) at 1000 and 2000 ppm. In the respiratory nasal mucosa, subacute or chronic inflammation (slight to moderately severe) and hyperplasia of goblet cells (slight to moderately severe) and hyperplasia of the respiratory epithelium (slight to moderately severe) was increased in females at 1000 and 2000 ppm and in males

at 2000 ppm. In the olfactory nasal mucosa, increased degeneration of the epithelium (slight to moderate) occurred in males at 2000 ppm and in females at 1000 and 2000 ppm. In females, there was an increase in the replacement of the epithelium with ciliated and non-ciliated columnar epithelial (slight to moderate severe), and hyperplasia of ciliated and non-ciliated columnar epithelial cells (slight to moderate severe) at 1000 and 2000 ppm. In the lung, oedema (minimal to moderate), subacute-chronic interstitial and purulent-chronic purulent inflammation (minimal to moderate) and foreign body granulomas (minimal to moderate) occurred at 2000 ppm in males and at 1000 and 2000 ppm in females. In the middle ear, subacute (chronic active)/chronic inflammation was accompanied by the accumulation of inflammatory cells or cells debris within the tympanic spaces at 1000 ppm in females and at 2000 ppm in both sexes. Collectively these effects were attributable to inhaled food particles resulting in tissue injury and inflammation to the nasal cavity, with secondary effects in the lungs and middle ear.

Table 41. Non-neoplastic findings in male and female rats exposed to malaoxon for 2 years

Parameter	No. per dietary concentration			
	0 ppm	20 ppm	1 000 ppm	2 000 ppm
Stomach – mineral deposits in the muscularis				
Males	2/65	1/55	13/55	25/64
Females	0/64	0/55	5/53	23/65
Nasal lumen – presence of foreign material				
Males	6/65	10/65	9/65	28/64
Females	1/65	6/63	17/64	27/65
Nasal lumen – inflammatory cell debris				
Males	13/65	21/65	15/65	31/64
Females	6/65	6/63	20/64	27/65
Nasal mucosa (respiratory) – subacute (chronic active) or chronic inflammation				
Males	11/65	11/65	10/65	21/64
Females	6/65	6/63	20/64	27/65
Nasal mucosa (respiratory) – epithelial hyperplasia				
Males	11/65	18/65	13/65	20/64
Females	3/65	5/63	27/64	20/65
Nasal mucosa (respiratory) – epithelium squamous or squamoid metaplasia				
Males	3/65	4/65	8/65	6/64
Females	0/65	1/63	6/64	5/65
Nasal mucosa (olfactory) – epithelium degeneration				
Males	4/65	6/65	5/65	12/64
Females	2/65	0/63	17/64	10/65
Nasal mucosa (olfactory) – olfactory epithelium replaced by ciliated and non-ciliated columnar epithelial cells				
Males	5/65	6/65	7/65	7/64
Females	2/65	2/63	11/64	10/65
Nasal mucosa (olfactory) – hyperplasia of ciliated and non-ciliated columnar epithelial cells				
Males	5/65	2/65	4/65	7/64
Females	1/65	1/63	11/64	7/65
Lung – oedema				

Parameter	No. per dietary concentration			
	0 ppm	20 ppm	1 000 ppm	2 000 ppm
Males	5/65	5/55	9/55	16/65
Females	1/64	3/55	22/55	17/65
Lung – inflammation of the interstitium				
Males	12/65	9/55	12/55	23/65
Females	14/64	15/55	29/55	34/65
Lung – purulent/chronic purulent inflammation or abscess(es)/chronic abscess(es)				
Males	4/65	2/55	7/55	17/65
Females	2/64	2/55	22/55	19/65
Lung – granulomatous inflammation/granulomas				
Males	8/65	3/55	11/55	12/65
Females	2/64	6/55	29/55	29/65
Middle ear (tympanic cavity/epithelial lining) – subacute (chronic active)/chronic inflammation/inflammatory cells/cell debris				
Males	8/54	5/16	7/22	15/58
Females	2/54	3/8	17/20	19/50

No.: number; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

Results expressed as the absolute number of rats / number of rats examined.

Source: Daly (1996b).

There was a significant dose-related trend in mononuclear cell leukaemia in males ($P = 0.03$) based on either the Cox's test and Gehan-Breslow test, but pairwise comparisons did not reveal any significant differences (20%, 22%, 35% and 25% at 0, 20, 1000 and 2000 ppm, respectively). Subsequently, Nicolich (1998b) undertook additional statistical analysis using the method Peto et al. (1980) described; this indicated that mononuclear cell leukaemia was increased in high-dose males. However, as the incidences were within the historical control range of both the NTP (10–72%) and the performing laboratory (15–36%), and as there was no dose–response relationship, this significant increase is not considered treatment related.

The NOAEL for chronic toxicity was 20 ppm (equal to 1 mg/kg bw per day in both sexes) based on mortality and the inhibition of brain acetylcholinesterase activity at 1000 ppm (equal to 57 mg/kg bw per day in males and 68 mg/kg bw per day in females). The NOAEL for carcinogenicity was 2000 ppm (equal to 110 mg/kg bw per day in males and 140 mg/kg bw per day in females), the highest test dose (Daly, 1996b).

Genotoxicity

The results of genotoxicity studies on malathion metabolites are described in section 2.4.

(g)   Studies on cholinesterase inhibition

Comparative studies on the inhibition of acetylcholinesterase activity in juvenile and adult rats by malathion metabolites are described in section 2.6(b).

3. Observations in humans

3.1 Dosing studies in volunteers

A randomized double-blind placebo-controlled ascending single-dose study was undertaken in human volunteers to determine the NOAEL for the inhibition of plasma and erythrocyte cholinesterase activities. The participants (38 men and 10 women, aged 18–50 years) were given a gelatine capsule containing malathion (purity 95.8%) over seven sessions at 0, 0.5, 1.5, 5, 10 or 15 mg/kg bw. In the first session, three men were given malathion at a dose of 0.5 mg/kg bw. In the second session, another three men were given malathion at a dose of 1.5 mg/kg bw. Subsequently, seven men were given malathion at a dose of 5.0 mg/kg bw. Three and four men received malathion at a dose of 10 mg/kg bw in two separate sessions. Over three separate sessions, three male, four male and seven female participants all received doses of 15 mg/kg bw. In each session, one or more participants received a placebo, which contained lactose.

The participants were kept under close observation from before dosing until 72 hours after dosing. Any symptoms or clinical signs were recorded and blood pressure, pulse rate, respiratory rate and body temperature monitored from the day before dosing, immediately before dosing and 2, 4, 8 and 24 hours after dosing. Twelve-lead electrocardiograms were performed 30 minutes before dosing and 2, 4, 8 and 24 hours after dosing, and single channel continuous electrocardiograms were performed from 30 minutes before dosing until 4 hours after dosing. Blood collected at screening, prior to dosing and 24 hours after dosing was analysed for haematological and clinical chemistry parameters. Urine was collected at screening and 24 hours after dosing for urine analysis. Plasma cholinesterase activity and erythrocyte acetylcholinesterase activity were analysed in blood samples collected on days 9, 7, 5, 2 and 1 and 30 minutes prior to dosing, and then at 1, 2, 4, 8, 12, 24 and 48 hours and days 4, 7 and 14 after dosing.

There were no treatment-related clinical effects, changes in haematological or clinical chemistry parameters or inhibition of plasma or erythrocyte cholinesterase activities. The NOAEL was 15 mg/kg bw, the highest tested dose (Gillies & Dickson, 2000).

Healthy male and female volunteers ingested a single gelatine capsule containing malathion (purity 95.8%) at a dose of 0, 0.5, 1.5, 5.0, 10.0 or 15.0 mg/kg bw after a light meal. For each of the two lowest dose groups, three volunteers received malathion and one received the placebo. All remaining groups consisted of seven treated and three control volunteers. Volunteers were observed closely for 48 hours in a clinic and were then followed-up for two weeks. Blood was sampled six times prior to dosing and at 1, 2, 4, 8, 12, 24 and 48 hours and 4, 7 and 14 days after dosing to analyse plasma and erythrocyte cholinesterase activities. There were no treatment-related adverse events or effects on plasma or erythrocyte cholinesterase activity (Jellinek, Schwartz & Connolly Inc., 2000).

The potential of malathion to effect cutaneous vasculature was examined by applying 0.5 mL of a 10 mg/mL malathion solution to the forearms of human volunteers for 5 hours under an occlusive dressing. Malathion increased blood flow within the region of application, and pretreatment with 10 mmol/L atropine attenuated this effect. Iontophoretic delivery of acetylcholinesterase within 1 hour of the removal of malathion or its vehicle caused a significant increase in blood flux at both the malathion and control-treated sites. Malathion had no effect on the concentration-dependent blood flux response to sodium nitroprusside. There was no treatment-related effect on nitric oxide or histamine levels (Boutsiouki & Clough, 2004).

The efficacy and clinical effects of malathion in the treatment of head lice was examined in school-age children (7–14 years old) and their families. Of a cohort of 629 students, 48 were found to have live head lice. Students who had lice and/or eggs were treated with 1% malathion shampoo by applying it to the dry scalp and hair for 10 minutes before washing it off. No estimates of the systemic doses were calculated. The shampoo was also given to family members and students without lice on

request. The participants were treated twice with the shampoo. Blood was collected from 32 volunteers before treatment and after the second treatment to analyse erythrocyte acetylcholinesterase activity. Reported side-effects among the 43 treated students included nausea (4%), a burning sensation (7%) and irritation (2%). Pairwise comparisons indicated that mean erythrocyte acetylcholinesterase activity was significantly decreased (P = 0.03; −7.5%) after two applications; 26 of the 32 participants showing a decrease in erythrocyte acetylcholinesterase ranging from −5 to −26% of pretreatment activity, with the remaining 6 students showing an increase of between +3 and +29% (Wananukul et al., 2011).

3.2 Occupational exposure studies

In a summary report by Nielsen (1994), no poisoning incidents and no inhibition of plasma cholinesterase was observed in workers involved in the manufacture of malathion over a 20-year period. In a subsequent summary report by Ravn Nielsen (1999), biological monitoring of workers employed at plants manufacturing dimethoate and malathion from 1994 to 1999 detected no work-related reduction in plasma cholinesterase activity.

3.3 Poisoning case reports

A workman was hit on his clothes by a mixture of MP-1 (O,O-dimethyldithiophosphate) and malathion during operations at a malathion plant in November 1998. There was no direct contact with his skin as his clothes absorbed the chemical, and he immediately took a shower. No clinical symptoms were noted. Subsequent assessment of cholinesterase showed no reduction in blood cholinesterase levels and the specific activity (activity/concentration) was normal (Ravn Nielsen, 1999).

3.4 Epidemiological studies

The pre-agreed evaluation process and Tier 1 screening criteria used to evaluate epidemiological studies on malathion (and diazinon and glyphosate) are described in "Section 2.2: Methods for the evaluation of epidemiological evidence for risk assessment" of the meeting report[13].

This evaluation considered several aspects of each study and of all studies combined in this evaluation, including factors that decrease the level of confidence in the body of evidence, including risk of bias, unexplained inconsistency, and imprecision, and factors that increase the level of confidence, including large magnitude of effect, dose–response and consistency (Guyatt et al., 2008; Morgan et al., 2016).

The findings for each study are summarized in Table 42, with findings for non-quantitative exposure assessment (predominantly ever-use vs never-use) shown in forest plots below.

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[13] Pesticide residues in food 2016: Special session of the joint FAO/WHO meeting on pesticide residues May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/).

Table 42. Results of Tier 1 evaluation and summary of cancer epidemiological studies on malathion

Study / Location	Details	Reference
Malathion/NHL		
Meta-analysis	Qualitative exposure only – ever-use/never-use of malathion. Meta risk ratio: 1.8 (95% CI: 1.4–2.2)	Schinasi & Leon (2014)
	Meta-analysis includes Waddell et al., 2001; Mills, Yang & Riordan, 2005; Pahwa et al., 2012. Does not include AHS (i.e. Bonner et al., 2007) or Eriksson et al., 2008. *N*s for each meta-analysis not presented	
AHS	Qualitative (ever/never) Risk estimates – aRR (95% CI) Ever-use: 0.64 (0.41–0.99) *N* = 34 exposed cases	Lerro et al. (2015)
AHS	Quantitative exposure – LEDs and IW-LEDs given Risk estimates – aRR (95% CI) Ever- vs never-use 0.9 (0.8–1.1) No exposure 1.0 (ref) LED ≤ 8.75 0.97 (0.7–1.3) LED >8.75–38.75 0.7 (0.5–1.1) LED > 38.75–737.5 0.9 (0.6–1.3) *P* for trend 0.63 No exposure 1.0 (ref) IW-LED – Low 1.0 (0.7–1.4) IW-LED – Med 0.8 (0.6–1.1) IW-LED – High 0.9 (0.6–1.2) *P* for trend 0.46 Total *N* = 26 737 with 269 incident NHL cases *N* = 179 exposed cases (LED analysis), 178 exposed cases (IW-LED analysis)	Alavanja et al. (2014)
AHS	Exclude - Alavanja et al. (2014) has longest follow-up	Bonner et al. (2007)
United States Midwest case–control studies	The study population overlaps with Waddell et al. (2001) and total *N* is smaller, but this study is not excluded as it provides more fully adjusted risk estimates for ever-use vs never-use analyses. Qualitative (ever/never) Risk estimates – aRR (95% CI) From a logistic regression model: Exposed 1.1 (0.6–1.8) From a hierarchical regression model: Exposed 1.1 (0.7–1.7) Both adjusted for other pesticides Total *N* = 2 583 (650 NHL cases, 1 933 controls) *N* = 53 exposed cases and 100 exposed controls	De Roos et al. (2003)

Study / Location	Details	Reference
United States Midwest case–control studies	The study population overlaps with De Roos et al. (2003) above. Quantitative exposure – days of use per year – for Nebraska only Risk estimates – aOR (95% CI) Exposed 1.6 (1.2–2.2) N = 91 exposed cases and 147 exposed controls. Restricted to direct respondent farmers: Exposed 1.2 (0.9–1.8) Adjusted for diazinon 1.1 (0.7–1.8) Adjusted for fonofos 1.1 (0.7–1.6) N = 68 exposed cases and 121 exposed controls Risk estimates – aOR (95% CI) Non-farmers: 1.0 (ref.) < 5 days/year: 2.1 (0.7–6.1) 5+ days/year: 1.5 (0.5–5.2) N = 12 exposed cases and 15 exposed controls (7/8 for < 5 days and 5/7 for 5+ days for exposed cases/controls, respectively)	Waddell et al. (2001)
Cross-Canada Study of Pesticides and Health	NB. Study population is almost the same as for McDuffie et al. (2001), minus N = 4 cases excluded due to pathology review. This study is not excluded as outcome classification is expected to be more accurate, and thus it provides the most robust risk estimate for ever-use vs never-use analysis. Qualitative (ever/never) Risk estimates – aOR (95% CI) Ever-use 1.96 (1.42–2.70) NB. Estimates also reported stratified by no/asthma; no/allergies; no/asthma or allergies or hay fever N = 72 exposed cases and 127 exposed controls	Pahwa et al. (2012)
Cross-Canada Study of Pesticides and Health	Exclude – Analysis focuses on malathion use in combination with other pesticides. Multiple 'malathion only' ORs reported but they vary in sample size by each 'combination' analysis. The largest 'malathion only' analysis gives the following result: aOR for ever-use: 1.75 (1.22–2.52) Total N = 158 (52 cases, 106 controls) However, these analyses were conducted in a very small selected subset of the Cross-Canada Study of Pesticides and Health with exposure to both malathion and another pesticide Because McDuffie et al. (2001) reports on the largest sample size, Hohenadel et al. (2011) is excluded	Hohenadel et al. (2011)
Cross-Canada Study of Pesticides and Health	The study population overlaps with Pahwa et al. (2012) (see above). Quantitative exposure – days of use per year (3 categories – cutpoints are given). Risk estimates – aOR (95% CI) Ever-use 1.83 (1.31–2.55) Unexposed 1.00 (ref) >0– <=2 days/year 1.82 (1.25–2.68) > 2 days/year 1.75 (1.02–3.03) Total N = 2 023 517 cases, 1 506 controls (overall); 179 cases, 456 controls (with telephone interview data, i.e. detailed pesticide information) N = 72 exposed cases, 127 exposed controls	McDuffie et al. (2001)

| Study / Location	Details	Reference
United Farm Workers of America	Qualitative (high vs low – no cutpoints; the researchers used work history and crop-exposure-matrix coupled with pounds applied) Risk estimates – aOR (95% CI) High vs low 1.77 (0.99–3.17) Total 131 cases, 651 controls. *N* exposed cases/controls not reported	Mills, Yang & Riordan (2005)
Sweden	Qualitative Risk estimates – aOR (95% CI) Ever-use 2.81 (0.54–14.7) *N* = 5 and 2 exposed cases and controls, respectively (few cases; not reported in main results but only in discussion)	Eriksson et al. (2008)
Malathion/Prostate cancer		
AHS	Quantitative exposure – quartiles – median values for quartiles given (results for IW-LEDs shown, results for LEDs not presented) Risk estimates – aRR (95% CI) Total prostate cancer Non-exposed 1.0 (ref.) Q1 IW-LED 1.03 (0.84–1.26) Q2 IW-LED 1.13 (0.94–1.36) Q3 IW-LED 1.11 (0.93–1.34) Q4 IW-LED 1.08 (0.90–1.29) *P* for trend 0.62 Aggressive prostate cancer Non-exposed 1.0 (ref.) Q1 IW-LED 1.19 (0.89–1.59) Q2 IW-LED 1.27 (0.97–1.67) Q3 IW-LED 1.28 (0.98–1.68) Q4 IW-LED 1.43 (1.08–1.88) *P* for trend 0.04 Total *N* = 54 412 (746 exposed and 328 non-exposed prostate cases; 374 and 140 exposed and non-exposed aggressive prostate cancers)	Koutros et al. (2013)
United Farm Workers of America	Quantitative – Quartiles of exposure, based on ecological exposure assessment (total pesticide use in the county in which the cases/controls were employed on a farm). Risk estimates – aOR (95% CI) High vs low: 0.96 (0.66–1.40) Quartile 1 (low): 1.00 (ref.) Quartile 2: 0.93 (0.62–1.39) Quartile 3: 1.01 (0.61–1.67) Quartile 4 (high): 1.04 (0.59–1.85) *P* for trend = 0.89 Total *N* = 1284 (222 cases, 1062 controls) *N* = 129 exposed cases (i.e. High or Quartiles 2–4)	Mills & Yang (2003)
Case–control study of prostate cancer in British Columbia	Quantitative – Ever vs low vs high exposure – based on median of lifetime cumulative exposure level for controls as a cutpoint (i.e. < 7.67, and > 7.67). However, no units are given for this exposure metric. Risk estimates – aOR (95% CI) Ever exposure: 1.34 (1.01–1.78) Unexposed: 1.0 (ref.) Low (< 7.67): 1.18 (0.78–1.78) High (> 7.67): 1.49 (1.02–2.18) *P* for trend = 0.03 Total *N* = 5152 (1153 cases, 3999 controls) *N* = 82 exposed cases, 210 exposed controls	Band et al. (2011)

AHS: Agricultural Health Study; aOR: adjusted odds ratio; aRR: adjusted risk ratio; CI: confidence interval; IW-LED: intensity-weighted lifetime-exposure days, defined as number of years of use × number of days used per year × personal protective equipment use reduction factor × intensity level score (a unit-less score which reflects a combination of self-reported pesticide exposure modifiers, e.g. pesticide mixing status, application method, equipment repair activities); LED: lifetime-exposure days (defined as number of years of use × number of days used per year); *N*: size of sample; NHL: non-Hodgkin lymphoma; NB.: *nota bene*; OR: odds ratio; Q: quartile

A.   Malathion / NHL

The evaluation included seven studies (Lerro et al., 2015; Alavanja et al., 2014; Waddell et al., 2001; Pahwa et al., 2012; McDuffie et al., 2001; Mills, Yang & Riordan, 2005; Eriksson et al., 2008) and one meta-analysis (Schinasi & Leon, 2014). The Agricultural Health Study (AHS)[14] found no evidence of elevated risk of NHL associated with malathion exposure in either men (Alavanja et al., 2014) or women (Lerro et al., 2015). In contrast, various case–control studies reported elevated risks of NHL associated with use of malathion: Waddell et al. (2001) report significant elevated risk of NHL associated with ever-use versus never-use of malathion (OR: 1.6; 95% CI: 1.2–2.2) from the United States Midwest pooled case–control studies; however, risk estimates were attenuated and no longer significant when a) proxy respondents were excluded, and b) analyses were mutually adjusted for other pesticides (diazinon, fonofos). Similarly, in a further analysis of the United States Midwest pooled case–control studies limited to just direct respondents, De Roos et al. (2003) found no association in risk estimates adjusted for all other pesticides. Significant elevated risks of NHL were reported from the Cross-Canada Study of Pesticides and Health for ever-use versus never-use of malathion (OR: 1.96; 95% CI: 1.42–2.70 by Pahwa et al., 2012; McDuffie et al., 2001), and when examining annual days of exposure. However, there was no clear exposure–response relationship across quantitative exposure categories (McDuffie et al., 2001). [NB. McDuffie et al. (2001) and Pahwa et al. (2012) report on the same study population, but are both included in this evaluation because Pahwa et al. (2012) provides the most robust risk estimate for 'ever-use' for this population whereas McDuffie et al. (2001) provide risk estimates for quantitative exposure categories]. Non-significant elevated risks of NHL were reported by two other case–control studies (Mills, Yang & Riordan, 2005; Eriksson et al., 2008). The report by Eriksson et al. (2008) is limited by the extremely small number of exposed cases and controls (5 and 2 respectively); the resulting uncertainty around the risk estimate reported means it adds little to the evidence base. Schinasi & Leon (2014) report a meta risk ratio of 1.8 (95% CI: 1.4–2.2) for ever-use versus never-use of malathion, but this was based only on Waddell et al. (2001), Mills, Yang & Riordan (2005), and Pahwa et al. (2012) and excluded the large AHS cohort (the relevant publication at the time would have been Bonner et al. (2007) and Eriksson et al. (2008)).
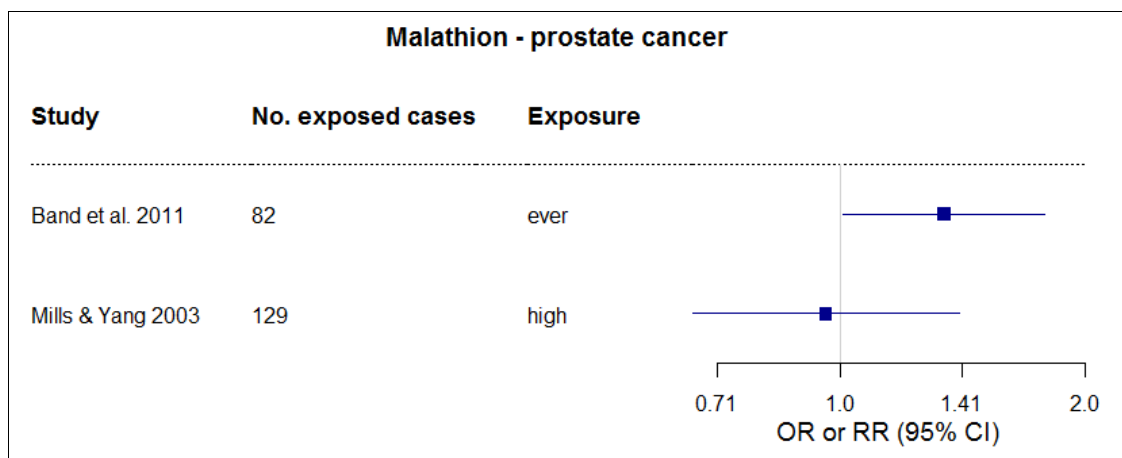
Fig. 3. Forest plot for risk estimates of studies with qualitative exposure categories
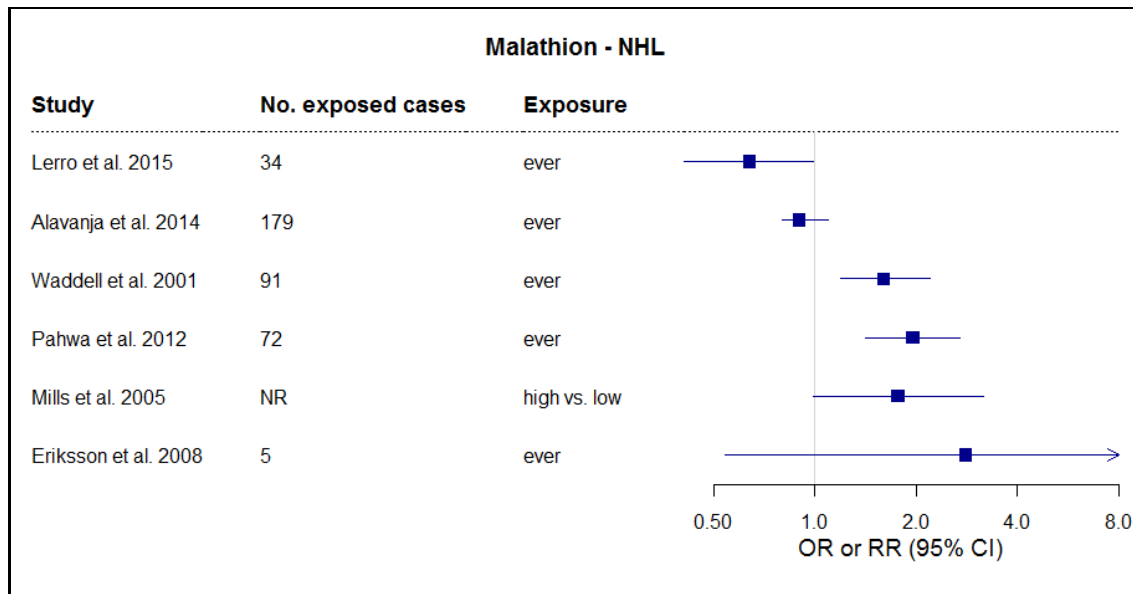
B.   Malathion / prostate cancer

The evaluation included three studies (Koutros et al., 2013; Band et al., 2011; Mills & Yang, 2003). There was no evidence of an association with all prostate cancer and malathion exposure in the AHS (Koutros et al., 2013). However, Koutros et al. (2013) observed a significant excess risk of aggressive prostate cancer (RR: 1.43; 95% CI: 1.08–1.88) in the highest exposure category (highest quintile of intensity-weighted lifetime days of malathion exposure) along with a significant exposure–response relationship (P for trend = 0.04). Band et al. (2011) observed a significant elevated risk of all prostate cancer in a case–control study for ever-use (OR: 1.34; 95% CI: 1.01–1.78) and for high exposure versus no exposure (OR: 1.49; 95% CI: 1.02–2.18) with a significant linear trend across exposure categories (P = 0.03). Both studies were large. However, the interpretation of results by Band et al. (2011) is limited by a potential for exposure misclassification in the assessment based job–exposure matrix as well as the potential for confounding due to other pesticides as there was no adjustment for their use. There was no evidence of an association between prostate cancer and malathion exposure in the United Farm Workers of America study (Mills & Yang, 2003); however, this study is limited by the use of ecological exposure assessment, with potential for considerable exposure misclassification.

Fig. 4. Forest plot for risk estimates of studies with qualitative exposure categories

Malathion - prostate cancer

Study	No. exposed cases	Exposure
Band et al. 2011	82	ever
Mills & Yang 2003	129	high

OR or RR (95% CI): 0.71, 1.0, 1.41, 2.0

Comments

Biochemical aspects

In a study conducted in rats using [^{14}C]malathion (Reddy, Freeman & Cannon, 1989), gastrointestinal absorption was at least 77% in males and 86% in females. The majority (up to 90%) of radioactivity was excreted in urine within 24 hours. Less than 1% of radioactivity was detected in tissues, with the highest proportions in the liver, skin, fat and gastrointestinal tract. There was no evidence that malathion or its metabolites accumulated in any tissue.

Malathion is extensively metabolized via desulfuration, oxidation, hydrolysis, dealkylation and demethylation reactions. In particular, the oxidative desulfuration of malathion in the liver generates malaoxon, which is a more potent inhibitor of acetylcholinesterase compared with malathion. The major metabolites detected in rat urine (> 80% of urinary radioactivity) were α- and β-monocarboxylic acids (MMCA) and the dicarboxylic acid (MDCA) of malathion. Other urinary metabolites include desmethyl malathion, *O,O*-dimethyl phosphorothioic acid, fumaric acid, 2-mercaptosuccinic acid, *O,O*-dimethyl phosphorodithioic acid, monoethyl fumarate and malaoxon. Malaoxon was observed only in urine samples and accounted for less than 2% of total urinary radioactivity. Similar metabolites were detected in human studies (Aston, 2000; Jellinek, Schwartz & Connolly Inc., 2000).

Published in vitro studies have further investigated the metabolism of malathion. In human liver microsomes, the metabolism of malathion to malaoxon was catalysed by CYP1A2, CYP2B6 or CYP3A4, their respective contributions depending on the concentration of malathion (Buratti et al., 2005). Isomalathion, a storage impurity, was a potent non-competitive inhibitor of hepatic carboxylesterase activity, important for the formation of MMCA by human liver microsomes (Buratti & Testai, 2005).

Estimates of in vitro dermal absorption through human skin ranged from 1.44% to 8.74% (de Ligt, 2004) and from 8% to 20.7% (Moody et al., 2007). In a volunteer study (Wester et al., 1983), dermal absorption was 4.48% following a single application and 3.53% following a second application.

Toxicological data

Consistent with other organophosphorus insecticides, the most sensitive toxicological effect following acute and repeated exposures to malathion is the inhibition of acetylcholinesterase activity in erythrocytes and brain. At higher doses, cholinergic signs become evident.

In rats, the oral LD_{50} ranged from 1539 to 8227 mg/kg bw (Terrell, 1979a,b; Kynoch, 1986a; Fischer, 1991a,b; Kuhn, 1996; Moore, 2002, 2003), the dermal LD_{50} was greater than 2000 mg/kg bw (Kynoch, 1986b; Bollen, 2003a) and the inhalation LC_{50} was greater than 5.2 mg/L (Jackson, 1986; Decker, Knuppe & Ullrich, 2003). The dermal LD_{50} in rabbits was 8790 mg/kg bw (Parke, 1978). Malathion was slightly irritating to rabbit skin (Liggett & Parcell, 1985a) and eyes (Liggett & Parcell, 1985b; Bollen, 2003c). In a Buehler test conducted in guinea-pigs, malathion did not cause skin sensitization (Kynoch & Smith, 1986), whereas malathion caused skin sensitization in the guinea-pig maximization test (Bollen, 2003d). Malathion was not sensitizing in the mouse local lymph node assay (Wang-Fan, 2003; Lowe, 2011a).

In a 14-day range-finding study conducted in juvenile rats, which tested gavage malathion doses of 0, 250, 450 and 600 mg/kg bw per day, salivation occurred at 450 and 600 mg/kg bw per day. In males, erythrocyte and brain acetylcholinesterase activities were reduced at every dose, whereas in females, erythrocyte and brain acetylcholinesterase activities were reduced at 450 and 600 mg/kg bw per day.

In a 28-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 5000 and 10 000 ppm (equal to 0, 9.2, 46.1, 457.5 and 947.8 mg/kg bw per day for males and 0, 9.4, 47.4, 461.3 and 910.1 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 46.1 mg/kg bw per day) for the inhibition of erythrocyte and brain acetylcholinesterase activities at 5000 ppm (equal to 457.5 mg/kg bw per day). Nasal toxicity, consisting of goblet cell depletion and hyperplasia of the olfactory epithelium, was noted at the highest dose (Barnett, 2012a).

In a 30-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 50, 100, 500, 10 000 and 20 000 ppm (equal to 0, 5.1, 10.4, 51.9, 1036 and 2008 mg/kg bw per day for males and 0, 5.7, 11.6, 57.6, 1134 and 2193 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 51.9 mg/kg bw per day) for the inhibition of brain acetylcholinesterase activity at 10 000 ppm (equal to 1036 mg/kg bw per day) (Daly, 1993a).

The overall NOAEL from these two 1-month repeated-dose toxicity studies in rats was 500 ppm (equal to 51.9 mg/kg bw per day), with an overall lowest-observed-adverse-effect level (LOAEL) of 5000 ppm (equal to 457.5 mg/kg bw per day).

In a 90-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 5000, 10 000 and 20 000 ppm (equal to 0, 7, 34, 340, 680 and 1390 mg/kg bw per day for males and 0, 8, 39, 384, 784 and 1597 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 34 mg/kg bw per day) for the inhibition of brain acetylcholinesterase activity at 5000 ppm (equal to 340 mg/kg bw per day) (Daly, 1993b).

In a second 90-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 5000 and 10 000 ppm (equal to 0, 7.2, 35.0, 353.6 and 733.8 mg/kg bw per day for males and 0, 7.5, 35.9, 363.1 and 719.0 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 7.2 mg/kg bw per day) for goblet cell depletion at 500 ppm (equal to 35.0 mg/kg bw per day) (Barnett, 2012b). This is considered to be an atypical result, as the effect is likely to have arisen through non-dietary exposure.

In a 13-week neurotoxicity study in rats, which tested dietary malathion concentrations of 0, 50, 5000 and 20 000 ppm (equal to 0, 4, 352 and 1486 mg/kg bw per day for males and 0, 4, 395 and 1575 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 4 mg/kg bw per day), based on the inhibition of erythrocyte acetylcholinesterase activity at 5000 ppm (equal to 352 mg/kg bw per day) (Lamb, 1994b).

The overall NOAEL for the 90-day (neuro)toxicity studies in rats was 500 ppm (equal to 34 mg/kg bw per day) for effects at 5000 ppm (equal to 340 mg/kg bw per day).

In a 28-day range-finding study in dogs in which malathion was administered orally in capsules at doses of 0, 125, 250 and 500 mg/kg bw per day, inhibition of erythrocyte

acetylcholinesterase occurred at 250 and 500 mg/kg bw per day, with deaths, cholinergic signs and reduced body weight and feed consumption occurring at the highest dose (Fischer et al., 1988).

In a 12-month repeated-dose toxicity study in dogs in which malathion was administered orally in capsules at doses of 0, 62.5, 125 and 250 mg/kg bw per day, the NOAEL was 125 mg/kg bw per day for reduced body weight and haematological changes at 250 mg/kg bw per day. Inhibition of erythrocyte acetylcholinesterase activity occurred at every dose but was of marginal toxicological significance in the absence of brain acetylcholinesterase inhibition (Shellenberger & Billups, 1987).

In a 3-week repeated-dose dermal toxicity study in rabbits, which tested malathion doses of 0, 50, 300 and 1000 mg/kg bw per day, the NOAEL was 300 mg/kg bw per day for the inhibition of brain acetylcholinesterase activity at 1000 mg/kg bw per day (Moreno, 1989).

In a 21-day repeated-dose dermal toxicity study in rabbits, which tested malathion doses of 0, 75, 100, 150 and 500 mg/kg bw per day, the NOAEL was 150 mg/kg bw per day for the inhibition of brain acetylcholinesterase activity at 500 mg/kg bw per day (Barnett, 2006d).

In a 13-week repeated-dose inhalational toxicity study in which rats were exposed whole body to an aerosol malathion concentration of 0, 0.1, 0.45 or 2.0 mg/L, a no-observed-adverse-effect concentration (NOAEC) was not determined, as laryngeal hyperplasia and degeneration and/or hyperplasia of the olfactory epithelium occurred at every concentration (Beattie, 1994).

In an 18-month pre-GLP study conducted in mice, which tested dietary malathion concentrations of 0, 8000 and 16 000 ppm (equivalent to 0, 1200 and 2400 mg/kg bw per day, respectively), a NOAEL for chronic toxicity was not identified, because clinical signs during the second year of exposure and reduced body weight occurred at both doses. Although no treatment-related tumours were observed, this study was considered unreliable for assessing carcinogenicity because of the small number of concurrent control mice ($n = 10$) compared with the treated groups ($n = 50$) (NCI, 1978).

In a second 18-month study conducted in mice, which tested dietary malathion concentrations of 0, 100, 800, 8000 and 16 000 ppm (equal to 0, 17, 143, 1476 and 2978 mg/kg bw per day for males and 0, 21, 167, 1707 and 3448 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 800 ppm (equal to 143 mg/kg bw per day) for the inhibition of brain acetylcholinesterase activity at 8000 ppm (equal to 1476 mg/kg bw per day). Increases in liver carcinomas in males at the low dose and second-highest dose were not considered treatment related because of the lack of a dose–response relationship, the lack of corroboration in females and the fact that liver carcinomas are a common age-related tumour in this strain of mouse (B6C3F1). The NOAEL for carcinogenicity was 800 ppm (equal to 143 mg/kg bw per day) for an increased incidence of liver adenomas at 8000 ppm (equal to 1476 mg/kg bw per day) (Slauter, 1994).

In an 80-week pre-GLP study conducted in rats, which tested dietary malathion concentrations of 0, 4700 and 8150 ppm (equivalent to 0, 1200 and 2400 mg/kg bw per day, respectively), it was not possible to identify a NOAEL for chronic toxicity because of the lack of reporting detail (NCI, 1978). While there was an increase in proliferative lesions of the thyroid in both sexes at both doses, these increases were not statistically significant in males and were significant in females only in a trend test and not by pairwise comparison when compared with groups of pooled controls. Overall, this study is not considered acceptable for the assessment of carcinogenicity because of the small number of rats in the concurrent control group (15 versus 50 in the treated groups) and the short duration of exposure.

In a subsequent 24-month pre-GLP study conducted in rats, which tested dietary malathion concentrations of 0, 100, 1000 and 5000 ppm (equivalent to 0, 5, 50 and 250 mg/kg bw per day, respectively, as calculated by a previous Meeting), the NOAEL was 100 ppm (equivalent to 5 mg/kg bw per day) for the inhibition of erythrocyte acetylcholinesterase activity at 1000 ppm (equivalent to 50 mg/kg bw per day) (Rucci, Becci & Parent, 1980; Seely, 1991). The NOAEL for carcinogenicity was 5000 ppm (equivalent to 250 mg/kg bw per day), the highest dose tested.

In a 24-month chronic toxicity and carcinogenicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 6000 and 12 000 ppm (equal to 0, 7, 29, 359 and 729 mg/kg bw per day for males and 0, 8, 35, 415 and 868 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 500 ppm (equal to 29 mg/kg bw per day) for reduced red cell parameters, inhibition of brain acetylcholinesterase activity and the occurrence of nasal toxicity at 6000 ppm (equal to 359 mg/kg bw per day) (Daly, 1996a). The nasal toxicity was characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, oedema and inflammation. Four nasal adenomas were observed, one in each sex at the two highest doses. In females, but not males, the incidence of liver adenomas was increased slightly at 6000 and 12 000 ppm, but the incidences were within the performing laboratory's historical control range. A NOAEL of 500 ppm (equal to 29 mg/kg bw per day) was identified for carcinogenicity, based on the increase in nasal adenomas at 6000 ppm (equal to 359 mg/kg bw per day) (Daly, 1996a, 1999).

The Meeting concluded that there is some evidence that malathion is carcinogenic in rats and mice.

The Meeting noted that the mouse liver adenomas observed in the second 18-month study (Slauter, 1994) occurred at doses exceeding the maximum tolerated dose and were not replicated in other mouse studies. The increases in liver adenomas in rats observed in the 24-month chronic toxicity and carcinogenicity study (Daly, 1996a) occurred only in females and were within the performing laboratory's historical control range. Whereas the rodent liver adenomas were coincident with liver hypertrophy, there were no findings in these or other studies to suggest a possible mode of action, such as liver enzyme induction or cytotoxicity. Malathion showed no peroxisome proliferator–activated receptor alpha or gamma (Takeuchi et al., 2006) activity and also showed no aryl hydrocarbon receptor activity (Takeuchi et al., 2008). Overall, the Meeting considered that there was equivocal evidence to suggest a tumorigenic response in the liver, but this had a clear threshold and was likely to be secondary to the effects on the liver of prolonged exposure to very high dietary concentrations of malathion.

Based on consistent observations of nasal toxicity in dietary studies of various durations ranging from 28 days to 2 years and in a short-term inhalational toxicity study, the Meeting concluded that the formation of nasal adenomas in rats was due to a local mechanism of irritancy and cytotoxicity caused by prolonged exposure of the nasal epithelium to high concentrations of malathion absorbed via inhaled food particles or as a vapour arising from food. This produces a state of reactive hyperplasia, a causative factor in tumour formation. Scenarios of prolonged, direct and excessive exposure of human nasal tissue to malathion or malathion metabolites following ingestion of residues is unlikely, and therefore these tumours would not occur in humans following exposure to malathion in the diet.

Malathion has been extensively tested for genotoxicity using a broad range of in vitro and in vivo assays. In 1997, the Meeting evaluated the available unpublished and published genotoxicity studies and noted that the majority of studies indicated that malathion is not genotoxic, although a small number of studies indicated that it can induce chromosomal aberrations and sister chromatid exchanges in vitro. However, there was no evidence that malathion induced chromosomal aberrations in vivo. Therefore, the 1997 Meeting concluded that malathion does not induce genotoxic damage in vivo. The 2003 Meeting evaluated supplementary genotoxicity studies and found that malathion caused chromosomal aberrations in cultured human lymphocytes and gene mutations in the mouse lymphoma assay at cytotoxic concentrations, but did not cause unscheduled DNA synthesis in vivo in male rats. The 2003 Meeting reaffirmed its previous conclusion that although the results of some in vitro tests were positive, malathion was not considered to induce genotoxic damage in vivo.

In addition to the studies considered at previous meetings, the current Meeting considered a number of new published and unpublished genotoxicity studies, including studies that involved the assessment of genotoxic damage in exposed workers. Many of the published studies do not provide

adequate experimental detail, do not specify the purity of the malathion tested or were conducted on commercial formulations, or used in vivo test systems or exposure routes less relevant to the risk assessment of dietary residues of pesticides. The following discussion is limited to studies that evaluated technical malathion or malathion at purities above 90% and provided adequate experimental and data analysis details to allow interpretation of the findings.

Using standard genotoxicity test systems, malathion was not mutagenic in assays using prokaryotes or lower eukaryotes when tested with or without metabolic activation (USEPA, 1977; Haworth et al., 1983; Traul, 1987; Machado, 1996; Bowles, 2005; Taylor, 2008c; Beevers, 2009; Thompson, 2013; Schreib, 2015b). In contrast, in in vitro assays using either human or non-human cells, malathion was generally positive for the induction of (1) chromosome damage, as measured by increased frequencies of chromosomal aberrations (Galloway et al., 1987; Herath et al., 1989; Garry et al., 1990; Edwards, 2001a; Lloyd, 2009) or micronuclei (Titenko-Holland et al., 1997; Josse et al., 2014); (2) mutations (Edwards, 2001b; Pluth et al., 1996, 1998); and (3) DNA damage, as measured by increases in DNA migration in the alkaline comet assay (Lu et al., 2012) and increased frequencies of sister chromatid exchanges (Nicholas, Vienne & van den Berghe, 1979; Chen et al., 1981; Nishio & Uyeki, 1981; Herath et al., 1989). Negative findings were reported for the induction of micronuclei in Molt-4 T-lymphocytes (Szekely, Goodwin & Delaney, 1992), unscheduled DNA synthesis in WI-38 cells (USEPA, 1977) and primary rat liver hepatocytes (Pant, 1989), and mutations in a mouse lymphoma assay (reported to be equivocal without metabolic activation and negative with metabolic activation, in Chemical Effects in Biological Systems [CEBS]; NTP 2016).

Using in vivo nonmammalian systems, malathion was active for micronucleus induction in a bird model (Hussain et al., 2015) and for induction of reciprocal translocations and sex-linked recessive lethals in one *Drosophila melanogaster* study (Foureman et al., 1994), but not for sex-linked recessive lethals, sex chromosome loss or wing-spot mutations in another study (Valencia, 1981).

Based on the criteria mentioned in section 2.1 of the main JMPR meeting report[15], very few of the 34 in vivo mammalian study/end-point combinations were considered adequate for this review. In reports submitted by the sponsor, malathion was negative in a rat liver unscheduled DNA synthesis study when administered by gavage (Meerts, 2003), in a rat bone marrow chromosomal aberration study when administered by gavage (Gudi, 1990) and in a mouse bone marrow erythrocyte micronucleus assay when administered intraperitoneally (Navarro, 1995). However, the unscheduled DNA synthesis assay is insensitive for detecting genotoxic compounds; the micronucleus assay, as conducted, suffers from concerns about scoring criteria; and the chromosomal aberration test appears to be significantly underpowered, based on the frequency of chromosomal aberrations detected among control and treated animals. A negative mouse dominant lethal test was also reported when malathion was administered in feed for 7 weeks (USEPA, 1977), and a negative mouse bone marrow chromosomal aberration study was reported in intraperitoneally treated mice (Degraeve, Chollet & Moutschen, 1984b). In contrast, malathion-induced micronuclei and chromosomal aberrations were reported in bone marrow immature erythrocytes and proliferating cells, respectively (Dulout et al., 1982, 1983). A positive alkaline comet assay using blood leukocytes sampled from rats treated intraperitoneally once a day for 5 days was reported (Moore, Patlolla & Tchounwou, 2011).

The Meeting evaluated a number of human studies that examined genotoxicity end-points. Patients treated for acute intoxication with a malathion-based product exhibited increased levels of chromosomal damage in lymphocytes (van Bao et al., 1974). The frequency of micronuclei and glycophorin A mutations in erythrocytes or micronuclei in lymphocytes was not increased in workers exposed selectively to malathion (Titenko-Holland et al., 1997; Windham et al., 1998). However, DNA damage and chromosomal aberrations have been reported in workers exposed to a mixture of pesticides, including malathion (Yoder, Watson & Benson, 1973; Páldy et al., 1987; Rupa et al., 1988, 1991; De Ferrari et al., 1991; Pluth et al., 1996; Garaj-Vrhovac & Zeljezic, 2000, 2001; Omari, 2011;

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[15] Pesticide residues in food 2016: Special session of the joint FAO/WHO meeting on pesticide residues. May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/).

Singh et al., 2011; Benedetti et al., 2013; Varona-Uribe et al., 2016). These studies are of limited value for examining the specific effect of malathion on genotoxicity end-points in humans.

The Meeting noted that malathion has been reported to have genotoxic activity in multiple assay systems at multiple genetic end-points. In several studies where evaluated, reactive oxygen species appear to have been responsible for the increased damage, as demonstrated by the detection of malathion-induced 8-hydroxy-2′-deoxyguanosine and increased malondialdehyde concentrations in isolated human peripheral blood mononuclear cells treated in vitro, an effect attenuated by co-treatment with *N*-acetylcysteine or curcumin (Ahmed et al., 2011); by increased intracellular levels of reactive oxygen species and reduced levels of catalase, superoxide dismutase and glutathione in rat PC12 cells treated in vitro (Lu et al., 2012); and by the detection of oxidative damage using the comet assay in isolated rat lymphocytes treated in vitro with malathion (Ojha & Srivastava, 2014). Supportive of this hypothesis, malathion appears to selectively induce markers of oxidative stress in Tox21/ToxCast high-throughput screening assays (USEPA interactive Chemical Safety for Sustainability Dashboard, 2016). The Meeting concluded that the observed genotoxic effects occur secondary to the formation of reactive oxygen species, which will exhibit a threshold.

The Meeting concluded that malathion is unlikely to be genotoxic at anticipated dietary exposures.

In the multigeneration and developmental toxicity studies, cholinesterase activity was not measured.

In a two-generation reproductive toxicity study conducted in rats, which tested dietary malathion concentrations of 0, 550, 1700, 5000 and 7500 ppm (equal to 0, 43, 130, 393 and 595 mg/kg bw per day for males and 0, 50, 152, 438 and 655 mg/kg bw per day for females, respectively), the NOAEL for both reproductive toxicity and parental toxicity was 7500 ppm (equal to 595 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 1700 ppm (equal to 130 mg/kg bw per day) for reduced pup weights at 5000 ppm (equal to 393 mg/kg bw per day) (Schroeder, 1990).

Two published studies reported potential testicular toxicity in rats exposed to malathion orally (Uzun et al., 2009; Geng et al., 2015), but these studies had a number of methodological limitations that reduced their utility. Further, the reported observations are not corroborated by the preceding GLP-compliant multigenerational rat study in which no effects on the testes were observed (Schroeder, 1990).

A variety of in vivo and in vitro assays in mammalian and nonmammalian models indicated that malathion is unlikely to affect the endocrine system (Barnett, 2011b,c,d; Palmer, 2011a,b,c; Wagner, 2011; Wilga, 2011; Willoughby, 2011b; Kjeldsen, Ghisari & Bonefeld-Jørgensen, 2013).

In a pilot developmental toxicity study in rats, which tested gavage malathion doses of 0, 300, 600, 800 and 1000 mg/kg bw per day from days 6 to 15 of gestation, no embryo or fetal toxicity occurred, whereas maternal toxicity occurred at and above 600 mg/kg bw per day (Lochry, 1988). In the main developmental toxicity study in rats, which tested gavage doses of 0, 200, 400 and 800 mg/kg bw per day from days 6 to 15 of gestation, the NOAEL for maternal toxicity was 400 mg/kg bw per day for clinical signs and reduced body weight gain and feed consumption at 800 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 800 mg/kg bw per day, the highest dose tested (Lochry, 1989).

In a range-finding developmental toxicity study in rabbits, which tested gavage malathion doses of 0, 25, 50, 100, 200 and 400 mg/kg bw per day from days 6 to 18 of gestation, no embryo or fetal toxicity occurred, whereas maternal toxicity occurred at 200 and 400 mg/kg bw per day (Siglin, Voss & Becci, 1985). In the main study, which tested malathion doses of 0, 25, 50 and 100 mg/kg bw per day from days 6 to 18 of gestation, the NOAEL for maternal toxicity was 25 mg/kg bw per day for a marginal effect on body-weight gain at 50 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested.

The Meeting concluded that malathion is not teratogenic.

In a study conducted in hens, there was no evidence that malathion caused delayed peripheral neuropathy (Fletcher, 1989).

In an acute neurotoxicity study in rats, which tested gavage malathion doses of 0, 500, 1000 and 2000 mg/kg bw, the NOAEL was 1000 mg/kg bw for reduced erythrocyte acetylcholinesterase activity in females and reduced ambulatory activity in males at 2000 mg/kg bw (Lamb, 1994a).

A 13-week neurotoxicity study in rats (Lamb, 1994b) is described above together with the other 13-week toxicity studies in rats, and an overall NOAEL is identified for these studies.

In a developmental neurotoxicity study in rats, which tested gavage malathion doses of 0, 5, 50 and 150 mg/kg bw per day from day 6 of gestation to day 10 of lactation, the NOAEL for both maternal toxicity and offspring toxicity was 50 mg/kg bw per day for clinical signs at 150 mg/kg bw per day (Fulcher, 2002b; Reiss, 2004).

Administration of malathion from day 6 of gestation to day 21 of lactation had no effect on the thickness of the corpus callosum in rat pups at doses up to 150 mg/kg bw per day (Myers, 2003, 2004).

The Meeting concluded that malathion is neurotoxic.

Studies in rats have examined the time to peak effect and compared the effects of malathion and malaoxon on the inhibition of acetylcholinesterase activity. The time to peak effect in juvenile rats following dosing with malathion ranged from 30 to 90 minutes for the inhibition of erythrocyte acetylcholinesterase activity and from 60 to 90 minutes for the inhibition of brain acetylcholinesterase activity (Stannard, 2006a,b,c; Barnett, 2008a,b,c). Malaoxon was a more potent inhibitor of acetylcholinesterase activity compared with malathion (Barnett, 2006c, 2008d). Comparison of BMDs following acute oral dosing indicated that the TAF for malaoxon was 21.6 in males and 17.8 in females for the inhibition of erythrocyte acetylcholinesterase activity and 14.8 in males and 11.0 in females for the inhibition of brain acetylcholinesterase activity (Reiss, 2008). Comparison of BMDs for the inhibition of erythrocyte acetylcholinesterase activity from chronic toxicity studies indicated that TAFs for malaoxon ranged from 37 to 38 in males and from 65 to 69 in females (Reiss, 2006a).

In a 6-week immunotoxicity study in female rats, which tested dietary malathion concentrations of 0, 50, 100, 700 and 7000 ppm (equal to 0, 8.9, 17.6, 126.8 and 1215.8 mg/kg bw per day, respectively), the NOAEL for immunotoxicity was 7000 ppm (equal to 1215.8 mg/kg bw per day), the highest dose tested (Barnett, 2011d).

The Meeting concluded that malathion is not immunotoxic.

An extensive literature search did not identify any potential adverse effects on intestinal microbiota or any evidence that intestinal microbiota can metabolize malathion.

Toxicological data on metabolites, degradates and/or impurities

Current FAO specifications for malathion prescribe maximum limits for isomalathion (CAS No. 3344-12-5), malaoxon (CAS No. 1634-78-2), *O*,*O*,*S*-trimethyl phosphorothioate (CAS No. 2953-29-9) and *O*,*S*,*S*-trimethyl phosphorodithioate (CAS No. 152-18-1).

Toxicity tests were conducted on malaoxon, isomalathion, desmethyl malathion, desmethyl-malathion monocarboxylic acid, MMCA, MDCA and desmethyl-malaoxon dicarboxylic acid.

Malaoxon

The oral LD$_{50}$ in rats for malaoxon was 50 mg/kg bw (Lowe, 2011b).

In a 14-day range-finding study in rats, which tested malaoxon at dietary concentrations of 0, 10, 25, 100, 2500 and 3500 ppm (equal to 0, 1.1, 3.0, 12.1, 293 and 387 mg/kg bw per day for males and 0, 1.1, 3.1, 12.5, 281.6 and 294.7 mg/kg bw per day for females, respectively), inhibition of erythrocyte acetylcholinesterase activity occurred at and above 100 ppm (equal to 12.1 mg/kg bw per day). At the two highest doses, inhibition of brain acetylcholinesterase activity and reduced body-weight gain and feed consumption occurred (Daly, 1995).

In a 103-week carcinogenicity study conducted in mice, which tested dietary malaoxon concentrations of 0, 500 and 1000 ppm (estimated by a previous Meeting to be equal to 0, 75 and 150 mg/kg bw per day, respectively), survival and body weight were reduced at the highest dose. There were no treatment-related neoplastic or non-neoplastic lesions (NCI, 1979b). In a parallel study conducted in rats, which tested the same dietary concentrations of malathion (equal to 0, 25 and 50 mg/kg bw per day, respectively), the combined incidence of C-cell adenomas and carcinomas of the thyroid in females was increased, although this was comparable to historical control values. The incidence of gastric ulcers, commonly observed in the forestomach, was increased in treated rats.

In a 24-month toxicity study in rats, which tested malaoxon at dietary concentrations of 0, 20, 1000 and 2000 ppm (equal to 0, 1, 57 and 110 mg/kg bw per day for males and 0, 1, 68 and 140 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 20 ppm (equal to 1 mg/kg bw per day), based on mortality and the inhibition of brain acetylcholinesterase activity at 1000 ppm (equal to 57 mg/kg bw per day). The NOAEL for carcinogenicity was 2000 ppm (equal to 110 mg/kg bw per day), the highest dose tested. Similar to studies conducted on malathion, inflammatory changes in the nasal mucosa occurred at 1000 and 2000 ppm; these changes were likely attributable to inhaled food particles containing malaoxon, resulting in tissue injury and inflammation of the nasal cavity, with secondary effects on the lungs and middle ear (Daly, 1996b).

The Meeting concluded that malaoxon is not carcinogenic in mice or rats.

Malaoxon was negative for mutagenicity in bacterial assays (Zeiger et al., 1988; Schreib, 2015a) and in lower eukaryotes, both with and without metabolic activation (USEPA, 1977; Gilot-Delhalle et al., 1983). Malaoxon was reported to be active for induction of sister chromatid exchanges but not chromosomal aberrations in CHO cells, with or without metabolic activation (Ivett et al., 1989). An increase in sister chromatid exchanges when tested in the absence of metabolic activation only was also reported (Nishio & Uyeki, 1981); it was also reported that malaoxon was more potent than malathion in this assay. Malaoxon was also reported to induce DNA damage as measured by the comet assay in rat adrenal gland PC12 cells when tested in the absence of metabolic activation only (Lu et al., 2012) and was mutagenic in mouse lymphoma (L5178Y) cells in the absence but not the presence of metabolic activation (Myhr & Caspary, 1991). In this study, there seemed to be a preference for the induction of small colonies, generally considered to be indicative of chromosomal damage rather than gene mutations.

Malaoxon induced DNA damage in isolated lymphocytes in the absence of metabolic activation, as measured by the alkaline comet assay; studies with metabolic activation were not conducted (Blasiak et al., 1999). Further, a follow-up study concluded that the malaoxon-mediated damage was likely induced by reactive oxygen species (Blasiak & Stankowska, 2001). Also, malaoxon is more potent than malathion in inducing intracellular levels of reactive oxygen species and reducing levels of catalase, superoxide dismutase and glutathione in rat PC12 cells treated in vitro (Lu et al., 2012). When provided in food, malaoxon induced an increase in reciprocal translocations and sex-linked recessive lethals in *D. melanogaster*, but not for sex-linked recessive lethals when administered by injection (Foureman et al., 1994). Malaoxon was reported negative for the induction of chromosomal aberrations and sister chromatid exchanges in the bone marrow cells of male mice following a single intraperitoneal injection (NTP, 2016).

The Meeting concluded that the observed genotoxic effects occur secondary to the formation of reactive oxygen species, which will exhibit a threshold.

The Meeting concluded that malaoxon is unlikely to be genotoxic at anticipated dietary exposures.

Other metabolites

The oral LD_{50} in rats was greater than 2000 mg/kg bw for desmethyl malathion, desmethyl-malathion monocarboxylic acid, MMCA, MDCA and desmethyl-malaoxon dicarboxylic acid (Pratt, 2005; Sanders, 2008a,b; Leoni, 2012). The oral LD_{50} in rats for desmethyl-malaoxon dicarboxylic acid, trisodium salt, was greater than 2000 mg/kg bw (Allingham, 2015).

There are a limited number of genotoxicity studies on other metabolites of malathion. MDCA (Taylor, 2008b), MMCA (Taylor, 2008a), desmethyl-malathion monocarboxylic acid, potassium salt (Donath, 2012), and desmethyl-malaoxon dicarboxylic acid, trisodium salt (Schreib, 2015a), as well as isomalathion, *O,O,O*-trimethyl phosphorothioate, *O,O,S*-trimethyl phosphorothioate and *O,S,S*-trimethyl phosphorodithioate (Imamura & Talcott, 1985), were reported negative for bacterial mutagenicity, with and without metabolic activation. Isomalathion induced DNA damage in isolated lymphocytes in the absence of metabolic activation, as measured by the alkaline comet assay; studies with metabolic activation were not conducted (Blasiak et al., 1999). Isomalathion was also reported to induce micronuclei in the human liver–derived HepaRG cell line (Josse et al., 2014).

Using QSAR, the storage impurity, 2-mercaptosuccinic acid diethyl ester, was determined to have no greater toxicity than malathion (Clerkin, 2015).

The potential of malathion metabolites to inhibit acetylcholinesterase activity has been studied in rats. Comparisons of erythrocyte acetylcholinesterase activities indicated that desmethyl malathion, MMCA and MDCA are at least 2.75-, 1.9- and 4.6-fold less potent than malathion.

Based on a comparison of the inhibitions of acetylcholinesterase activities over acute and chronic exposure durations and a comparison of BMDs (see above), the Meeting concluded that malaoxon is approximately 30-fold more potent than malathion.

Human data

As in laboratory animals, the inhibition of acetylcholinesterase activity is the most sensitive adverse effect in humans exposed to malathion, mediated through the metabolite malaoxon, which is a more potent inhibitor of acetylcholinesterase activity compared with malathion. A comparative in vitro study (Rodriguez et al., 1997) indicated that malaoxon was a slightly less potent inhibitor (less thatn threefold) of human compared with rat acetylcholinesterase activity.

In a study conducted in male and female volunteers, which tested single oral doses of malathion at 0, 0.5, 1.5, 5, 10 and 15 mg/kg bw, the NOAEL was 15 mg/kg bw, the highest dose tested, based on the absence of any adverse effects, including the inhibition of erythrocyte acetylcholinesterase activity (Gillies & Dickson, 2000). In a subsequent study conducted in male and female volunteers, which tested single oral doses of malathion of 0, 0.5, 1.5, 5.0, 10.0 and 15.0 mg/kg bw, There were no treatment-related adverse events or effects on erythrocyte acetylcholinesterase activity (Jellinek, Schwartz & Connolly Inc., 2000).

In a published study, application of malathion to the forearm of human volunteers increased blood flow, mediated via the inhibition of acetylcholinesterase activity (Boutsiouki & Clough, 2004).

In a published non-blinded study (Wananukul et al., 2011), slight inhibition of erythrocyte acetylcholinesterase activity occurred in most of the children following two applications of a 1% malathion shampoo used to treat head lice.

In a 1994 summary report (Nielsen, 1994), there were no poisoning incidents and no inhibition of plasma cholinesterase activity in workers involved in the manufacture of malathion over

a 20-year period. In a subsequent summary report (Ravn Nielsen, 1999), biological monitoring of workers employed at dimethoate and malathion manufacturing plants from 1994 to 1999 detected no reduction in plasma cholinesterase activity.

Several epidemiological studies on cancer outcomes in relation to occupational exposure to malathion were available. The evaluation of these studies focused on the occurrence of NHL and prostate cancer, as outlined in section 2.2 of the meeting report. One meta-analysis was available, as well as one prospective cohort study, the AHS, with a large sample size and detailed exposure assessment. Cohort studies are considered a powerful design, as recall bias is avoided. All other studies were case–control studies, usually retrospective, which are more prone to recall and selection biases.

The AHS found no evidence of a positive association of NHL with malathion exposure or of an exposure–response relationship (Alavanja et al., 2014; Lerro et al., 2015). In contrast, various case–control studies reported excess risks of NHL associated with use of malathion. In a large pooled case–control study, the unadjusted estimates showed a significant increased risk of NHL (RR: 1.6; 95% CI: 1.2–2.2) associated with ever-use versus never-use of malathion (Waddell et al., 2001). However, these were attenuated and/or no longer significant when proxy respondents were excluded and analyses were mutually adjusted for other pesticides (Waddell et al., 2001; De Roos et al., 2003). Significant elevated risks of NHL were reported from the Cross-Canada Study of Pesticides and Health for ever-use versus never-use of malathion (OR: 1.96; 95% CI: 1.42–2.70) (McDuffie et al., 2001; Pahwa et al., 2012) and when examining annual days of use, although there was no clear exposure–response relationship across exposure categories (McDuffie et al., 2001). Non-significant increased risks of NHL were reported by two other case–control studies (Mills, Yang & Riordan, 2005; Eriksson et al., 2008), one of which had limited statistical power based on only five exposed cases (Eriksson et al., 2008). The meta-analysis, which did not include the AHS, found a significant 80% excess risk ratio for ever-use versus never-use of malathion (Schinasi & Leon, 2014).

Overall, there is some very weak evidence of a positive association between malathion exposure and NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS (Alavanja et al., 2014), which is the only cohort study and is large and of high quality, found no evidence of an association at any exposure level.

There was no evidence of an association with all prostate cancers and malathion exposure in the AHS (Koutros et al., 2013). However, a significant excess risk of aggressive prostate cancer (RR: 1.43; 95% CI: 1.08–1.88) in the highest exposure category (highest quintile of intensity-weighted lifetime days of malathion exposure), along with a significant exposure–response relationship (P for trend = 0.04), was observed (Koutros et al., 2013). A significant elevated risk of all prostate cancer was observed in a case–control study (Band et al., 2011) for ever-use (OR: 1.34; 95% CI: 1.01–1.78) and for highest lifetime cumulative exposure versus those unexposed (OR: 1.49; 95% CI: 1.02–2.18). A significant trend across exposure categories (P = 0.03) was also reported. However, interpretation of results from this study is limited by potential for exposure misclassification in the job–exposure matrix used for exposure assessment and by the potential for residual confounding from lack of adjustment for other pesticide exposures (Band et al., 2011). There was no evidence of an association between prostate cancer and malathion exposure in the United Farm Workers of America study (Mills & Yang, 2003), which was limited by the use of ecological rather than individual-level exposure assessment.

Overall, the evidence is suggestive of a positive association between malathion exposure and risk of aggressive prostate cancer; however, the evidence base is limited to the one large AHS cohort study.

Based on a consideration of the results of animal bioassays, genotoxicity assays and epidemiological data from occupational exposures, the Meeting concluded that malathion and its metabolites are unlikely to pose a carcinogenic risk to humans from exposure via the diet.

The Meeting concluded that the existing database on malathion was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The current Meeting reaffirmed the acceptable daily intake (ADI) of 0–0.3 mg/kg bw per day, based on the NOAEL of 500 ppm (equal to 29 mg/kg bw per day) in the 2-year study of toxicity and carcinogenicity in rats for the inhibition of brain acetylcholinesterase and using a 100-fold safety factor, established by the 1997 Meeting. The margins of exposure between this ADI and the doses causing liver adenomas in mice and nasal adenomas in rats are 5000-fold and 1200-fold, respectively.

The current Meeting reaffirmed the acute reference dose (ARfD) of 2 mg/kg bw, based on the NOAEL of 15 mg/kg bw for the inhibition of erythrocyte acetylcholinesterase activity in a study conducted in male and female volunteers with the application of a 10-fold safety factor, established by the 2003 Meeting. This ARfD is supported by the NOAEL of 15 mg/kg bw in a second study conducted in male and female volunteers. The ARfD is considered to be a conservative value, because human acetylcholinesterase is slightly less sensitive (< 3-fold) than rat acetylcholinesterase to malaoxon.

The Meeting concluded that the metabolite malaoxon is approximately 30-fold more toxic than malathion. On this basis, a 30-fold potency factor should be applied to the residue levels for use in both the acute and chronic dietary exposure estimates for malaoxon, and these should be added to the dietary exposures for malathion and compared with the ARfD and ADI for malathion, respectively.

Both the ADI and ARfD are established for the sum of malathion and malaoxon (corrected for its potency), expressed as parent malathion. The other metabolites of malathion considered by the present Meeting are less potent than the parent compound and therefore would be covered by the ADI and ARfD for malathion. The impurity isomalathion may need to be taken into consideration in the risk assessment depending on its concentration in food commodities.

Levels relevant to risk assessment of malathion

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	800 ppm, equal to 143 mg/kg bw per day	8 000 ppm, equal to 1 476 mg/kg bw per day
		Carcinogenicity	800 ppm, equal to 143 mg/kg bw per day	8 000 ppm, equal to 1 476 mg/kg bw per day
Rat	Acute neurotoxicity study[b]	Toxicity	1 000 mg/kg bw per day	2 000 mg/kg bw per day
	One-month studies of toxicity[a,c]	Toxicity	500 ppm, equal to 51.9 mg/kg bw per day	5 000 ppm, equal to 457.5 mg/kg bw per day
	Thirteen-week studies of toxicity and neurotoxicity[a,c]	Toxicity	500 ppm, equal to 34 mg/kg bw per day	5 000 ppm, equal to 340 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 29 mg/kg bw per day	6 000 ppm, equal to 359 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 29 mg/kg bw per day	6 000 ppm, equal to 359 mg/kg bw per day
	Two-generation study of reproductive toxicity[a,e]	Reproductive toxicity	7 500 ppm, equal to 595 mg/kg bw per day[d]	–
		Parental toxicity	7 500 ppm, equal to 595 mg/kg bw per day[d]	–

Species	Study	Effect	NOAEL	LOAEL
		Offspring toxicity	1 700 ppm, equal to 130 mg/kg bw per day	5 000 ppm, equal to 393 mg/kg bw per day
	Developmental toxicity study[a,c]	Maternal toxicity	400 mg/kg bw per day	800 mg/kg bw per day
		Embryo and fetal toxicity	800 mg/kg bw per day[d]	–
	Developmental neurotoxicity study[b,e]	Maternal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
		Offspring toxicity	50 mg/kg bw per day	150 mg/kg bw per day
Rabbit	Developmental toxicity study[a,c]	Maternal toxicity	25 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day[d]	–
Dog	One-year study of toxicity[f]	Toxicity	125 mg/kg bw per day	250 mg/kg bw per day
Human	Acute volunteer studies[c,f]	Cholinesterase inhibition	15 mg/kg bw[d]	–

[a] Dietary administration.

[b] Gavage administration.

[c] Two or more studies combined.

[d] Highest dose tested.

[e] Acetylcholinesterase activity not measured.

[f] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–0.3 mg/kg bw (for sum of malathion and malaoxon, adjusted for its potency and expressed as malathion)

Estimate of acute reference dose (ARfD)

2 mg/kg bw (for sum of malathion and malaoxon, adjusted for its potency and expressed as malathion)

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Results from in vivo genotoxicity studies investigating oral dosing, because malathion genotoxicity data are highly variable and inconsistent and there is a lack of robust in vivo rodent studies using the oral route of exposure

Critical end-points for setting guidance values for exposure to malathion

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; > 77%
Dermal absorption	Estimates vary (1.44–20.7% in human skin)
Distribution	Rapid tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and complete

Metabolism in animals	Extensive; oxidation, hydrolysis, dealkylation and demethylation reactions
Toxicologically significant compounds in animals and plants	Malathion, malaoxon, desmethyl malathion, desmethyl malaoxon, MMCA, MDCA, isomalathion

Acute toxicity

Rat, LD_{50}, oral	> 1 539 to < 8 227 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.2 mg/L
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Slightly irritating
Guinea-pig, dermal sensitization	Not sensitizing (Buehler assay)
	Sensitizing (maximization assay)
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)

Short-term studies of toxicity

Target/critical effect	Acetylcholinesterase inhibition
Lowest relevant oral NOAEL	51.9 mg/kg bw per day (28 days; rat)
Lowest relevant dermal NOAEL	150 mg/kg bw per day (21 days; rabbit)
Lowest relevant inhalation NOAEC	< 0.1 mg/L (13 weeks; rat)

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Acetylcholinesterase inhibition
Lowest relevant NOAEL	29 mg/kg bw per day (rat)
Carcinogenicity	Some evidence of carcinogenicity in mice and rats[a]

Genotoxicity

	Genotoxic, possibly due to the generation of reactive oxygen species[a]

Reproductive toxicity

Reproduction target/critical effect	No effect on reproduction
Lowest relevant parental NOAEL	595 mg/kg bw per day (rat; highest dose tested)[b]
Lowest relevant offspring NOAEL	130 mg/kg bw per day (rat)[b]
Lowest relevant reproduction NOAEL	595 mg/kg bw per day (rat; highest dose tested)[b]

Developmental toxicity

Developmental target/critical effect	Marginally reduced maternal body-weight gain
Lowest maternal NOAEL	25 mg/kg bw per day (rabbit)[b]
Lowest embryo/fetal NOAEL	100 mg/kg bw per day (rabbit; highest dose tested)[b]

Neurotoxicity

Acute neurotoxicity NOAEL	1 000 mg/kg bw
Subchronic neurotoxicity NOAEL	4 mg/kg bw per day[c]
Developmental neurotoxicity NOAEL	50 mg/kg bw per day[b]
Delayed neurotoxicity	No evidence

Other toxicological studies

Immunotoxicity NOAEL	1 216 mg/kg bw per day (rat; highest dose tested)
	Not immunotoxic

Toxicological studies on malaoxon

Rat, LD_{50}, oral	50 mg/kg bw
Lowest relevant long-term NOAEL	1 mg/kg bw per day (rat)
Carcinogenicity	No evidence of carcinogenicity (mouse, rat)
Genotoxicity	Some evidence of genotoxicity, secondary to the formation of reactive oxygen species
Toxicological studies on desmethyl-malathion, sodium salt	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on desmethyl-malathion monocarboxylic acid, potassium salt	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on MMCA	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on MDCA	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on desmethyl-malaoxon dicarboxylic acid	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Human data	Acetylcholinesterase inhibition:
	Acute NOAEL: 15 mg/kg bw, highest dose tested
	No adverse effects in manufacturing personnel

[a] Unlikely to pose a carcinogenic risk to humans from the diet.

[b] Acetylcholinesterase activity not measured.

[c] Ninety-day neurotoxicity study in rats is covered by the overall oral NOAEL for repeated-dose studies of toxicity.

Summary

	Value	Studies	Safety factor
ADI	0–0.3 mg/kg bw	Two-year chronic toxicity and carcinogenicity study (rat)	100
ARfD	2 mg/kg bw	Single-dose studies (humans)	10

ADI: acceptable daily intake; AFfD: acute reference dose

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Appendix 1. Mode of action analysis – rodent liver tumours

INTRODUCTION

Malathion is an organophosphorus insecticide, and like all members of this chemical class, its mechanism of toxic action is the inhibition of acetylcholinesterase (AChE) activity mediated via the metabolite, malaoxon.

CARCINOGENICITY OF MALATHION IN ANIMALS

Nine rodent carcinogenicity studies have been conducted on either malathion or malaoxon. Two of these studies reported an increase in liver adenomas in male and female B6C3F1 mice (Slauter, 1994) and female CDF(F-344)/CrlBr rats (Daly, 1996a) at high dietary concentrations of malathion. Two other studies conducted in B6C3F1 mice did not detect liver adenomas at similar doses (NCI, 1978, 1979) while studies conducted in Osborne–Mendel rats (NCI, 1978), F344 rats (NCI, 1979a,b), Sprague-Dawley rats (Rucci, Becci & Parent, 1980) and Fischer 344 (CD®(F-344)/CrlBR) rats (Daly 1996a) also found no liver tumours.

Only the study by Slauter (1994), conducted in B6C3F1 mice, demonstrated a clear, treatment-related increase in adenomas at very high dietary concentrations of malathion exceeding the maximum tolerated dose (8000 and 16 000 ppm – equal to 1476 and 2978 mg/kg bw per day in males and 1707 and 3448 mg/kg bw per day in females). The occurrence of these adenomas was coincident with liver masses, nodules and tan or yellow foci observed macroscopically, increased liver weight and hypertrophy of hepatocytes – no increase in carcinomas was observed (Table A2.1). At 8000 and 16 000 ppm, absolute body weight was significantly lower than the control over the entire period of exposure. Statistically and toxicologically significant inhibition of erythrocyte acetylcholinesterase activity occurred in both sexes at and above 800 ppm. Brain acetylcholinesterase activity was

inhibited by greater than 20% at 8000 and 16 000 ppm, however, only inhibition at the highest dose at termination was statistically significant.

Table A2.1. Liver findings in B6C3F1 mice exposed to malathion in the diet for up to 18 months

Parameter	No. and incidence per dietary concentration (ppm)				
	0 ppm	100 ppm	800 ppm	8 000 ppm	16 000 ppm
Liver masses – 18 month necropsy					
Males	0/50	8/51 (16%)	4/48 (8%)	5/54 (9%)	18/50 (36%)
Females	1/55 (2%)	0/52	3/52 (6%)	2/53 (4%)	10/51 (20%)
Liver nodules - 18 month necropsy					
Males	5/50 (10%)	2/51 (4%)	3/48 (6%)	10/54 (19%)	19/50 (38%)
Females	1/50 (2%)	2/51 (4%)	0/48	9/54 (17%)	29/50 (58%)
Tan or yellow liver foci - 18 month necropsy					
Males	0/50	0/51	1/48 (2%)	2/54 (4%)	18/50 (36%)
Females	0	0	0	2/54 (4%)	9/50 (18%)
Absolute liver weight (g) – 12 months					
Males	1.62	1.71	1.78	1.98** (+22%)	2.38** (+47%)
Females	1.55	1.68	1.56	1.66	1.92** (+24%)
Relative liver weight (%) – 12 months					
Males	5.15	5.19	5.42	6.95** (+35%)	8.30** (+61%)
Females	5.22	5.39	5.22	6.21** (+19%)	7.56** (+45%)
Absolute liver weight (g) – 18 months					
Males	1.90	2.90	1.96	2.26** (+19%)	2.66** (+40%)
Females	1.93	1.77	1.96	1.92	2.18
Relative liver weight (%) – 18 months					
Males	5.59	6.15	5.82	7.51** (+34%)	9.38** (+68%)
Females	6.19	5.76	6.26	6.90	8.51** (+37%)
Hypertrophy of hepatocytes – 12 months					
Males	0/10	0/10	0/10	7/10	10/10
Females	0/10	0/10	0/10	5/10	10/10
Hypertrophy of hepatocytes – 18 months					
Males	0/50	1/51	0/48	1/54	3/50
Females	0/55	0/52	0/52	53/53	51/51
Hepatocellular adenoma – 18 months					
Males	1/50	6/51	2/48	13/54*	49/50**
Females	0/55	1/52	0/52	9/53*	42/51**
Hepatocellular carcinoma – 18 months					
Males	0/50	6/51*	2/48	6/54*	1/50
Females	1/55	0/52	2/52	1/53	2/51

no.: number; ppm: parts per million; *$P < 0.05$; **$P < 0.01$

Results expressed as the mean, with the % increase (+) or decrease (-) relative to the control in parentheses.

Source: Slauter (1994)

Two other carcinogenicity studies have been conducted in B6C3F1 mice – both are pre-GLP studies conducted by the National Cancer Institute; one on malathion (NCI, 1978) and the other on malaoxon (NCI, 1979b). Neither study found an increase in either adenomas or carcinomas. In the study conducted on malathion (NCI, 1978), there was a slight increase in liver nodules observed macroscopically at the highest dietary concentration in males (16 000 ppm – equal to 2400 mg/kg bw per day; 6/49 versus 3/49 in the control), but this increase was not statistically significant. When the incidence of hepatocellular carcinoma and nodules was combined, a significant linear trend was determined when either the matched control (P = 0.019) or pooled control (P = 0.019) was used; pairwise comparisons of either neoplasm were not statistically significant. In addition, the incidence of these findings was consistent with historical control data from the same laboratory where the incidence of spontaneous liver tumours in males was 19%. Rueber (1985) re-examined the slides from this study and concluded that malathion caused an increase in neoplasms in the liver of male mice. However, this re-evaluation is considered unreliable because no methodological details were provided in the paper. The USA National Toxicology Program (Huff et al., 1985) also re-evaluated the same slides and confirmed the conclusion of the original study authors that there was no evidence of carcinogenicity. Overall this study is not considered acceptable because of the small number of mice in the concurrent control group.

Only one of the six studies conducted in rats reported an increase in liver tumours in female CDF(F-344)/CrlBr rats (Daly 1996a). In male rats, liver adenomas and carcinomas occurred with similar frequency across all groups. In females, the incidence of liver adenomas was significantly increased at 6 000 and 12 000 ppm (0, 1.8, 1.8, 5.5 and 4.3% at 0, 100, 500, 6,000 and 12,000 ppm, respectively), while the incidence of liver carcinomas was significantly increased at 12,000 ppm (0, 1.8, 1.8, 0 and 4.3%, respectively). The occurrence of liver adenomas in females was within the performing laboratory's historical control range (0–5.4%), while the occurrence of carcinomas was outside the historical control range (0–2.4%). There were a number of independent re-evaluations of the slides from this study, with Hardisty (2000) confirming the increase in hepatocellular adenomas at 6 000 and 12 000 ppm in females but determining that no hepatocellular carcinomas were present at any dose in females. Given that the incidence of liver adenomas in females was within the performing laboratory's historical control range and as there was a poor dose–response relationship, the increase in liver adenomas in females is unlikely to be treatment-related.

IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH AN MOA IN ANIMALS?

a.   *Postulated MOA*

The proposed mode of action (MOA) for the occurrence of liver adenomas in B6C3F1 mice is prolonged exposure to excessive dietary concentrations of malathion leading to sustained metabolic activity of the liver resulting in hypertrophy and the formation of benign liver adenomas.

b.   *Key events*

- *Metabolism of malathion in the liver*. Malathion is metabolized to malaoxon in the liver by the mitochondrial cyctochrome P450-monooxygenase system, microsomal carboxylesterases and the cytosolic glutathione-*S*-transferases (Ketterman, 1987). The metabolite profile is qualitatively similar in rats (Reddy, Freeman & Cannon, 1989) and humans (Jellinek, Schwartz & Connolly Inc., 2000).

- *Liver enzyme induction*. In SD rats, continuous exposure to 200 mg/kg bw per day malathion administered intraperitoneally was required to induce epoxide hydrolase and glutathione-*S*-transferase activities – no induction of cytochrome P450 monooxygenase activity occurred (Reidy et al., 1987). There is in vitro evidence of CYP1A2, 2B6 and 3A4 involvement in the metabolism of malathion by human microsomes, with 2B6 and 3A4 playing more of a role at high malathion concentrations (Buratti et al. 2005). Malathion showed no peroxisome proliferator-activated receptor (PPAR)α or PPARγ (Takeuchi et al. 2006) activity and also

showed no aryl hydrocarbon receptor activity (Takeuchi et al. 2008). There is no evidence available to indicate the activation of the constitutive androstane receptor or pregnane X receptor.

- *Proliferative changes in the liver*. There are no short-term studies in mice to indicate the occurrence of liver hypertrophy. In the pivotal rat study (Daly, 1996a), increased liver weight and hepatocellular hypertrophy occurred after 12 months of exposure, with no additional histopathological changes indicative of liver toxicity. Studies in rats consistently show increases in liver weight and changes in liver function (increased cholesterol, total protein, albumin and GGT). There was no evidence of proliferative changes or cytotoxicity in the liver, including hyperplasia or neoplasia in mice or rats.

- *Development of liver adenomas.* Benign liver adenomas occurred in mice after two years of dietary exposure to very high doses of malathion (i.e. greater than the maximum tolerated dose).

c. Dose–response relationship

In the pivotal mouse study (Slauter, 1994), there was a clear dose-related increase in liver hypertrophy, nodules or discolouration observed macroscopically and adenomas in both sexes at 8000 and 16 000 ppm (Table A2.1).

d. Temporal relationship

There is a paucity of data on the early to middle events in the postulated MOA for the formation of liver adenomas in mice – specifically around the induction of liver enzymes in B6C3F1 mice, adverse effects on liver function and the occurrence of pre-neoplastic changes. In the pivotal rat study (Daly, 1996a), increased liver weight and microscopic evidence of liver hypertrophy was observed after 12 months of dietary exposure to malathion suggestive of an adaptive response to high doses of malathion. After 2 years of dietary exposure, these same changes remained evident at the same doses but were coincident with macroscopic changes (nodules and tan or yellow foci) in addition to adenomas. It is noted that the liver hypertrophy was graded as more severe after 12 than 18 months. An analysis of this study by Hardisty (2000) confirmed the increase in hepatocellular adenomas at 6000 and 12 000 ppm in females but determined that no hepatocellular carcinomas were present at any dose in females.

e. Strength consistency and specificity of association of the tumour response with key events

Studies conducted in the same mouse strain over comparable doses and timescales did not reproduce the treatment-related increase in liver adenomas following dosing with either malathion (NCI, 1978) or malaoxon (NCI, 1979b). There are no data on the potentially reversibility of liver weight increases or hypertrophy in either mice or rats.

f. Biological plausibility and coherence

Notwithstanding the absence of data to support some of the key events, the proposed MOA is considered biologically plausible based on the liver being a target organ.

g. Other MOAs

There is no evidence to suggest other possible modes of action such as cytotoxicity, hormonal perturbation, immunosuppression or porphyria.

h. Uncertainties, inconsistencies and data gaps

The main areas of uncertainty and/or data gaps are studies conducted in B6C3F1 mice demonstrating increases in liver metabolism, such as the induction of CYP enzymes or proliferative processes, including the time frame and doses over which these events might happen. Similar tumours were not observed in mouse and rat studies conducted on malaoxon, which is more toxic than parent malathion. There are no studies that malathion is cytotoxic to hepatocytes.

i. Assessment of the postulated MOA

The level of confidence in the proposed MOA is considered low based on the absence of experimental data indicating proliferative processes in the liver of rodents, observations of precursor lesions or corroborative evidence across similarly conducted studies on malathion or malaoxon in mice and rats.

CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF FUNDAMENTAL, QUALITATIVE DIFFERENCES IN KEY EVENTS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

Given the similar metabolic profile between humans and experimental animals, and evidence of the involvement of CYP enzymes in metabolism by human liver microscomes, the key early metabolic events in the proposed MOA cannot be excluded as relevant to humans. However, scenarios of prolonged human exposure to very high doses of malathion, essential to the proposed MOA, are considered highly unlikely because:

- the use pattern of malathion limits such prolonged, excessive exposure;

- overt inhibition of acetylcholinesterase activity occurs at lower doses and is rate-limiting for the proposed MOA to occur in humans.

CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF QUANTITATIVE DIFFERENCES IN EITHER KINETICS OF DYNAMIC FACTORS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

Hepatocellular adenomas are the most common age-related neoplasm that occurs in B6C3F1 mice and on this basis one might reasonably exclude this mouse strain as a suitable model for humans. Published data (Haseman, Hailey & Morris, 1998) indicates that the combined incidence of these tumours is 10–68%, with the range for adenomas and carcinomas individually being 4–60 and 6-29%, respectively. In addition, the occurrence of liver adenomas in mice occurred only following prolonged exposure to excessive doses of malathion and such a scenario is unlikely to occur in humans. Furthermore, adenomas in mice occurred at doses at least an order of magnitude higher than the lowest doses causing toxicologically significant inhibition of acetylcholinesterase activity.

CONCLUSION: STATEMENT OF CONFIDENCE, ANALYSIS AND IMPLICATIONS

The MOA for malathion-induced liver adenoma formation in B6C3F1 mice cannot be determined with confidence and hence cannot be completely dismissed as relevant to humans. However, the occurrence of these adenomas is considered a likely secondary effect of prolonged exposure to excessive dietary concentrations of malathion and to have a clear threshold; on this basis the risk of carcinogenicity in humans is negligible.

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Appendix 2. Mode of action analysis – rodent nasal tumours

INTRODUCTION

Malathion is an organophosphorus insecticide, and like all members of this chemical class, its mechanism of toxic action is the inhibition of acetylcholinesterase (AChE) activity. Malathion is metabolized to malaoxon in the liver, which is a more potent inhibitor of AChE activity.

CARCINOGENICITY OF MALATHION IN ANIMALS

A total of nine rodent carcinogenicity studies have been conducted on either malathion or malaoxon. In only one of these studies (Daly, 1996a), one nasal adenoma and one nasal carcinoma were observed microscopically in male rats at 6000 ppm and 12 000 ppm, respectively (equal to 359 and 729 mg/kg bw per day, respectively). There were a number of independent re-evaluations of this study to more closely examine the microscopic findings in nasal tissue. Swenberg (1999b) confirmed that dietary exposure to malathion at 6000 or 12 000 ppm caused significant nasal toxicity characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, oedema, and inflammation. No treatment-related increases in neoplasms were apparent in nasoturbinal or nasopharyngeal tissues. A total of four nasal epithelial cell tumours were observed, one in each of the two highest doses of each sex; all were adenomas. Bolte (1999a) examined additionally prepared slides and concluded that the carcinoma originally observed in the respiratory epithelium of one high-dose male was more appropriately diagnosed as an adenoma of the respiratory epithelium. No nasal neoplasms have been observed in any other studies including the counterpart study on malaoxon (Daly, 1996b).

Malathion-induced nasal toxicity has been observed in mice or rats consistently following oral or inhalational exposure of various durations. These observations are summarized in Table A3.1, with a more detailed description of each study following. While specific examination of the nasal cavity and respiratory tract has occurred in all studies of toxicity and carcinogenicity, routine histopathological examination of these sites has not occurred in studies conducted prior to 1996 or conducted over less than lifetime durations.

A.3.1. Summary of nasal toxicity findings in mice and rats

Study	Observations	Reference
28-day dietary Crl:CD[SD] rats	Goblet cell depletion on the nasal septum and hyperplasia of the olfactory epithelium at 10 000 ppm in both sexes (equal to 947.8 and 910.1 mg/kg bw per day, respectively).	Barnett Jr (2012a)
90-day dietary Crl:CD[SD] rats	At and above 500 ppm (35 mg/kg bw per day in males and 35.9 mg/kg bw per day in females), depletion of goblet cells in the nasal cavity occurred. Small numbers of cells with abundant non-staining cytoplasm were interspersed where there was depletion of goblet cells. Hyperplasia of the olfactory epithelium was also noted at the same doses consisting of increased numbers of nuclei.	Barnett Jr (2012b)
13-week inhalation Crl:CD[SD]BR rats	At every tested concentration (at and above 0.1 mg/L), laryngeal hyperplasia, and degeneration and/or hyperplasia of the olfactory epithelium occurred in the nasal cavity.	Beattie (1994)
18-month dietary B6C3F1 BR mice	At 8 000 and 16 000 ppm (equal to 1 476 and 2 978 mg/kg bw per day in males and 1 707 and 3 448 mg/kg bw per day in females), degeneration and loss of cellularity of the olfactory epithelium, loss of olfactory nerves in the submucosa, increased glandular secretion in the lumen due to the retention of mucus and atrophy of the olfactory epithelium adjacent to the retained mucus were observed at both 12 and 18 months.	Slauter (1994)

Study	Observations	Reference
24-month dietary CDF[F-344]/CrlBr rats	At 6 000 and 12 000 ppm (equal to 359 and 729 mg/kg bw per day in males and 415 and 868 mg/kg bw per day in females) dilated mucosal glands, subacute or chronic inflammation of the nasal mucosa, degeneration of the epithelium, epithelium cysts in the nasal mucosa and glandular and epithelium hyperplasia occurred. Nasal adenomas occurred in one male and one female each at 6 000 and 12 000 ppm.	Daly (1996a)
24-month dietary Fischer 344 (CDF[F-344]/CrlBR) rats	The presence of foreign material and inflammatory cell debris was increased at 1 000 and 2 000 ppm. In the respiratory nasal mucosa, subacute or chronic inflammation and hyperplasia of goblet cells and hyperplasia of the respiratory epithelium was increased in females at 1 000 and 2 000 ppm and in males at 2 000 ppm. In the olfactory nasal mucosa, increased degeneration of the epithelium occurred in males at 2 000 ppm and in females at 1 000 and 2 000 ppm. In females, there was an increase in the replacement of the epithelium with by ciliated and non-ciliated columnar epithelial, and hyperplasia of ciliated and non-ciliated columnar epithelial cells at 1 000 and 2 000 ppm. In the lung, oedema, subacute-chronic interstitial and purulent-chronic purulent inflammation and foreign body granulomas occurred at 2 000 ppm in males and at 1 000 and 2 000 ppm in females. In the middle ear, subacute inflammation was accompanied by the accumulation of inflammatory cells or cells debris within the tympanic spaces at 1 000 ppm in females and at 2 000 ppm in both sexes.	Daly (1996b)

bw: body weight; ppm: parts per million

In a 28-day repeat-dose toxicity study in Crl:CD[SD] rats (Barnett Jr, 2012a), which tested dietary concentrations of malathion at 0, 100, 500, 5000 or 10 000 ppm (equal to 0, 9.2, 46.1, 457.5 and 947.8 mg/kg bw per day in males and 0, 9.4, 47.4, 461.3 and 910.1 mg/kg bw per day), minimal to marked goblet cell depletion on the nasal septum and minimal to moderate hyperplasia of the olfactory epithelium (consisting of increased numbers of nuclei) occurred at 10 000 ppm (Table A3.2). The authors proposed that these findings were the result of continued nasal exposure to malathion in the diet.

Table A3.2. Nasal finding in rats exposed to malathion for 28 days in the diet

	Males		**Females**	
Finding	0 ppm	10 000 ppm	0 ppm	10 000 ppm
N	15	15	15	15
Nose, Level 2 – Goblet cell depletion				
Minimal	0	1	0	4
Mild	0	3	0	6
Moderate	0	9	0	4
Marked	0	2	0	0
Total	0	15	0	14
Nose, Level 3 – Hyperplasia of the olfactory epithelium				
Minimal	0	3	0	6
Mild	0	12	0	9
Nose, Level 4 – Hyperplasia of the olfactory epithelium				
Minimal	0	0	0	2
Mild	0	3	0	9
Moderate	0	12	0	4

Finding	Males		Females	
	0 ppm	10 000 ppm	0 ppm	10 000 ppm
Total	0	15	0	15
Nose, Level 5 – Hyperplasia of the olfactory epithelium				
Minimal	0	0	0	1
Mild	0	2	0	5
Moderate	0	12	0	8
Total	0	14	0	14

ppm: parts per million

Results expressed as the absolute number of rats with the finding.

Source: Barnett Jr (2012a)

In a 90-day repeat-dose toxicity study in Crl:CD[SD] rats (Barnett Jr, 2012b), which tested dietary concentrations of malathion of 0, 100, 500, 5000 or 10 000 ppm (equal to 0, 7.2, 35.0, 353.6 and 733.8 mg/kg bw per day in males and 0, 7.5, 35.9, 363.1 and 719.0 mg/kg bw per day in females), minimal to mild depletion of goblet cells in the nasal cavity was observed at and above 500 ppm (Table A3.3). Small numbers of cells with abundant non-staining cytoplasm were interspersed where there was depletion of goblet cells. Minimal to moderate hyperplasia of olfactory epithelium was also noted at these same doses consisting of increased numbers of nuclei.

Table A3.3. Microscopic nasal findings in rats

Finding	Males					Females				
	0	100	500	5 000	10 000	0	100	500	5 000	10 000
N	10	10	10	10	10	10	10	10	10	10
Goblet cell depletion – nose Level 2										
Minimal	0	0	5	1	3	0	0	3	4	1
Mild	0	0	0	7	2	0	0	2	3	2
Moderate	0	0	0	2	4	0	0	0	1	6
Marked	0	0	0	0	0	0	0	0	0	1
Total	0	0	5	10	9	0	0	5	8	10
Hyperplasia of olfactory epithelium – nose Level 3										
Minimal	0	0	0	6	7	0	0	0	5	3
Mild	0	0	0	3	3	0	0	0	4	6
Moderate	0	0	0	0	0	0	0	0	0	1
Total	0	0	0	9	10	0	0	0	9	10
Hyperplasia of olfactory epithelium – nose Level 4										
Minimal	0	0	0	0	0	0	0	0	1	0
Mild	0	0	0	7	7	0	0	0	4	5
Moderate	0	0	0	3	3	0	0	0	5	5
Total	0	0	0	10	10	0	0	0	10	10

Results expressed as the number of rats with the finding.

Source: Barnett Jr (2012b)

In a 13-week inhalational toxicity study in Crl:CD[SD]BR rats (Beattie, 1994), which tested nominal concentrations of 0, 0.24, 1.10 or 4.94 mg/L (analytical concentrations of 0, 0.1, 0.45 and 2.0 mg/L, respectively), laryngeal hyperplasia and degeneration and/or hyperplasia of the olfactory epithelium in the nasal cavity occurred at all doses, with a dose-related increase in severity (Table A3.4).

Table A3.4. Microscopic findings in rats exposed to aerosols of malathion for 13 weeks

Parameter	Aerosol concentration (mg/L)			
	0	0.1	0.45	2.0
Laryngeal hyperplasia				
Males (*n* = 15)				
Incidence	0	13	15	15
Severity	–	1.1	2.4	2.9
Females (*n* = 15)				
Incidence	0	15	15	15
Severity	–	1.4	2.7	2.5
Degeneration and/or hyperplasia of the olfactory epithelium				
Males (*n* = 15)				
Incidence	1	15	15	14
Severity	0.1	1.6	1.7	2.6
Females (*n* = 15)				
Incidence	1	10	15	14
Severity	0.1	0.7	1.6	2.6

Source: Beattie (1994)

In an 18-month study of toxicity and carcinogenicity conducted in B6C3F1 BR mice (Slauter, 1994; re-examination by Swenberg, 1999c) which tested dietary concentrations of 0, 100, 800, 8000 and 16 000 ppm (equal to 0, 17, 143, 1476 and 2978 mg/kg bw per day in males and 0, 21, 167, 1707 and 3448 mg/kg bw per day in females), degeneration and loss of cellularity of the olfactory epithelium, loss of olfactory nerves in the submucosa, increased glandular secretion in the lumen due to the retention of mucus and atrophy of the olfactory epithelium adjacent to the retained mucus occurred at 8000 and 16 000 ppm. This nasal toxicity was observed at both 12 and 18 months.

In a 24-month study of toxicity and carcinogenicity conducted in CDF[F-344]/CrlBr rats (Daly, 1996a), which tested dietary concentrations of malathion at 0, 100, 500, 6000 or 12 000 ppm (equal to 0, 7, 29, 359 and 729 mg/kg bw per day in males and 0, 8, 35, 415 and 868 mg/kg bw per day in females at 0, 100, 500, 6000 or 12 000 ppm, respectively), dilated mucosal glands (the majority graded as slight), subacute or chronic inflammation of the nasal mucosa (the majority graded as slight to moderate), degeneration of the epithelium (the majority graded as moderate to moderately severe), epithelium cysts in the nasal mucosa (mainly graded as minimal to slight), and glandular and epithelium hyperplasia (mainly graded as slight) occurred at 6000 and 12 000 ppm (Table A3.5).

Table A3.5. Histopathological findings in nasal tissue

Parameter	Dietary concentration (ppm)				
	0	**100 or 50**	**500**	**6 000**	**12 000**
N	90	90	90	90	90
Nasal mucosa (olfactory) – glands dilated					
Males	2	1	0	31	27
Females	2	1	0	38	33
Nasal mucosa (olfactory) – subacute/chronic inflammation					
Males	6	1	7	52	35
Females	0	3	2	42	20
Nasal mucosa (olfactory) – epithelium degeneration					
Males	4	2	5	66	69
Females	2	2	1	69	66
Nasal mucosa (olfactory) – epithelium cysts					
Males	0	0	0	43	55
Females	0	0	0	58	62
Nasal mucosa (olfactory) – glandular hyperplasia					
Males	0	0	0	17	18
Females	0	0	0	24	14
Nasal mucosa (olfactory) – epithelium hyperplasia					
Males	0	0	0	42	51
Females	0	0	0	57	54
Nasal mucosa (olfactory) – olfactory epithelium replaced by ciliated and non-ciliated columnar epithelial cells					
Males	6	1	7	43	43
Females	2	2	1	50	25
Nasal mucosa (olfactory) – hyperplasia of ciliated and non-ciliated columnar epithelial cells					
Males	3	1	4	18	22
Females	2	1	0	33	21
Nasal mucosa (respiratory) – subacute/chronic inflammation					
Males	10	2	12	41	21
Females	7	4	5	34	10
Nasal mucosa (respiratory) – glands dilated					
Males	18	0	13	28	24
Females	8	4	6	14	20
Nasal mucosa (respiratory) – hyperplasia					
Males	13	2	12	44	41
Females	7	3	7	44	33
Nasal lumen – cell/cell debris/metachromatic basophilic amorphous material					
Males	15	5	22	69	63
Females	10	7	9	64	58
Nasopharynx – epithelial hyperplasia					
Males	10	0	15	22	14

Parameter	Dietary concentration (ppm)				
	0	100 or 50	500	6 000	12 000
Females	4	1	14	26	21

Results expressed as the number of rats with the finding.

Source: Daly (1996a)

Neoplasms observed microscopically in nasoturbinal tissue included an adenoma in one male at 6000 ppm and a carcinoma in one male at 12 000 ppm. The occurrence of spontaneous neoplasms of nasoturbinal tissue is a rare finding in F344 rats and one not observed by the performing laboratory in six previous studies (0/238 males and 0/241 females). In addition, in eight National Toxicology Program studies only six neoplasms were detected in approximately 4000 control males. There were a number of independent pathological re-evaluations conducted after this study was completed to more closely examine the microscopic findings in nasal tissue. Swenberg (1999b) confirmed that dietary exposure to malathion at 6000 or 12 000 ppm caused significant nasal toxicity characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, oedema, and inflammation. No treatment-related increases in neoplasms were apparent in the nasoturbinal and nasopharyngeal tissues. A total of four nasal epithelial cell tumours were observed, one in each of the two highest doses of each sex; all were adenomas. Bolte (1999a) examined additionally prepared slides and concluded that the carcinoma originally observed in the respiratory epithelium of one high-dose male was more appropriately diagnosed as an adenoma of the respiratory epithelium.

A 24-month study of toxicity and carcinogenicity was conducted in Fischer 344 (CDF (F-344)/CrlBR) rats, which tested dietary concentrations of malaoxon of 0, 20, 1000 or 2000 ppm (equal to 0, 1, 57 and 110 mg/kg bw per day in males and 0, 1, 68 and 140 mg/kg bw per day in females). Nasal findings are summarized in Table A3.6. In the nasal lumen, the presence of foreign material (minimal to severe) and inflammatory cell debris was increased (minimal to moderately severe) at 1000 and 2000 ppm. In the respiratory nasal mucosa, subacute or chronic inflammation (slight to moderately severe) and hyperplasia of goblet cells (slight to moderately severe) and hyperplasia of the respiratory epithelium (slight to moderately severe) was increased in females at 1000 and 2000 ppm and in males at 2000 ppm. In the olfactory nasal mucosa, increased degeneration of the epithelium (slight to moderate) occurred in males at 2000 ppm and in females at 1000 and 2000 ppm. In females, there was an increase in the replacement of the epithelium with ciliated and non-ciliated columnar epithelial (slight to moderate severe), and hyperplasia of ciliated and non-ciliated columnar epithelial cells (slight to moderate severe) at 1000 and 2000 ppm. In the lung, oedema (minimal to moderate), subacute-chronic interstitial and purulent-chronic purulent inflammation (minimal to moderate) and foreign body granulomas (minimal to moderate) occurred at 2000 ppm in males and at 1000 and 2000 ppm in females. In the middle ear, subacute (chronic active)/chronic inflammation was accompanied by the accumulation of inflammatory cells or cells debris within the tympanic spaces at 1000 ppm in females and at 2000 ppm in both sexes. Collectively these effects were attributable to inhaled food particles resulting in tissue injury and inflammation to the nasal cavity, with secondary effects in the lungs and middle ear.

Table A3.6. Non-neoplastic findings in rats exposed to malaoxon for 2 years

Parameter	Dietary concentration (ppm)			
	0	**20**	**1 000**	**2 000**
Nasal lumen – presence of foreign material				
Males	6/65	10/65	9/65	28/64
Females	1/65	6/63	17/64	27/65
Nasal lumen – inflammatory cell debris				
Males	13/65	21/65	15/65	31/64
Females	6/65	6/63	20/64	27/65
Nasal mucosa (respiratory) – subacute (chronic active) or chronic inflammation				
Males	11/65	11/65	10/65	21/64
Females	6/65	6/63	20/64	27/65
Nasal mucosa (respiratory) – epithelial hyperplasia				
Males	11/65	18/65	13/65	20/64
Females	3/65	5/63	27/64	20/65
Nasal mucosa (respiratory) – epithelium squamous or squamoid metaplasia				
Males	3/65	4/65	8/65	6/64
Females	0/65	1/63	6/64	5/65
Nasal mucosa (olfactory) – epithelium degeneration				
Males	4/65	6/65	5/65	12/64
Females	2/65	0/63	17/64	10/65
Nasal mucosa (olfactory) – olfactory epithelium replaced by ciliated and non-ciliated columnar epithelial cells				
Males	5/65	6/65	7/65	7/64
Females	2/65	2/63	11/64	10/65
Nasal mucosa (olfactory) – hyperplasia of ciliated and non-ciliated columnar epithelial cells				
Males	5/65	2/65	4/65	7/64
Females	1/65	1/63	11/64	7/65
Lung – oedema				
Males	5/65	5/55	9/55	16/65
Females	1/64	3/55	22/55	17/65
Lung – inflammation of the interstitium				
Males	12/65	9/55	12/55	23/65
Females	14/64	15/55	29/55	34/65
Lung – purulent/chronic purulent inflammation or abscess(es)/chronic abscess(es)				
Males	4/65	2/55	7/55	17/65
Females	2/64	2/55	22/55	19/65
Lung – granulomatous inflammation/granulomas				
Males	8/65	3/55	11/55	12/65
Females	2/64	6/55	29/55	29/65
Middle ear (tympanic cavity/epithelial lining) – subacute (chronic active)/chronic inflammation/inflammatory cells/cell debris				
Males	8/54	5/16	7/22	15/58

Parameter	Dietary concentration (ppm)			
	0	20	1 000	2 000
Females	2/54	3/8	17/20	19/50

*P < 0.05; **P < 0.01

Results expressed as the absolute number of rats / number of rats examined.

Source: Daly (1996b)

IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH A MOA IN ANIMALS?

a. Postulated MOA

The proposed MOA for the occurrence of nasal adenomas in rats is direct exposure to malathion vapours when present in feed or to inhalation of food particles containing malathion. Direct and repeated contact of malathion or its metabolites with nasal tissue results in irritation, which over prolonged periods causes inflammation, pre-neoplastic changes and tumour formation.

b. Key events

- *Distribution of malathion or malathion metabolites to nasal tissue*. It is considered unlikely that malathion or its metabolites could accumulate directly in nasal tissue following systemic exposure via the diet. The Reddy, Freeman & Cannon (1989) study showed that malathion undergoes extensive metabolism, is rapidly excreted and does not accumulate in any tissue. However, no studies have specifically examined the distribution of malathion or its metabolites in nasal tissue. The vapour pressure of malathion is relatively low and therefore it is unlikely that rats would inhale malathion vapours from feed. The most likely exposure pathway is by inhaling malathion-containing food particles. Evidence to support this exposure pathway comes from 2-year rat studies on malathion (Daly, 1996a) and malaoxon (Daly, 1996a), where the occurrence of inflammatory and hyperplastic changes was coincident with the presence of inhaled food particles or debris in the nasal passage. Similar tissue changes observed in rats following direct inhalational exposure to malathion aerosols confirm that direct exposure of nasal tissue to malathion causes hyperplastic changes (Beattie 1994).

- *Irritation of nasal tissue by repeated exposure to malathion*. Studies conducted in rats indicated that malathion was only slightly irritating to rabbit skin and eyes. The respiratory and olfactory epithelium of rats contains high concentration of carboxylesterases that could metabolize malathion to MMCA and MDCA. Prolonged irritation by either of these two metabolites could induce a reactive hyperplasia. The absence of nasal tumours in rats exposed continuously to malaoxon is consistent with this hypothesis because the metabolism of malaoxon does not involve the formation of these acids.

- *Development of inflammatory changes in nasal tissue*. Goblet cell depletion of the nasal septum and hyperplasia of the olfactory epithelium was observed following 28 days of dietary exposure (Barnett Jr, 2012a). Similar changes were also observed in subchronic studies (Barnett Jr, 2012b; Beattie, 1994). Longer-term exposure to malathion or its metabolites results in more severe changes including dilated mucosal glands, chronic inflammation, epithelial degeneration, epithelium cysts and squamous metaplasia (Daly, 1996a,b).

- *Development of benign nasal adenomas*. Continuous exposure of nasal tissue to malathion or its metabolites results in the formation of benign adenomas at high doses in rats as a secondary effect of continuous inflammation and regeneration of nasal tissue.

c. Dose–response relationship

In the pivotal rat study, single adenomas occurred in both sexes at the two highest doses. Nasal tumours are an apparently rare finding in rats and therefore the modest dose–response relationship is considered unremarkable. In relation to nasal toxicity preceding the possible development of

adenomas, there is a clear and consistent, dose-related increase in the numbers of animals affected in addition to the severity of nasal toxicity.

d. Temporal relationship

Over time there was an increase in the severity of nasal toxicity (first evident after 28 days of dietary exposure) as the duration of dietary exposure increased to the point where the development of adenomas occurred only after two years of continuous exposure to malathion.

e. Strength consistency and specificity of association of the tumour response with key events

In the pivotal rat study (Daly, 1996a), the occurrence of nasal adenomas was coincident with nasal toxicity. Where histopathological examination of nasal tissue was incorporated into the study protocol, malathion-induced nasal toxicity was observed consistently across multiple studies where rats or mice were exposed to malathion in the diet or via inhalation from 28 days to 24 months.

f. Biological plausibility and coherence

Notwithstanding the absence of data demonstrating that direct exposure of nasal tissue is necessary to induce nasal toxicity (rather than systemic exposure), the proposed MOA is considered biologically plausible based on the consistency of observations and the increase in severity of toxicity over prolonged periods of exposure.

g. Other MOAs

No other modes of action are proposed

h. Uncertainties, inconsistencies and data gaps

The main data gaps relate to tissue distribution and metabolism studies indicating that malathion or its metabolites do not preferentially distribute to nasal tissue systemically. Such data would support the hypothesis that direct and prolonged exposure of the upper respiratory tract to inhaled food particles containing malathion is necessary for tumour development.

i. Assessment of the postulated MOA

The level of confidence in the proposed MOA is considered moderate to high based on the consistency of nasal toxicity observed across studies of various durations. The MOA is qualitatively possible in humans, though quantitatively unlikely due to functional and anatomical differences in the respective respiratory systems (Frederick et al., 2002). There is no risk from human dietary exposure as there is negligible potential for prolonged and direct contact with nasal tissue.

CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF FUNDAMENTAL, QUALITATIVE DIFFERENCES IN KEY EVENTS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

No. It is plausible that direct contact of human nasal tissue with malathion over lifetime exposures could induce nasal toxicity.

CAN HUMAN RELEVANCE OF THE MOA BE REASONABLY EXCLUDED ON THE BASIS OF QUANTITATIVE DIFFERENCES IN EITHER KINETICS OF DYNAMIC FACTORS BETWEEN EXPERIMENTAL ANIMALS AND HUMANS?

The human olfactory epithelium is better protected from vapours of organic esters than is rat olfactory epithelium due to differences in nasal anatomy, nasal and systemic metabolism, systemic physiology and airflow (Frederick et al., 2002).

CONCLUSION: STATEMENT OF CONFIDENCE, ANALYSIS AND IMPLICATIONS

The MOA for malathion-induced nasal adenomas in rats was considered quantitatively implausible for humans on the basis that nasal tissue would not be exposed directly to the prolonged and excessive doses of malathion necessary to induce tumours in rats.

REFERENCES (ADDITIONAL TO THOSE CITED IN THE MONOGRAPH)

Frederick CB, Lomax LG, Black KA, Finch L, Scribner HE, Kimbell JS et al. (2002). Use of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry comparisons of ester vapours. Toxicol Appl Pharmacol. 183:23-40.