Pesticide residues in food — 2016

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2016

Part II — Toxicological

Food and Agriculture Organization of the United Nations

World Health Organization

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

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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 × *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 Koutros et al. (2015); Lerro et al. (2015)
	Scopus (limited to 2014–2016)	28	
(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.

[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

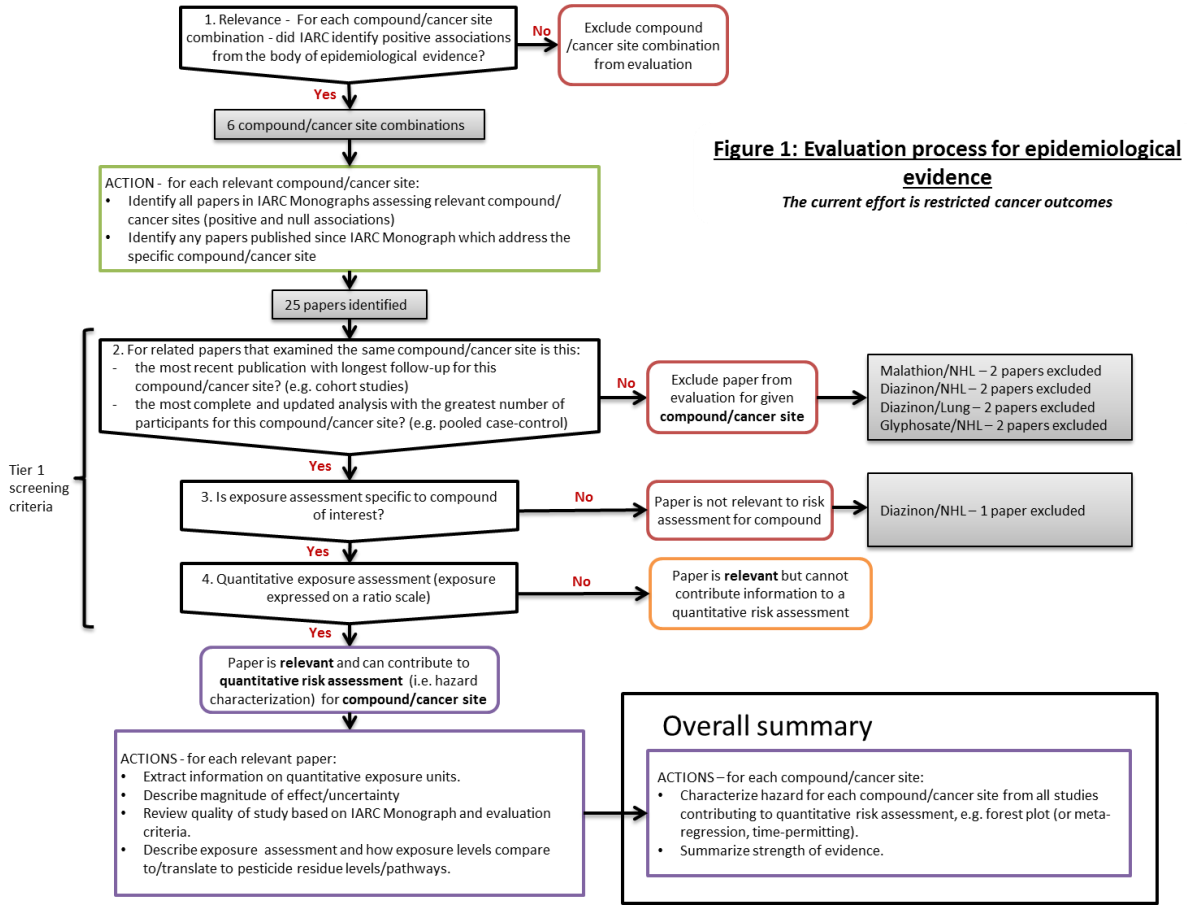

Figure 1: Evaluation process for epidemiological evidence

The current effort is restricted cancer outcomes

(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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TOXICOLOGICAL MONOGRAPHS
AND MONOGRAPH ADDENDA

DIAZINON

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Explanation

Diazinon (Fig. 1) is the common name approved by the International Organization for Standardization (ISO) for *O*,*O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service (CAS) number 333-41-5.

Diazinon is a contact organophosphorus insecticide with a wide range of insecticidal activity. It is effective against adult and juvenile forms of flying insects, crawling insects, acarians and spiders. Diazoxon, the biologically active metabolite of diazinon, inhibits the activity of cholinesterases.

Diazinon is used mainly as a pesticide in agriculture and as a drug in veterinary medicine. Thus, the major source of diazinon residues in edible crops is from its use as an agricultural pesticide; residues in meat, offal and other animal products arise from its use as a veterinary drug containing active ingredient.

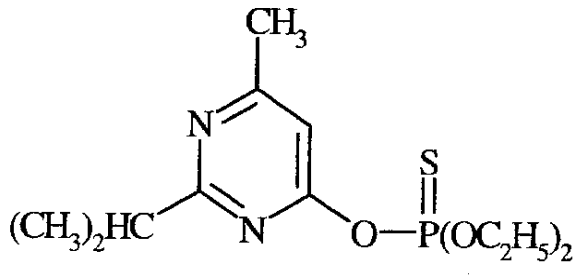
Diazinon has been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) on several occasions since the first evaluation in 1963. In the most recent evaluation, in 2006, the Meeting established an acceptable daily intake (ADI) of 0 to 0.005 mg/kg body weight (bw), based on a no-observed-adverse-effect level (NOAEL) of 0.5 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in a 92-day repeated-dose toxicity study in rats. The 2006 Meeting reaffirmed the acute reference dose (ARfD) of 0.03 mg/kg bw, established by the 2001 JMPR, based on a NOAEL of 2.5 mg/kg bw observed in a study of acute neurotoxicity in rats.

Diazinon was scheduled within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR) for 2021. The compound was placed on the agenda by the JMPR Secretariat following the recommendation of an electronic task force of the World Health Organization (WHO) Core Assessment Group on Pesticide Residues that it be re-evaluated due to public health concern 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 considered toxicological data in addition to new published or unpublished toxicological studies and published epidemiological studies on cancer outcomes. Several study reports evaluated at previous JMPR meetings were not available to the present Meeting, as they were not submitted in the sponsor's dossier; for these studies, the evaluations in this monograph were taken from the 1993 JMPR monograph without further review.

All critical unpublished studies contained statements of compliance with good laboratory practice (GLP), unless otherwise specified. The studies on human volunteers were conducted in accordance with the principles expressed in the Declaration of Helsinki or equivalent ethical standards.

Fig. 1. Structure of diazinon



Evaluation for acceptable intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The effect of oral and topical administration of diazinon was studied in various animal species using unlabelled and radiolabelled diazinon. Additional studies were performed in vitro using tissue slices or cell fractions from different tissues and species to investigate the biotransformation of the compound.

(a) Oral route

Rats

According to a study by Mücke, Alt & Esser (1970):

After the application of a single oral dose of 0.8 mg/rat (4 mg/kg bw) ^{14}C-labelled diazinon (labels at 2-^{14}C, 4-^{14}C or ethoxy-^{14}C) to four male and two female Wistar rats, radioactivity was eliminated practically completely during the 168-hour observation period with either label. Excretion of applied radioactivity amounted to 65–80% in urine, and 16–24% in faeces. The excretion half-time was estimated to be 12 hours for the pyrimidine-labelled and 7 hours for the ethoxy-labelled compound. After [applying the] side-chain labelled material, about 6% of the applied dose was eliminated as $^{14}CO_2$. The absence of radioactive CO_2 in the expired air after application of ring-labelled diazinon shows that no cleavage of the pyrimidine ring occurred.

After feeding male rats [for] 10 days 0.1 mg/rat per day (0.5 mg/kg bw per day) of 2-^{14}C-diazinon, 2.9% of the applied dose was found in the essential organs 6 hours after the final application, whereas 2 days after cessation of treatment the radioactivity was below the detection limit (< 0.1%). These results indicate that no accumulation of diazinon or its metabolites occurs in the body (Mücke, Alt & Esser, 1970; study evaluation copied from 1993 JMPR without further evaluation).

In the low-dose group, 93% of the applied radioactivity was excreted in the urine [of] male [rats] and 86% in [the urine of] female [rats] within 24 hours. At the high-dose level, an average of 91% and 58% in males and females, respectively, was excreted in the first 24 hours. After preconditioning, about 90% of the ^{14}C-dose was excreted within 24 hours in both sexes. The total amounts eliminated over the seven-day observation period did not differ between the various dosing regimens. Total urinary excretion amounted to 96%, and faecal elimination to 3%. These results suggest that complete absorption occurs following intragastric administration and supports the hypothesis that the small amount of faecal radioactivity found may be of biliary origin. Tissue concentrations of ^{14}C-diazinon and metabolites seven days after dosing were mostly less than 0.05 ppm [parts per million] for low-dose and preconditioned animals. In high-dose animals, residue levels in different organs varied between 0.1 and 0.4 ppm (red blood cells) with female rats showing consistently slightly higher tissue residues than males. The ^{14}C in the blood was associated primarily with the cellular fraction, suggesting that binding occurred (Capps et al., 1989; Craine, 1989; study evaluations copied from the 1993 JMPR without further evaluation).

Guinea-pigs

^{32}P-Labelled diazinon was administered orally or subcutaneously to male guinea-pigs at a dose level of 45 mg/kg bw. After oral dosing, 80% of the applied radioactivity was excreted in the urine within 48 hours, whereas faecal excretion was 10% mostly eliminated within the first day. After subcutaneous dosing, urinary excretion was 53%, faecal excretion was minimal. Highest residues were found in the caecum corresponding to 36% and 5% of the radioactivity administered 16 hours after oral and subcutaneous dosing, respectively. About 1–2% of the radioactivity was found in the liver after 16 hours. During the 7-day observation period, over 87% of the dose was eliminated in the excreta, mainly in the urine, indicating that the large amounts found in the caecum after oral treatment ultimately left the body via the kidneys. The accumulation of radioactivity in the caecum also following subcutaneous injection indicates that this tissue may play a role in metabolism or elimination of diazinon and/or its metabolites in guinea-pigs (Kaplanis, Louloudes & Roan, 1962; study evaluation copied from the 1993 JMPR without further evaluation).

Hens

The following studies measured the elimination of ^{14}C-labelled diazinon by hens:

After oral administration of ^{14}C-diazinon to 4 laying hens by capsule for 7 consecutive days at daily doses of 2.8 mg/animal corresponding to 25 ppm, 79% of the total dose applied was eliminated in the excreta and 0.1% was found in the tissues at sacrifice, about 24 hours after the final dose. Highest tissue levels of 0.15 ppm were found in the kidney. Maximum residues in eggs amounted to 0.07 ppm. The treatment caused some reduction in body weight in most animals but had no influence on the general health condition (Simoneaux, 1988b; Simoneaux et al., 1988; Burgener & Seim, 1988; study evaluations copied from the 1993 JMPR without further evaluation).

Dogs

In an intravenous study, ^{14}C-ethoxy-labelled diazinon was injected at a dose level of 0.2 mg/kg bw [into dogs]. After 24 hours, 58% of the applied radioactivity was recovered in urine (Iverson, Grant & Lacroix, 1975; study evaluation copied from the 1993 JMPR without further evaluation).

Goats

Two lactating goats were treated daily with ^{14}C-labelled diazinon by capsule for four consecutive days at a dose of 150 mg/animal (4 mg/kg bw). Urinary excretion amounted to 64% of the applied dose, whereas faeces contained an average of 10%. Highest residues of 2 ppm ^{14}C were found in kidneys whereas the levels in the other tissues ranged from 0.2 to 1.2 ppm. Highest levels in milk were 0.5 ppm (Pickles & Seim 1988; Simoneaux, 1988a,c; Simoneaux et al., 1988).

Cows

According to a study by Robbins, Eddy & Hopkins (1957):

A lactating cow was orally treated by capsule with a dose level of 20 mg/kg bw of ^{32}P-labelled diazinon. About 74% of the applied dose was excreted in the urine within 36 hours, and 6% in the faeces. The cumulative percentage of total dose in milk 36 hours after treatment was less than 0.08% (Robbins, Eddy & Hopkins, 1957; study evaluation copied from 1993 JMPR without further evaluation).

In summary, diazinon is rapidly and almost completely absorbed and eliminated after oral application. Excretion occurs mainly via the kidneys. Diazinon or its metabolites do not accumulate in the body.

(b) Dermal route

Rats

According to a study by Ballantine, Marco & Williams (1984):

Groups of 4 rats were dermally treated with dose levels of 1 or 10 mg/kg bw ^{14}C-diazinon dissolved in tetrahydrofuran. Renal excretion ranged from 70–80% of the applied dose at both dose levels over the 6 days observation period; elimination in faeces was usually less than 10%. Most of the radioactivity was eliminated within 48 hours after application. After 72 hours, less than 1% of the applied dose was found on the skin. Highest tissue residues were measured 8 hours after treatment varying between 0.1 and 0.4 ppm in the low-dose animals. Residues in the high-dose animals were correspondingly higher. These results show that diazinon is easily absorbed through the skin (Ballantine, Marco & Williams, 1984; study evaluation copied from 1993 JMPR without further evaluation).

Sheep

The following studies assessed the effects of dermal exposure in sheep:

Two sheep were dermally exposed for 3 days to diazinon at a daily dose of 40 mg/kg bw. The sheep were sacrificed six hours after the final application. Radiolabelled residues were observed in all tissues at levels ranging from 2.2 to the highest value of 13 ppm in kidney. Because the study was designed to allow the identification of metabolites in sheep tissue after topical application of ^{14}C-diazinon, no results were presented with respect to elimination of the compound (Capps et al., 1990; Pickles & Seim 1990; study evaluations copied from 1993 JMPR without further evaluation).

1.2 Biotransformation

Biotransformation of diazinon has been extensively studied in mammalian and avian species. Considerable breakdown occurs in all the species studied.

(a) In vitro studies

The following study evaluations were copied from the 1993 JMPR without further evaluation:

Diazinon was incubated with liver microsomes from different avian species, rat, guinea-pig, pig, sheep and cow. Metabolites identified included hydroxydiazinon, isohydroxydiazinon, dehydroxydiazinon, their oxons and diazoxon. Yields and rates of production of these metabolites varied between the different species (Machin et al., 1975).

In vitro studies using microsomal preparations from rat liver showed that diazinon was converted rapidly to water-soluble metabolites (Dahm, 1970). The oxidation of diazinon by the microsomal enzyme system fortified with NADPH [nicotinamide adenine dinucleotide phosphate (reduced)] or NADH [nicotinamide adenine dinucleotide (reduced)] occurred through hydroxylation of the ring alkyl side-chain, desulfuration, and cleavage of the arylphosphate bound. The major metabolic products of diazinon were hydroxydiazinon, diazoxon and hydroxydiazoxon. Other metabolites identified were 2-isopropyl-4-methyl-6-hydroxypyrimidine, 2-(2'-hydroxy-2'-propyl)-4-methyl-6-hydroxypyrimidine, diethyl-phosphorothioic acid and diethylphosphoric acid, which were all produced by the cleavage of the arylphosphate bound (Shishido et al., 1972).

Diazoxon, the active toxicant, formed by the oxidation of diazinon through desulfuration was degraded mainly by hydrolysis resulting in the two metabolites diethylphosphoric acid and 2-isopropyl-4-methyl-6- hydroxypyrimidine (Shishido & Fukami, 1972).

A glutathione conjugate *S*-(2-isopropyl-4-methyl-6-pyrimidinyl) glutathione was also identified. This compound was formed by conjugation of reduced glutathione and the pyrimidinyl moiety of diazinon with the simultaneous cleavage of the phosphate ester bound (Shishido, Usui & Fukami, 1972). In insects, diazoxon is degraded slowly by the microsomal mixed function oxidase system, while no diazoxon-hydrolyzing enzyme was found (Shishido & Fukami 1972; Yang, Hodgson & Dautermann, 1971).

(b) In vivo studies

Rats

In a metabolic study, Mücke, Alt & Esser (1970) showed that

… rats treated with ^{14}C-labelled diazinon (2-^{14}C, 4-^{14}C or ethoxy-^{14}C labels) by the oral route (4 mg/kg bw) showed that the parent compound is degraded rapidly yielding the 3 pyrimidinols, 2-isopropyl-6-methyl-4(1*H*)-pyrimidinone, 2-(alpha-hydroxyisopropyl)-6-methyl-4(1*H*)-pyrimidinone and its beta isomer as main urinary metabolites. A number of polar unidentified substances was also found. In addition to small amounts of unchanged diazinon the same metabolites were also found in faeces. Diazoxon as a labile and transient intermediate was absent in the extracts of urine and faeces. The absence of radiolabelled CO_2 indicated that no cleavage of the pyrimidine ring took place. The intravenous application of these main metabolites revealed that the pyrimidinols are further degraded yielding some unidentified polar metabolites (Mücke, Alt & Esser, 1970; study evaluation copied from the 1993 JMPR without further evaluation).

A similar metabolic pattern was found when

…[one group of rats was] treated with single oral doses of 10 or 100 mg/kg bw of ^{14}C-labelled diazinon. Another group was preconditioned for 14 days with unlabelled diazinon and then dosed with 10 mg/kg bw of ^{14}C-labelled diazinon…. In urine, the three pyrimidinols accounted for 65% of the applied radioactivity, whereas 15% consisted of polar non-identified metabolites and trace amounts of diazinon (0.11%), hydroxydiazinon (0.12%) and diazoxon (0.14%) (Capps et al., 1989).

Dogs

Metabolic studies in dogs were performed after oral and intravenous administration of ring-labelled and ethoxy-labelled diazinon, respectively. After [intravenous administration] application the ^{14}C-ethoxy-labelled diazinon, diethylphosphoric acid (DEP) and diethylphosphorothioic acid (DETP) were detected in urine, whereas after the oral dosing with the ^{14}C-ring-labelled diazinon, the pyrimidinols 2-isopropyl-6-methyl-4(1*H*)-pyrimidinone and 2-(alpha-hydroxyisopropyl)-6-methyl-4(1*H*)-pyrimidinone could be identified. In contrast to the results of an in vitro study (Shishido & Fukami, 1972; study evaluation copied from 1993 JMPR without further evaluation) no evidence was given from this dog study that the cleavage of the ester bond was glutathione-mediated (Iverson, Grant & Lacroix, 1975; study evaluation copied from the 1993 JMPR without further evaluation).

Cows, sheep and other mammalian and nonmammalian species

In a metabolic study with cows treated with an oral dose of 20 mg/kg bw ^{32}P-labelled diazinon, DEP and DETP were found as urinary end-products of diazinon metabolism (Robbins, Eddy & Hopkins, 1957; study evaluation copied from 1993 JMPR without further evaluation).

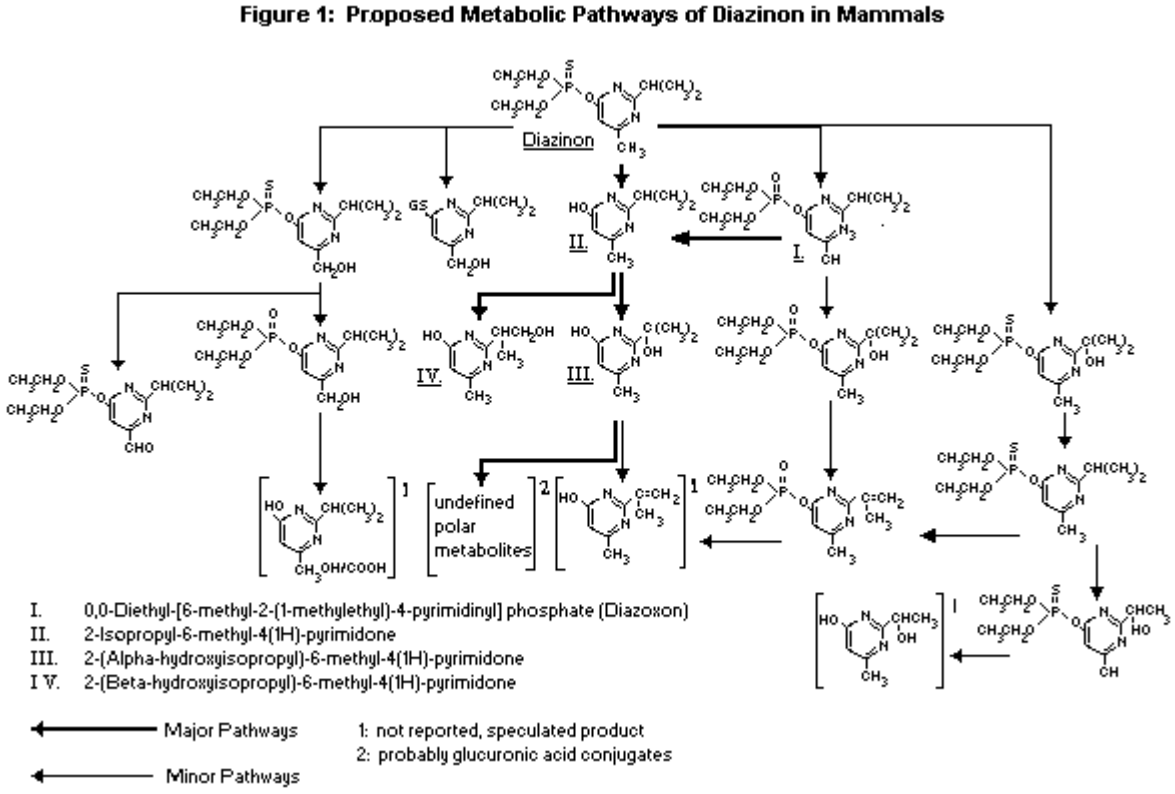
The pyrimidinols were also identified as the major metabolites in urine and tissues of dermally treated sheep (Capps et al., 1990; study evaluation copied from 1993 JMPR without further evaluation) and orally treated goats and hens (Simoneaux 1988c,d; Simoneaux et al., 1988; study evaluations copied from 1993 JMPR without further evaluation). The respective glucuronides were also identified in these species (Sachsse & Bathe, 1976; Simoneaux 1988c,d; Simoneaux et al., 1989; Capps et al., 1990; study evaluations copied from the 1993 JMPR without further evaluation).

In summary, diazinon was found to be readily degraded and the metabolites formed were mainly eliminated via the kidneys. The main degradative pathway of diazinon in mammals includes the oxidase/hydrolase-mediated cleavage of the ester bond leading directly and via diazoxon to the pyrimidinol derivative 2-isopropyl-6-methyl-4(1*H*)-pyrimidinone, which is further oxidized at the isopropyl substituent resulting in the hydroxy pyrimidinols either excreted as such or further degraded to more polar metabolites. Metabolites with an intact pyrimidinyl phosphorus ester bond such as hydroxydiazinon, diazoxon and its hydroxy derivative are only transient products which are ultimately cleaved to their corresponding pyrimidine analogs. The uncleaved products were found only in in vitro

studies (Hagenbuch & Mücke, 1985; study evaluation copied from the 1993 JMPR without further evaluation).

[Fig. 2] presents the proposed metabolic pathway of diazinon in mammals.

Fig. 2. Proposed metabolic pathway for diazinon in the rat



2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity tests on diazinon are summarized in Table 1.

Table 1. Summary of acute toxicity studies of diazinon

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Mouse	N/S	M + F	Oral	N/S	LD_{50} 187 mg/kg bw	Bathe (1972a) [a]
Mouse	ICR	M + F	Oral	96.0	LD_{50} 177 mg/kg bw (males) and 178 mg/kg bw (females)	Ishige et al. (1986)
Rat	N/S	M + F	Oral	97.1	LD_{50} 422 mg/kg bw	Bathe & Gfeller (1980) [a]
Rat	Sprague Dawley	M + F	Oral	87.9	LD_{50} 1 350 mg/kg bw (males) and 1 160 mg/kg bw (females)	Kuhn (1989a)
Rat	Sprague Dawley	M + F	Oral	94.6	LD_{50} 1 129 mg/kg bw (males) and 1 155 mg/kg bw (females)	Dreher (1997)
Rat	N/S	M + F	Oral	95.7	LD_{50} 300 mg/kg bw	Piccirillo (1978) [a]
Rat	N/S	M + F	Oral	96.1	LD_{50} 1 031 mg/kg bw (males) and 870 mg/kg bw (females)	Schoch & Gfeller (1985) [a]
Rat	N/S	M + F	Oral	N/S	LD_{50} > 2 150 mg/kg bw	Bathe (1972b) [a]
Rabbit	Albino	M + F	Dermal	94	LD_{50} > 2 000 mg/kg bw	Nissimov (1984a)
Rabbit	New Zealand White	M + F	Dermal	87.9	LD_{50} > 2 020 mg/kg bw	Kuhn (1989b)
Rat	N/S	M + F	Inhalation	87.9	LC_{50} > 2.327 mg/L	Holbert (1989) [a]
Rat	Sprague Dawley	M + F	Inhalation	88	LC_{50} > 5.44 mg/L	Holbert (1994)
Rat	Wistar	M + F	Inhalation	96.02	LC_{50} 3.1 mg/L	Jackson (1987)
Rat	Sprague Dawley	M + F	Inhalation	93.0	LC_{50} > 5.00 mg/L (males) and 4.873 mg/L (females)	Cummins (1985)

bw: body weight; LC_{50}: median lethal concentration; F: female; LD_{50}: median lethal dose; M: male; N/S: not stated

[a] Study evaluation copied from 1993 JMPR without further evaluation.

(a) Dermal irritation

Diazinon was tested for its potential as an irritant on rabbit skin. No skin irritation was observed in any of the six animals in one study (Nissimov, 1984b), and very slight to well-defined erythema and very slight oedema was observed in all six animals in another study; no effects on the skin could be seen after 14 days (Kuhn, 1989c).

(b) Ocular irritation

Diazinon was evaluated for its potential as an irritant in rabbit eyes. In one study, conjunctival redness and/or chemosis was observed in four out of six animals 1 hour after instillation. No effects were seen after 24 hours (Nissimov, 1984c). In another study, conjunctival redness, chemosis and discharge was observed in all six animals 1 hour after instillation. No effects were seen after 72 hours (Kuhn, 1989d).

(c) Dermal sensitization

In a skin-sensitization study, 10 guinea-pigs were topically treated with undiluted diazinon for three treatments and with a 10% volume per volume (v/v) solution in ethanol for each of the seven successive treatments (every 2–3 days). One animal died on day 7. Two weeks after the last induction treatment, the animals were challenged with a 10% v/v solution in ethanol. No skin reactions were observed after the challenge treatment (Kuhn, 1989e).

Diazinon caused delayed hypersensitivity after a Magnusson–Kligman maximization test in Dunkin–Hartley guinea-pigs (Cummins, 1987a).

2.2 Short-term studies of toxicity

Although reported in the summaries of the following studies, inhibition of plasma cholinesterase was not utilized as a criterion for the NOAEL; inhibition of erythrocyte or brain acetylcholinesterase activity may be used as an indicator of an adverse effect of anticholinesterase pesticides. Inhibition of activity greater than 20% compared with that of controls is considered adverse and toxicologically significant.

(a) Oral administration

Mice

In a 90-day dose range-finding toxicity study, groups of 10 male and 10 female B6C3F1 mice were fed diazinon (purity 98%) ad libitum in the diet at concentrations of 0, 50, 100, 200, 400, 800, 1600 or 3200 ppm for 13 consecutive weeks. The study was of limited value since only mortality, body weight and necropsy findings were reported.

Mortality was observed in males and females at 200 ppm (1/10 animals per sex), 1600 ppm (10/10 animals per sex) and 3200 ppm (10/10 animals per sex). At week 13, body weights were reduced by more than 10% for males (84% of control value) and females (78% of control value) at 800 ppm. No gross abnormalities were observed in necropsied animals at any dose, and microscopic examination of the tissues of the animals that survived the highest doses showed no pathological changes (Angel et al., unknown year; also referenced as NTP, 1979, or NCI, 1979).

Rats

In a 28-day cholinesterase inhibition feeding study, groups of 15 male and 15 female Sprague Dawley Crl:CD[BR] rats were fed diazinon (purity 88%) in the diet at concentrations of 0, 0.3, 30, 300 or 3000 ppm (equal to 0.02, 2.3, 23 and 213 mg/kg bw per day in males and 0.02, 2.4, 23 and 210 mg/kg bw per day in females, respectively). All the test animals were examined twice daily for clinical signs and mortality. Individual body weights and feed consumption were recorded weekly. Five animals per sex per group were terminated at weeks 1, 2 and 4. The cerebellum, cerebral cortex, hippocampus, cerebrum, striatum and thoracic spinal cord were dissected to assess any differences in regional sensitivity. Plasma and erythrocyte cholinesterase activities were measured at 0, 1, 2 and 4 weeks after exposure; central nervous system cholinesterase activity was measured at termination (4 weeks after exposure).

There were no deaths and the predominant treatment-related clinical sign was muscle fasciculations first observed on day 8 in both sexes (3/15 males and 14/15 females) at 3000 ppm. Diarrhoea was also observed in three females from day 8. Body-weight gain in males was significantly reduced ($P < 0.01$) by 54%, 29%, 26% and 26%, respectively, after each week of treatment at 3000 ppm. Females at the same dose also had a reduced weekly body-weight gain of 103%, 40%, 45% and 40% ($P < 0.01$) with all except the gain during week 3 being statistically significant. Feed consumption at 3000 ppm tended to be lower for females, although this reduction was not significant ($P < 0.01$) except during week 1 for males (21%) and females (27%). The mean

percentage reductions in cholinesterase activity for each treatment group and averaged over the three measurements are shown in Table 2.

Table 2. Change in cholinesterase activity in rats exposed to diazinon in the diet for 28 days

		Mean per cent change in cholinesterase activity per dose of diazinon							
		Males				**Females**			
Tissue	**Exposure (weeks)**	**0.02 mg/kg bw per day**	**2.3 mg/kg bw per day**	**23 mg/kg bw per day**	**213 mg/kg bw per day**	**0.02 mg/kg bw per day**	**2.4 mg/kg bw per day**	**23 mg/kg bw per day**	**210 mg/kg bw per day**
Plasma	Week 1	−14*	−59**	−88**	−96**	−10	−81**	−95**	−98**
	Week 2	−17	−59**	−84**	−91**	−2	−81**	−93**	−97**
	Week 4	−5	−51**	−77**	−87**	−32	−81**	−94**	−96**
Erythrocytes	Week 1	−1	−39**	−89**	−94**	−9	−38**	−86**	−94**
	Week 2	0	−55**	−83**	−85**	−8	−59**	−79**	−89**
	Week 4	−4	−58**	−64**	−74**	+5	−57**	−88**	−82**
Cerebellum	Week 1	+4	−2	−8*	−67**	+12	+14	−49**	−88**
	Week 2	−6	−1	−22**	−72**	+6	−6	−60**	−81**
	Week 4	+2	+6	−15*	−70**	−1	−6	−60**	−94**
Cerebral cortex	Week 1	−15	−7	−14	−77**	+5	−1	−47**	−92**
	Week 2	−2	+16	0	−80**	+6	+13	−62**	−91**
	Week 4	+4	+3	−10	−84**	+7	−6	−72**	−91**
Striatum	Week 1	−1	+4	−13	−82**	−6	0	−58**	−95**
	Week 2	−11	−2	−15	−82**	−4	+3	−73**	−95**
	Week 4	+3	+5	−5	−84**	−4	−9	−78**	−97**
Hippocampus	Week 1	+5	+6	+2	−79**	+3	+1	−51**	−92**
	Week 2	+5	0	−14	−79**	−5	−2	−72**	−94**
	Week 4	−5	−9	−9	−84**	+11	+2	−71**	−94**
Thoracic spinal cord	Week 1	+15	+10	−12	−73**	−10	−8	−44**	−89**
	Week 2	+1	−1	−8	−72**	+4	−1	−76**	−96**
	Week 4	+3	−3	−11	−71**	+13	+21	−62**	−89**

bw: body weight; *: $P \leq 0.05$; **: $P \leq 0.01$ (Dunnett t-test)

Results expressed as mean increase (+) or mean decrease (−) in cholinesterase activity relative (%) to the control.

Source: Chang (1994)

Significant and dose-related inhibition of plasma and erythrocyte cholinesterase was evident in males and females at concentrations equal to and greater than 30 ppm (2.3 mg/kg bw per day) from week 1. Cholinesterase inhibition in the brain showed little regional variation although females appeared to be more sensitive with significant dose-related inhibition ($P < 0.01$) observed in all tested brain regions from week 1 at 300 ppm (23 mg/kg bw per day), while significance at the same dose was only apparent in the cerebellum of males. Therefore, in both sexes cholinesterase inhibition in plasma and erythrocytes was at least an order of magnitude more sensitive to treatment with diazinon than that observed in regional areas of the brain.

The NOAEL was 0.3 ppm (equal to 0.02 mg/kg bw per day) based on the inhibition of erythrocyte cholinesterase activity at 30 ppm (equal to 2.3 mg/kg bw per day) (Chang, 1994).

In a published study:

Groups of 50 male and 50 female Wistar rats (aged 6 weeks) were given semi-purified diets containing diazinon (purity, 99.2%) at a concentration of 0 or 2 ppm (equivalent to 0.2 mg/kg bw per day) for 7 days, or, 0 or 25 ppm (equivalent to 2.5 mg/kg bw per day) for 30 days. The diet was prepared before the start of the studies by mixing diazinon suspended in corn oil with a semisynthetic diet. Food consumption and body weight were recorded twice weekly and clinical signs were monitored daily. Blood for measurements of plasma and erythrocyte cholinesterase activity were collected from random groups of 10 rats at various times (at 3–5 days intervals) except for those at 0 or 25 ppm in the 30-day study, where the same rats were sampled at each bleed. At days 15 and 30 in the 30-day study, brain cholinesterase activity was measured in 6 sacrificed rats per group. All cholinesterase activities were measured using a radiometric method, with tritiated acetylcholine as the substrate.

There were no clinical signs observed at any dose. Treated rats at 2 ppm or 25 ppm had a similar food consumption and body-weight gain except for female rats at 25 ppm for which mean food consumption increased by 9% from day 15 onwards. The maximum mean percentage reductions in cholinesterase activity measured in the two studies are shown in Table 3.

Table 3. Maximum inhibition of cholinesterase activity in rats given diets containing diazinon

Dietary concentration (ppm)	Duration (days)	Mean percentage reduction in cholinesterase activity					
		Plasma		Erythrocyte		Brain	
		Males	Females	Males	Females	Males	Females
2	7	5	29*	[12]	3	ND	ND
25	30	52*	76*	44*	85*	[3]	6

ND, not determined; ppm: parts per million; *: $P \leq 0.05$

Values in square brackets indicate the extent (%) to which the measured activity was greater than that in controls.

Source: Davies & Holub (1980a), copied from the 2006 JMPR without further evaluation

Relative to males, cholinesterase activity in females appeared to be more sensitive to inhibition after exposure to diet containing diazinon. The NOAEL for this study was 2 ppm (equivalent to 0.2 mg/kg bw per day) based on a statistically significant (> 20%) inhibition of erythrocyte acetylcholinesterase activity at the next highest dose of 25 ppm (equivalent to 2.5 mg/kg bw per day) (Davies & Holub, 1980a; study evaluation copied from the 2006 JMPR without further evaluation).

It should be noted that 2 ppm was only fed for 7 days, whereas 25 ppm was fed for 30 days.

In a 90-day dose range-finding toxicity study, groups of 10 male and 10 female F344 (Fischer) rats were fed diets containing 0, 50, 100, 200, 400, 800, 1600 or 3200 ppm diazinon (purity 98%) (equivalent to 0, 5, 10, 20, 40, 80, 160 and 320 mg/kg bw per day) for 13 consecutive weeks. The study was of limited value since only mortality, body weight and necropsy findings were reported.

At 3200 ppm, three males and four females died. Body weights were reduced at week 13 at 1600 and 3200 ppm (93% and 78% of control body weight for males, and 85% and 67% for females, respectively). No gross abnormalities were observed in necropsied animals at any dose, and microscopic examination of tissues of animals surviving the high doses showed no pathological changes (NTP, 1979; also referenced as Angel et al., unknown year, or NCI, 1979).

In a 3-month toxicity study, groups of 15 male and 15 female Sprague Dawley Crl:CD[SD]BR rats were fed diets containing diazinon (purity 87.7%) at concentrations of 0, 0.5, 5, 250 or 2500 ppm (equal to 0.03, 0.3, 15 and 168 mg/kg bw per day in males 0.04, 0.4, 19 and 212 mg/kg bw per day in females, respectively). All the animals were observed at least once daily for clinical signs and mortality. Body weights and feed consumption were recorded at predosing and weekly thereafter. Ophthalmoscopic, haematological and clinical chemistry observations, including cholinesterase activity (by colorimetric assay), were conducted during week 13. Organs were weighed and macroscopic pathology and histopathology conducted. Urine analysis was performed prior to study termination on weeks 12 and 13.

There were no treatment-related deaths. Potentially treatment-related clinical changes occurred only at 2500 ppm, with soft faeces (10/15 males and 15/15 females) and a degree of hypersensitivity to touch and sound intermittently observed throughout treatment in both sexes (12/15 males and 15/15 females); aggressive behaviour was also noted in three males. Treatment-related body-weight loss was observed at 2500 ppm; reduced body-weight gain was most evident (statistically significant, $P < 0.01$) from day 14 to day 42 in males and day 7 to day 49 in females so that at the end of treatment males were 6% lighter and females 13% lighter than their controls. Although no significant changes in water consumption occurred, feed consumption was reduced at 2500 ppm, but only during the first week of treatment in males (17%; $P < 0.01$) and for the first 2 weeks in females (31% and 13% respectively; $P < 0.01$).

Ophthalmoscopic examination did not reveal any treatment-related changes. Haematological assessments showed dose-dependent changes in erythrocytic parameters in females (i.e. erythrocyte count: 1.3%, 1.8%, 2.8% and 9.5%, respectively; haemoglobin concentration: 1.5%, 2%, 3.6% and 4.1% respectively; erythrocyte volume fraction, 0.018, 0.018, 0.044 and 0.077 respectively), although only erythrocyte volume fraction at 250 ($P < 0.05$) and 2500 ppm ($P < 0.01$) achieved statistical significance. A corresponding significant ($P < 0.01$) increase in reticulocytes (3.3-fold) was also observed in females at 2500 ppm (changes at lower concentrations were not examined). Increased leukocyte count ($P < 0.05$) in females at 2500 ppm and eosinophil count ($P < 0.05$) in males at 0.5 ppm were probably incidental findings as there did not appear to be any dose–response relationship. Clinical chemistry changes were also characterized by a lack of any dose–response relationship so that in males the reduced cholesterol (by 18%; $P < 0.05$), elevated alanine aminotransferase (by 16%; $P < 0.05$) at 2500 ppm, and reduced sodium (by 1%; $P < 0.05$) observed at 5 ppm may not be attributable to treatment. Similarly, in females, reduced alanine aminotransferase (by 46%: $P < 0.01$), sodium concentration (1.2%; $P < 0.05$) and chloride concentration (3.4%; $P < 0.05$) and elevated phosphorus concentration (24%; $P < 0.01$) at 2500 ppm, and reduced alanine aminotransferase activity at 250 (by 36%; $P < 0.05$) and 0.5 ppm (by 39%; $P < 0.05$) may also be unrelated to treatment. However, reduced cholinesterase activity in erythrocytes, plasma and brain was attributed to the treatment. These mean percentage reductions in cholinesterase activities are shown in Table 4.

Cholinesterase activity was significantly inhibited ($P < 0.01$) in the erythrocytes of females and the plasma of both males and females at 5 ppm; brain cholinesterase activity was significantly inhibited once dietary diazinon concentrations reached 250 ppm in females and 2500 ppm in males. Apart from a significantly increased specific gravity of urine (males: 2.2%; females: 1.6%; $P < 0.01$ for both) at 2500 ppm that was associated with nonsignificant reductions in urine volume (males: 20%; females: 17%) and water consumption (males: 17%; females: 12%), urine analysis was similar across treatment groups.

Table 4. Change in cholinesterase activity in rats exposed to diazinon in the diet for three months

Dietary concentration of diazinon (ppm)	Mean per cent change in cholinesterase activity (%)					
	Plasma		Erythrocytes		Brain	
	Males	Females	Males	Females	Males	Females
0.5	+10	−12	+4	−4	+7	+2
5	−26**	−78**	−4	−17**	+5	0
250	−89**	−97**	−27**	−41**	−4	−41**
2500	−97**	−98**	−26**	−42**	−49**	−57**

ppm: parts per million; **: $P \leq 0.01$

Results expressed as mean increase (+) or mean decrease (−) in cholinesterase activity relative to the control as a percentage (%).

Source: Singh, Arthur & McCormick (1988)

Macroscopic inspection of organs at necropsy showed no gross abnormalities, although the absolute (15%; $P < 0.05$) and relative-to-body weight (20%; $P < 0.01$) of the liver was significantly increased in females at 2500 ppm. The absolute weights of the livers in the males at the same dose also increased (4%) as did the relative-to-body weight (7%), although neither change was significant. Although neither males nor females at 2500 ppm had significantly increased liver weights relative to brain weights, an increase of 6.5% and 12% respectively suggests a physiological adaptation, an assertion consistent with centrilobular hepatocellular hypertrophy observed in 13 out of 15 females at 2500 ppm (and 3 out of 15 at 250 ppm). The only other significant ($P < 0.05$) organ-weight change was the relative increase in weight of the kidneys (12%) in females at 2500 ppm.

In conclusion, rats fed diets containing diazinon at 2500 ppm lost body weight, were hypersensitive to touch and sound, and excreted soft faeces. Increased liver weight resulting from hepatocellular hypertrophy was also observed in females at 2500 ppm. The NOAEL was 5 ppm (equal to 0.3 mg/kg bw per day) based on the inhibition of erythrocyte and brain cholinesterase activity at 250 ppm (equal to 15 mg/kg bw per day) (Singh, Arthur & McCormick, 1988).

In a 3-month study, groups of 15 male and 15 female Sprague Dawley Crl:CD[SD]BR rats were fed diets containing diazinon (purity 88%) at concentrations of 0, 0.3, 30, 300 or 3000 ppm for 13 weeks (equal to 0.017, 1.7, 17 and 177 mg/kg bw per day in males and 0.019, 1.9, 19 and 196 mg/kg bw per day in females). Feed consumption and body weight were measured weekly and clinical monitoring performed twice daily. All the rats were palpated weekly (although the justification for this in a 3-month study was not given). Ophthalmoscopy was performed before and at the completion of treatment (though not for the satellite group). In each group, five rats were used exclusively as the source of blood to assess the extent of cholinesterase inhibition (by colorimetric assay) during weeks 4, 8 and 13. Regional brain cholinesterase activity in these five rats was then measured at week 13. Neurological tests, namely functional observational battery and figure-eight maze motor activity, were performed in the presence of white noise for 10 rats in each group 1 week before treatment and again at week 4, 8 and 13 of treatment. The functional observational battery comprised observations in the home cage, manipulative measurements, open-field and reflex responses, neuromuscular tests and physiological functions.

After each functional observational battery, the rats in each group were individually tested in a figure-eight maze for spontaneous activity (measured by light-beam interruption). After 13 weeks of treatment, the functional observational battery–tested rats were anaesthetized, terminated by whole-body perfusion (with glutaraldehyde) and necropsied. The following tissues were histopathologically examined: brain, spinal cord with ganglia (at each level, i.e. cervical, thoracic, lumbar and sacral), peripheral nerves (left and right sciatic, fibular, tibial, lateral cutaneous sural), trigeminal (Gasserian) ganglia, eyes with associated optic nerves, skeletal muscle and any gross

lesions detected. Since a preliminary histopathological assessment of sections from the controls and rats at 3000 ppm showed no significant differences other than for some lesions in the nerve roots of the sacral spinal cord, this tissue was the only one examined in detail from rats at 0.3 and 300 ppm.

All the rats survived treatment, although clinical signs consistent with organophosphate toxicity were observed in males (i.e. hypersensitivity to touch and sound) and females (i.e. muscle fasciculations and tremors) at 3000 ppm. At this concentration, body-weight gain was significantly ($P < 0.01$) reduced for the first 6 weeks in males and 12 weeks in females. This reduced weight gain, associated with a reduction in feed consumption that achieved significance ($P < 0.01$) during weeks 1 and 2 in males (average, 15%) and weeks 1, 2 and 4 (average, 14%) in females, resulted in a generally reduced body weight that achieved significance during weeks 1 to 6 (average, 11%) in males and weeks 1 to 9 (average, 11%) and 11 in females. Significant changes in body weight and feed consumption that occurred at other concentrations were considered not treatment-related because they were transient, lasting no more than 1 week, and had no apparent trend. There were no treatment-related ophthalmoscopy findings.

Clear treatment-related functional observational battery findings were observed at 3000 ppm. For males, forelimb and hindlimb grip strength was reduced (average for both, 16%) throughout treatment (i.e. weeks 4, 8 and 13) but did not achieve significance. These reductions in limb grip strength, which averaged 25% in females, were significant at weeks 4, 8 and 13 for forelimb grip strength ($P < 0.01$) and at week 4 for hindlimb grip strength ($P < 0.05$). Hindlimb foot splay in females was also significantly reduced ($P < 0.01$) by 32% at week 4 and by 26% and 23% (both not statistically significant) at weeks 8 and 13 respectively. Rectal temperature in females was significantly reduced ($P < 0.01$) at week 13, and four had signs of dehydration at week 4. Functional observational battery changes observed at other concentrations were probably unrelated to treatment because they did not appear to be dose related. Similarly, figure-eight maze activity in all groups appeared to be unchanged by treatment.

As shown in Table 5, cholinesterase activity in plasma and erythrocytes was significantly reduced ($P < 0.01$) as a function of dose so that, as anticipated, no inhibition was observed at the lowest tested concentration of 0.3 ppm. Inhibition of plasma cholinesterase activity was generally more pronounced than that of erythrocyte cholinesterase activity at 30, 300 and 3000 ppm in males and females, except in males at 30 and 300 ppm. Cholinesterase activity in all regions of the brain was significantly ($P < 0.01$) reduced at 300 and 3000 ppm in females and at 3000 ppm in males. The spurious values observed in the striatum of males at 30 ppm and 300 ppm were attributed to an inconsistent dissection technique for this small brain region.

Table 5. Change in cholinesterase activity in rats exposed to diazinon in the diet for 13 weeks

Dietary concentration of diazinon (ppm)	Time point (weeks)	Mean per cent change in cholinesterase activity (%)									
		Plasma		Erythrocytes		Cerebellum[a]		Cortex hippocampus[a]		Striatum[a]	
		M	F	M	F	M	F	M	F	M	F
0.3	4	+5	+12	−16	−19	–	–	–	–	–	–
	8	+2	+3	+11	+7	–	–	–	–	–	–
	13	+5	+5	−11	+9	0	0	+2	+10	−4	−26
Mean	4–13	+4	+7	−5	−1	–	–	–	–	–	–
30	4	−37*	−79*	−60**	−60**	–	–	–	–	–	–
	8	−39*	−83*	−37*	−53*	–	–	–	–	–	–
	13	−45*	−86*	−75*	−59*	−10	+2	+21	−25*	+89	−18
Mean	4–13	−40	−83	−57	−57	–	–	–	–	–	–
300	4	−72*	−91*	−71**	−84**	–	–	–	–	–	–
	8	−78*	−94*	−86*	−81*	–	–	–	–	–	–

Dietary concentration of diazinon (ppm)	Time point (weeks)	Mean per cent change in cholinesterase activity (%)									
		Plasma		Erythrocytes		Cerebellum[a]		Cortex hippocampus[a]		Striatum[a]	
		M	F	M	F	M	F	M	F	M	F
	13	−79*	−95*	−84*	−75*	−14	−55**	−6	−75**	+138	−74**
Mean	4–13	−76	−93	−80	−80	–	–	–	–	–	–
3000	4	−81**	−94**	−72**	−85**	–	–	–	–	–	–
	8	−85**	−96**	−80**	−88**	–	–	–	–	–	–
	13	−85**	−97**	−86**	−79**	−64**	−89**	−77**	−92**	−62**	−96**
Mean	4–13	−84	−96	−79	−84	–	–	–	–	–	–

F: female; M: male; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$

Results expressed as mean increase (+) or decrease (−) in cholinesterase activity relative to the control as a percentage (%).

[a] Brain cholinesterase activity was measured after termination at week 13.

Source: Pettersen & Morrissey (1994)

No treatment-related findings were observed after gross necropsy, and there were no histopathological lesions apart from the mild focal degeneration of the sacral spinal-cord nerve root axons detected in the preliminary screen in two females at 3000 ppm.

In conclusion, rats fed diets containing diazinon at concentrations of 0.3, 30, 300 or 3000 ppm for 13 weeks lost body weight and had characteristic clinical signs of organophosphate poisoning at the highest concentration tested (3000 ppm). Functional observational battery studies showed a reduction in fore- and hindlimb grip strength throughout the treatment in all the rats at 3000 ppm; with reduced hindlimb foot splay and rectal temperature in females. The NOAEL was 0.3 ppm (equal to 0.017 mg/kg bw per day) based on significant inhibition of erythrocyte acetylcholinesterase activity at 30 ppm (equal to 1.7 mg/kg bw per day) (Pettersen & Morrissey, 1994).

Groups of 50 female Wistar rats were fed semi-purified diets containing diazinon (purity 99.2%) at concentrations of 0, 5, 10 or 15 ppm (equivalent to 0, 0.5, 1 and 1.5 mg/kg bw per day, respectively) for 92 days. Female rats were selected for this study because a previous short-term study had shown them to be more sensitive than males to inhibition of cholinesterase (Davies & Holub, 1980a). In order to increase the accuracy for determining a NOAEL based on inhibition of cholinesterase activity in plasma, erythrocytes and brain, a second and a third study, each with a reduced duration and concentration of diazinon, were performed. The second study involved groups of 16 rats (mean body weight, 149 g) fed diets containing diazinon at concentrations of 0, 1, 2, 3 or 4 ppm for 42 days. In the third study, groups of 10 rats (mean body weight, 143 g) were fed diets containing diazinon at concentrations of 0, 0.1, 0.5, 1 or 2 ppm for 35 days. Food consumption and body weight were recorded twice weekly and clinical signs were monitored daily.

In the three studies, blood for determining plasma and erythrocyte cholinesterase activity was collected from groups of 8 to 10 rats at various time points (at intervals of approximately 2–5 days). Determination of brain cholinesterase activity was confined to the two longer studies in which satellite groups of six rats were sacrificed at various time points (intervals of 5–14 days).

There were no clinical signs observed at any dose in any of the three studies. Treated rats in all studies had similar food consumption and body-weight gain except for rats at 0.5 ppm (third study) where mean body-weight gain was 12% and food consumption 10% less than values for controls. The maximum mean percentage reductions in cholinesterase activity measured in the three studies are shown in Table 6.

Table 6. Maximum inhibition of cholinesterase activity in rats exposed to diazinon in the diet

Dietary concentration of diazinon (ppm)	Duration (days)	Mean per cent reduction in cholinesterase activity (%)		
		Plasma	Erythrocyte	Brain
0.1	35	−4	0	–
0.5	35	−16*	0	–
1	35	−28*	−16	–
2	35	−42*	−12	–
1	42	−33*	−8	NS
2	42	−51*	−9	NS
3	42	−65*	−8	NS
4	42	−61*	−4	NS
5	92	−75*	−18*	−2
10	92	−80*	−38*	−6
15	92	−85*	−55*	−2

NS: not significant (approximately equivalent to controls); ppm: parts per million; *: $P \leq 0.05$ (Duncan test)

Results expressed as mean decrease (−) in cholinesterase activity relative to the control as a percentage (%).

The apparent significant reduction in cholinesterase activity, due to an unexplained increase in cholinesterase activity in controls on day 42, was discounted.

Source: Davies & Holub (1980b)

The NOAEL for cholinesterase inhibition in the first study was 5 ppm (equivalent to 0.5 mg/kg bw per day) based on a statistically significant (> 20%) inhibition of erythrocyte acetylcholinesterase activity at the next higher dose of 10 ppm (equivalent to 1 mg/kg bw per day) after dosing for 92 days. The NOAEL for females in the second and third studies can be established at the highest tested doses of 4 ppm (equivalent to 0.4 mg/kg bw per day) and 2 ppm (equivalent to 0.2 mg/kg bw per day) after dosing for 42 and 35 days respectively (Davies & Holub, 1980b).

In another 3-month oral toxicity GLP study conducted in accordance with the test guidelines of the Ministry of Agriculture, Forestry and Fisheries of Japan, 28 male and 28 female Sprague Dawley Crl:SD rats were fed diazinon (purity 95.0–95.5%) in the diet at concentrations of 0, 5, 125 or 3000 ppm (equal to 0, 0.3, 7.8 and 198 mg/kg bw per day in males and 0, 0.3, 8.9 and 247 mg/kg bw per day in females, respectively). Ten male and female rats per group were used to examine subacute toxicity using common parameters, and six male and female rats per group in both sexes euthanized at week 2, 4 or 8 to measure erythrocyte cholinesterase activity. The animals in the main groups were checked weekly; at week 13, grip strength was checked and cholinesterase activities in erythrocyte and brain measured.

A male in the 125-ppm group died on day 74; the death was not considered treatment related because other animals in this and higher-dose groups survived until termination. There were no treatment-related clinical signs. The treatment-related changes are summarized in Table 7. Forelimb grip strength was weakened at 3000 ppm in females, and motor activities decreased at 3000 ppm in both sexes. Body weights were significantly ($P < 0.01$) decreased by about 10% at 3000 ppm in both sexes during the first 4 weeks for males and 7 weeks for females. These consistent depressions were considered treatment related. No treatment-related ophthalmological abnormalities were observed. Urine analysis found lower pH, increased specific gravity and decreased urine volume in both males and females at 3000 ppm. The female rats at 3000 ppm were found to have slight anaemia. Statistically nonsignificant increases in alkaline phosphatase and gamma-glutamyltransferase in males at 3000 ppm were considered to be related to the changes in the liver. The increases in glucose in

males at 125 and 3000 ppm and urine nitrogen in females at 3000 ppm were potentially treatment related although the increases were slight and no corresponding histopathological changes were observed. Other slight changes in blood chemistry at 3000 ppm were not considered treatment related. Slight hepatocellular hypertrophy was seen microscopically in males at 3000 ppm. Similarly, incidences and intensities of hyaline droplets and eosinophils in the kidney proximal tubule epithelium were increased at 3000 ppm in males only. These changes in males only may have been a result of accumulation of α_{2u}-globulin, a protein specific to the male rat. However, no additional histopathological examination was conducted to identify the changes.

Table 7. Summary of treatment-related changes observed in a 90-day oral toxicity study of diazinon in rats

Treatment-related measures	Treatment-related changes per dose of diazinon							
	Males				Females			
	0 ppm	5 ppm	125 ppm	3 000 ppm	0 ppm	5 ppm	125 ppm	3 000 ppm
No. of rats	10	10	9	10	10	10	10	10
Functional observation								
Forelimb grip strength (g)	1 656.23	1 723.07	1 787.04	1 624.67	1 411.85	1 442.76	1 384.93	1 082.07**
Motor activity (count), total of 6 trials	1 124.2	1 167.8	922.4	636.9	2 362.2	2 541.5	2 345.5	1 572.1
Haematology								
Erythrocyte ($10^4 \times \mu L$)	903.6	881.0	891.2	882.2	855.7	841.8	837.3	825.0*
Haematocrit (%)	46.21	46.22	44.94	44.70	45.43	45.76	44.60	41.58**
Haemoglobin (g/dL)	15.94	15.81	15.58	15.28	15.72	15.76	15.39	14.45**
Blood chemistry								
Aspartate aminotransaminase (IU/L)	71.0	75.3	77.8	84.4	64.9	76.6	81.5	72.1
Alkaline phosphatase (IU/L)	266.6	258.4	253.8	376.8	126.3	130.4	150.2	163.4
Gamma-glutamyltransferase (IU/L)	0.48	0.78	0.59	1.81	0.88	0.78	0.75	1.34
Glucose (mg/dL)	159.0	165.7	173.6*	178.1*	149.8	149.5	160.7	144.4
Urinary nitrogen (mg/dL)	15.19	13.91	13.66	14.02	14.04	14.10	14.95	16.20*
Histopathology								
Lung – aggregation, macrophage, alveolar	1	3	2	3	3	2	2	8
Liver – hypertrophy, hepatocyte, centrilobular	0	0	0	0	0	0	0	7[#]
Kidney – hyaline droplet, proximal tubule epithelium	3	3	3	9	0	0	0	0
Kidney – eosinophilic body, proximal tubule epithelium	3	3	3	9	0	0	0	0

IU: International Unit; no.: number; ppm: parts per million; *: $P < 0.01$ (Dunnett *t*-test, one-tailed); #: $P < 0.01$ (Fisher exact test)

Source: Sunaga (2010)

Erythrocyte and brain acetylcholinesterase activities are summarized in Table 8. Erythrocyte acetylcholinesterase activity was decreased consistently by over 20% at 125 and 3000 ppm at each time point in both sexes. Brain acetylcholinesterase activity was reduced by over 20% at 3000 ppm for males and at 125 and 3000 ppm for females.

The NOAEL for 90-day oral rat toxicity was 5 ppm (equal to 0.3 mg/kg bw per day) based on significant inhibition of erythrocyte acetylcholinesterase activity at 125 ppm (equal to 7.8 mg/kg bw per day) (Sunaga, 2010).

Table 8. Summary of acetylcholinesterase activities in a 90-day oral toxicity study of diazinon in rats

Site of AChE activity / Time point	AChE activity per dose of diazinon							
	Males				Females			
	0 ppm	5 ppm	125 ppm	3 000 ppm	0 ppm	5 ppm	125 ppm	3 000 ppm
Erythrocytes								
Week 2[a]	389.3	388.2 (99.7)	225.7# (58.0)	123.7# (31.8)	374.0	361.0 (96.5)	224.7# (60.1)	124.8# (33.4)
Week 4[a]	460.7	452.0 (98.1)	155.2# (33.7)	108.8# (23.6)	383.8	364.2 (94.9)	159.5# (41.6)	105.3# (27.4)
Week 8[a]	330.7	296.2 (89.6)	169.2# (51.2)	116.2# (35.1)	317.7	355.7 (112.0)	192.0* (60.4)	118.2* (35.5)
Week 13[b]	337.1	332.5 (98.6)	175.1# (51.9)	94.4# (16.3)	262.2	264.1 (100.7)	124.3# (47.4)	84.8# (32.3)
Brain[c]	157.5	150.5 (95.6)	147.8# (93.8)	45.3# (28.8)	166.6	161.2 (96.8)	115.4# (69.3)	24.5# (14.7)

AChE: acetylcholinesterase; IU: International Unit; ppm: parts per million; *: $P < 0.01$ (Dunnett *t*-test, one-tailed); #: $P < 0.01$ (Steel test, one-tailed)

Results shown as AChE activity in IU/L with the mean activity relative to control activity as a percentage (%) in parentheses.

[a] Six male and six female rats per group were examined at weeks 2, 4 and 8.

[b] Ten, ten and nine male rats at 0, 5, 125 and 3000 ppm, respectively, were examined at week 13. Ten female rats were examined in each group and at each time point.

Source: Sunaga (2010)

Dogs

In a 90-day toxicity study, groups of four male and four female beagle dogs were fed diets containing diazinon (purity 87.7%) at concentrations of 0, 0.1, 0.5, 150 or 300 ppm (equal to 0.0034, 0.020, 5.9 and 10.9 mg/kg bw per day in males and 0.0037, 0.021, 5.6 and 11.6 mg/kg bw per day in females, respectively). Feed consumption and body weight were measured weekly for 2 weeks prior to treatment and weekly during treatment. Clinical observations were performed daily. Urine was collected during weeks 5, 9 and 14. Ophthalmoscopic and auditory/physical examinations were performed before treatment and during weeks 14 and 13, respectively. Haematological and clinical chemistry, including blood cholinesterase activity, were assessed during treatment weeks 5 and 9 and then prior to termination (week 13), and brain cholinesterase activity was measured after the terminal

kill (week 14). Urine analysis and macroscopic pathology and microscopic pathology were analysed and organs weighed.

No treatment-related mortalities occurred. The treatment did not adversely affect feed consumption, organ weights or ophthalmoscopic, haematological, urine analysis or macroscopic findings. Clinical signs such as emesis or bloody faeces were sporadically observed in males at 300 ppm and in females at 150 ppm. Reduced body-weight gain was observed in females at 150 ppm and at 300 ppm in both sexes. In males, inhibition of serum cholinesterase activity was 70%, 20% and 15% that of controls at the end of the study at 0.5, 150 and 300 ppm, respectively. Erythrocyte cholinesterase activity at 75% and 69% of control activity was measured at 150 and 300 ppm, while brain cholinesterase activity was 69% and 58% of control activity at 150 and 300 ppm, respectively. In females, a reduction in cholinesterase activity was observed at 150 and 300 ppm: serum and erythrocyte cholinesterase activities were about 18% and 70% of control activity, respectively, at both doses, and brain cholinesterase activity was 70% and 55% of control activity at 150 and 300 ppm, respectively. Other changes in biochemical parameters consisted of a decrease in total protein at 300 ppm in males. The only microscopic alteration that may potentially have been treatment related was atrophy of the pancreatic acini in one male dog at the highest dose.

The NOAEL was 0.5 ppm (equal to 0.020 mg/kg bw per day) based on greater than 20% inhibition of erythrocyte and brain cholinesterase activities at a dietary concentration of 150 ppm (equal to 5.6 mg/kg bw per day) (Barnes, Arthur & Hazelette, 1988).

In a second 90-day oral dog study conducted as a GLP study in accordance with the test guidelines of the Ministry of Agriculture, Forestry and Fisheries of Japan, four male and four female beagle dogs (Nasan: Beagle, Japan) per group were administered diazinon (purity 95.0–95.5%) by gavage in gelatine capsules at 0, 0.3, 3 or 10 mg/kg bw per day for 90 days. (The highest dose was changed from 15 mg/kg bw per day to 10 mg/kg bw per day on day 8 because of the decreased body weight and feed consumption in one male and two females; moreover, this male dog was not treated on days 14 to 28, one of these female dogs was not treated on days 11 to 28, 37 to 50 and 72 to 78 and the other female was not treated on days 11–28.) All the dogs were monitored daily for clinical signs; detailed observations and body weight and feed consumption measurement were carried out every week. Erythrocyte acetylcholinesterase activity was measured at weeks 2, 4, 8 and 13 and brain activity at week 13. At week 13, all dogs were necropsied and all tissues or organs microscopically examined.

Treatment-related changes are summarized in Table 9. Vomiting pre- and post-capsule administration was observed in all dogs at the highest dose, and the frequency increased in three males and all the females. Other clinical signs included diarrhoea or mucous stool. Body weights and feed consumption were comparable across all treated groups except in the high-dose dogs that were temporarily taken off the treatment due to their body weight loss and decreased feed consumptions (one male and two females). There were no treatment-related changes in ophthalmology or urine analysis. Although anaemia indicating decreased erythrocytes, haemoglobin or haematocrit was observed in the dogs that were temporarily removed from treatment at 10 mg/kg bw, other animals in the treated group showed no treatment-related changes in haematology. Blood biochemistry parameters were also not affected except in the dogs that were temporarily taken off the treatment at 10 mg/kg bw: total protein was lower in one male and one female, and the one male had increased aspartate aminotransaminase, alanine aminotransaminase and alkaline phosphatase levels. Erythrocyte acetylcholinesterase activity was continuously and dose-dependently reduced in both sexes by over 20% at 3 mg/kg bw and above. Brain acetylcholinesterase was also reduced by 20% or more at 3 mg/kg bw and above in both sexes. Microscopically, atrophic change in the pancreas, thymus spleen or prostate was observed in those dogs temporarily taken off the treatment. These were considered secondary changes because of the dogs' decreased body weight at 10 mg/kg bw. Any changes suggesting primary organ toxicity was not found in the treated group.

Table 9. Summary of a 90-day oral toxicity study of diazinon in dogs

Parameter	No. and incidence per dose of diazinon							
	Males				Females			
	0 mg/kg bw per day	0.3 mg/kg bw per day	3 mg/kg bw per day	10 mg/kg bw per day [a]	0 mg/kg bw per day	0.3 mg/kg bw per day	3 mg/kg bw per day	10 mg/kg bw per day [a]
Clinical signs[b]								
Vomiting, after treatment	0/4	0/4	1/4	3/4	0/4	1/4	0/4	4/4
Vomiting, before treatment	1/4	1/4	0/4	4/4	0/4	1/4	0/4	4/4
Diarrhoea	0/4	0/4	1/4	4/4	0/4	0/4	0/4	3/4
Mucous stool	0/4	0/4	1/4	4/4	0/4	1/4	0/4	2/4
AChE activity[c]								
Erythrocytes								
Pretreatment	863.0	736.5 (85.3)	876.3 (101.5)	864.7[d] (100.2)	967.3	884.3 (91.4)	970.3 (100.3)	965.5[e] (99.8)
Week 2	1038.8	933.0 (89.8)	554.5* (53.4)	256.0[d] (24.6)	1006.3	895.8 (89.0)	720.0 (71.5)	253.5[e] (25.2)
Week 4	1126.5	944.3 (83.8)	447.0** (39.7)	118.3[d] (16.8)	1053.3	1061.5 (100.5)	609.8[#] (52.9)	277.5[e] (21.6)
Week 8	1126.5	890.5 (98.6)	804.5 (90.3)	320.0*[,d] (36.2)	949.5	854.8 (90.0)	481.0[#] (50.7)	166.0[e] (17.5)
Week 13	1097.3	868.0 (79.1)	318.8** (29.0)	197.7[d] (18.2)	1141.0	1012.8 (88.8)	484.5[#] (50.7)	195.5[e] (17.1)
Brain	122.0	146.8 (120.3)	73.0 (59.8)	39.7[d] (32.5)	139.8	161.3 (115.4)	85.8 (61.4)	32.5[e] (23.2)

AChE: acetylcholinesterase; bw: body weight; IU: International Unit; no.: number; *: $P < 0.05$ (Dunnett *t*-test); [#]: $P < 0.01$ (Fisher exact test)

[a] The starting high dose of 15 mg/kg bw per day was lowered to 10 mg/kg bw per day on day 8.

[b] Results for clinical signs shown as number of dogs affected /number of dogs examined.

[c] Results for AChE activity expressed in IU/L and as a percentage (%) relative to the control in parentheses.

[d] Three dogs were tested; one male was removed from the calculation of mean value because of a shortened exposure period.

[c] Two dogs were tested; two females were removed from the calculation of mean value because of a shortened exposure period.

Source: Ichido (2010)

The NOAEL for 90-day oral toxicity in dogs was 0.3 mg/kg bw per day based on a greater than 20% reduction in erythrocyte and brain acetylcholinesterase activity at 3.0 mg/kg bw per day (Ichido, 2010).

In a 1-year toxicity study, groups of four male and four female pure-bred beagle dogs were fed diazinon (purity 87.7%) admixed into their normal diet (for approximately 3 hours/day) at concentrations of 0, 0.1, 0.5, 150 or 300 ppm (equal to 0.0032, 0.015, 4.7 and 7.7 mg/kg bw per day in males and 0.0037, 0.020, 4.5 and 9.1 mg/kg bw per day in females, respectively) over a period of 52 weeks. Dietary concentration was reduced from 300 to 225 ppm after 14 weeks of treatment (test day 99) due to a general lack of body-weight gain in this test group. All animals were observed daily for mortality and clinical signs. Body weight and feed consumption were recorded before treatment and then weekly for the first 16 weeks and monthly thereafter. Physical and auditory examinations

were performed 4 weeks before testing and then during weeks 12, 26, 39 and 51. Ophthalmoscopic examinations were performed 3 weeks before testing and at week 25 and 53. All the dogs were terminated and necropsied during week 53. Haematology and biochemistry parameters, including plasma, erythrocyte and brain cholinesterase activities, and urine analysis were assessed 4 weeks before treatment and then at weeks 13, 26, (27 & 38, urine analysis only), 39 and 52. At termination, body weight was measured and the main organs removed and weighed before fixation. All the dogs including the one that died and another that was terminated in poor condition early in the dosing period underwent a complete necropsy.

Mortality was not affected by treatment, and haematology, urine analysis, gross pathology and histopathology assessments showed no treatment-attributable changes. Overt clinical signs of dehydration and emaciation became evident in one male at 300/225 ppm and the symptoms remained despite a reduction in the initial dose. Reductions in body-weight gain were observed at 150 ppm and higher doses in males and at 300/225 ppm in females. However, no clear-cut dose–response relationship was evident and the differences attained statistical significance relative to the control group only at certain observation times. Feed consumption was reduced at dietary concentrations of 150 ppm and higher doses, again without a clear dose–response relationship, most probably owing to reduced palatability of the feed admixtures. Serum cholinesterase activity was reduced at 0.5 ppm and higher doses in males and at 150 ppm and higher doses in females to about 20% of the control group activity at 150 and 300/225 ppm in both sexes. Erythrocyte cholinesterase activity in both sexes was also reduced at 150 and 300/225 ppm to about 70% of the control activity measured. Brain cholinesterase activity was inhibited to 75% of control activity in females at 150 ppm, and at 300/225 ppm to 65% and 75% of control activity in females and males, respectively.

The NOAEL was 0.5 ppm (equal to 0.015 mg/kg bw per day) based on inhibition of erythrocyte (males and females) and brain (females only) cholinesterase activity at 150 ppm (equal to 4.5 mg/kg bw per day) (Rudzki, Arthur & McCormick, 1991).

(b) Dermal application

Rats

In a 2-week dose range-finding study, groups of four male and four female Sprague Dawley Crl:CD[SD] rats were topically treated with diazinon (purity 87.4%) suspended in Epoxol (epoxidized soybean oil; the control vehicle) at daily doses of 0, 5, 25, 125 or 250 mg/kg bw through a porous gauze dressing. Clinical conditions, body weights, feed consumption, blood (erythrocyte and plasma) and brain acetylcholinesterase activities, blood chemistry, brain weight and macropathology were assessed.

Treatment with diazinon at 125 or 250 mg/kg bw per day resulted in reduced plasma cholinesterase activity in females (31% and 26% of controls, respectively) and males (68% and 63% of controls, respectively) on day 1 of treatment. By day 14 of treatment, there were clear reductions of over 20% in both plasma and erythrocyte enzyme activity in males at doses equal and greater than 25 mg/kg per day and in females at doses equal and greater than 5 mg/kg bw per day, with the degree of response dose related and generally greater in females. Erythrocyte enzyme activity was 97%, 44%, 27% and 21% of controls in males and 76%, 48%, 19% and 14% of controls in females. Treatment of males with diazinon at 125 or 250 mg/kg bw per day and of females at doses equal to and greater than 25 mg/kg bw per day resulted in a clear reduction (16–72% of controls) in brain cholinesterase activity on day 14 of treatment; the degree of response was dose related and higher in females. Males at 125 and 250 mg/kg bw per day and females at 5 mg/kg bw per day and greater showed a local reaction to the treatment, namely slight- to well-defined erythema. Males at the higher dose of 250 mg/kg bw per day showed greater mean body-weight loss during the first week of treatment compared with controls. The treatment had no effect on feed consumption or brain weights; macroscopic examination did not reveal any treatment-related changes.

No NOAEL could be determined; the lowest-observed-adverse-effect level (LOAEL) in this dose range-finding study was considered to be 5 mg/kg bw per day based on a greater than 20% inhibition of erythrocyte cholinesterase activity in females (Brennan, 2010).

In a 90-day toxicity study, groups of 10 male and 10 female Sprague Dawley Crl:CD[SD] rats were topically treated with diazinon (purity 87.4%) suspended in Epoxol (epoxidized soybean oil; the control vehicle) at dermal doses of 0, 0.3, 1, 3 or 25 (for males) and 0, 0.3, 1, 3 or 10 (for females) mg/kg bw through a porous gauze dressing. Treatment was daily, for 6 hours for 13 weeks. Five more males and five more female rats were assigned to each group and similarly treated for 13 weeks, followed by a 4-week period without treatment to assess recovery. Sensory reactivity, grip strength, motor activity, body weight, feed consumption, ophthalmology, haematology, blood chemistry, including erythrocyte and plasma activity, brain acetylcholinesterase activity, urine analysis, organ weights, macropathology and histopathology were assessed.

There were no treatment-related signs after dose administration or at the routine weekly physical examinations; sensory reactivity, grip strength and motor activity as well as body weight and feed consumption were unchanged at the end of the treatment period. Four deaths occurred during the study but these were all clearly incidental to treatment. There were no treatment-related ophthalmoscopic findings.

Plasma acetylcholinesterase activity was reduced in females at 1 mg/kg bw per day and higher doses. In males, plasma acetylcholinesterase activity was only affected at the highest dose, 25 mg/kg bw per day. Recovery was complete, though in the first week of the recovery period a small reduction in plasma acetylcholinesterase activity remained in those females dosed with 3 or 10 mg/kg bw per day. Erythrocyte acetylcholinesterase activity was reduced in high-dose males and females, although there was a wide variation in the magnitude of the response. In males at 25 mg/kg bw per day, erythrocyte cholinesterase inhibition was 1% to 38% compared with controls; in females at 10 mg/kg bw per day, erythrocyte cholinesterase inhibition was 10% to 44%, reaching statistical significance at several occasions. After the 13 weeks of treatment, brain acetylcholinesterase activity was reduced in males at 25 mg/kg/day (10% inhibition) and in females at 3 or 10 mg/kg bw per day (6% and 11% inhibition, respectively). After 13 weeks of treatment, there was no effect on organ weights and no treatment-related macroscopic or histopathological findings.

The NOAEL was 3 mg/kg bw per day for males and females, based on greater than 20% inhibition of erythrocyte cholinesterase activity at 25 mg/kg bw per day for males and 10 mg/kg bw per day for females (Brennan, 2011).

Rabbits

In a 21-day toxicity study, groups of five male and five female albino HAR:PF/CF(NZW)BR rabbits were topically treated with diazinon (purity 97.1%) suspended in 50% aqueous polyethylene glycol 300 (the control vehicle), through a porous gauze dressing, at daily doses of 0, 1, 5 or 100 mg/kg bw diazinon. Treatment was daily, for 6 hours, 5 days per week for 3 weeks. As mortality in the male rabbits at 100 mg/kg was high (4/5), the highest dose was reduced to 50 mg/kg after 7 study days (5 treatment days). Both eyes of each rabbit were examined prior to dosing and at study termination. Dermal reactions were graded. Evaluations were conducted once daily immediately prior to applying the test substance. Each animal was monitored at least twice daily, in the morning and the afternoon, for appearance, mortality and overt toxicological and/or pharmacological effects. Haematological and biochemical measurements, including cholinesterase activity, were made prior to dosing and at termination. Necropsies were performed on every animal, and tissues included all gross lesions, liver, skin (treated and untreated areas), tissue masses, kidneys and brain harvested.

As a result of four of the male rabbits dying at 100 mg/kg during the first test week (days 3 and 6), the highest dose was reduced to 50 mg/kg at the end of the first week. All the other animals survived the scheduled experimental period. Anorexia, ataxia, fasciculations, tremors, diarrhoea,

hypoactivity, hypotonia and salivation were generally observed in high-dose animals during the first week. All groups, including vehicle controls, consistently exhibited very slight erythema throughout the study. Well-defined erythema was occasionally observed at all doses. In addition, dry, flaky areas of skin were observed among high-dose female rabbits during the second test week. No statistically significant differences in weekly mean body weights or feed consumption between treated and control groups were noted. Terminal ocular examination did not reveal treatment-related effects.

Slight, but statistically significant ($P < 0.01$) decreases in eosinophil and basophil percentages (male) and platelet counts (females) were observed in the high-dose animals, although the absolute values of these parameters were within the normal range. Dose-dependent inhibition of serum, erythrocyte and brain cholinesterase activities were observed in both male and female rabbits. Statistically significant ($P < 0.01$) reductions in mean serum cholinesterase occurred only in high-dose males and females. Serum cholinesterase was reduced by 64.3% in the single surviving male rabbit and by an average of 56.5% in females. There were no statistically significant reductions in low- or mid-dose groups. Erythrocyte cholinesterase activity was reduced in high-dose males and females relative to baselines, but the reduction was statistically significant ($P < 0.01$) only in high-dose females. Dose-related reduction in mean brain cholinesterase relative to controls occurred in both sexes but was statistically significant only in mid-dose (18.1%; $P < 0.05$) and high-dose (43.3%; $P < 0.01$) females. Slight but statistically significant ($P < 0.05$) increases in serum inorganic phosphorus concentrations (in males) and serum glucose (in females) occurred in all dose groups. Similarly, slight but statistically significant ($P < 0.05$) increases in serum albumin and albumin-to-globulin ratio and reductions in serum sodium concentrations were observed in high-dose females. In all instances, the absolute values of these parameters were well within the normal range. Mean organ weights and organ-weight ratios were comparable in treated and control groups. Macroscopic examinations did not reveal any gross internal lesions directly associated with the dermal application of diazinon; however, one high-dose male exhibited slight gastric irritation. Except for minimal hyperkeratosis of the treated skin among high-dose rabbits (60% of each sex), no histological changes were observed in the other tissues examined (brain, kidney and liver).

The NOAEL was 5 mg/kg bw based on greater than 20% inhibition of erythrocyte and brain cholinesterase at 100 mg/kg bw per day (Tai & Katz, 1984). It should be noted that the cholinesterase measurements were compared to baseline and not to controls.

(c) Exposure by inhalation

In a 21-day toxicity study

… groups of rats (9/sex/group) were exposed to an aerosol of diazinon (purity 97.1%; droplet size < 1 µm 30–40%, 1–7 µm 50%) for 6 hours a day, 5 days per week for three weeks. Only the animals' snouts were exposed to the aerosol. The mean concentrations were 0, 151, 245 or 559 mg/m^3. The treatment did not cause changes in the mortality rate, in haematology, macroscopical and histopathological findings or organ weights that were attributable to the inhalation of diazinon. Exophthalmos and diarrhoea were observed in animals at all dose levels and tonic-clonic muscle spasms occurred in the high-dose animals. The symptoms were reversible. Food intake at the highest dose level was reduced at the beginning of the treatment period. Body-weight gain was reduced in male rats at 245 and 559 mg/m^3, and in female rats at 559 mg/m^3. Plasma cholinesterase was inhibited at the intermediate and high levels, resulting in values corresponding to 56% and 37% of the activity in control animals, respectively. Erythrocyte cholinesterase was inhibited only at the highest dose level (34% of the control activity). Brain cholinesterase activity was dose-dependently reduced at all dose levels resulting in activities of 81, 56 and 37% of the control activity in both sexes at the low-, medium- and high-dose levels, respectively. The cholinesterase values returned to normal at the end of the 25-day recovery period.

The NOAEL was [less than] 151 mg/m^3 (corresponding to an estimated dose of 55 mg/kg bw/day), based on lack of significant brain cholinesterase inhibition at the lowest dose level (Zak et al., 1973; study evaluation copied from the 1993 JMPR without further evaluation).

The relevance of the inhibition of brain cholinesterase activity at low doses is unclear as plasma and erythrocyte cholinesterase inhibition did not occur. Moreover, it is in contrast to the Hartmann (1990) study described below, which used much lower doses.

In a 21-day toxicity study, groups of 10 male and 10 female rats were exposed by inhalation (nose only) to measured concentrations of 0 (filtered air), 0 (vehicle control), 0.05, 0.46, 1.57 or 11.6 mg/m^3 diazinon (purity 88%) for 6 hours a day, 5 days per week. The mass median aerodynamic diameter of the aerosol particles ranged from 0.7 to 1.4 µm. All animals were assessed for mortality, clinical signs, body weight, feed consumption and ophthalmological, haematological and blood chemistry measured, including plasma and erythrocyte cholinesterase activity. At study termination, a gross pathology was performed, with brain cholinesterase activity and organ weights assessed and histopathology determined.

There were no deaths attributable to treatment. Apart from piloerection in most animals at all doses, no signs of systemic toxicity were observed. No concentration-dependant difference in the body-weight change or feed consumption could be observed. No treatment-related ophthalmoscopic effects were recorded. Minimal lower values of erythrocyte parameters (erythrocyte count, haemoglobin and packed cell volume) were recorded in high-dose females. At the end of the exposure period, plasma cholinesterase activity was decreased in both males and females at 1.57 and 11.6 mg/m^3, and also in females at 0.46 mg/m^3, indicating exposure. Greater than 20% inhibition of erythrocyte cholinesterase activity was observed in both sexes at 11.6 mg/m^3; the inhibition in females exposed to 1.57 mg/m^3 diazinon was lower (10%). A statistically significant but not dose-related decrease value of cholinesterase activity was noted in the brains of females at 0.05 mg/m^3, 1.57 mg/m^3 and 11.6 mg/m^3, with less than 20% inhibition at 0.46 mg/m^3, when compared to both the air and the vehicle control. Minimally lower plasma glucose levels were recorded in males at 1.57 and 11.6 mg/m^3. A significantly higher lung-to-body-weight ratio was recorded in females at 0.46 mg/m^3 and 1.57 mg/m^3, but not in the highest dose group. No dose–response relationship or other deviations in organ weights and organ-to-body-weight ratios in comparison to the controls could be seen, and no treatment-related effects in macroscopic pathology or histopathology were recorded.

The NOAEL was 0.46 mg/m^3, based on a statistically significant reduction in cholinesterase activity (> 20%) in the brains of the female group dosed at 1.57 and 11.6 mg/m^3. In the absence of corroborative changes in plasma and erythrocyte cholinesterase activity, the statistically significant lower value for brain cholinesterase activity noted in females dosed 0.05 mg/m^3 was not considered biologically relevant (Hartmann, 1990).

2.3 Long-term studies of toxicity and carcinogenicity

A study report (NTP, 1979; also referenced as Angel et al., unknown year, or NCI, 1979) described a bioassay of diazinon for possible carcinogenicity conducted by the National Cancer Institute in the United States of America. This study is evaluated by test animal species in the subsections below. Subchronic feeding studies are also described in the reports; these 13-week studies in rats and mice are described in section 2.2 on short-term studies of toxicity.

Mice

In a pre-GLP carcinogenicity study, groups of 50 male and 50 female B6C3F1 mice were fed diazinon (purity 98%) admixed in the diet at concentrations of 100 or 200 ppm for 103 consecutive weeks; control groups of 25 males and 25 females were fed the basal diet only. The mice were observed for an additional 2 to 3 weeks at the end of treatment. The prepared diets were used within 1 week. All the animals were examined twice a day for signs of toxicity, weighed at 2-week intervals, palpated for tissue masses at each weighing and subjected to gross and histopathological examination.

The study was performed according to National Cancer Institute–established guidelines for carcinogen bioassays, but deviates from Organisation for Economic Co-operation and Development (OECD) guidelines 451, 452 and 453 for the following reasons:

- two dose groups rather than the prescribed three were used in the chronic study;
- feed consumption was not measured, so the intake of the test material could not be calculated; and
- haematological or clinical chemistry tests were not conducted.

Since the critical effect of diazinon on cholinesterase activity were not measured, and histopathological information about non-neoplastic findings were limited, the study was inappropriate for the evaluation of chronic toxicity. Nevertheless, the study provides useful information on carcinogenicity.

Compared with the controls, mortality was not increased in any of the dosed groups of mice; survival was 84% or greater in all dosed and control groups at week 78 (84%, 90% and 98% of survival in males and 96%, 100% and 98% in females of the control, low- and high-dose groups, respectively). There was no appreciable effect on mean body weights of either sex, except for the last 20 weeks of the study, when the mean body weights of the dosed females were lower than those of the controls. Hyperactivity was observed in the dosed groups of mice but was rare in the control groups.

No tumours clearly related to diazinon administration occurred in any of the dosed groups of mice of either sex (Table 10). Incidence of hepatocellular carcinoma increased to statistically significant levels in low-dose males; the majority of carcinomas were trabecular carcinomas. The incidence at the high dose was similar to that in the control group. The incidences of adenoma did not increase at either dose.

Table 10. Incidence of hepatic tumours in 2-year study of carcinogenicity of diazinon in mice

	Incidence of hepatic tumours per dose of diazinon					
	Control		**100 ppm**		**200 ppm**	
Morphology	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Hepatocellular adenoma	1/21 (5)	1/23 (4)	0 /46 (0)	0/47 (0)	3/48 (6)	1/49 (2)
Hepatocellular carcinoma	4/21 (19)	1/23 (4)	20/46* (43)	0/47 (0)	10/48 (21)	2/49 (4)
Carcinoma and adenoma combined	5/21 (24)	2/23 (9)	20/46 (43)	0/47 (0)	13/48 (27)	3/49 (6)

ppm: parts per million; *: $P < 0.05$ (Fisher exact test)

Results shown as number of tumour-bearing animals / number of animals examined, with incidence relative to the control as a percentage (%) in parentheses.

Source: NTP (1979)

A NOAEL for systemic toxicity could not be set due to the deficiencies in study design. No treatment-related tumours were observed in male or female mice (NTP, 1979; also referenced as Angel et al., unknown year, or NCI, 1979).

In a 2-year non-GLP carcinogenicity study, male and female B6C3F1 mice (59–61 mice/group for both sexes) were fed diazinon (lot no. P-604; purity unknown,) in the diet at concentrations of 0, 100, 200 or 300 (for males) or 400 (for females) (equal to 0, 16, 31 and 46 mg/kg bw per day for males and 0, 22, 43 and 86 mg/kg bw per day for females, respectively). Clinical signs were checked daily and the results of palpating for tumours recorded. Body weight and feed consumption were measured weekly for the first 13 weeks and every 2 weeks thereafter. Water intake was measured at 1, 12, 25, 51 and 71 weeks. At 52 and 104 weeks, blood was drawn from the orbital vein for haematological assessment. Acetylcholinesterase was not measured. Ten mice per group were allocated for interim necropsy at 52 weeks, and all the survivors were necropsied at

termination at 104 weeks. Representative organs were weighed at the interim and terminal kills. All the mice were histopathologically examined.

There were no treatment-related clinical signs or increased mortality throughout the study period. At the highest dose, body weights in males were slightly (about 5%) but significantly ($P < 0.05$) decreased for the first 41 weeks and in females (about 10%) throughout the study. At the same dose, feed consumption was slightly decreased in males throughout the study and in females during the first year, but the changes were not statistically significant at many points. The consistent changes in body weight and feed consumption at the highest dose were considered treatment related. No treatment-related haematological changes were observed. The pathological examination, including organ weights and macroscopic or microscopic examinations, also showed no treatment-related changes. No increases in incidences or malignancy, or early occurrence of tumours, were observed in the treated groups compared to the control group. The types of tumour were common in this strain mouse.

Although this study did not comply with GLP or any test guideline, the major parameters used were adequate for detecting chronic toxicity and carcinogenicity. The NOAEL for 2-year chronic toxicity in mice was 200 ppm (equal to 31 mg/kg bw per day) based on the slight decreases in body weight and feed consumption at 300 ppm (equal to 46 mg/kg per day). No carcinogenicity was observed in this study. Cholinesterase activity was not measured (Goldsmith, 1983).

Rats

In a pre-GLP carcinogenicity study, groups of 50 male and 50 female F344 (Fischer) rats were fed diazinon (purity 98%) admixed in the diet at concentrations of 400 or 800 ppm for 103 consecutive weeks; control groups of 25 males and 25 females were fed the basal diet only. The rats were observed for an additional 1 to 2 weeks after the treatment ended. The prepared diets were used within 1 week. All animals were observed twice a day for signs of toxicity, weighed at 2-week intervals, palpated for tissue masses at each weighing, and subjected to gross and histopathological examination.

The study was performed according to guidelines for carcinogen bioassays established by the NCI (USA) but this method does not comply with the OECD guidelines:

- two dose groups, rather than the prescribed three, were used in the chronic study;
- feed consumption was not measured, so the achieved intake of test material could be not be calculated; and
- haematology or clinical chemistry data were not recorded.

Since the critical effect of diazinon on cholinesterase activity were not measured, the study cannot be used to determine chronic toxicity. However, the study provides useful information on carcinogenicity.

Mortality was not increased at any of the doses compared to the controls, and survival was 88% or greater in all dosed and control groups of animals at week 78 (96%, 98% and 98% of survival in males and 92%, 88% and 88% in females in the control, low- and high-dose groups, respectively). Diazinon did not appreciably affect mean body weights of either sex. Clinical signs observed included hyperactivity, discoloured urine, bloating, vaginal bleeding and vaginal discharge, tissue masses and tachypnoea.

There were no treatment-related tumours in any of the dosed groups of rats of either sex (Tables 11 and 12). Leukaemia or lymphoma occurred in dosed and control groups of rats of each sex at an incidence higher in low-dose males (25/50 [50%]) than in the controls (5/25 [20%]) or high-dose males (12/50 [24%]). Malignant lymphoma is rare in F344 rats and is systemically spread from the lymph node through lymph ducts (Frith, Ward & Chandra, 1993). On the other hand, mononuclear cell leukaemia (synonium: F344 rat leukaemia or large granular lymphocyte leukaemia) are very common tumours, occurring at high incidences in ageing F344 rats (Haseman, Arnold & Eustis,

1990). The tumour cell of mononuclear cell leukaemia in rats is understood to derive from NK-cells in the marginal zone of the spleen (Ward, 1983; Losco & Ward, 1984); leukaemia proliferates in the red pulp of the spleen and systemically spreads out through blood vessels (Frith, Ward & Chandra, 1993). Mononuclear cell leukaemia in rats has a different profile to that of human NK-cell large granular lymphocyte leukaemia, although some similarities in histogenesis or pathological features have been reported (Thomas et al., 2007).

Criteria for diagnosis of mononuclear cell leukaemia in F344 rats were not yet established when this NTP study (1979) was reported, but the descriptions of leukaemia or lymphoma were considered to match those of mononuclear cell leukaemia. As a result, the Meeting evaluated the incidence of leukaemias and lymphomas combined as that of mononuclear cell leukaemia. This combined incidence was within the historical control data of NTP studies in F344 rats (rate: 33.6%; range: 10–72% in untreated control male rats in an NTP study from 1977 to 1987; Haseman, Eustis & Arnold, 1990) and no dose dependency was observed, indicating that the incidence at high dose was not treatment related.

Endometrial stromal polyps were observed at a higher incidence in the low-dose (8/43; 19%) and high-dose (11/49; 22%) groups than those in the control group (2/23; 9%). This finding is common in ageing F344 rats, and both incidences were within the historical control range of NTP studies from 1977 to 1987 (rate 21.4%; range 8–37%) (Haseman, Eustis & Arnold, 1990).

Table 11. Summary of tumour incidences in rats administered diazinon in the diet

	Incidence of tumours per dose of diazinon					
	Control		**400 ppm**		**800 ppm**	
Morphology	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Haematopoietic system, liver, Peyer patch						
Lymphoma or haematopoietic system leukaemia	5/25 (20)	2/25 (8)	25/50* (50)	6/50 (12)	12/50 (24)	6/50 (12)
Uterus						
Endometrial stromal polyp	–	2/23 (9)	–	8/43 (19)	–	11/49 (22)

–: not evaluated; *: $P < 0.05$ (Fisher exact test)

Results shown as number of tumour-bearing animals / number of animals examined, with incidence relative to the control expressed as a percentage (%) in parentheses.

Source: NTP (1979)

Table 12. Combined incidence of each leukaemia or lymphoma in the haematopoietic system, liver and Peyer patch in male rats

	Incidence per dose of diazinon		
Morphology	**Control**	**400 ppm**	**800 ppm**
Haematopoietic system			
Leukaemia	5/25 (20)	16/50 (32)	10/50 (20)
Lymphocytic leukaemia	0/25	1/50 (2)	0/50
Monocytic leukaemia	0/25 (0)	6/50 (12)	2/50 (4)
Liver			
Leukaemia	0/24 (0)	1/49 (2%)	0/49 (0)
Peyer patch			
Malignant lymphoma	0/22 (0)	1/44 (2%)	0/48 (0)

ppm: parts per million; *: $P < 0.05$ (Fisher exact test)

Results shown as number of tumour-bearing animals / number of animals examined, with incidence relative to the control expressed as a percentage (%) in parentheses.

Source: NTP (1979)

A NOAEL for systemic toxicity could not be set due to the deficiencies in study design. No treatment-related tumours were observed in male or female rats (NTP, 1979; also referenced as Angel et al., unknown year, or NCI, 1979).

In a chronic toxicity study, groups of 30 to 40 male and 30 to 40 female Sprague Dawley Crl:CD[SD]BR VAF/Plus rats were fed diazinon (purity 87.7%) admixed in the diet at concentrations of 0, 0.1, 1.5, 125 or 250 ppm (equal to 0, 0.004, 0.06, 5 and 10 mg/kg bw per day for males and 0, 0.005, 0.07, 6 and 12 mg/kg bw per day for females, respectively) for 98/99 weeks. The vehicle-control group was fed a diet containing 26.5 ppm of epoxidized soybean oil at the same concentration as that in the highest diazinon dose group.

Up to 10 randomly selected animals per sex from each group were terminated and necropsied at 1 year, and up to 10 per sex per group from both control groups and the high-dose group were terminated and necropsied after a 4-week recovery period (1 year plus 4 weeks). The rats were approximately 6 weeks of age at initiation of treatment, and their body weights ranged from 158.3 to 241.1 grams for males and 126.4 to 216.5 grams for females. Body weight and feed consumption were measured before starting the treatment and then weekly through weeks 1 to 13 of the dosing period and monthly thereafter. Haematology and biochemistry assessments were conducted during weeks 13 to 14, 26 to 27, 51 to 52, 56, 79 to 80 and 97 to 98. All the surviving animals were used to determine haematological effects at each time point, and 10 animals per sex per group were used to determine clinical chemistry, including cholinesterase activity. The study was performed in accordance with GLP and USEPA Guidelines 83-1/83-5 (1982), which are equivalent to OECD Guideline 453 (1981) and Ministry of Agriculture, Forestry and Fisheries of Japan (1985) guidelines, and which do not differ significantly from the method prescribed by the European Union (B.33). However, the USEPA, European Union and OECD guidelines require group sizes of 100 animals (50 males and 50 females) for carcinogenicity studies. The Kirchner, McCormick & Arthur (1991) study used group sizes of 20 animals per sex, excluding satellite groups, a group size sufficiently large to determine the chronic toxicity, but not the carcinogenicity, of diazinon.

During week 97, increased mortality (6/20) was observed in males at 0.1 ppm. The major cause of moribundity or death was senile nephropathy and/or pituitary adenomas, both unrelated to treatment but associated with senescence in this strain of rats. The study was terminated during weeks 98 to 99 because of the need to have at least five animals per sex per group to provide a meaningful

evaluation of cholinesterase activity data. This early termination was not considered to affect the quality or integrity of the study or any interpretation of the data.

Body-weight gain was increased in males at 0.1 ppm and higher doses and, in some instances, in females at 125 ppm and higher doses compared with the untreated control rats, but because body-weight gain in the control group also showed an increase compared with the untreated controls, these increases in body weight may reflect increased palatability of the feed. In fact, the increases in mean body-weight gain generally coincided with increases in mean feed consumption in these groups.

Decreases in serum cholinesterase activities were observed at 1.5 ppm and higher doses in both sexes. A dose-dependent reduction in erythrocyte cholinesterase activity was observed at 125 and 250 ppm, resulting in activities of 80% and 75% of controls in males and females, respectively, at either dose at the end of the study. Brain cholinesterase activity was also inhibited to 76% and 71% of the control value in males and females, respectively, at 125 ppm, and to 58% and 52% of the control value at 250 ppm in males and females, respectively. Similar inhibition was observed after the first year of the study. A slight reduction of less than 9% was still found after the 4-week recovery period in erythrocyte and brain cholinesterase activity at 250 ppm (Table 13).

Table 13. Changes in mean erythrocyte and brain cholinesterase activities in rats administered diazinon in the diet

Concentration of diazinon (ppm)	Mean per cent change in cholinesterase activity (%)					
	Erythrocyte			Brain		
	One year	One year + 4 weeks recovery	Two years	One year	One year + 4 weeks recovery	Two years
Males						
0.1	+6	–	+7	−4	–	−3
1.5	+7	–	−5	+1	–	−2
125	−16*	–	−21*	−2	–	−24*
250	−11*	−1	−22*	−10	+5	−42*
Females						
0.1	+5	–	−3	+5	–	+1
1.5	0	–	−3	+6	–	+6
125	−22*	–	−26*	−26*	–	−29*
250	−20*	−7*	−25*	−40*	−9*	−48*

–: not evaluated; ppm: parts per million; *: $P < 0.05$ (two-tailed Student *t*-test)

Results expressed as mean increase (+) or decrease (−) in cholinesterase activity as a percentage of control cholinesterase activity.

Source: Kirchner, McCormick & Arthur (1991)

No treatment-related increases in non-neoplastic findings were observed in any diazinon doses. The incidence of neoplasms in the vehicle- and diazinon-treated groups was comparable to that seen in the untreated controls.

The NOAEL was 1.5 ppm (equal to 0.06 mg/kg bw per day) based on the inhibition of erythrocyte and brain cholinesterase activity at dietary concentrations of 125 ppm (equal to 5 mg/kg bw per day). From the available data, there was no evidence of a tumorigenic response; however, the group size ($N = 20$) was too small to allow a conclusion to be reached on carcinogenicity (Kirchner, McCormick & Arthur, 1991; also referenced as EPA, 1993).

In accordance with USEPA Guidelines 83-5 and GLP, chronic toxicity and carcinogenicity of diazinon was examined in rats with the test compound (purity 96.2–97.02%) admixed in the diet of male and female F344 rats (75 rats/group in both sexes in the first study, 15 rats/group in both sexes in the second) at concentrations of 0, 0.1, 1.5 or 22.5 mg/kg bw per day (in the first study) and 0 or 0.025 mg/kg bw per day (in the second study). The second study, started a year after the first, was conducted to measure erythrocyte cholinesterase activity at 13-week intervals and brain acetylcholinesterase activity at termination (after 108 weeks of treatment). Mortality and clinical signs were checked daily. Body weights were measured weekly for 26 weeks after study commencement and every 2 weeks thereafter. Feed consumption was measured weekly for 13 weeks after study commencement, and every 13 or 26 weeks thereafter. Water intakes were measured at 1, 12, 25, 51, 77 and 101 weeks. At 12, 25, 51 and 102 weeks, blood was drawn from ocular vein (10 rats/group in both sexes at each time point) for haematological and blood biochemistry analysis of plasma. Plasma and erythrocyte acetylcholinesterase activity and plasma butyrylcholinesterase activities were measured at the same time points as haematology and at 17 and 38 weeks in the first study and 13, 26, 39, 52, 66, 78, 91 and 104 weeks in the second study. Brain acetylcholinesterase and butyrylcholinesterase were measured at interim kills at 18, 27, 52 (5 rats/group in both sexes) and 104 weeks (6–8 rats/group in both sexes) in the first study. Urine analysis was performed at the same time points as the haematological analyses. Ophthalmological assessments of 20 rats per group in both sexes were made at commencement of treatment and at 25, 50, 78 and 102 weeks. At each interim kill and at termination at 120 weeks, the adrenals, brain, heart, kidneys, lungs, pituitary, spleen, ovaries/testes and thyroids with parathyroids were weighed. Macroscopic and microscopic examinations were conducted.

In terms of clinical signs, incidences and frequencies of urogenital wetness or staining and perianal staining associated with loose stools were increased in females at 22.5 mg/kg bw as was the incidence and frequency of periorbital staining. These symptoms, which progressed with treatment, were considered treatment-related cholinergic inhibitory effects. No consistent and dose-dependent changes were observed in body weight, feed consumption, haematology, blood biochemistry or urine analysis. Erythrocyte and brain acetylcholinesterase activities are shown in Table 14. Erythrocyte acetylcholinesterase activities were inhibited by over 20% overall at 1.5 mg/kg bw and higher in a dose-dependently in both sexes. Brain acetylcholinesterase activity was inhibited by over 20% at 22.5 mg/kg bw in males from 27 week onwards. In females, brain acetylcholinesterase activity was reduced by over 20% at 22.5 mg/kg bw at all time points and at 1.5 mg/kg bw after 52 weeks.

In the second study, erythrocyte and brain acetylcholinesterase activities at 0.0025 mg/kg bw were comparable to the control except at 52 weeks when there was a significant inhibitory effect on erythrocyte acetylcholinesterase activity. The decrease in activity was within 20% of the controls (86% inhibition), indicating no toxicologically significant effect.

Table 14. Summary of erythrocyte and brain AChE activity in rats administered diazinon in the diet

		Concentration of AChE per dose of diazinon							
		Male				Female			
Parameter	**Duration (weeks)**	**0 mg/kg bw per day**	**0.1 mg/kg bw per day**	**1.5 mg/kg bw per day**	**22.5 mg/kg bw per day**	**0 mg/kg bw per day**	**0.1 mg/kg bw per day**	**1.5 mg/kg bw per day**	**22.5 mg/kg bw per day**
Erythrocyte AChE (IU/L)	12	592	623 (105)	450 (76)***	247 (42)***	677	704 (104)	496 (72)**	261 (39)***
	17	518	420*** (81)	212*** (41)	130*** (25)	420	420 (100)	254*** (60)	136*** (32)
	25	598	664* (111)	429* (72)	194*** (32)	619	623 (101)	526* (85)	198*** (32)
	38	630	680 (108)	298*** (47)	158*** (25)	645	601* (93)	313*** (49)	127*** (20)
	51	646	778* (120)	273*** (42)	104*** (16)	685	621 (91)	370*** (54)	184*** (27)
	102	752	740 (98)	386*** (51)	235** (31)	594	539 (91)	369*** (62)	140*** (24)
Brain AChE (IU/L)	18	7 084	6 939 (98)	7 001 (99)	6 043* (85)	6 295	6 315 (100)	5 815 (93)	3 308*** (53)
	27	6 146	6 986 (114)	6 578 (107)	4 446 (72)*	5 399	4 898 (91)	5 669 (105)	3 118 (58)
	52	5 390	5 573 (103)	5 108 (95)	4 446*** (75)	6 371	5 598 (88)	4 746 (74)**	2 243 (36)***
	104	4 896	4 696 (96)	4 754 (97)	1 384*** (28)	6 371	5 598 (88)	5 956* (114)	1 939*** (37)

AChE: acetylcholinesterase; IU: International Unit; *: P < 0.05; **: P < 0.01; ***P: < 0.001

Results shown as concentration of AChE with per cent activity relative to the control (%) in parentheses.

Source: Ashby & Danks (1987)

Absolute and relative thyroid weights were statistically significantly increased at 1.5 and 22.5 mg/kg bw in males at 104 and 120 weeks. The lack of corresponding histopathological change or ageing periods only (no increases at 27 or 52 weeks) indicated the increases were not treatment related. Histopathological examinations showed that several treatment-related changes were increased (Table 15). Continuous stimulating changes, such as ulcer, acanthosis, hyperkeratosis, granulation tissue or hyperplasia of mucosal epithelium, were increased in the forestomach of males and females at 22.5 mg/kg bw 2 years after commencing the oral treatment, and these changes in the forestomach were considered treatment-related. Continuous irritative effects might be involved, because the test chemical was a slight irritant to the rabbit skin (Brennan, 2010). The incidences of fatty change in adrenal cortex were increased in males at 1.5 and 22.5 mg/kg bw. As the increases were not dose dependent or were found in moribund or dead animals only, they were not considered treatment related. No increased incidence, earlier occurrence or increased malignancy was observed in neoplastic changes at all treated groups. Their incidences were comparable to those in controls.

Table 15. Summary of histopathological changes in rats administered diazinon in the diet

			No. of histopathological findings per dose of diazinon							
			Male				Female			
Morphology	**Outcome**	**Duration (weeks)**	**0 mg/kg bw per day**	**0.1 mg/kg bw per day**	**1.5 mg/kg bw per day**	**22.5 mg/kg bw per day**	**0 mg/kg bw per day**	**0.1 mg/kg bw per day**	**1.5 mg/kg bw per day**	**22.5 mg/kg bw per day**
Adrenal										
Cortical fatty vacuolation	ke/fd	105–120	3/16	7/18	10/17*	13/21*	11/17	4/17*	5/12	4/21*
	Tk	120	9/22	8/17	5/19	6/13	16/22	17/21	19/28	19/23
Forestomach										
Ulcer	ke/fd	105–120	4/16	5/18	8/17	12/21	3/17	2/17	4/12	9/21
	Tk	120	5/22	2/17	6/19	3/13	0/22	1/21	2/28	4/23
Acanthosis	ke/fd	105–120	6/16	11/18	8/17	15/21	9/17	4/17	4/12	12/21
	Tk	120	7/22	4/17	7/19	4/13	0/22	2/21	3/28	9/23**
Hyperkeratosis	ke/fd	105–120	6/16	9/18	8/17	15/21	9/17	4/17	3/12	14/21
	Tk	120	7/22	4/17	7/19	4/13	0/22	2/21	3/28	8/23**
Granulation tissue	ke/fd	105–120	6/16	11/18	9/17	16/21*	6/17	3/17	5/12	11/21
	Tk	120	6/22	3/17	7/19	5/13	0/22	1/21	3/28	8/23**
Hyperplasia of epithelium	ke/fd	105–120	0/16	3/18	0/17	7/21*	1/17	0/17	1/12	6/21
	Tk	120	0/22	0/17	1/19	0/13	0/22	0/21	0/28	1/23

ke/fd: killed in extremis or found dead; no.: number; Tk: terminal kill; *: $P < 0.05$; **: $P < 0.01$

Results shown as number of rats with an outcome / number of rats examined.

Source: Ashby & Danks (1987)

The NOAEL for long-term toxicity was 0.1 mg/kg bw per day based on the inhibition of erythrocyte acetylcholinesterase activity at 1.5 mg/kg bw per day. No treatment-related tumours were observed in male or female rats (Ashby & Danks, 1987).

2.4 Genotoxicity

A variety of genotoxicity studies were evaluated. These included published scientific studies as well as unpublished guideline studies. Studies that examined the effects of the oral route of exposure were considered to be the most relevant for assessing risk from low-level dietary exposure, whereas those administered by parenteral routes or at near-lethal doses, those that used a mixture including diazinon, such as formulations, or those that used nonmammalian test species were considered much less relevant. The studies, presented by the JMPR's evaluation of quality and relevance, are presented below. The results of acceptable and relevant studies of genotoxicity, consisting of unpublished and published studies, are summarized in Table 16. Other studies from open literature were considered to be less relevant for the evaluation; these are shown in Tables 17 to 18.

Table 16. Results of acceptable studies of diazinon-related genotoxicity (from open literature or submitted by companies)

End-point	Test object	Concentration	Purity (%)	Results	Comments	Reference
In vitro studies						
Reverse mutation	*Salmonella typhimurium* TA1535, TA1536, TA1537, TA1538	1 000 µg/plate (±S9)	Pure form from stock solution, standard for residue analysis	Negative	Similar to guideline, toxic at higher concentrations	Marshall, Dorough & Swim (1976)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, T1537, TI1538; *Escherichia coli* WP2 uvrA	10–5 000 µg/plate (+S9), 10–1 000 µg/plate (−S9)	96.16	Negative	Guideline study, GLP	Bootman & May (1986)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, T1537; *E. coli* WP2 uvrA	0, 313, 625, 1250, 2 500 and 5 000 µg/mL (±S9)	88	Negative	Guideline study, GLP	Geleick (1990)
Yeast deletion (DEL) assay	*Saccharomyces cerevisiae* RS112	0, 1 000, 5 000 and 10 000 µg/mL (±S9)	NP, obtained from Aldrich	Negative	Adequately described, sufficient details provided	Kirpnick et al. (2005)
Mammalian cell gene mutation	Mouse lymphoma L5178Y TK+/-	12, 24, 48, 72, 96, 108 and 120 µg/mL (+S9) 6, 12, 24, 36, 48, 54 and 60 µg/mL (−S9)	97.2	Negative	Guideline study, GLP	Dollenmeier (1986)
Chromosome damage (chromosomal aberrations)	Human lymphocytes	5–20 µg/mL (±S9)	96.16	Negative	Guideline study, GLP	Bootman, Hodson-Walker & Dance (1986)
Chromosome damage (chromosomal aberrations)	Human lymphocytes	0, 175, 200 and 250 µg/mL (+S9) 0, 100, 150, 175 and 200 µg/mL (−S9)	96	Negative	Guideline study, GLP	Allais (2002)
Unscheduled DNA synthesis	Rat hepatocytes	0, 1.1, 3.3, 10, 30, 60 and 120 µg/mL	88	Negative	Guideline study, GLP	Hertner & Arni (1990)
In vivo studies						
Chromosome damage (micronucleus formation)	Mouse (8Tif: MAGF) bone marrow erythrocytes	0 and 120 mg/kg (oral) sampled 16, 24, 48 h after treatment 0, 30, 60 and 120 mg/kg (oral) sampled 24 h after treatment	87.5	Negative	Guideline study, GLP	Ceresa (1988)
Chromosome damage	Mouse (ICR) bone marrow	31.3, 62.5 and125 mg/kg bw per	95.4	Negative	Guideline study, GLP	Kawamura (2006)

End-point	Test object	Concentration	Purity (%)	Results	Comments	Reference
(micronucleus formation)	erythrocytes	day, on 2 consecutive days (oral)				
Chromosome damage (micronucleus formation)	Mouse (albino, NMRI derived) spermatocyte	10.5, 21 and 63 mg/kg bw (oral); 5 treatments over 10 days	NP	Negative	Pre-Guideline study, pre-GLP	Hool (1981a)
Chromosome damage (micronucleus formation)	Mouse (albino, NMRI derived) spermatocyte	10.5, 21 and 63 mg/kg bw/day (oral) on 5 consecutive days	NP	Negative	Pre-Guideline study, pre-GLP	Hool (1981b)
Dominant lethal assay	Mice (albino, NMRI derived)	0, 15 and 45 mg/kg (oral)	Technical grade, analytical results not available	Negative	Pre-Guideline study, pre-GLP	Fritz (1975)
DNA damage (SCE)	Mouse (ICR, male & female) bone marrow cells	10, 50 and 100 mg/kg gavage	88.0	Negative in males and females	Industry sponsored GLP study; USEPA considered female results unacceptable due to insufficiently high dose	USEPA 1992 (review of Murli & Haworth, 1990)

bw: body weight; GLP: good laboratory practice; NP: not provided; S9: 9000 × *g* fraction from Aroclor 1254 or phenobarbital/5,6-benzoflavone–induced pretreated rat liver homogenate; SCE: sister chromatid exchange; *TK*: thymidine kinase locus; USEPA: United States Environmental Protection Agency

Table 17. Results of less relevant studies or studies deemed inadequate for use in human risk assessment of diazinon

End-point	Test object	Concentration / dose	Purity (%)	Results	Evaluation (Comments)	Reference
In vivo human biomonitoring studies						
Chromosome damage (chromosomal aberrations)	Lymphocytes of workers exposed to pesticides in producing factory	0.45 mg/m^3 in working place air	Commercial product (Basudin E)	Positive	Inadequate and not informative. Inappropriate statistical analyses. Data pooled from all individuals in an exposure group prior to analysis. Mixed, largely negative, results seen when compared with factory controls; more positive results seen when compared with non-factory controls	Kiraly et al. (1979)
Chromosome damage (chromosomal aberrations – structural and numerical)	Lymphocytes of workers in the flower industry	No data	No data	Positive	Not informative. Exposed to ~48 different pesticides	De Ferrari et al. (1991)
DNA damage (SCE)	Human blood lymphocytes from non-smoking students	Not determined, exposure level did not affect erythrocyte AChE activity	Sheep dip (Probably commercial product)	Positive	Not informative. Confounded by exposure to other organophosphorus compounds as evidenced by the presence of higher levels of dimethyl and dimethyl-thiophosphate metabolites than diazinon-derived diethyl- and diethyl-thiophosphate metabolites in urine	Hatjian et al. (2000)
In vivo studies in mammals – oral administration						
Chromosome damage (micronucleus formation)	Rat (Wistar, male) blood erythrocyte	20 mg/kg bw per day for 4 weeks	99	Positive	Inadequate. Scoring of MN in rat blood erythrocytes is problematic due to the ability of the spleen to screen out micronuclei in erythrocytes. Untreated animals exhibited unusually high mean MN frequency of 1%	Hariri et al. (2011)

End-point	Test object	Concentration / dose	Purity (%)	Results	Evaluation (Comments)	Reference
DNA damage (*AP* sites)	Liver and kidney in female rabbits (New Zealand White)	2.64 and 5.28 mg/kg bw per day	NP	Positive	Inadequate. Compound administered every 2 days for 3 months, followed by 8 months without exposure, followed by 1 month of exposure. Doses associated with organ pathology and oxidative stress. Concern about quality and interpretation due to small sample size (*N*=2/group), very low variability in controls, and lack of key details	Tsitsimpikou et al. (2013)
In vivo studies in mammals – intraperitoneal administration						
Chromosome damage (micronuclei)	Mouse (strain 615, sex NP)	0.8, 0.4, 0.2 and 0.1 × LD_{50} per bw per day for 4 days	98	Positive	Inadequate; less relevant route; insufficient data for genotoxicity evaluation including those on dose relationship or positive controls [in Chinese]	Ni et al. (1993)
DNA damage (sperm head decondensation)	Mouse (CF1, male) spermatozoa	22 and 43 mg/kg bw	60 from Exmark Laboratory (Chile)	Positive on day 1; negative on day 32	Less relevant route and test used a formulation. Mice terminated on day 1 and 32 postinjection. Positive response at lowest dose tested (equivalent to 1/3 LD_{50})	Sarabia et al. (2009a)
Chromosome damage (micronucleus formation)	Mouse (CF1, male) bone marrow cells	22 and 43 mg/kg bw	60 from Exmerk Laboratory (Chile)	Positive	Less relevant route, test used a formulation, and extremely high MN frequencies in control (5%) and treated (>25%) animals. Mice terminated on day 1 postinjection. Positive response at lowest dose tested (equivalent to 1/3 LD_{50}). Small number of PCEs scored	Sarabia et al. (2009b)
Chromosome damage (micronucleus formation)	Rat (Wistar, male) blood lymphocyte	20 mg/kg bw per day for 30 days	NP	Positive	Less relevant route and single dose. Very large (13.5 times) increase in MN in binucleated lymphocytes reported	Shadboorestan et al. (2013)
Chromosome damage (micronucleus formation)	Rat (Wistar, male) blood lymphocyte	20 mg/kg bw per day for 30 days	NP	Positive	Eliminated from consideration because it uses the same data as published in Shadboorestan et al. (2013)	Shokrzadeh et al. (2013)

End-point	Test object	Concentration / dose	Purity (%)	Results	Evaluation (Comments)	Reference
In vitro studies in bacteria and yeast						
DNA damage (Rec assay)	*Bacillus subtilis* H17 Rec, M45 Rec	NP	NP	Negative (10 mm paper disk containing 0.02 mL of solution)	Inadequate; no data provided	Shirasu et al. (1976)
Reverse mutation (Ames test)	*S. typhimurium* TA98, TA102, TA1535, TA1537	20 ppm (non-toxic) to 80 ppm (50% toxic)	≥ 90	positive (TA98 +S9 only)	Inadequate due to lack of experimental information and data presentation. Negative control values and variability not shown No positive controls	Wong et al. (1989)
Electrochemical study of interaction with purified DNA	Calf thymus DNA	0.492, 0.984, 1.476, 1.968, 2.46, 3.444, 4.428, 5.421 and 6.396 $\times 10^{-5}$ mol/L in HEPES buffer:methanol (50:50%)	Analytical reagent grade from Merk	Positive for DNA binding	Less relevant as it is a non-validated method for assessing DNA binding	Kashanian et al. (2008)
Reverse mutation (Ames test)	*S. typhimurium* TA98, TA100	0.01 to 1 mmol/L (±S9)	NP, compound provided by the Ministry of Environment of Japan	Negative (1 mmol/L with/without rat liver S9)	Inadequate; no data provided.	Kubo, Urano & Utsumi (2012)
In vitro studies in mammalian cells						
DNA damage (Comet assay-alkaline)	Primary nasal mucosa (epithelium from patients by surgery at inferior and middle turbunate)	0.5, 0.75 and 1.0 mmol/L (= 152, 228, 304 µg/mL) (−S9)	99.5	Positive (0.5 mmol/L)	No information about quality of cells collected from patients and used for the in vitro experiments. Data presented only as % undamaged cells	Tisch et al. (2002)
DNA damage (Comet assay –alkaline)	Primary nasal mucosa cells)	0.05, 0.1, 0.5, 0.75 and 1.0 mmol/L (= 15.2, 30.4, 152, 228, 304 µg/mL) (−S9)	99.2	Positive (dose–response)	No information about quality of cells collected from patients and used for the in vitro experiments. Graphical data only	Tisch, Faulde & Maier (2007)

End-point	Test object	Concentration / dose	Purity (%)	Results	Evaluation (Comments)	Reference
DNA damage (sperm chromatin structure assay (SCSA))	Human spermatozoa	0.05, 0.3, 0.5 and 0.7 mmol/L (= 15, 91, 152, 213 µg/mL) (−S9)	NP (Chem Service)	Weak positive (> 500 µmol/L)	Inadequate. Non-standard test. Figures are mislabelled. From the text Fig. 2 presents the diazinon results. Positive results also seen for diazoxon at ≥ 300 µmol/L	Salazar-Arredondo et al. (2008)
Chromosome damage (chromosomal aberrations)	Human blood lymphocytes	5, 10, 20 and 30 µg/mL (−S9)	NP, obtained from Ciba-Geigy	Negative	Inadequate, no positive control; high and variable aberration frequencies in negative control and treated cells	Lopez et al. (1986)
DNA damage (SCE)	LAZ-007 (human lymphoid cell line, B-cell origin)	0.02, 0.2, 2 and 20 µg/mL (−S9); 20 µg/mL (+S9)	NP	Negative (−S9), positive (+S9)	Data on +S9 limited to a single concentration tested only	Sobti, Krishan & Pfaffenberger (1982)
DNA damage (SCE)	Human blood lymphocytes	0.02, 0.2, 2.0 and 20.0 µg/mL (−S9)	98 or 45	Positive	Inadequate. Vehicle-control value falls within the middle of the dose–response curve. Questionable repeated measures and statistical method used. Significance of individual treatments not reported. Formulation more cytotoxic and top two concentrations could not be scored	Hatjian et al. (2000)
Chromosome damage (micronuclei in binucleate cells)	Human blood lymphocytes	750 µmol/L (= 228 µg/mL) (−S9)	NP	Positive	Inadequate. Questionable, very high induced MN frequency (>10%); no information on source or purity of diazinon; single concentration; no indication of cytotoxicity; similarity of the results to Karamian et al. (2013) in spite of very different treatment protocols raises questions	Shokrzadeh et al. (2014)
Chromosome damage (micronuclei in binucleate cells)	Human blood lymphocytes	750 umol/L (= 228 µg/mL) (−S9)	NP	Positive	Inadequate. Questionable very high induced MN frequency (~10%) occurring after a 1-hour treatment with diazinon prior to mitogenic stimulation; single concentration; no indication of cytotoxicity; similarity of the results to Shokrzadeh et al. (2014) in spite of very different treatment protocols raises questions	Karamian et al. (2013)

End-point	Test object	Concentration / dose	Purity (%)	Results	Evaluation (Comments)	Reference
Chromosome damage (micronuclei in binucleate cells)	MCF-7 cell	10^{-12}, 10^{-10} and 10^{-8} mol/L (−S9)	NP, obtained from Sigma-Aldrich	Positive	Inadequate. No dose–response with same induction reported at extremely low concentrations; number of cells with multiple micronuclei is unusually high	Ukpebor et al. (2011)
Chromosome damage (micronuclei in binucleate cells)	Human blood lymphocytes and skin fibroblasts	2×10^{-8}, 2×10^{-7}, 2×10^{-6} and 2×10^{-5} mol/L (−S9)	97.3	Positive	2- to 4-fold increases seen at all tested concentrations; UV-degraded diazinon showed increased clastogenicity	Colovic et al. (2010)
Chromosome damage (micronuclei in binucleate cells)	Human blood lymphocytes	0.04 and 0.4, 4 µg/mL (−S9)	NP	Weak positive (≥ 0.04 ug/mL)	Weak ≤ 2-fold increases at all concentrations, not concentration related	Bianchi-Santamaria et al. (1997)
Chromosome damage (chromosomal aberrations)	CHO cells	NP	NP	Positive	Not informative; the test material was the organic material isolated from the urine of orchard workers using various pesticides	See, Dunn & San. (1990)
Mammalian cell gene mutation	Mouse lymphoma cell L5178Y *TK* +/-	0, 6.25, 12.5, 25, 50 and 100 µg/mL (−S9 1st trial); 0, 20, 40, 60 and 80 µg/mL (−S9 2nd trial)	NP	Positive	Very different levels of cytotoxicity and mutation frequencies were seen in the 2 trials; no explanation provided. The positive control did not produce an increase in mutation frequency in the second trial	McGregor et al. (1988)
Micronuclei	Cultured primary rat hepatocytes from Sprague Dawley male rats	2, 6, 18 and 54 µg/mL	NP	Negative	Hepatocytes stimulated to proliferate using epidermal growth factor [in Italian]	Frölichsthal & Piatti (1996)
Chromosome damage (chromosomal aberrations)	Chinese hamster lung cells	0.1 mg/mL (0.33 mmol/L) ±S9	NP	Positive (+S9)	Diazinon was excessively toxic in the absence of S9. Inadequate due to inclusion of gaps in the analysis	Matsuoka, Hayashi & Ishidate (1979)
DNA damage (SCE)	V79 Chinese hamster cells	0, 0.05, 0.1, 0.2 and 0.4 µg/mL	99, Wako Pure Chemicals	Negative	Adequate, 0.4 µg/mL caused excessive cell cycle delay	Kuroda, Yamaguchi & Endo (1992)
DNA damage (SCE)	V79 Chinese hamster cells -S9 (Chen et al., 1981) and +S9 (Chen, Sirianni & Huang, 1982)	10, 20, 40 and 80 µg/mL	99.2 Ciba-Geigy	Negative	Adequate –S9 study since 80 µg/mL associated with excessive cytotoxicity; Inadequate +S9 as no data on cytotoxicity provided	Chen et al. (1981) Chen, Sirianni & Huang (1982)

End-point	Test object	Concentration / dose	Purity (%)	Results	Evaluation (Comments)	Reference
DNA damage (SCE)	CHO cells	0.03, 0.1, 0.3 and 1.0 mmol/L (= 9, 30, 90, 300 µg/mL)	89, Analytical grade	Negative	Adequate	Nishio & Uyeki (1981)
In vivo studies in nonmammalian species						
Mutation	Drosophila (wing spot test)	1,3,5,7 and 10 ppm fed to larvae	95	Positive	Not informative due to use of a nonmammalian species	Cakir & Sarikaya (2005)
Chromosome loss (partial and entire)	Drosophila (repair deficient cross)	100 ppb feeding to larvae	Commercial product	Negative	Not informative as test used a formulation in a nonmammalian species	Woodruff, Phillips & Irwin (1983)
DNA damage (comet assay, alkaline)	Freshwater mussels (*Utterbackia imbecillis*) glochida	0.28, 0.55 µg/mL	22.5	Positive at 0.28 µg/mL only	Not informative as test used a formulation in a nonmammalian species	Conners & Black (2004)
DNA damage (SCE)	Central mudminnow (*Umbra limi*) intestinal tissue	0, 5.4×10^{-11}, 5.4×10^{-10} and 5.4×10^{-9} mol/L for 11 days	48.72	Positive	Not informative as test used a formulation in a nonmammalian species	Vigfusson et al. (1983)

AP: apurinic/apyrimidinic locus; bw: body weight; CHO: Chinese hamster ovary; LD_{50}: median lethal dose; MN: micronuclei; NP: not provided; PCE: polychromatic erythrocyte; ppb: parts per billion; ppm: parts per million; S9: 9000 × *g* fraction from Aroclor 1254 or phenobarbital/5,6-benzoflavone–induced pretreated rat liver homogenate; SCE: sister chromatid exchange; SCSA: sperm chromatin structure assay; *TK*: thymidine kinase locus; UV: ultraviolet

Table 18. Results of less relevant in vitro genotoxicity studies or those deemed inadequate for use in human risk assessment of diazinon

Metabolite	End-point	Test object	Concentration	Purity (%)	Results	Comments	Reference
2-Isopropyl-6-methyl-4-pyrimidinol (IMP)	Chromosome damage (micronuclei in binucleate cells)	Human blood lymphocyte/skin fibroblast	2×10^{-5} mol/L	97.3	Positive	Inadequate, dose-related increase in MN reported; positive results also reported for UV-irradiated compound but with no negative control	Colovic et al. (2010)
Diethylthiophosphate	DNA damage (Comet assay) (alkaline at pH 12.1 and >13)	Blood lymphocytes (mitogen-stimulated) and WRL-68, HepG2, HeLa cell lines	1, 10, 25, 50, 100, 500 µmol/L	NP, obtained from Sigma	Positive (1 or 10 µmol/L in HepG2 or WRL-68 cell lines only at either pH with more migration with pH 12.1 as stated in text)	Inadequate. Quality issues in data presentation and interpretation. pH 12.1 detects single strand breaks, pH>13 detects single strand breaks and alkali labile sites. Interpretation in text does not match data in figure but increase in DNA migration under both conditions. Effect diminished by the addition of a inhibitor of P450 enzymes	Vega et al. (2009)
Diazoxon	DNA damage (SCE)	Chinese hamster ovary cells	0.03, 0.1, 0.3, 1.0 mmol/L (= 9, 30, 90, 300 µg/mL)	97	Positive at the highest concentration tested only	Concentration-dependent response but all within the control range for other tested chemicals	Nishio & Uyeki (1981)

NP: not provided; MN: micronuclei; SCE: sister chromatid exchange; UV, ultraviolet

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a two-generation study on reproductive toxicity, groups of 30 male and 30 female Sprague Dawley (CR CD) rats were fed diazinon (purity 94.9%) in the diet at concentrations of 0, 10, 100 or 500 ppm over two generations (F_0 and F_1). Mean diazinon intakes for the F_0 generation during the premating period were 0, 0.77, 7.48 and 32.85 mg/kg bw per day for males and 0, 0.77, 7.48 and 40.26 mg/kg bw per day for females, respectively. After 10 weeks, the animals were mated (1:1) within each dose group and allowed to rear the ensuing F_1 litters to weaning. Litters were culled to four male and four female pups, where possible, on day 4 postpartum. The breeding programme was repeated with the F_1 parents selected from the F_1 offspring. The test diet was fed continuously throughout the study. Parental feed consumption and body weights were measured throughout the study. Reproductive performance, pup survival and developmental parameters were assessed. Gross necropsy findings and histopathological observations in target organs of parental animals and pups not selected for mating were recorded.

Treatment-related clinical symptoms consisted of tremors in three high-dose F_0 females, and dystocia followed by death or termination of two high-dose females, both on gestational day 26. In addition, one intermediate-dose female was found dead on day 24 of gestation after being moribund and with chromodacryorrhoea and a nasal discharge. The cause of death of this female was unknown but may have been related to difficulties in delivery. In the F_1 generation, four high-dose females showed tremors during post-mating. Feed consumption was increased in F_0 females at 500 ppm, whereas in the F_1 generation, a dose-related reduction of feed consumption was observed at 100 and 500 ppm in males only. Body-weight gain was lower in F_0 females at 500 ppm during gestation. In the F_1 generation, reduced body-weight gain was observed at 100 and 500 ppm in males and at 500 ppm in females.

There were no treatment-related effects on mating behaviour and the reproductive parameters (including mating, fertility, gestation indices, number of viable pups and number of stillborn pups) were comparable in the control and treatment groups in the F_0 generation and at the low and intermediate doses in the F_1 generation. At 500 ppm, a greater proportion of females in both generations had a longer gestation. A decrease in the number of pregnancies and viable pups as well as reduced fertility and mating indices was seen in high-dose F_1 females. F_1 and F_2 litter sizes were smaller on lactation day 0. A dose-related reduction in survival of F_1 pups was observed at 100 ppm and 500 ppm and in F_2 at 500 ppm. Weights of F_0 pups were reduced in the 100 and 500 ppm groups, and in F_2 pups at 500 ppm. No treatment-related malformations were found in the pups.

The NOAEL for reproductive effects was 100 ppm (equal to 7.48 mg/kg bw per day for males and females) based on prolonged gestation duration, decrease in the number of pregnancies, and reduced fertility and mating indices at 500 ppm (equal to 32.85 mg/kg bw per day for males and 40.26 mg/kg bw per day for females).

The NOAEL for parental effects was 10 ppm (equal to 0.77 mg/kg bw per day for males and females), based on reduced parental body-weight gain at 100 ppm (equal to 7.48 mg/kg bw per day for males and females).

The NOAEL for offspring toxicity was 10 ppm (equal to 0.77 mg/kg bw per day for males and females), based on reduced viability of pups and pup weights at 100 ppm (equal to 7.48 mg/kg bw per day for males and females) (Giknis, 1989).

In another two-generation study on reproductive toxicity (Weatherholtz, 1982), groups of 13 to 15 male and 26 to 30 female Fischer 344 rats were fed diazinon (purity 97.36%) in the diet at concentrations of 0, 0.1, 1.0 or 10 mg/kg (equivalent to 0, 0.0067, 0.067 and 0.67 mg/kg bw per day, assuming concentrations are in mg/kg feed or ppm) over two generations (F_0 and F_1). Rationales for the dose selection or mean diazinon intakes were not provided. After the growth period, the animals were mated (1:2) within each dose group and allowed to rear the ensuing F_1 litters to weaning. Litters

were culled to 10 pups, where possible, on day 7 postpartum. The breeding programme was repeated with the F_1 parents selected from the F_1 offspring. The test diet was fed continuously throughout the study. Parental feed consumption and body weights were measured throughout the study. Reproductive performance, pup survival and developmental parameters were measured. Gross necropsy findings and histopathological observations in target organs of parental animals and pups not selected for mating were recorded.

There were no treatment-related effects observed in F_0 or F_1 parental animals or pups. The NOAEL for reproductive, parental and offspring effects was 10 ppm (equal to 0.67 mg/kg bw per day for males and females), the highest dose tested (Weatherholtz, 1982).

(b) Developmental toxicity

Mice

In the following non-GLP and non-guideline study,

> [g]roups of mice (6 females/dose) were given oral daily doses of 0, 0.18 or 9 mg/kg bw diazinon throughout gestation. Treated animals gained less weight during gestation compared to control animals. Weight gain of pups born to mothers receiving 9 mg/kg bw/day was reduced. Daily testing for physiological and behavioural development of the pups revealed some evidence of retarded development among the offspring of high-dose animals (e.g. retardation of eye and ear opening). Measures of endurance and coordination also gave some evidence of impairment (e.g. increased rod cling endurance in both groups, reduced rotarod endurance, impaired running performance in a maze inclined plane test). Impaired reactions were sometimes observed at both dose levels but without a clear dose-effect relationship. Examination of brain tissue of only 8 of a total number of 132 offspring at the high-dose level revealed morphological abnormalities in the forebrain. The relationship of these findings to the observed behavioural changes is unknown (Spyker & Avery, 1977; study evaluation copied from the 1993 JMPR without further evaluation). The evidence for morphological effects of diazinon on the developing brain was considered insufficient by an expert (Krinke, 1991; copied from the 1993 JMPR without further evaluation).

It should be noted that the group size was rather small: six dams per group, with eight pups per dam (48 pups per dose group).

Rats

> In a teratogenicity study in Sprague-Dawley rats at dose levels of 0, 15, 50 or 100 mg/kg bw [per] day administered orally on days 6 through 15 of gestation, dams at the 100 mg/kg bw/day dose level showed a marked decrease in food consumption correlating with weight loss at the beginning of the treatment period. Skeletal assessment showed a slightly higher incidence of incomplete ossification at different sites in the fetuses at 100 mg/kg bw/day. Visceral examination revealed a dystopia cordis in association with hypoplasia of lungs in 1/105 fetuses at 100 mg/kg bw/day. This anomaly has been reported to occur spontaneously in control animals. Because of the single occurrence this anomaly was not considered to be due to a direct action of diazinon but to be secondary to maternal toxicity (Fritz, 1974; study evaluation copied from the 1993 JMPR without further evaluation).

In another teratogenicity study (Infurna & Arthur, 1985), groups of 27 mated female rats (Charles River Crl:COBS CD[SD] BR) were administered doses of diazinon (purity 97.4%) by gavage at 0, 10, 20 or 100 mg/kg bw per day in 0.20% carboxymethyl cellulose containing 0.5% Tween 80 during gestational days 6 through 15. The study was terminated on day 20 of gestation. Body weight, feed consumption, mortality and abortion were continuously checked. All the dams were necropsied, the ovaries examined and corpora lutea counted. Approximately one out of three of the fetuses from each litter was dissected (organs and glands examined included the brain, eyes, spinal cord, heart and major blood vessels, nasal passages, trachea, lungs, diaphragm, oral cavity, tongue, oesophagus, stomach, intestines, liver, pancreas, thymus, spleen, kidneys, ureters, bladder, adrenals,

ovaries, uterus or testicles). The skeletons of the remaining two of three fetuses were examined for abnormalities, size, shape, location and relationship to adjacent ossification centres).

The diazinon treatment had no effect on the mortality of the dams. During the first half of the dosing period (gestation days 6–10), the high-dose dams lost weight (−11 grams), and body weights and body-weight gains were significantly reduced throughout gestation (*P* values between 0.01 and 0.05). Feed consumption was reduced on gestation days 6 to 9 at a dose of 100 mg/kg bw per day. No compound-related clinical signs and no abnormal gross pathological findings were observed in the dams. At 100 mg/kg bw per day some reproductive parameters differed from the control values but no statistical significance was achieved (e.g. increase in number of resorptions and increased per cent pre- and post-implantation loss, reduction in number of live fetuses). However, at 20 mg/kg bw per day, the number of resorptions and post-implantation losses were significantly ($P \leq 0.05$) reduced. Male and female fetuses in the high-dose group weighed significantly (approximately 6%) more than control fetuses. This effect can be attributed to the slightly decreased number of fetuses in the high-dose group.

On gross observation, one fetus in the intermediate-dose group exhibited exencephaly. In the high-dose group, 3 of the 262 (1%) fetuses from three litters showed external malformations (single occurrences of a umbilical hernia, filament tail, sublingual extraneous soft tissue), and there were no similar effects in the controls. However, umbilical hernia and tail abnormalities are routine spontaneous malformations in this strain of rat, and these malformations were morphologically unrelated; as a result, they were considered to be a consequence of the marked maternotoxicity at this dose level and not due to a teratogenic effect of the test compound. An increased incidence in rudimentary ribs (T-14) was observed in all treatment groups, attaining statistical significance in the high-dose group (5% versus 0%). The increased incidence of the skeletal variations observed at the highest dose level was considered to be related to maternotoxicity at this dose level.

The NOAEL for maternal toxicity was 20 mg/kg bw per day, based on body weight loss on gestation days 6 to 10, reduced body weight and body-weight gains throughout treatment, and decreased feed consumption on gestation days 6 to 9 at 100 mg/kg bw per day.

The NOAEL for embryo/fetal toxicity was 20 mg/kg bw per day, based on increased incidence of rudimentary T14 ribs at 100 mg/kg bw per day (Infurna & Arthur, 1985).

Rabbits

Diazinon (purity 89.2%) was administered to groups of 18 to 22 pregnant rabbits (New Zealand White) by gavage at 0, 7, 25 or 100 mg/kg bw per day in 0.2% carboxymethyl cellulose or methyl cellulose from gestation days 6 to 18 (inclusive). The females were monitored daily for clinical signs. Body weights and feed consumption were recorded at selected intervals during gestation. On gestation day 30, the rabbits were terminated and all the thoracic and abdominal cavity organs examined for macroscopic changes. The ovaries, uterus and cervix were weighed and examined. Corpora lutea were counted and the number and distribution of fetuses and intrauterine deaths determined. All the fetuses were sexed, weighed and examined for soft tissue and skeletal malformations and variations. A transverse section of the brain was made, and organ structure was examined.

An increase in maternal mortality was observed at 100 mg/kg bw per day (9/22, 41% versus 0% in all other groups). Overt clinical signs of maternal toxicity at 100 mg/kg bw per day included tremors, convulsions, hypoactivity and anorexia. Reduced body-weight gain was found at the highest dose level. The treatment did not affect the number of corpora lutea, number of implantation sites, live fetuses per litter or fetal weight. The incidence of visceral and skeletal malformations and variations showed no treatment-attributable differences between the groups. The study therefore gave no evidence for an embryotoxic or teratogenic activity of diazinon.

The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on mortality, tremors, convulsions, hypoactivity, anorexia and reduced body-weight gain observed at 100 mg/kg bw per day.

The NOAEL for embryo/fetal toxicity was 100 mg/kg bw per day, the highest dose tested (Harris & Holson, 1981).

In another developmental toxicity study, diazinon (purity 96.2%) was administered to groups of 17 to 20 pregnant rabbits (New Zealand White) by gavage at dose levels of 0, 2.5, 10 or 40 mg/kg bw per day in 5% aqueous solution of gum arabic from gestation days 6 to 18 (inclusive). The females were monitored daily for clinical signs. Body weights and feed consumption were recorded at selected intervals during gestation. Cholinesterase activity was not measured. On gestation day 29, the rabbits were terminated and examined for macroscopic changes. Corpora lutea were counted. The uterus was opened and examined for the number and distribution of live fetuses and intrauterine deaths. All fetuses were weighed and examined for external, soft tissue and skeletal variations and malformations. Clinical signs, body weights and feed consumption were measured regularly and plasma and erythrocyte cholinesterase measured in blood samples obtained 2 hours after dosing on day 13, the final day of dosing.

Clinical signs, including unsteadiness, body tremors and abnormal movement and posture were observed in high-dose females. Feed consumption and body-weight gain were also reduced in this dose group. Mean litter and fetal weights were lower than control values in high-dose fetuses, the difference in mean fetal weight being statistically significant ($P < 0.05$). Litter size and pre- and post-implantation losses were not affected. There was no obvious adverse effect on incidence of malformation anomalies or skeletal variants. In the preliminary study, where groups of six non-mated females were administered 0, 10, 30 and 50 mg/kg bw per day, qualitatively similar but more severe findings were observed in the high-dose females. In addition, plasma and erythrocyte cholinesterase activity was dose-dependently inhibited at all dose levels compared to controls (Table 19).

Table 19. Cholinesterase estimation in preliminary study

	Mean cholinesterase levels on last day of dosing period (µmol/mL per min)	
Dose level of diazinon	**Blood plasma**	**Erythrocyte**
Control	0.70	1.92
10 mg/kg bw per day	0.17	0.59
30 mg/kg bw per day	0.14	0.35
50 mg/kg bw per day	0.08	0.39

bw: body weight; min: minute

N = 6 per group.

Source: Edwards & Falconer, 1987

The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on clinical signs, decreased body weight and reduced feed consumption.

The NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day, based on decreased fetal weight at 40 mg/kg bw per day.

No NOAEL could be derived in the range-finding study as inhibition of erythrocyte cholinesterase activity was observed at the lowest dose of 10 mg/kg bw per day (Edwards & Falconer, 1987).

2.6 Special studies

(a) Acute neurotoxicity

In an acute neurotoxicity study, male and female Sprague Dawley rats (10 rats/group) were administered diazinon by gavage (lot no. 510530; purity 95.4%) at 0, 100, 300 or 500 mg/kg bw. This

study was conducted in compliance with GLP and in accordance with Ministry of Agriculture, Forestry and Fisheries of Japan test guidelines. Cholinesterase activity was not measured.

Four hours after dosing, decreased motor activity, respiratory rate and body temperature as well as mucous stool or stained fur (perianal, perigenital, perioral, periocular) was observed in one male at 300 mg/kg bw and in both males and females at 500 mg/kg bw. Motor activity was also decreased at 300 and 500 mg/kg bw in both sexes. Fore- and hindlimb grip strength was weakened at 500 mg/kg bw. No histopathological changes were found.

The NOAEL was 100 mg/kg bw, based on systemic toxicity and clinical signs of neurotoxicity observed at 300 or 500 mg/kg bw (Sunaga, 2007a).

Groups of 15 male and 15 female Sprague Dawley rats were administered single doses of diazinon (purity 88%) by gavage at doses of 0, 2.5, 150, 300 or 600 mg/kg bw. Of the 15 rats, 10 were used for neurological testing and five for measuring cholinesterase activity. Dose selection was based on the results of three preliminary studies that assessed median lethal doses (LD_{50}) and determined the lowest lethal dose to be 750 mg/kg bw and the highest non-lethal dose to be 500 mg/kg bw. As such, significant neurotoxic effects were expected at the highest selected dose of 600 mg/kg bw. The selected intermediate doses (150 and 300 mg/kg bw) were anticipated to give intermediate or minimal effects, while a previous investigation (Glaza, 1993) had found no reduction in erythrocyte and brain cholinesterase activity at the lowest dose of 2.5 mg/kg bw. Groups of 10 male and 10 female rats were administered triadimefon (purity 99%) by gavage at 150 mg/kg bw as a positive control for neurological effects. On the day of treatment, all rats were observed before and after dosing and twice daily thereafter for general appearance, behaviour, signs of toxicity, morbidity and mortality. Each week, all the rats were examined in detail, including palpation for tissue masses. Body weights were estimated before dosing and weekly thereafter, and feed consumption was estimated weekly. A functional observational battery was performed 1 week before administering the single dose, at the time of peak effect after dosing (9–11 hours for diazinon, 1 hour for triadimefon) and 7 and 14 days after dosing with diazinon on the 10 rats intended for neurological testing. Blood samples were obtained at the estimated time of peak effects and at 14 days for the five rats designated for cholinesterase testing in each group. Plasma and erythrocyte cholinesterase activity was determined by colorimetric assay, while brain cholinesterase activity was estimated in whole brain samples at termination 14 days after administration. The rats used for neurological examination (survivors to termination and decedents) were necropsied. Sections were made at 10 levels of the brain, cervical, thoracic, lumbar and sacral spinal cord with ganglia and right and left sciatic nerves, right and left fibular nerves, right and left tibial nerves and right and left lateral cutaneous sural nerves, and the Gasserian ganglion. Sections were also taken of skeletal muscle from the right thigh, eyes with the optic nerve and any gross lesions identified. Only five rats per group in the groups receiving diazinon at 600 mg/kg bw, the control group and those treated with triadimefon were processed for histopathological examination.

During the study, two males and one female treated at 600 mg/kg bw died. A single accidental death occurred in the group of five rats at 300 mg/kg bw intended for cholinesterase measurement during blood sampling. One control animal was removed from the study because it was thought to have been wrongly dosed, as it showed signs of cholinergic poisoning and low cholinesterase activity. The clinical observations included chromodacryorrhoea, reduced activity and tremors at a dose of 300 mg/kg bw, while the group at 600 mg/kg bw also had chromorhinorrhoea, diarrhoea and pallor. Significant decreases in body-weight gain were observed in males at doses greater than or equal to 300 mg/kg bw, while no effects were observed on body weight in females. Feed consumption was decreased in males at doses greater than or equal to 300 mg/kg bw and in females at doses greater than or equal to 150 mg/kg bw. In both males and females, effects on functional observational battery parameters were seen only at the estimated time of peak effect (9–11 hours for diazinon, 1 hour for triadimefon) and not on day 7 or 14 after exposure.

The autonomic parameters affected by diazinon at the time of peak effects in males were faecal consistency and soiled fur at doses greater than or equal to 150 mg/kg bw; these effects were

dose related. Increased salivation, staining of the nose and repeated opening and closing of the mouth were observed at 300 mg/kg bw, and impaired respiration, lachrymation and staining of the mouth at 600 mg/kg bw. In females, repeated opening and closure of the mouth were observed at doses greater than or equal to 150 mg/kg bw, and altered faecal consistency, soiled fur and staining of the nose were observed at doses greater than or equal to 300 mg/kg bw. Impaired respiration and lachrymation were observed at the highest dose. The neuromuscular parameters affected in males were abnormal gait at 150 mg/kg bw and higher doses; ataxic gait, impaired righting reflex, impaired hindlimb extensor reflex and decreased hindlimb footsplay at 300 mg/kg bw and higher doses and reduced forelimb grip strength at 600 mg/kg bw. In the females, ataxic and abnormal gait was observed at 150 mg/kg bw and higher doses and impaired righting reflex at 300 mg/kg bw and higher doses. Impaired hindlimb extensor reflex, abnormal hindlimb positioning when held by the tail and reduced forelimb and hindlimb grip strength were observed at 600 mg/kg bw. Central nervous system excitability parameters were also affected. In males, tremors were observed in the home cage and open field at in animals at 300 mg/kg bw and higher, as was twitching or muscle fasciculation. At 600 mg/kg bw, the arousal level was decreased. Females had tremors in the home cage at the highest dose only and in the open field at 300 mg/kg bw and higher doses. Twitching in the open field was seen at the highest dose, and a lowered arousal level at 300 mg/kg bw and higher doses. Touch response was reduced in females at the highest dose, and the tail-pinch response was reduced in both sexes at this dose. Reduced body temperature was observed in males at doses greater than or equal to 300 mg/kg bw and in females at greater than or equal to 150 mg/kg bw. In addition, females at 300 mg/kg bw and higher doses were dehydrated. Locomotor activity in the figure-eight maze decreased over time in all groups. At the estimated time of peak effect, the activity of males at doses 300 mg/kg bw and higher and of females at 150 mg/kg bw and higher was decreased. At 7 and 14 days after dosing, the mean total activity counts were similar for all groups.

At the time of peak effect after diazinon intake (9–11 hours), plasma cholinesterase activity was reduced in all the treated groups; however, no differences between groups were observed 14 days after dosing. Erythrocyte cholinesterase activity was inhibited at 150 mg/kg bw and higher doses in both males and females at the time of peak effect. At 150, 300 and 600 mg/kg bw, the activity was 18%, 17% and 15% of controls in males and 24%, 23% and 24% of controls in females, respectively. Partial recovery was seen 14 days after dosing: in males, the activity at 150 mg/kg bw was 91% that of concurrent controls, while it was 66% and 53% that of concurrent controls at 300 and 600 mg/kg bw, respectively; in females, the activity was 89%, 57%, 74% and 65% that of concurrent controls at 2.5, 150, 300 and 600 mg/kg bw, respectively. No significant differences in brain cholinesterase activity were seen in the groups of males. Significantly reduced brain cholinesterase activity (92% of control value) was seen at termination in females at 150 mg/kg bw; however, as activity in the groups at the higher doses was comparable to that in the controls, this finding is of no biological significance. No gross or microscopic treatment-related abnormalities were seen at necropsy. Triadimefon decreased weight gain and feed consumption during week 1 of the study. At the time of peak effect after exposure (1 hour), changes in central nervous system excitability parameters (increased incidence of rearing; increased arousal level) were the only changes seen in the functional observational battery.

The NOAEL was 2.5 mg/kg bw based on decreased erythrocyte cholinesterase concentrations and behavioural changes at 150 mg/kg bw (Chow & Richter, 1994).

Groups of albino Crl:CD[BR] VAF/Plus rats were administered diazinon (purity 87.9%) by gavage. In phase 1 of the study, five males and five females were given diazinon at 100, 250 or 500 mg/kg bw. Subsequently, in order to identify an NOAEL, groups with five females dosed with diazinon at 25 or 50 mg/kg bw were added. In phase 2 of the study, groups of five males each were administered diazinon at 0.05, 0.5, 1.0, 10.0, 100 or 500 mg/kg bw by gavage, and groups of five females received doses of 0, 0.05, 0.12, 0.25, 2.5, 25 or 250 mg/kg bw. In phase 1, clinical observations were carried out 1, 2, 4 and 8 hours after administration of the test material and daily thereafter. Body weights were determined before treatment and on days 7 and 14. The rats that died after day 1 were also weighed; cholinesterase activity in these animals was not measured. In phase 2,

clinical observations were made 1, 2 and 4 hours after administration of diazinon, while body weights were measured before treatment and on day 1. The surviving rats from phase 1 were terminated on day 14 and subjected to gross necropsy; abnormal tissues were retained for possible histopathological examination. In phase 2, plasma and erythrocyte cholinesterase activity was measured by colorimetric assay 24 hours after treatment. The rats were then terminated and subjected to gross necropsy, with abnormal tissues retained for possible histopathological examination; in addition, the right half of the brain was removed to determine cholinesterase activity.

One high-dose female died during phase 1 of the study. There was no treatment-related effect on body weight. Clinical signs were seen in males at 250 and 500 mg/kg bw and in females at 50 mg/kg bw and higher doses; the signs included miosis, hypoactivity, absence of the pain reflex, red-stained face, yellow-stained urogenital region and soft stools. No pathological changes were observed that could be attributed to treatment. In phase 2, no deaths occurred. Body-weight loss within 24 hours of dosing was greater in males at 500 mg/kg bw and in females at 250 mg/kg bw than in concurrent controls. Clinical signs of toxicity were seen in males at 500 mg/kg bw and in females at 250 mg/kg bw; these signs included miosis, hypoactivity, absent pain reflex, staggering gait, excessive salivation, red-stained face and yellow-stained and/or wet urogenital region. The only gross pathological findings of note were yellow staining of the perineum and red paranasal discharge in males at 500 mg/kg bw and in females at 250 mg/kg bw.

Plasma cholinesterase activity was reduced in males at 10 mg/kg bw and higher and in females at 2.5 mg/kg bw and higher. A statistically significant ($P \leq 0.05$) and biologically significant reduction in erythrocyte cholinesterase activity was seen in males at 100 mg/kg bw (to 51% of control value) and 500 mg/kg bw (to 64% of control value). In females, erythrocyte cholinesterase activity was statistically and biologically significantly reduced at 25 mg/kg bw (to 65% of control value) and 250 mg/kg bw (55% of concurrent control value). Brain cholinesterase activity was statistically and biologically significantly reduced in males at 500 mg/kg bw (to 31% of control value) and in females at 250 mg/kg bw (to 30% of concurrent control). In females at 25 mg/kg bw, brain cholinesterase activity was 64% that of controls; while this difference was not statistically significant, it is considered biologically significant.

At necropsy in phase 2, staining of the perineum and red paranasal discharge were seen in rats of each sex at the highest dose. No treatment-related histological lesions were seen.

The NOAEL was 2.5 mg/kg bw based on the inhibition of brain and erythrocyte acetylcholinesterase activities in females at 25 mg/kg bw (Glaza, 1993).

One study reported on the time course of acute inhibition of cholinesterase activity. Groups of 15 male and 15 female rats were administered diazinon (purity 88%) at 0, 2.5, 150, 300 or 600 mg/kg bw. Doses were selected based on the results of a range-finding study (Glaza, 1993) in which whole brain cholinesterase activity was inhibited 24 hours after dosing in males at 500 mg/kg bw and females at 250 mg/kg bw by about 70%. Clinical observations were made immediately before blood sampling from the orbital plexus to determine serum and erythrocyte cholinesterase activity. Five rats of each sex were terminated at 3, 5 and 9 hours and the remainder at 24 hours to determine brain and spinal-cord cholinesterase activity. Acetylcholinesterase activity was measured in the cerebellum, cerebral cortex, striatum and hippocampus and in the thoracic spinal cord using a modification of the method of Ellman et al. (1961).

Survival was unaffected by treatment. At the highest dose, clinical signs were seen at 3 hours, with maximum effect at 9 hours in males and some recovery after 24 hours, and maximum effect after 24 hours in females. No significant differences were seen in body weight. Plasma cholinesterase activity was decreased by more than 20% at the lowest dose at 3 hours and 9 hours, with maximum reduction at 9 hours in males and females. At 24 hours, the cholinesterase activity at the lowest dose was decreased by 17% in males and 42% in females compared with that of the controls. Although erythrocyte cholinesterase activity was significantly decreased in females 9 hours after dosing at 2.5 mg/kg bw, the absence of any inhibition at 3 and 24 hours and at any time in males suggests that

this result may be an anomalous finding. In an analysis of cholinesterase activity in regions of the central nervous system at the lowest dose, cholinesterase activity in males was never less than 80% that of controls (although it was equal to 80% that of controls in the cerebral cortex at 9 hours). In females at the lowest dose, no substantial decrease in central nervous system cholinesterase activity was observed. At the higher doses, significantly decreased activity was observed in all regions and at all times but was usually greater at 9 hours and 24 hours than at 3 hours (Table 20).

The NOAEL was 2.5 mg/kg bw, based on inhibition of brain and erythrocyte acetylcholinesterase activities at 150 mg/kg bw. Inhibition was observed beginning at 3 hours post-dosing, with maximal inhibition at 9 hours post-dosing (Potrepka, 1994).

Table 20. Change in cholinesterase activity in rats administered diazinon

		Mean per cent change in cholinesterase activity per dose of diazinon							
		Male				**Female**			
Sample	**Interval (hour)**	**2.5 mg/kg bw**	**150 mg/kg bw**	**300 mg/kg bw**	**600 mg/kg bw**	**2.5 mg/kg bw**	**150 mg/kg bw**	**300 mg/kg bw**	**600 mg/kg bw**
Plasma	3	−21**	−66**	−71**	−72**	−57**	−74**	−77**	−79**
	9	−30**	−79**	−80**	−77**	−60**	−82**	−85**	−73**
	24	−17**	−76**	−84**	−88**	−42**	−89**	−89**	−91**
Erythrocyte	3	0	−66**	−82**	−74**	+1	−42**	−50**	−73**
	9	+1	−76**	−78**	−81**	−40**	−68**	−78**	−74**
	24	−11	−68**	−77**	−76**	−11	−70**	−68**	−71**
Cerebellum	3	−1	−51**	−76**	−80**	−7	−54**	−48**	−66**
	9	−6	−59**	−78**	−84**	+2	−65**	−79**	−77**
	24	+3	−45**	−60**	−80**	0	−68**	−74**	−81**
Cerebral cortex	3	+16	−31	−67**	−75**	+4	−34*	−35**	−56**
	9	−20*	−62**	−82**	−85**	−5	−63**	−75**	−78**
	24	+23	−45**	−60**	−80**	−1	−73**	−77**	−85**
Striatum	3	0	−28*	−69**	−75**	+13	−26*	−31**	−50**
	9	+10	−65**	−77**	−85**	+9	−66**	−81**	−83**
	24	+12	−43**	−58**	−85**	−5	−68**	−84**	−87**
Hippocampus	3	+5	−40**	−70**	−80**	−10	−46**	−47**	−56**
	9	−5	−57**	−76**	−84**	+5	−68**	−81**	−83**
	24	+25	−45**	−62**	−85**	−1	−65**	−74**	−81**
Thoracic spinal cord	3	+10	−27	−65**	−77**	+8	−39**	−33*	−49**
	9	+8	−51**	−76**	−85**	+4	−63**	−73**	−78**
	24	+9	−42**	−50**	−81**	+3	−51**	−46**	−81**

bw: body weight; *:$P \leq 0.05$; **:$P \leq 0.01$ (Dunnett *t*-test)

Results expressed as mean increase (+) or decrease (−) in cholinesterase activity relative to the control (%).

Source: Potrepka (1994)

(b) Subacute neurotoxicity

A 90-day repeated neurotoxicity study in rats was conducted in accordance with test guidelines and GLP. Ten male and female Sprague Dawley rats were fed diazinon in the diet at concentrations of 0, 25, 125 or 1000 ppm (equal to 0, 1.7, 8.4 or 69.1 mg/kg bw per day in males and 0, 1.8, 9.3 or 82.4 mg/kg bw per day in females) for 90 days. All the rats were checked for mortality and clinical signs twice a day. Body weights and feed consumption were measured and chemical intakes calculated on day 1, 4 and 8 and weekly thereafter. Detailed clinical signs and behaviour were monitored before the treatment and 2, 4, 8 and 13 weeks after starting the treatment. An ophthalmological examination was conducted before the treatment for all the rats and at week 13 for the rats at 1000 ppm and the controls. Plasma, erythrocyte and brain acetylcholinesterase activities were measured at termination. At termination, all the rats were necropsied. Five rats per group in both sexes were fixed by perfusion of glutaraldehyde and paraformaldehyde solutions under deep anaesthesia. The fixed tissues and organs in the central and peripheral nervous systems underwent histopathological examination.

Mortality was unaffected by the treatment. No treatment-related clinical signs were observed. Body weight were slightly decreased (within 10% of the control) in females on days 4 to 15, but the decrease was not considered treatment related because it was slight (<10%), transient and bore no similarity to the results of the preliminary study. No treatment-related changes were noted in functional observational battery or neurological, ophthalmological or pathological analyses. Cholinesterase activities in the plasma, erythrocyte and brain are summarized in Table 21. Erythrocyte acetycholinesterase activity was inhibited by over 20% at 25 ppm and above in both sexes; brain acetycholinesterase activities were inhibited by 20% and above at 125 ppm and 1000 ppm.

Table 21. Summary of AChE activity in 90-day neurotoxicity study in rats administered diazinon in the diet

	AChE activity per dose of diazinon							
	Male				**Female**			
	0 ppm	**25 ppm**	**125 ppm**	**1 000 ppm**	**0 ppm**	**25 ppm**	**125 ppm**	**1 000 ppm**
Erythrocyte AChE (IU/L)	544.9	396.0$^{\#}$ (73)	223.1$^{\#\#}$ (41)	155.0$^{\#\#}$ (28)	601.4	313.5$^{\#\#}$ (52)	175.0$^{\#\#}$ (29)	152.4$^{\#\#}$ (25)
Brain AChE (mU/mg)	127.4	126.0 (99)	117.8 (92)	117.8** (49)	125.4	124.0 (99)	80.4** (64)	21.6** (17)

AChE: acetylcholinesterase; IU: International Unit; ppm: parts per million; U: enzyme unit; $^{\#}$: $P \leq 0.05$; $^{\#\#}$: $P \leq 0.01$ (Mann–Whitney *U*-test); *: $P \leq 0.05$; **: $P \leq 0.01$ (Dunnett *t*-test)

Results shown as concentration in IU/L for erythrocyte AChE and mU/mg for brain AChE, with activity relative to controls as a percentage (%) in parentheses.

Source: Sunaga (2007b)

The NOAEL for subacute neurotoxicity was not determined based on cholinesterase inhibitions in the erythrocytes and brain at 25 ppm (equal to 1.7 mg/kg bw per day), the lowest dose tested (Sunaga, 2007b).

(c) Delayed neurotoxicity

An oral dose of 28 mg/kg bw of diazinon technical (87% purity) was administered to a group of 18 hens (the target dose was 13 mg/kg bw per day as the approximate LD_{50} was doubled due to a preparation error). The schedule to protect the hens from acute cholinergic effects of diazinon consisted of a 10 mg/kg bw atropine pretreatment by intramuscular injection and an additional intramuscular injection of 2-pyridinealdoxime methiodide (2-PAM) at the time of diazinon dosing.

Post-treatment consisted of concurrent doses of atropine and 2-PAM 1 and 5 hours after dosing. Since there were no neurotoxic responses in the test group in the 3 weeks following the treatment, the hens were treated again with 13 mg/kg bw of diazinon on day 21. One test group hen was found dead on day 5 after the first dosing and one vehicle control hen was found dead on day 7 after the first dosing, after exhibiting a slight unsteadiness when walking. After the second dosing on day 21, one test group hen was found dead 6 hours after the second treatment, and one test group hen exhibited a slight unsteadiness in walking only on day 41. No neurotoxic signs were apparent during the 3-week observation period after the second treatment. Histopathological examination showed no lesions in the brain, spinal cord or peripheral nerves in the diazinon-treated animals, whereas animals from the positive control (tri-orthocresyl phosphate [TOCP] treatment) showed multiple lesions (axonal degeneration) consistent with peripheral neuropathy (Jenkins, 1988).

In another delayed neurotoxicity study, groups of 12 hens were administered an oral dose of 0, 10, 30 or 100 mg/kg bw per day of diazinon technical (purity 96.3%) in peanut oil, the high dose being twice the estimated LD_{50} of 50 mg/kg bw. TOCP at 500 mg/kg bw was used as a positive control. To protect the hens against cholinergic toxicity, hens were treated with intramuscular physostigmine and/or atropine for 24 to 48 hours after the diazinon doze. The hens were observed for clinical signs and ataxia over 21 days. After perfusion fixation, the peripheral nerves, spinal cord, brainstem and cerebellum were examined histologically. For biochemical assessment of esterase activities 24 and 48 hours after administration, satellite animals were dosed accordingly.

One hen at 30 mg/kg bw died on day 1 and was replaced by a reserve animal. In addition, 3 of 10 hens in the 100 mg/kg bw group died within 3 days of administration. These early decedents were not necropsied because delayed neuropathy requires longer to develop. Dose-related signs of cholinergic toxicity, including diarrhoea, salivation, vomiting or dyspnoea and reduced activity, impaired gait and recumbency, lasting for up to 6 days, were observed in the animals dosed 30 and 100 mg/kg bw. Body-weight gains were reduced in 30 and 100 mg/kg bw animals. Severe inhibition of plasma cholinesterase was observed at all doses. Brain and spinal-cord cholinesterase activity was reduced in a dose-related manner in hens treated at 30 and 100 mg/kg bw, while those at 10 mg/kg bw were not affected. Neither erythrocyte activity nor neurotoxic esterase activity in brain and spinal cord was affected by diazinon. TOCP-treated controls showed signs consistent with organophosphate ester-induced delayed neuropathy (Classen, 1996).

In a third delayed neurotoxicity study, groups of 12 Sterling Ranger hybrid hens were administered an oral dose of 20 mg/kg bw of diazinon technical (96.2% purity) in maize oil, this being the approximate oral median lethal dosage. The hens were treated on day 1 and again 21 days later. Two other groups of six hens were similarly treated with maize oil (vehicle control) or with TOCP at 700 mg/kg bw (positive control).

The observed marked cholinergic responses and associated motor dysfunction were controlled using atropine sulfate and pralidoxime therapy. One bird with severe cholinergic signs – body weight loss and deteriorating condition – was terminated in extremis 3 days after the second administration of diazinon. The surviving birds generally fully recovered within 2 to 4 days of treatment, and the majority showed no abnormalities during the remainder of the observation period. One bird showed reduced activity and slight body weight loss from day 22 onwards and episodes of perianal soiling, unsteady stance and resting on hocks. Other signs seen in three birds were largely restricted to transient reduced activity and unsteady stance. The positive control animals showed the expected signs of delayed neurotoxicity in four of the six animals (disturbed balance, unsteady gait/stance). Minimal axonal swelling and eosinophilic accumulations within the axons of the upper cervical or mid-thoracic region of the spinal cord were observed in five of the 12 birds treated with diazinon and in all the birds treated with TOCP. The lesions were generally less severe but qualitatively similar to those observed in the upper spinal cord of TOCP-treated birds. Neuromotor changes observed in diazinon-treated birds (also observed in TOCP-treated birds) largely comprised reduced activity and/or unsteady stance (four birds) and axonal swelling and eosinophilic accumulation within the

axons of the upper cervical or mid-thoracic region of the spinal cord (five birds). However, these was no intra-animal correlation between the in vivo and histopathological findings. In view of the minimal nature of both types of change, it was concluded that there was no unequivocal evidence that diazinon caused acute delayed neurotoxicity in the hen (Cummins, 1987b).

(d) Mechanistic studies

Special study of effects on the pancreas

A study was conducted using a canine model to investigate the induction of pancreatic ductal hypertension following cholinesterase inhibitor intoxication known to be an important triggering mechanism in the pathogenesis of acute and chronic pancreatitis. Diazinon was intravenously administered (25 mg/kg bw) to the pancreatic ampulla of dogs and the tissue cholinesterase activity of the canine pancreatic sphincters was determined. Two enzymes are responsible for the total cholinesterase activity of whole blood: a membrane-bound AChE associated with the erythrocyte membrane, and a soluble enzyme, pseudocholinesterase or butyrylcholinesterase (BChE) in the serum. In tissues, which also contain the two forms of cholinesterase, AChE is important in the regulation of neuromuscular activity and parasympathetic ganglion transmission, while the role of BChE is unknown. The observation of the negative correlation between serum BChE activity and intraductal pancreatic pressure supports the hypothesis that the pancreatic ductal hypertension which occurs following cholinesterase inhibitor intoxication is due to a selective reduction in pancreatic BChE activity (Dressel et al., 1980; study evaluation copied from the 1993 JMPR without further evaluation).

The induction of acute pancreatitis by [organophophates] found in dogs was confirmed in guinea-pigs but not in cats. These results may reflect species-related differences in the distribution of pancreatic BChE (Frick et al., 1987; study evaluation copied from the 1993 JMPR without further evaluation).

Special studies on antidotes

Previous reports with respect to the usefulness of PAM [*sic*] and other oxine reactivators against this organophosphate have been published (Sanderson & Edson, 1959; Wills, 1959; study evaluations copied from the 1993 JMPR without further evaluation). A study was undertaken to provide information on the antidotal effectiveness of pyridine-2-aldoxime methochloride (2-PAM) [*sic*] against diazinon poisoning in animals. The administration of atropine (16 mg/ kg bw; [intramuscular]) or 2-PAM (30 mg/kg bw; [intravenous]) alone, 10 minutes after poisoning rats with doses of 235 mg/kg bw (corresponding to the approximate oral LD_{50} or higher) provided little or no protection. Best protection was achieved when the oxime was given orally in conjunction with [intramuscular] atropine or followed by a subsequent dose of 2-PAM orally or [intravenously]. Administration of 2-PAM to diazinon-poisoned rabbits (1600 mg/kg bw) resulted in reactivation of inhibited blood ChE [cholinesterase] activity concurrent with a decrease in signs of poisoning. Within 2 hours, however, the animals were again weak and ataxic and blood ChE showed renewed inhibition. The authors suggested that effective therapy of diazinon intoxication requires repeated doses of oxime to maintain effective antidote levels in the body (Harris et al., 1969; study evaluation copied from the 1993 JMPR without further evaluation). In dogs and guinea-pigs, pretreatment with atropine protected the animals against diazinon-induced pancreatitis (Frick et al., 1987; study evaluations copied from the 1993 JMPR without further evaluation).

(e) Estrogenic or androgenic activities

Estrogen receptor transcriptional activation

Agonistic activity of diazinon to human estrogen alpha (hER) was investigated using the transcriptional activation assays using the hERα-HeLa-9903 cell line (HeLa-9903). This assay was conducted as outlined in the USEPA Office of Prevention, Pesticides & Toxic Substances (OPPTS) Endocrine Disruptor Screening Program Test Guidelines 890.1300 and in accordance with GLP. A preliminary study was conducted for cytotoxicity and precipitation to identify a suitable top concentration of diazinon (purity 98.7%; batch no. 00896074) for use. The final concentrations were 10^{-11} to 10^{-3} mol/L. For each concentration, additional replicates were prepared that incorporated the

hERα antagonist ICI 182,780. The transcriptional activation assay was also performed for four reference compounds (17β-estradiol, 17α-estradiol, corticosterone and 17α-methyltestosterone). The optimum top concentration of diazinon for use in the transcriptional activation assays was 10^{-5} mol/L, based on excessive cytotoxicity (≥ 20% reduction in cell viability) at concentrations greater than or equal to 10^{-4} mol/L.

In the assay, diazinon did not result in an increase in luciferase activity (maximum response < 10%) at any of the viable concentrations tested. Diazinon was not an agonist of human estrogen receptor alpha (hERα) in the HeLa-9903 model system (Willoughby, 2011a).

Aromatase inhibitory activity

The inhibitory effect of diazinon on aromatase activity were investigated using a human recombinant aromatase assay using human CYP19 (aromatase) and P450 reductase supersomes. This study was conducted as outlined in USEPA OPPTS 890.1200 test guideline and in accordance with GLP. Final concentrations of diazinon (purity 98.7%; batch no. 00896074) tested by the aromatase assay were 10^{-10} to 10^{-3} mol/L. Four independent runs of the aromatase assay were conducted. In addition, the positive control inhibitor 4-hydroxyandrostenedione was included each time the aromatase assay was performed.

Increasing concentrations of 4-hydroxyandrostenedione decrease the aromatase activity in a concentration-dependent manner. In four independent runs of the assay, increasing concentrations of diazinon resulted in a decrease in aromatase activity (at 10^{-4} and 10^{-3} mol/L); however, visual inspection of diazinon concentrations showed particulate matter and cloudiness at 10^{-3} mol/L that was determined to be insoluble test substance. Thus, the top concentration of diazinon suitable for use in the aromatase assay was established at 10^{-4} mol/L. According to the data-interpretation procedure outlined by the USEPA for aromatase inhibition, diazinon was classified as 'equivocal'. It had a mean (standard deviation [SD]) value of 63.7% (± 14.3%) control activity at 10^{-4} mol/L. Diazinon was determined to be equivocal for aromatase activity while a decrease in aromatase activity was identified at the highest concentration of diazinon (e.g. 10^{-3} mol/L). Solubility issues were observed so this concentration was not included in the data interpretation (Wilga, 2011).

Estrogen receptor binding assay using rat uterine cytosol

The ability of diazinon to interact with the estrogen receptors isolated from the rat uterus was investigated as outlined in the USEPA OPPTS 890.1250 test guideline and in accordance with GLP. In a preliminary study, the final concentrations of diazinon (purity 98.7%; batch no. 00896074) tested in the binding assays were 10^{-11} to 10^{-3} mol/L. Three independent runs of the binding assay were conducted. A complete concentration–response curve for the negative control, octyltriethoxysilane, and weak positive control, 19-norethindrone, was run each time the binding assay was performed.

The top concentration of diazinon suitable for use in the binding assays was 10^{-4} mol/L. No precipitation was observed at 10^{-3} mol/L in the first runs so the concentration range was shifted for the second and third runs. In all three valid independent runs, the mean specific binding was greater than 93% for the negative control, octyltriethoxysilane. In the first run, the mean specific binding was greater than 75% at every soluble concentration tested for diazinon. The mean specific binding for diazinon at 10^{-3} mol/L was 59.3% of control. Since precipitation was observed at this concentration, data were not evaluated, resulting in diazinon being classified as 'non-interacting'. In the second run, the mean specific binding was greater than 75% at every soluble concentration tested for diazinon, classifying it as 'non-interacting'. In the third run, the mean specific binding was 69.8% for 10^{-4} mol/L diazinon, classifying it as 'equivocal'. The mean relative binding affinity (RBA; calculated by dividing the log of the half maximal inhibitory concentration [$LogIC_{50}$] of the control/test material by the $LogIC_{50}$ of the positive control 17β-estradiol) was 0.6 for 19-norethindrone. There was no RBA to be calculated for diazinon. Diazinon was classified as 'non-interacting' in the first two independent

valid runs and as 'equivocal' in the third independent valid run, and was therefore considered to be non-interacting (Willoughby, 2011b).

Androgen receptor binding (rat prostate cytosol)

The ability of diazinon to interact with the androgen receptors isolated from rat prostates was investigated by a study conducted as outlined in the USEPA OPPTS 890.1150 test guideline and in accordance with GLP. Concentrations of diazinon (purity 98.7%; batch no. 00896074) for use in the binding assays were identified at 10^{-10} to 10^{-3} mol/L. Three valid independent runs were examined. In this assay, the classification of a chemical as a binder or non-binder was based on the average results of three non-concurrent runs, each of which met the performance criteria and, taken together, were consistent with each other. A run is classified as a 'non-binder' if the lowest point on the fitted response curve within the range of the data was above 75%. A run is classified as 'equivocal' if the average lowest point on the fitted response curves within the range of the data was above 50% but below 75% (USEPA, 2009d).

The top concentration of diazinon suitable for use in the binding assays was 10^{-3} mol/L. In the first run, the mean specific binding was 71.9% at 10^{-4} mol/L diazinon, classifying it as 'equivocal' for this run. At 10^{-3} mol/L, the mean specific binding was 44.1%, but precipitation of diazinon was observed at this concentration so the data were not assessed. In the second run, the mean specific binding was 41.6% at 10^{-3} mol/L and 80.5% at 10^{-4} mol/L diazinon, classifying it as a "binder" for this run. This resulted in a $LogIC_{50}$ of −3.6 mol/L and an RBA of 0.4 for diazinon. In addition, two of the three replicates for 10^{-10} mol/L diazinon were pulled out of the data analysis as outliers. In the third valid independent run, the mean specific binding was 36.7% at 10^{-3} mol/L and 79.0% at 10^{-4} mol/L diazinon, classifying it as a binder for this run. As diazinon was classified as a binder for only two of the three runs, the mean RBA could not be calculated, though the mean based on RBA for those two runs alone was 0.4.

Diazinon was classified as 'equivocal' in the first valid independent run and as a 'binder' in the second and third runs (Willoughby, 2012).

The uterotrophic assay

A uterotrophic assay was performed to evaluate the estrogenic effects of diazinon on ovariectomized rats. This study was conducted as outlined in the USEPA OPPTS 890.1600 test guideline and OECD 440 test guideline and conducted in accordance with GLP. Ovariectomized Sprague Dawley rats were allocated to four dose groups. The animals were administered dose levels of 78 and 250 mg/kg bw per day of diazinon, the vehicle control, or 17α-ethinylestradiol (positive control) for three consecutive days by gavage. Dose levels of diazinon were selected based the LD_{50} and available toxicity data. Following administration of 17α-ethinylestradiol, wet and blotted uterine weights were significantly increased.

Under the conditions of this uterotrophic assay, which utilized the ovariectomized rat model, oral administration of diazinon at dose levels of 78 and 250 mg/kg bw per day (maximum tolerated dose) did not increase uterine weights, indicating an absence of estrogenic effects (Davis & Lea, 2011).

Hershberger bioassay

The Hershberger bioassay was used to screen diazinon for its androgen agonist/antagonist activity and 5α-reductase inhibition properties. This study was conducted as outlined in the USEPA OPPTS 890.1400 and OECD 441 test guidelines and in accordance with GLP. Castrated Sprague Dawley rats were allocated to eight groups: diazinon (purity 98.7%; batch no. 00896074) at 47 or 150 mg/kg bw per day, corn oil (the vehicle control), testosterone propionate at 0.4 mg/kg bw per day as positive control, co-administration of diazinon at 15, 47 or 150 mg/kg bw per day and co-

administration of flutamide at 3.0 mg/kg bw per day. Diazinon and flutamide were administered by gavage for 10 consecutive days and testosterone propionate was subcutaneously injected. In the testosterone propionate group, the rats were administered testosterone propionate for 5 days and then co-administered testosterone propionate and corn oil (the vehicle) for 5 days. Twenty-four hours after the final treatment, androgen-dependent organs, including the glans penis, ventral prostate, levator ani plus bulbocavernosus muscle complex, Cowper's glands and seminal vesicles with coagulating gland with fluid were weighed.

The rats did not show any severe signs of toxicity during the treatment and their final body weight at 150 mg/kg bw was approximately 90% of the control. Diazinon treatment at all doses did not show statistically significant changes in any androgen-dependent organs. However, diazinon co-administered testosterone propionate at all doses showed statistically significant and dose-dependent decreases in the weights of these organs though their anti-androgenic activities were weak compared to those of flutamide. The data on organ weights are summarized in Table 22.

Table 22. Summary of mean organs weights in the Hershberger bioassay in rats administered diazinon for 10 consecutive days

		Body-weight changes and final organ weight					
Dose group	**No. of rats**	**Body-weight changes (g)**	**Glans penis (mg)**	**Cowper's glands (mg)**	**LABC (mg)**	**Ventral prostate (mg)**	**Seminal vesicles (mg)**
Control (corn oil)	8	43.6	40.6	6.4	137.3	15.7	37.0
Diazinon at 47 mg	8	42.6	43.1	5.7	121.7	14.5	30.7
Diazinon at 150 mg	8	15.6*	43.2	4.6	108.7	16.1	35.6
Corn oil + TP	7	66.1	76.4*	49.2*	476.7*	205.6*	647.0*
Diazinon at 15 mg + TP	8	69.4	77.3	35.4	469.0	152.2#	461.1#
Diazinon at 47 mg + TP	8	59.2	68.9	38.0	376.2#	118.2#	409.6#
Diazinon at 150 mg + TP	7	38.2	66.2#	25.8#	265.6#	82.6#	246.3#
Flutamide + TP	7	67.5	61.0§	15.1§	262.2§	46.6§	91.6§

bw: body weight; LABC: levator ani plus bulbocavernosus muscle complex; no.: number; TP: testosterone propionate; *: $P < 0.05$ compared to the vehicle control (*t*-test); #: $P < 0.05$ compared to the corn oil + TP group (Dunnett *t*-test); §: $P < 0.05$ compared to the corn oil +TP group (*t*-test)

Source: Davis (2011)

In the Hershberger bioassay, diazinon did not show any androgen agonist activity; however, it did show antagonist activity (Davis, 2011).

Steroidogenesis (Human Cell Line – H295R)

The steroidogenesis laboratory proficiency assay to evaluate the ability of diazinon to affect the testosterone and estradiol/estrone steroidogenic pathway was performed using the H295R human adrenocortical carcinoma cell line. Forskolin or prochloraz was used as a known hormone inducer or inhibitor, respectively. This study was conducted in accordance with GLP and as outlined in USEPA OPPTS 890.1550 test guideline (USEPA, 2009). Diazinon (purity 98.7%; batch no. 00896074) was tested in the assay at 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 µmol/L. Six independent runs of the steroidogenesis assay were conducted. Test chemicals and reference chemicals (forskolin and prochloraz) were tested in replicates of three per plate, while six solvent control wells were analysed on the quality control plate. The H295R supplemented medium used in the assay at the time of plating, dosing and harvest contained 10 µmol/L 22R-hydroxycholesterol to increase basal hormone production. The duration of exposure was 48 ± 2 hours. A quality control plate containing two doses

of the reference chemicals forskolin and prochloraz was run each time the assay was performed. Cell viability was assessed after the approximately 48-hour exposure using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Testosterone and estradiol levels were measured using high-performance liquid chromatography with mass spectrometry (HPLC/MS-MS).

The highest concentration of diazinon that could be analysed in the steroidogenesis assay was 10 µmol/L in all three independent runs because of precipitation seen under the microscope at the 100 µmol/L diazinon concentration in two runs (run 1 and run 2) as well as cytotoxicity greater than 20% at the 100 µmol/L concentration in run 3. The results are summarized in Table 23. A statistically significant decrease in testosterone was observed after exposure with 10 µmol/L diazinon in all three runs of the assay. In run 1 of estradiol, statistically significant decreases were observed at the 0.0001, 0.001, 0.01 and 0.1 µmol/L diazinon exposure concentrations, but the decreases were not dose dependent; an increase in estradiol was observed at 10 µmol/L diazinon. The levels of estradiol at 10 µmol/L in runs 2 and 3 were similar to that in run 1, but not statistically significant.

Table 23. Fold changes[a] for testosterone or estradiol in steroidogenesis using H295R

Concentration of diazinon (µmol/L)	Fold changes[a]					
	Testosterone			Estradiol		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3
0.000 1	1.08	1.11*	1.01*	0.85*	0.99	0.87
0.001	0.98	1.04	1.07*	0.82*	0.91	0.89
0.01	0.97	1.04	1.06*	0.86*	0.97	0.90
0.1	0.99	1.03	1.00	0.86*	0.94	0.94
1	0.93	1.05	1.06	0.98	1.12	1.03
10	0.76*	0.85*	0.92*	1.30*	1.40	1.30
100	N/A	N/A	N/A	N/A	N/A	N/A

N/A: not analysed because of precipitation and/or cytotoxicity; *: $P > 0.05$

[a] Fold changes over solvent control.

Source: Wagner (2012)

A statistically significant decrease in testosterone in all three runs indicated that the decrease was induced by the 10 µmol/L diazinon exposure. The decreases in estradiol at multiple concentrations in run 1 were considered not treatment related as there was no dose relationship or reproducibility. The increase in estradiol at 10 µmol/L diazinon was seen in run 1 only (Wagner, 2012).

Pubertal development and thyroid function in intact juvenile/peripubertal female and male rats

An assay was conducted to identify the effects of diazinon on pubertal development and thyroid function in intact juvenile/peripubertal female and male rats. The studies were conducted as outlined in USEPA OPPTS 890.1450 (females) or OPPTS 890.1500 (males) test guidelines and conducted in accordance with GLP. The rats were orally administered diazinon at 50 or 100 mg/kg bw per day (purity 95.9%; batch no. 00896074) or the vehicle control (corn oil) from postnatal day 22 for 21/22 or 31/32 days in females or males, respectively. The high dose was selected following a review of previous study reports, including a study that showed no abnormal clinical signs and a decrease in body-weight gain (males only) following 14 days of administration of 100 mg/kg bw per day diazinon (Green, 1983). Approximately 2 hours after the final dose, all the animals were examined. While mean terminal body weights at 100 mg/kg bw per day were 96.2% that of the controls, they were

92.7% of mean control body weights on postnatal day 30 (day 8), suggesting the maximum tolerated dose was approached during the course of the study.

Diazinon treatment did not affect pubertal development of female rats, including the day of vaginal opening, estrus onset, estrus cyclicity or pituitary, ovarian or uterine weights. At termination, the rats showed decreases in circulating serum thyroxine concentrations at both dose levels (3.04 ± 0.73, 2.35 ± 0.58, 2.51 ± 0.68 µg/dL, vehicle control and diazinon at 50 and at 100 mg/kg bw per day, respectively). However, the decreases were not dose related, and there were no corresponding changes in thyroid-stimulating hormone, thyroid gland weight or thyroid gland histopathology. Therefore, the decreases in thyroxine levels were not considered treatment related.

Terminal body weights in male rats at 100 mg/kg bw per day diazinon were 99.4% that of the vehicle-control body weights; however, on postnatal day 30, after 7 days of dose administration, body weights were 93.1% that of mean control body weights. Diazinon did not affect pubertal development including organ weights of androgen-dependent tissues, serum testosterone concentrations or pituitary weights. After 31/32 days of diazinon administration, there was a decrease in circulating serum thyroxine concentrations at 50 mg/kg bw per day only. No corresponding changes in thyroid-stimulating hormone concentrations or thyroid gland weight or histopathology were observed at either dose levels, and so the decrease was not considered treatment related. In conclusion, administration of diazinon to intact juvenile/peripubertal female and male rats resulted in no changes in end-points that suggest an effect on pubertal development (Davis, 2012).

(f) Studies on metabolites or impurities

Several studies with tetraethyl pyrophosphate (TEPP), an impurity no longer permitted in diazinon, and oxypyrimidine, a metabolite, were submitted (see Table 24).

Table 24. Summary of studies of acute toxicity of diazinon impurities and metabolites

Test substance	Species	Strain	Sex	Route	Purity (%)	Result	Reference
O,*O*-TEPP	Rat	Sprague Dawley	M + F	Oral	N/S	LD_{50} 0.947 mg/kg bw (M) and 0.658 mg/kg bw (F)	Kuhn (1995a)
O,*S*-TEPP	Rat	Sprague Dawley	M + F	Oral	N/S	LD_{50} 0.711 mg/kg bw (M) and 0.460 mg/kg bw (F)	Kuhn (1995b)
S,*S*-TEPP	Rat	Sprague Dawley	M + F	Oral	N/S	LD_{50} >10 mg/kg bw (M) and 3.48 mg/kg bw (F)	Kuhn (1995c)
oxypyrimidine	Rat	Sprague Dawley	M + F	Inhalation	93.0	LC_{50} > 5.32 mg/L	Maedgen (1987)

bw: body weight; F: female; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; M: male; N/S: not stated; TEPP: tetraethyl pyrophosphate

In a 5-week toxicity study, groups of 10 male and 10 female Sprague Dawley rats were administered oxypyrimidine (purity unknown) in aqueous 3% cornstarch with 0.5% Tween 80 by gavage at a daily dose of 0, 20, 100, 500 or 1000 mg/kg bw per day. All test animals were observed daily for clinical signs and mortality. Individual body weights and feed consumption were recorded weekly. Physical and ophthalmoscopic examinations as well as haematological, clinical chemistry and histopathological (liver only) tests were performed on all the rats. Cholinesterase was not measured.

Body-weight gain in males was significantly and dose-dependantly reduced at all dose levels, from 89% of control values (not statistically significant) at low doses to 66% of control values at high doses. Feed consumption was statistically significantly decreased after 7 days in both males and

females at 500 or 1000 mg/kg bw per day. Changes in mean serum biochemistries in both males and females included increase in total bilirubin and calcium at 100 mg/kg bw per day and higher doses; decreased albumin-to-globulin ratio and chloride and increased alkaline phosphatase, alanine aminotransferase, total protein, albumin and phosphorus at 500 mg/kg bw per day and higher doses; and reduction in glucose at 1000 mg/kg bw per day. Absolute and/or relative liver weights were increased at 500 mg/kg bw per day and higher doses, and relative kidney weight were increased at 1000 mg/kg bw per day. No microscopic findings were observed in the liver.

A NOAEL could not be established, based on reduced body-weight gain in males at 20 mg/kg bw per day, the lowest dose tested (Spoede-Thompson, Batastini & Arthur, 1990).

(g) Intestinal microbiota effects

An extensive literature search did not show published reports on the potential adverse effects of diazinon on the intestinal microbiota. Based on the mode of action, diazinon is unlikely to affect the intestinal microbiota. In addition, no studies were found on the ability of intestinal microbiota to metabolize diazinon.

(h) Immunotoxic effects

No specific studies on immunotoxicity were submitted. A study in the open literature with intraperitoneal injection of diazinon in mice (Neishabouri et al., 2004) was not informative. The submitted repeated-dose toxicity studies do not indicate an immunotoxic potential for diazinon after oral exposure.

3. Observations in humans

3.1 Studies in volunteers

In a double-blind, placebo-controlled study, healthy, informed male volunteers were given single, ascending doses of diazinon (purity 97.8%) in corn oil in gelatine capsules. Some volunteers received a placebo of only corn oil in gelatine capsules. The lowest dose used was 0.03 mg/kg bw, given initially to one volunteer receiving the test material and one the placebo. In the next phase, one volunteer received the placebo, six diazinon at 0.03 mg/kg bw and one diazinon at 0.12 mg/kg bw. In the next phase, one volunteer received the placebo, six diazinon at 0.12 mg/kg bw and one diazinon at 0.21 mg/kg bw. In the next phase, one volunteer received the placebo, six diazinon at 0.21 mg/kg bw and one diazinon at 0.30 mg/kg bw. In the final phase, three volunteers received the placebo and seven diazinon at 0.20 mg/kg bw. A complete physical examination, including an electrocardiogram, was carried out before dosing and 2 days and 15 days after dosing. Vital signs (respiratory parameters, oral temperature, blood pressure and pulse) were recorded before administering the test material and 1, 2, 4, 6, 8, 12, 24 and 48 hours and 4, 7 and 14 days afterwards. The volunteers were asked to report all adverse events. Blood was drawn for clinical chemistry and haematological examination before dosing and on days 1, 2 and 15 after dosing. Blood was also drawn 1 and 2 days before and immediately before dosing, and plasma and erythrocyte cholinesterase activity was determined using a modification of the method of Ellman et al. (1961). Further samples for determination of cholinesterase activity were taken 1, 2, 4, 6, 8, 12, 24 and 48 hours after dosing and on days 5, 8 and 15 after dosing. Urine samples were collected 24 hours before dosing and for 0–6, 6–12, 12–24 and 24–48 hours after dosing. Pharmacokinetic and urinary metabolite data collected during the study according to Protocol Parts B and C were reported separately (reports not submitted to the JMPR).

The only self-reported adverse event considered to be related to diazinon intake was back pain in a man at the highest dose. No treatment-related effects on haematological or clinical chemical parameters were observed, except for changes in cholinesterase activity. Plasma cholinesterase activity was inhibited by more than 20% at doses greater than 0.12 mg/kg bw. No

significant inhibition of erythrocyte cholinesterase activity was seen at any dose; at 0.21 mg/kg bw, 7% inhibition was observed at 4 hours, 4% inhibition at 8 hours and 6–7% inhibition at days 5, 8 and 15. At other times, the erythrocyte cholinesterase activity was greater than or equal to that of the group given the placebo. Furthermore, data for the man given the highest dose did not suggest any significant inhibition of erythrocyte cholinesterase activity.

The NOAEL was 0.21 mg/kg bw (the highest dose was ignored as only one volunteer received it) (Boyeson, 2000; final version reported as Anderson, 2000).

In a preliminary study, four healthy adult male volunteers (age not specified; body weight 79.5, 83.5, 84 and 95 kg) were given gelatine capsules containing either 2.47 mg or 2.85 mg of technical diazinon (purity 99.5%), depending on whether their body weight was closer to 85 or 95 kg. The study was conducted in accordance with principles expressed in the Declaration of Helsinki or equivalent statements prepared for use by national and/or multinational authorities (Cristie, 2000). The dose was 0.03 mg/kg bw per day for 28, 29 or 31 days, depending on the participant's availability. The dose was selected on the basis of the Payot study (Payot, 1966), but slightly increased because the purity of diazinon had increased with a concomitant reduction in its acute toxicity. The exclusion criteria included clinically relevant cardiovascular, renal, haematological or biochemical profiles (including plasma cholinesterase activity) and metabolic or gastrointestinal disorders likely to influence absorption. Individuals with an abnormal electrocardiogram or an HIV-positive or hepatitis B-positive diagnosis were also excluded. Blood was drawn to measure haemoglobin concentrations; erythrocyte, leukocyte and platelet counts; blood urea nitrogen, glucose, sodium, potassium, chloride, bicarbonate, cholesterol, creatinine, triglyceride and bilirubin concentrations; and alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, creatine kinase and gamma-glutamyltransferase activities before dosing. Blood for estimating cholinesterase activity was collected on four occasions before dosing (days −28, −27, −7 and −1) and then 6 hours after dosing. Thereafter, activity was measured on days 1, 2, 3, 8, 13/14, 20, 28, 29 or 30. Clinical investigations, haematology, clinical chemistry and urine analysis were performed on days 8, 13/14, 20, 28, 29 or 30.

No cholinergic signs were observed. Similarly, there were no treatment-related clinical changes in haematological or clinical chemical end-points, apart from cholinesterase activity during the study. Plasma cholinesterase activity was reduced by an average of 48% relative to pretest values in all four volunteers on day 20 of treatment. A significant reduction in plasma cholinesterase activity (> 20%) occurred by day 8 of treatment. There was no reduction in erythrocyte acetylcholinesterase activity at any time during treatment.

Based on the absence of cholinergic signs or inhibition of acetylcholinesterase activity in erythrocytes, the NOAEL was set at 0.03 mg/kg bw per day (Beilstein, 1998).

The results of the following study by Payot (1966) were reported by the JMPR in 1966, 2001 and 2006. Because of some inaccuracies in reporting the administered doses in the previous JMPR evaluations, the amended summary from the JMPR 2006 is given below.

Four adult male volunteers (age 30–45 years; body weight, 66, 74, 91 and 95 kg) were given gelatine capsules containing 0.5 mg of technical diazinon (purity 95.4–95.7%) postprandially. Since the amount of diazinon in each capsule was fixed and volunteers were given either four or five capsules depending on whether their body weight was closer to 75 or 100 kg, the actual administered doses varied slightly at 0.03, 0.027, 0.022/0.027 (alternate day treatment with 4 and 5 capsules) and 0.026 mg/kg bw per day, respectively. To ascertain reversibility of effects, after complete plasma cholinesterase inhibition in two of the four volunteers was observed after 1 day of treatment, dosing was suspended on days 5–10 of the 42-day regimen, while the other two (lighter) volunteers who commenced treatment a month later were treated uninterrupted for 34 days.

No plasma cholinesterase activity was measurable on the first 6 days of treatment in two of the volunteers. As a result, treatment was interrupted for 6 days to enable recovery. Subsequent treatment at the same dose did not reveal any inhibitory effects on plasma cholinesterase activity. The fluctuations observed during treatment were similar to those observed during the pretest period. At no time was erythrocyte acetylcholinesterase activity decreased compared with the pretest values. Other parameters investigated included haematology and blood chemistry, urine analysis and symptomatology. No changes were observed that could be attributed to treatment.

The NOAEL was 0.03 mg/kg bw per day on the basis of transitory depression of plasma cholinesterase activity, the only effect observed at this dose (Payot, 1966).

The results of the following study by Lazanas, Fancher & Calandra (1966) were reported by the JMPR in 2006 and in earlier monographs; the study evaluation is copied from the 2006 JMPR without further evaluation.

> Three healthy adult male volunteers (Nos. 4, 5 and 6) were given gelatine capsules containing a mixture of diazinon 50W (50% [weight per weight] wettable powder; purity 49.6%) and corn starch at a final dose of 0.025 mg/kg bw per day administered as three doses per day, taken before meals at 08:00, 12:00 and 18:00, for 43 days. A concurrent control group of three volunteers (Nos. 1, 2 and 3) were given gelatine capsules containing corn starch only. In a second dosing regimen, three different volunteers (Nos. 1, 7 and 8) were treated as above at a dose of 0.020 mg/kg bw per day for 37 days. In the recovery phase, after treatment at 0.025 mg/kg bw per day, volunteers were given capsules containing corn starch only (placebo) for 101 days; this recovery phase was reduced to 41 days for the group at 0.020 mg/kg bw per day. Plasma and erythrocyte cholinesterase activities in all eight volunteers were measured on five separate occasions before dosing and then at intervals of 1–5 days throughout treatment and recovery using an electrometric method (Δ pH/h). Body weight and clinical signs were monitored daily. Haematological parameters, including haemoglobin concentration, erythrocyte count, total and differential leukocyte count and prothrombin time were determined at intervals of 4–7 days during treatment and at 4–14 days during recovery, as were clinical chemistry parameters, i.e. cholinesterase activity (erythrocytes and plasma), blood urea nitrogen, alkaline phosphatase and alanine aminotransferase, and urine analysis, i.e. pH and microscopic elements.
>
> No clinical signs or changes in body weight were observed. No significant changes were detected in any of the haematological, urinary or clinical chemistry parameters measured, except for plasma cholinesterase activity (average inhibition, approximately 22%). By contrast, mean erythrocyte cholinesterase activity was not appreciably inhibited (maximum, 2%) at any time during treatment at 0.025 mg/kg bw per day or during the 22 days of recovery.
>
> Treatment at 0.020 mg/kg bw per day resulted in a combined nonsignificant mean plasma cholinesterase inhibition of 8% and recovery appeared to be complete after 16 days. Similarly, erythrocyte cholinesterase activity was not significantly affected by treatment at 0.020 mg/kg bw per day. The NOAEL was 0.025 mg/kg bw per day on the basis of depression of plasma cholinesterase activity, the only effect observed at this dose (Lazanas, Fancher & Calandra, 1966).

3.2 Case studies

The 1993 JMPR mentioned numerous reports on intentional and accidental intake of diazinon and other organophosphates. The text below is copied from the 1993 JMPR without further evaluation.

> Numerous reports [describe] the clinical manifestations of organophosphate toxicity and the usefulness of erythrocyte and serum cholinesterase activity assays for diagnosis as well as the efficacy of supportive and specific therapies (Kabrawala, Shah & Oza, 1965; Payot, 1966; Banerjee, 1967; Gupta & Patel, 1968; Zwiener & Ginsburg, 1988).
>
> A case of acute diazinon poisoning complicated by pericarditis and pneumonia followed by recovery has been reported. The intoxication caused the occurrence of cyanosis, tracheobronchial congestion, pulmonary oedema and pneumonia. The treatment included the intramuscular injection of atropine (Banerjee, 1967).

Another complication reported following accidental ingestion of diazinon consisted of severe pancreatitis and a pseudocyst (Dressel et al., 1979). The induction of pancreatitis was reproduced in dogs treated intravenously with a dose of 5 mg/kg bw diazinon. Pancreatitis may be the result of hypersecretion and ductal obstruction (Dressel et al., 1980).

A study of 60 poisoning cases with diazinon has been reported. The most common clinical manifestations were vomiting, giddiness, constricted pupils and signs of bronchoconstriction with pulmonary congestion. The amount of poison ingested varied from 4 ml to 15 ml with an average of 7.5 ml (% active ingredient not specified in publication). This was ingested by 55 patients with suicidal intention, whereas in 5 patients the compound was taken accidentally. Atropine injections were administered repeatedly (up to a total dose of 22.4 g over 42 hours). Five patients died in spite of intensive atropine therapy initiated more than 8 hours after ingestion. Other cases who had ingested the same amount of poison as the fatal cases received treatment within three hours of ingestion. These findings emphasize the importance of early treatment in diazinon poisoning (Gupta & Patel, 1968). In addition, these results are in agreement with an earlier observation that the combination of diazinon with cholinesterase occurs in two stages, an early reversible stage and a late irreversible stage (Grob, 1956).

In another study, cases of organophosphate and carbamate poisoning in 37 infants and children were reported, only 5 of which were associated with diazinon. Miosis, excessive salivation, muscle weakness, respiratory distress, lethargy and tachycardia were the most common clinical findings. Erythrocyte cholinesterase activities were determined from 24 patients showing erythrocyte cholinesterase activities that were less than 50% of the lower limit of the normal range. There were no differences between the erythrocyte and serum cholinesterase activity in 20 [of the] 24 patients from whom both tests were obtained, whereas in the remaining 4 cases either the erythrocyte or the serum activity was decreased. A combined atropine/pralidoxime therapy is recommended in case of organophosphate toxicity. Although the recommended dose for atropine in infants is 0.01 to 0.02 mg/kg bw, the dose is generally insufficient for treatment of signs and symptoms secondary to organophosphate poisoning (Zwiener & Ginsburg, 1988).

Neurobehavioural effects of short-term, low-level exposure to diazinon were investigated in 99 pest control workers. A computer assisted neurobehavioural test battery (including attention, vigilance, hand-eye coordination, visual perception, verbal ability) was used before and after their work shift. The diazinon metabolite diethylthiophosphate (DETP) was measured in urine samples collected from 46 diazinon applicators and 56 non-applicators. Post-shift DETP values were 24 and 3 [parts per billion] for applicators and non-applicators, respectively. The study failed to demonstrate any behavioural effects of diazinon under the conditions of this study (Maizlish et al., 1987).

3.3 Epidemiological data

Several epidemiological studies on diazinon exposure were available. The review of these studies focused on the occurrence of three cancer types: non-Hodgkin lymphoma (NHL), leukaemia and lung cancer, the cancer types that the IARC identified as having positive associations with diazinon in their recent monograph (IARC, 2015). One prospective cohort study was available, the Agricultural Health Study (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.

Details of the risk estimates are provided in Table 25.

Table 25. Results of Tier 1 details of risk estimates in epidemiological data

Study/ Location	Reference	Diazinon / NHL	Diazinon / Leukaemia	Diazinon / Lung cancer
Meta-analysis	Schinasi & Leon (2014)	Qualitative exposure only – ever- vs never-use of diazinon. Meta RR: 1.6 (95% CI: 1.2–2.2) Meta-analysis includes McDuffie et al. (2001); Waddell et al. (2001); and Mills, Yang & Riordan (2005a). Does not include AHS; *N* for each meta-analysis not presented		
AHS	Lerro et al. (2015)	Qualitative (ever/never) Risk estimates – aRR (95% CI) Ever-use 0.93 (0.53–1.56) *N* = 18 exposed cases		Qualitative (ever/never) Risk estimates – aRR (95% CI) Ever-use 0.92 (0.52–1.64) *N* = 15 exposed cases
AHS	Jones et al. (2015)			Quantitative exposure – LED and IW-LED (tertiles – cut-points given for both LED and IW-LED) Risk estimates – RR (95% CI) No exposure LED < 20: 1.11 (0.75–1.65) LED 20.0–38.8: 0.76 (0.44–1.3) LED > 38.8: 1.6 (1.11–2.31) *P* for trend: 0.02 No exposure IW-LED <368: 1.09 (0.61–1.53) IW-LED 368–1800: 0.99 (0.66–1.52) IW-LED >1800: 1.41 (0.98–2.04) *P* for trend 0.08 Total *N* = 22 830, with 283 lung cancer cases *N* = 84 exposed cases
AHS	Alavanja et al. (2014)	Quantitative exposure – LED and IW-LED (tertiles – cut-points only given for LED, not IW-LED) Risk estimates – RR (95% CI) Ever- vs never-use 1.0 (0.8–1.3) No exposure LED ≤ 8.75: 1.1 (0.7–1.6) LED 8.75–25: 1.0 (0.6–1.8) LED >25–457.25: 1.2 (0.7–1.9) *P* for trend: 0.52 Median days/year=2.5 Minimum days/year=1 Maximum days/year=7 Median days/year=3.0		

Study/ Location	Reference	Diazinon / NHL	Diazinon / Leukaemia	Diazinon / Lung cancer
		Minimum days/year=1 Maximum days/year=25 Median days/year=7 Minimum days/year=2 Maximum days/year=200 No exposure IW-LED – Low: 1.1 (0.7–1.8) IW-LED – Med: 0.9 (0.5–1.5) IW-LED – High: 1.3 (0.8–2.1) *P* for trend: 0.33 Median days/year=2.5 Minimum days/year=1 Maximum days/year=29.5 Median days/year=2.5 Minimum days/year=1 Maximum days/year=49.5 Median days/year=7 Minimum days/year=2 Maximum days/year=200 Total *N* = 24 211 with 257 incident NHL cases *N* = 70 exposed cases (LED analysis), 69 exposed cases (IW-LED analysis)		
AHS	Beane Freeman et al. (2005)	Exclude – Alavanja et al. (2014) has longest follow-up	Quantitative exposure – LEDs and IW-LEDs (tertiles – cut-points only given for LED, not IW-LED) Risk estimates – RR (95% CI) No exposure LED <20.0: 1.10 (0.32–3.72) LED 20.0–38.8: 2.62 (0.88–7.82) LED >38.8: 3.36 (1.08–10.49) *P* for trend: 0.026 Median days/year = 2.5 Minimum = 2.5 Maximum = 7.0 Median days/year = 7.0 Minimum = 2.5 Maximum = 29.5 Median days/year = 14.5 Minimum = 2.5 Maximum = 200 No exposure IW-LED – Tertile 1: 0.99 (0.23–4.24) IW-LED – Tertile 2: 2.46 (0.94–6.66) IW-LED – Tertile 3: 2.88 (0.92–9.03) *P* for trend: 0.053 Median days/year = 2.5 Minimum = 2.5 Maximum = 14.5 Median days/year = 2.5 Minimum = 2.5	Exclude – Jones et al. (2015) has longest follow-up

Study/ Location	Reference	Diazinon / NHL	Diazinon / Leukaemia	Diazinon / Lung cancer
			Maximum = 29.5 Median days/year = 7.0 Minimum = 2.5 Maximum = 200 Total *N* = 23 106, with 32 incident leukaemia cases. *N* = 11 exposed cases	
AHS	Alavanja et al. (2014)			Exclude – Jones et al. (2015) has longest follow-up
United States Midwest case–control studies	De Roos et al. (2003)	NB. Study population overlaps with Waddell et al. (2001) and total *N* is smaller, but as an exception this study is not excluded as it provides more fully adjusted risk estimates for ever- vs never-use analyses Qualitative (ever/never) Risk estimates – aRR (95% CI) Exposed: 1.9 (1.1–1.3.6), from a logistic regression model 1.7 (1.0–2.8), from the hierarchical regression. Both adjusted for other pesticides Total *N* =2 583 (650 NHL cases, 1 933 controls). *N* = 40 and 62 exposed cases and controls, respectively		
United States Midwest case–control studies	Waddell et al. (2001)	NB. Study population overlaps with De Roos et al. (2003) above. See comment above. Quantitative exposure – days of use per year (2 categories – cut-points are given) Risk estimates – aOR (95% CI) Exposed 1.7 (1.2–2.5) *N* = 60 and 93 exposed cases and controls, respectively Restricted to direct respondent farmers: Exposed: 1.3 (0.8–2.0) Adjusted for fonofos: 1.2 (0.7–2.1) Adjusted for malathion: 1.8 (0.9–3.5) 44/69 exposed cases/controls Risk estimates – aOR (95% CI) Non-farmers: 1.0 <5 days/year: 1.3 (0.5–3.9) 5+ days/year: 2.4 (0.7-8.0)		

Study/ Location	Reference	Diazinon / NHL	Diazinon / Leukaemia	Diazinon / Lung cancer
		N = 12 and 17 exposed cases and controls respectively. (6/11 for <5 days and 6/6 for 5+ days for cases/controls, respectively.)		
United States Midwest case–control studies	Zahm et al. (1993)	Exclude - not specific to diazinon		
United States Midwest case–control studies	Cantor et al. (1992)	Exclude – as this study is pooled in Waddell et al. (2001) and De Roos et al. (2003) Qualitative exposure only – ever-/never-use of diazinon. Risk estimates – OR (95% CI) Ever-use of diazinon = 1.5 (0.9–2.5) Ever-use before 1965 = 2.6 (1.2–5.9) Total *N* =1 867 (622 cases, 1 245 controls) *N* = 27 exposed cases		
United States Midwest case–control studies	Brown et al. (1990)		Quantitative exposure – days of use per year (3 categories – cut-points are given). Risk estimates – OR (95% CI) Ever-use: 1.2 (0.6–2.1) By days per year of diazinon handling: Never farmers (unexposed) 1–4 days: 2.1 (0.8–5.6) 5–9 days: 0.5 (0.1–2.4) ≥ 10 days – no cases Total *N* =1 867 (578 leukaemia cases, 1245 controls). *N* = 17 exposed cases in ever-/never-use analysis *N* = 10 exposed cases in days/year analysis	
Cross-Canada Study of Pesticides and Health	McDuffie et al. (2001)	Qualitative exposure only – ever-/never-use of diazinon Risk estimates – OR (95% CI) Ever-use: 1.69 (0.88–3.24) Total *N* = 2 023		

Study/ Location	Reference	Diazinon / NHL	Diazinon / Leukaemia	Diazinon / Lung cancer
		517 cases, 1506 controls (overall). 179 cases, 456 controls (with telephone interview data, i.e. detailed pesticide information) *N* = 18 exposed cases		
Florida Pest Control Worker Study	Pesatori et al. (1994)			Qualitative (ever/never) Risk estimates – aOR (95% CI) Ever-use: 2.0 (0.7–5.5) *deceased controls* Ever-use: 1.3 (0.6–3.1) *living controls* *N* = 17 exposed cases
United Farm Workers of America	Mills, Yang & Riordan (2005)	Qualitative (high vs low – no cut-points) Risk estimates – aOR (95% CI) High vs low: 1.39 (0.76–2.53) *Also reported sex-stratified results* Total 131 cases, 651 controls. *N* exposed cases/controls not reported	Qualitative (high vs low - no cut-points) Risk estimates – aOR (95% CI) High vs low 1.32 (0.65–2.65) *Also reported sex-stratified results* Total 131 cases, 651 controls. *N* exposed cases/controls not reported	
No. of publications after exclusions (not counting meta-analysis)		6	3	3
No. of publications eligible for quantitative risk assessment		2 – with different units	2 – with different units	1
Exclusions		3	0	2

AHS: Agricultural Health Study; aOR: adjusted odds ratio; aRR: adjusted risk ratio; CI: confidence interval; IW-LED, intensity-weighted lifetime-exposure days; LED: lifetime-exposure days; NHL: non-Hodgkin lymphoma; no.: number; OR: odds ratio; RR: risk ratio

Maximally adjusted risk estimates were extracted by the reviewers.

Diazinon / NHL

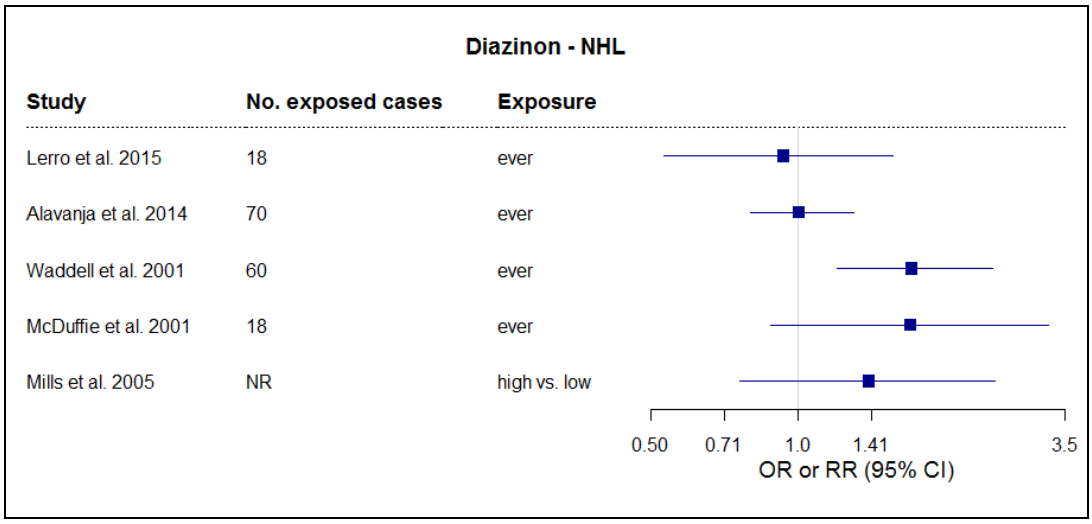
There was no significant evidence of a positive association between NHL and diazinon exposure and no evidence of an exposure–response relationship in the AHS (Alavanja et al., 2014; Lerro et al., 2015).

In a large pooled case–control study, the unadjusted estimates showed a significant elevated risk of NHL (relative risk [RR] = 1.7; 95% confidence interval [CI] = 1.2–2.5) associated with ever- versus never-use of diazinon (Waddell et al., 2001). However, these risks were attenuated and/or no longer significant when proxy respondents were excluded and analyses were mutually adjusted for other pesticides (e.g. malathion, fonofos). Although increasing risk across exposure–duration categories was observed, which was suggestive of a duration–response pattern, confidence intervals were nonsignificant, wide and overlapping between categories.

Two other studies reported elevated risks of NHL for ever- versus never-use of diazinon (McDuffie et al., 2001) or high versus low diazinon use (Mills, Yang & Riordan, 2005), but

confidence intervals were wide, reflecting uncertainty in the risk estimates, and chance could not be excluded as an explanation for the findings. Overall, there was no convincing evidence of a positive association between NHL and exposure to diazinon, as shown in the exposure–response plot below.

Fig. 3. Forest plot showing results of studies of risk of NHL on ever exposure to or high versus low exposure to diazinon

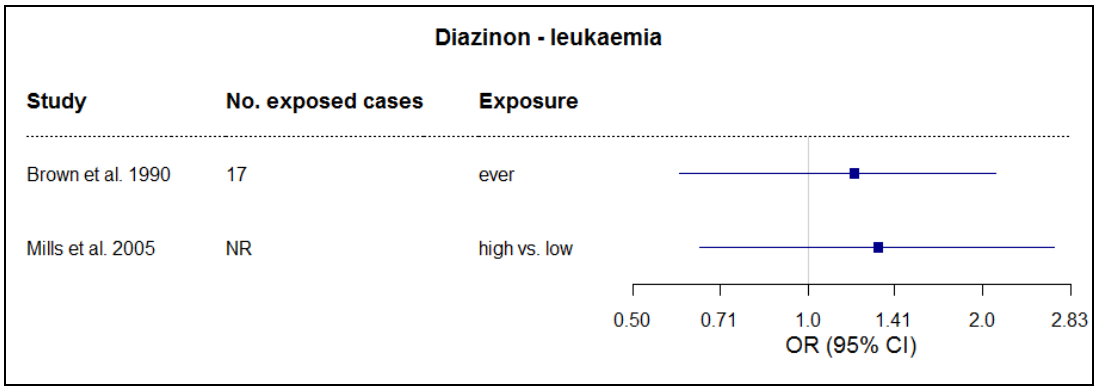


CI: confidence interval; NHL: non-Hodgkin lymphoma; OR: odds ratio; RR: relative risk

Diazinon / Leukaemia

A significantly increased risk of leukaemia in the highest exposure category (> 38.8 lifetime days of diazinon exposure; RR = 3.36; 95% CI = 1.08–10.49) and a significant exposure–response relationship were observed in the AHS. Findings for intensity-weighted lifetime-exposure days demonstrated a similar pattern, but did not reach significance (Beane Freeman et al., 2005). Two other studies reported nonsignificantly elevated risks of leukaemia for high versus low diazinon use (Mills, Yang & Riordan, 2005) and ever- versus never-use of diazinon (Brown et al., 1990), with a nonsignificant dose–response relationship observed using days of use per year (Brown et al., 1990). Overall, there is weak evidence of a positive association between leukaemia and exposure to diazinon from the AHS alone. Note that the number of diazinon-exposed cases was low or not reported in all three available studies.

Fig. 4. Forest plot showing results of studies of risk of leukaemia on ever exposure to diazinon or high versus low exposure to diazinon

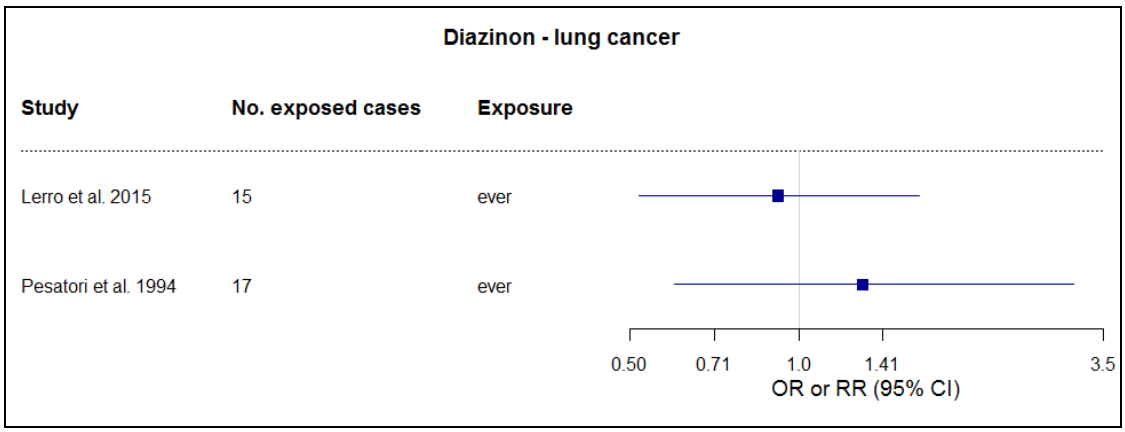


CI: confidence interval; OR: odds ratio

Diazinon / Lung cancer

A significant 60% excess risk of lung cancer in the highest exposure category (> 38.8 lifetime days of diazinon exposure) and a significant trend across exposure categories were observed in the AHS. Findings for intensity-weighted lifetime-exposure days demonstrated a similar pattern, but did not reach significance (Jones et al., 2015). A separate analysis of ever-use versus never-use of diazinon found no evidence of elevated risk of lung cancer among spouses of farmers or pesticide applicators; however, there were only 15 exposed cases (Lerro et al., 2015). One other study reported a nonsignificant elevated risk of lung cancer for ever- versus never-use of diazinon (based on 17 exposed cases) (Pesatori et al., 1994). Overall, there is weak evidence of a positive association between lung cancer and exposure to diazinon from the AHS cohort study only.

Fig. 5. Forest plot showing results of studies of risk of lung cancer on ever exposure to diazinon



CI: confidence interval; OR: odds ratio; RR: relative risk

Comments

Biochemical aspects

Following oral administration to rats, diazinon was almost completely absorbed and rapidly eliminated, mainly in the urine. There was no evidence of accumulation (Robbins, Eddy & Hopkins, 1957; Mücke, Alt & Esser, 1970; Burgener & Seim, 1988; Simoneaux, 1988b; Simoneaux et al., 1989; Capps et al., 1989; Craine, 1989).

Diazinon is metabolized by P450 to diazoxon, the active metabolite. The main degradative pathway includes the oxidase/hydrolase-mediated cleavage of the ester bond, leading to the pyrimidinol derivative 2-isopropyl-6-methyl-4(1*H*)-pyrimidinone, which is further oxidized to more polar metabolites (Mücke, Alt & Esser, 1970; Iverson, Grant & Lacroix, 1975; Capps et al., 1989).

Toxicological data

The oral LD_{50} for diazinon in rats ranged from 300 to greater than 2150 mg/kg bw, whereas the dermal LD_{50} was greater than 2000 mg/kg bw (Bathe, 1972b, 1980; Piccirillo, 1978; Nissimov, 1984a; Schoch & Gfeller, 1985; Kuhn, 1989a,b; Dreher, 1997). The inhalation median lethal concentration (LC_{50}) was 3.1 mg/L in rats (Cummins, 1985; Jackson, 1987; Holbert, 1989, 1994). Diazinon produced mild skin and eye irritation in rabbits (Kuhn, 1989c,d). It caused skin sensitization in the guinea-pig Magnusson–Kligman maximization test (Cummins, 1987a).

The most sensitive end-point observed in all species given single and repeated doses of diazinon was inhibition of cholinesterase activity. Brain acetylcholinesterase activity was generally decreased at doses higher than those that inhibited erythrocyte acetylcholinesterase activity. Clinical

signs of cholinergic toxicity occurred at doses causing more than 50% inhibition of brain acetylcholinesterase activity. Female rats were more sensitive than male rats.

Many repeated-dose toxicity studies are available. In both rats and dogs, no effects other than those related to cholinesterase inhibition have been observed at the LOAEL; in general, effects observed at the highest doses can be considered secondary to the cholinergic toxicity. In these studies, NOAELs ranged from 0.02 to 0.5 mg/kg bw per day, and LOAELs from 1 to 15 mg/kg bw per day, based on erythrocyte acetylcholinesterase inhibition (i.e. > 20%), with brain acetylcholinesterase inhibition (i.e. > 10%) generally appearing at the next higher dose and clinical cholinergic signs appearing at doses above 23 mg/kg bw per day.

In a 28-day acetylcholinesterase inhibition study, rats received diazinon by dietary administration at a concentration of 0, 0.3, 30, 300 or 3000 parts per million (ppm) (equal to 0, 0.02, 2.3, 23 and 213 mg/kg bw per day for males and 0, 0.02, 2.4, 23 and 210 mg/kg bw per day for females, respectively). The NOAEL was 0.3 ppm (equal to 0.02 mg/kg bw per day), on the basis of inhibition of erythrocyte acetylcholinesterase activity at 30 ppm (equal to 2.3 mg/kg bw per day) (Chang, 1994).

In a short-term toxicity study, rats were fed diazinon at a concentration of 0 or 2 ppm (equivalent to 0 and 0.2 mg/kg bw per day, respectively) for 7 days or at a concentration of 0 or 25 ppm (equivalent to 0 and 2.5 mg/kg bw per day, respectively) for 30 days. The NOAEL was 2 ppm (equivalent to 0.2 mg/kg bw per day), based on inhibition of erythrocyte acetylcholinesterase activity at 25 ppm (equivalent to 2.5 mg/kg bw per day) (Davies & Holub, 1980a).

In a 3-month toxicity study, rats were given diets containing diazinon at a concentration of 0, 0.5, 5, 250 or 2500 ppm (equal to 0, 0.03, 0.3, 15 and 168 mg/kg bw per day for males and 0, 0.04, 0.4, 19 and 212 mg/kg bw per day for females, respectively). The NOAEL was 5 ppm (equal to 0.3 mg/kg bw per day), on the basis of inhibition of erythrocyte and brain acetylcholinesterase activities at 250 ppm (equal to 15 mg/kg bw per day) (Singh, Arthur & McCormick, 1988).

In a second 3-month toxicity study, rats were fed diets containing diazinon at a concentration of 0, 0.3, 30, 300 or 3000 ppm (equal to 0, 0.017, 1.7, 17 and 177 mg/kg bw per day for males and 0, 0.019, 1.9, 19 and 196 mg/kg bw per day for females, respectively). The NOAEL was 0.3 ppm (equal to 0.017 mg/kg bw per day), on the basis of inhibition of erythrocyte acetylcholinesterase activity at 30 ppm (equal to 1.7 mg/kg bw per day) (Pettersen & Morrissey, 1994).

In a third 3-month toxicity study, female rats were fed diets containing diazinon at a concentration of 0, 5, 10 or 15 ppm (equivalent to 0, 0.5, 1 and 1.5 mg/kg bw per day, respectively) for 92 days. In the second phase, female rats were fed diets containing diazinon at a concentration of 0, 1, 2, 3 or 4 ppm (equivalent to 0, 0.1, 0.2, 0.3 and 0.4 mg/kg bw per day, respectively) for 42 days. In the third phase, female rats were fed diets containing diazinon at a concentration of 0, 0.1, 0.5, 1 or 2 ppm (equivalent to 0, 0.01, 0.05, 0.1 and 0.2 mg/kg bw per day, respectively) for 35 days. The NOAEL in the first phase was 5 ppm (equivalent to 0.5 mg/kg bw per day), based on inhibition of erythrocyte acetylcholinesterase activity at 10 ppm (equivalent to 1 mg/kg bw per day) after dosing for 92 days. The NOAEL for females in the second and third phases were the highest tested doses of 4 ppm (equivalent to 0.4 mg/kg bw per day) and 2 ppm (equivalent to 0.2 mg/kg bw per day) after dosing for 42 and 35 days, respectively (Davies & Holub, 1980b).

In a fourth 3-month toxicity study, rats were fed diets containing diazinon at a concentration of 0, 5, 125 or 3000 ppm (equal to 0, 0.3, 7.8 and 198 mg/kg bw per day for males and 0, 0.3, 8.9 and 247 mg/kg bw per day for females, respectively). The NOAEL was 5 ppm (equal to 0.3 mg/kg bw per day), on the basis of inhibition of erythrocyte acetylcholinesterase activity at 125 ppm (equal to 7.8 mg/kg bw per day) (Sunaga, 2010).

In a 90-day repeated-dose neurotoxicity study, rats were dosed in the diet at 0, 25, 125 or 1000 ppm (equal to 0, 1.7, 8.4 and 69.1 mg/kg bw per day for males and 0, 1.8, 9.3 and 82.4 mg/kg bw per day for females, respectively). A NOAEL could not be identified, as erythrocyte acetylcholinesterase activity was inhibited at 1.7 mg/kg bw per day, the lowest dose tested (Sunaga, 2007b).

In considering the NOAELs and LOAELs identified in the 28-day and 3-month (neuro)toxicity studies in rats measuring the inhibition of acetylcholinesterase activity, the Meeting concluded that the extent of acetylcholinesterase inhibition was not dependent on duration of dosing once steady state had been achieved (within 4 weeks). The overall NOAEL for the 28-day and 3-month (neuro)toxicity studies in rats was 5 ppm, based on inhibition of erythrocyte acetylcholinesterase activity at the overall LOAEL of 10 ppm. In studies where feed consumption data were used to calculate test substance intake, 5 ppm was equal to 0.3 mg/kg bw per day. These substance intake data are considered to be more accurate than those calculated using a default conversion factor, in which the NOAEL of 5 ppm is equivalent to 0.5 mg/kg bw per day.

In a 90-day toxicity study, dogs were given diets containing diazinon at a concentration of 0, 0.1, 0.5, 150 or 300 ppm (equal to 0, 0.0034, 0.020, 5.9 and 10.9 mg/kg bw per day for males and 0, 0.0037, 0.021, 5.6 and 11.6 mg/kg bw per day for females, respectively). The NOAEL was 0.5 ppm (equal to 0.020 mg/kg bw per day), on the basis of inhibition of erythrocyte and brain cholinesterase activities at a dietary concentration of 150 ppm (equal to 5.6 mg/kg bw per day) (Barnes, Arthur & Hazelette, 1988).

In a second 90-day toxicity study, dogs were given diazinon at 0, 0.3, 3 or 10 mg/kg bw per day by gelatine capsule. The NOAEL was 0.3 mg/kg bw per day, on the basis of inhibition of erythrocyte and brain acetylcholinesterase activities at 3 mg/kg bw per day (Ichido, 2010).

In a 1-year toxicity study in dogs given diazinon in the diet at a concentration of 0, 0.1, 0.5, 150 or 300 ppm (equal to 0, 0.0032, 0.015, 4.7 and 7.7 mg/kg bw per day for males and 0, 0.0037, 0.020, 4.5 and 9.1 mg/kg bw per day for females, respectively), the NOAEL was 0.5 ppm (equal to 0.015 mg/kg bw per day), on the basis of inhibition of erythrocyte (males and females) and brain (females only) acetylcholinesterase activities at 150 ppm (equal to 4.5 mg/kg bw per day) (Rudzki, Arthur & McCormick, 1991).

The overall NOAEL for the 90-day and 1-year toxicity studies in dogs was 0.3 mg/kg bw per day, based on inhibition of erythrocyte and brain acetylcholinesterase activities at 3 mg/kg bw per day.

In a pre-GLP carcinogenicity study in mice that was considered adequate to evaluate carcinogenicity but not chronic toxicity, diazinon was administered at a dietary concentration of 0, 100 or 200 ppm (equivalent to 0, 15 and 30 mg/kg bw per day, respectively) over 103 weeks. No treatment-related tumours were observed (NTP, 1979; also referenced as Angel et al., unknown year, or NCI, 1979).

In another pre-GLP carcinogenicity study in mice, diazinon was administered at a dietary concentration of 0, 100, 200, 300 (males) or 400 (females) ppm (equal to 0, 16, 31 and 46 mg/kg bw per day for males and 0, 22, 43 and 86 mg/kg bw per day for females, respectively) for 104 weeks. Cholinesterase activity was not measured in this study. The NOAEL for chronic toxicity was 200 ppm (equal to 31 mg/kg bw per day), based on depression of body weight and lower feed consumption at 300 ppm (equal to 46 mg/kg bw per day). No treatment-related tumours were observed (Goldsmith, 1983).

In a pre-GLP carcinogenicity study in rats that was considered adequate to evaluate carcinogenicity but not chronic toxicity, diazinon was administered at a dietary concentration of 0, 400 or 800 ppm (equivalent to 0, 20 and 40 mg/kg bw per day, respectively) over 103 weeks. No treatment-related tumours were observed (NTP, 1979; also referenced as Angel et al., unknown year, or NCI, 1979).

In a chronic toxicity study, rats received diazinon in the diet at a concentration of 0 (untreated and vehicle controls), 0.1, 1.5, 125 or 250 ppm (equal to 0, 0.004, 0.06, 5 and 10 mg/kg bw per day for males and 0, 0.005, 0.07, 6 and 12 mg/kg bw per day for females, respectively) for 98/99 weeks. The NOAEL was 1.5 ppm (equal to 0.06 mg/kg bw per day) on the basis of inhibition of erythrocyte and brain acetylcholinesterase activities at 125 ppm (equal to 5 mg/kg bw per day). From the available data, there was no evidence of a tumorigenic response; however, the group size (N = 20) was too small to allow a conclusion to be reached on carcinogenicity (Kirchner, McCormick & Arthur, 1991).

In a combined chronic toxicity and carcinogenicity study in rats, diazinon was fed in the diet at concentrations adjusted to achieve target concentrations of 0, 0.025, 0.1, 1.5 or 22.5 mg/kg bw per day for 104 weeks. The NOAEL for long-term toxicity was 0.1 mg/kg bw per day, based on inhibition of erythrocyte acetylcholinesterase activity at 1.5 mg/kg bw per day. No treatment-related tumours were observed (Ashby & Danks, 1987).

The overall NOAEL for chronic toxicity in rats was 0.1 mg/kg bw per day, based on inhibition of erythrocyte acetylcholinesterase activity at 1.5 mg/kg bw per day.

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

Given the similarity of the sensitivities of mammalian species, an overall NOAEL in all studies of repeated-dose (neuro)toxicity in rats and dogs could be identified. The overall NOAEL was 0.3 mg/kg bw per day, on the basis of inhibition of acetylcholinesterase activity in erythrocytes at 1 mg/kg bw per day.

In studies submitted by the sponsors, diazinon was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. In addition, many studies with diazinon were described in the published literature, but most of these were considered by the Meeting as inappropriate to evaluate the genotoxicity of diazinon, as they had major deficiencies in study design or reliability (e.g. lack of statistical analysis, testing of mixtures of diazinon with other chemicals and similarity between negative and positive control values). Overall, these studies provided no convincing evidence of genotoxic effects.

The Meeting concluded that diazinon is unlikely to be genotoxic.

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

In a two-generation study on reproductive toxicity, rats received diazinon in the diet at a concentration of 0, 10, 100 or 500 ppm over the course of two generations (F_0 and F_1). Mean diazinon intakes for the F_0 generation during the premating period were 0, 0.77, 7.48 and 32.85 mg/kg bw per day for males and 0, 0.77, 7.48 and 40.26 mg/kg bw per day for females, respectively. The NOAEL for reproductive effects was 100 ppm (equal to 7.48 mg/kg bw per day), based on prolonged gestation duration, decrease in the number of pregnancies, and reduced fertility and mating indices at 500 ppm (equal to 32.85 mg/kg bw per day). The NOAEL for parental effects was 10 ppm (equal to 0.77 mg/kg bw per day), based on reduced parental body-weight gain at 100 ppm (equal to 7.48 mg/kg bw per day). The NOAEL for offspring toxicity was 10 ppm (equal to 0.77 mg/kg bw per day), based on reduced viability of pups and pup weights at 100 ppm (equal to 7.48 mg/kg bw per day) (Giknis, 1989).

In another two-generation study on reproductive toxicity, rats received diazinon in the diet at a concentration of 0, 0.1, 1.0 or 10 mg/kg (equivalent to 0, 0.0067, 0.067 and 0.67 mg/kg bw per day, assuming concentrations are in mg/kg feed or ppm) over the course of two generations (F_0 and F_1). A rationale for the dose selection was not provided. There were no treatment-related effects observed in F_0 or F_1 parental animals or pups. The NOAEL for reproductive, parental and offspring toxicity was 10 ppm (equivalent to 0.67 mg/kg bw per day), the highest dose tested (Weatherholtz, 1982).

In a range of studies on estrogenic and androgenic activities, no estrogenic, androgenic or anti-androgenic activity was observed at concentrations relevant to human exposure via the diet (Davis, 2011; Davis & Lea, 2011; Wilga, 2011; Willoughby, 2011a,b, 2012; Wagner, 2012).

Overall NOAELs from the multigeneration studies in rats were identified. The overall NOAEL for reproductive effects was 100 ppm (equal to 7.48 mg/kg bw per day), based on effects at 500 ppm (equal to 32.85 mg/kg bw per day). The overall NOAEL for parental toxicity was 10 ppm (equal to 0.77 mg/kg bw per day), based on effects at 100 ppm (equal to 7.48 mg/kg bw per day). The overall NOAEL for offspring toxicity was 10 ppm (equal to 0.77 mg/kg bw per day), based on effects at 100 ppm (equal to 7.48 mg/kg bw per day).

In a study of developmental toxicity evaluated by the 1993 JMPR, rats were administered diazinon via gavage at a dose of 0, 15, 50 or 100 mg/kg bw per day. A marked decrease in maternal feed consumption correlating with weight loss at the beginning of the treatment period and a slightly higher incidence of incomplete ossification at different sites in the fetuses were observed at 100 mg/kg bw per day. As limited information was available from the previous JMPR monograph, the Meeting was unable to identify a NOAEL for this study (Fritz, 1974).

In a study of developmental toxicity, rats were administered diazinon via gavage at a dose of 0, 10, 20 or 100 mg/kg bw per day. The NOAEL for maternal toxicity was 20 mg/kg bw per day, based on body weight loss on gestation days 6 to 10, reduced body weight/body-weight gains throughout treatment and decreased feed consumption on gestation days 6 to 9 at 100 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 20 mg/kg bw per day, based on an increased incidence of rudimentary 14th ribs at 100 mg/kg bw per day (Infurna & Arthur, 1985).

In a study of developmental toxicity, rabbits were dosed with diazinon via gavage at 0, 7, 25 or 100 mg/kg bw per day. The NOAEL for maternal toxicity was 25 mg/kg bw per day, based on mortality, tremors, convulsions, hypoactivity, anorexia and reduced body-weight gain observed at 100 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 100 mg/kg bw per day, the highest dose tested (Harris & Holson, 1981).

In another developmental toxicity study, diazinon was administered to pregnant rabbits by gavage at a dose level of 0, 2.5, 10 or 40 mg/kg bw per day. The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on clinical signs, decreased body weight and reduced feed consumption. The NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day, based on decreased fetal weight at 40 mg/kg bw per day (Edwards & Falconer, 1987).

The overall NOAEL for maternal toxicity in developmental toxicity studies in rabbits was 25 mg/kg bw per day, based on effects at 40 mg/kg bw per day, and the overall NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day, based on effects at 40 mg/kg bw per day.

The Meeting concluded that diazinon is not teratogenic.

In a limited acute neurotoxicity study in which acetylcholinesterase activity was not measured, rats were dosed with diazinon at 0, 100, 300 or 500 mg/kg bw by gavage. The NOAEL was 100 mg/kg bw, based on systemic toxicity and clinical signs of neurotoxicity observed at 300 or 500 mg/kg bw (Sunaga, 2007a). In another acute toxicity study, rats were administered a single dose of diazinon by gavage at 0, 2.5, 150, 300 or 600 mg/kg bw. The NOAEL was 2.5 mg/kg bw, on the basis of depressed erythrocyte acetylcholinesterase activity and behavioural changes at 150 mg/kg bw (Chow & Richter, 1994). In a third study, rats were administered a single dose of diazinon by gavage at 100, 250 or 500 mg/kg bw for males or 0, 0.05, 0.12, 0.25, 2.5, 25 or 250 mg/kg bw for females. The NOAEL was 2.5 mg/kg bw, on the basis of inhibition of brain and erythrocyte acetylcholinesterase activities in females at 25 mg/kg bw (Glaza, 1993).

In a study that investigated the time course of acute inhibition of acetylcholinesterase activity, rats were given a single dose of diazinon by gavage at 0, 2.5, 150, 300 or 600 mg/kg bw, and brain and blood samples were collected at 3, 9 and 24 hours after dosing. The NOAEL was 2.5 mg/kg bw, based on inhibition of brain and erythrocyte acetylcholinesterase activities at 150 mg/kg bw. Inhibition was observed beginning at 3 hours post-dosing, with maximal inhibition at 9 hours post-dosing (Potrepka, 1994).

The overall NOAEL in all studies of acute toxicity was 2.5 mg/kg bw, on the basis of inhibition of acetylcholinesterase activity in erythrocytes and in the brain at 25 mg/kg bw in rats of both sexes.

Three studies were performed on delayed neurotoxicity in the hen. Oral doses of diazinon technical ranging from 10 to 100 mg/kg bw were administered to hens. Inhibition of cholinesterase activity was observed from 20 mg/kg bw, but there was no evidence that diazinon caused acute delayed neurotoxicity in the hen (Cummins, 1987b; Jenkins, 1988; Classen, 1996).

No specific studies on immunotoxicity were submitted. A study in the open literature with intraperitoneal injection of diazinon in mice (Neishabouri et al., 2004) was not informative. The submitted repeated-dose toxicity studies do not indicate an immunotoxic potential for diazinon after oral exposure.

Toxicological data on metabolites and/or degradates

No toxicological data were available on any metabolites of diazinon other than diazoxon, which is the active metabolite of diazinon. However, the Meeting concluded that none of the other metabolites would be of toxicological concern at the levels present in the diet.

Human data

In a study of acute toxicity in male volunteers given ascending doses of diazinon (seven volunteers per group given 0.03, 0.12, 0.20 or 0.21 mg/kg bw; one volunteer given 0.30 mg/kg bw), acetylcholinesterase activity was not inhibited in erythrocytes at 0.21 mg/kg bw, the second highest dose tested. The highest dose (0.30 mg/kg bw) was not informative, as it was tested in a single volunteer only. Plasma cholinesterase activity was inhibited by more than 20% at doses above 0.12 mg/kg bw (Boyeson, 2000; final version reported as Anderson, 2000).

Repeated-dose studies in four male volunteers given diazinon for 28–37 days showed that, although there was some inhibition of plasma cholinesterase activity at the highest tested dose of 0.03 mg/kg bw per day (actual administered doses varied slightly, i.e. 0.03, 0.027, 0.022/0.027 and 0.026 mg/kg bw per day), no inhibition of erythrocyte acetylcholinesterase activity was observed (Payot, 1966; Beilstein, 1998).

Diazinon was evaluated in four male volunteers who received diazinon in capsules at 0.025 mg/kg bw per day for 37–43 days. There were no consistent treatment-related effects on erythrocyte acetylcholinesterase activity, blood chemistry or urine analysis. No clinical effects were reported. The NOAEL was 0.025 mg/kg bw per day, the only dose tested (Lazanas, Fancher & Calandra, 1966).

The overall NOAEL from repeated-dose studies in humans was 0.03 mg/kg bw per day.

Several epidemiological studies on cancer outcomes following occupational exposure to diazinon were available. The review of these studies focused on the occurrence of three cancer types: NHL, leukaemia and lung cancer (see section 2.2 of the meeting report). One prospective cohort study was available, 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.

There was no significant evidence of a positive association of NHL with diazinon exposure and no evidence of an exposure–response relationship in the AHS (Alavanja et al., 2014; Lerro et al., 2015). In a large pooled case–control study, the unadjusted estimates showed a significant elevated risk of NHL (RR = 1.7; 95% CI = 1.2–2.5) associated with ever- versus never-use of diazinon (Waddell et al., 2001). However, these risks were attenuated and/or no longer significant when proxy respondents were excluded and analyses were mutually adjusted for other pesticides (malathion, fonofos). Although increasing risk across exposure duration categories was observed, which was suggestive of a duration–response pattern, confidence intervals were nonsignificant, wide and overlapping between categories. Two other studies reported elevated risks of NHL for ever- versus never-use of diazinon (McDuffie et al., 2001) or high versus low diazinon use (Mills, Yang & Riordan, 2005), but confidence intervals were wide, reflecting uncertainty in the risk estimates, and chance could not be excluded as an explanation for the findings. Overall, there was no convincing evidence of a positive association between NHL and exposure to diazinon.

A significantly increased risk of leukaemia in the highest exposure category (> 38.8 lifetime days of diazinon exposure; RR = 3.36; 95% CI = 1.08–10.49) and a significant exposure–response relationship were observed in the AHS. Findings for intensity-weighted lifetime-exposure days

demonstrated a similar pattern, but did not reach significance (Beane Freeman et al., 2005). Two other studies reported nonsignificantly elevated risks of leukaemia for high versus low diazinon use (Mills, Yang & Riordan, 2005) and ever- versus never-use of diazinon (Brown et al., 1990), with a nonsignificant dose–response relationship observed using days of use per year (Brown et al., 1990). Overall, there is weak evidence of a positive association between leukaemia and exposure to diazinon from the AHS only. It is noted that the number of diazinon-exposed cases was low or not reported in all three available studies.

A significant 60% excess risk of lung cancer in the highest exposure category (> 38.8 lifetime days of diazinon exposure) and a significant trend across exposure categories were observed in the AHS. Findings for intensity-weighted lifetime-exposure days demonstrated a similar pattern, but did not reach significance (Jones et al., 2015). A separate analysis of ever-use of diazinon versus never-use from the AHS found no evidence of elevated risk of lung cancer among spouses of farmers/pesticide applicators; however, there were only 15 exposed cases (Lerro et al., 2015). One other study reported a nonsignificant elevated risk of lung cancer for ever- versus never-use of diazinon (based on 17 exposed cases) (Pesatori et al., 1994). Overall, there is weak evidence of a positive association between lung cancer and exposure to diazinon from the AHS cohort study only.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats and considering the available epidemiological data from occupational exposure, the Meeting concluded that diazinon is unlikely to pose a carcinogenic risk to humans via exposure from the diet.

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

Toxicological evaluation

The Meeting identified inhibition of acetylcholinesterase activity as the most sensitive end-point after single or repeated doses of diazinon in all species. After considering all previously evaluated data and the new studies, the Meeting established an ADI of 0–0.003 mg/kg bw, based on the overall NOAEL of 0.3 mg/kg bw per day from all repeated-dose toxicity studies, and using a safety factor of 100. This ADI was supported by the NOAEL of 0.03 mg/kg bw per day, the highest dose tested, identified in repeated-dose studies that involved a limited number of male volunteers, with application of a safety factor of 10.

In 2006, the Meeting established an ADI of 0–0.005 mg/kg bw, based on the highest NOAEL of 0.5 mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity at 1 mg/kg bw per day in a 92-day repeated-dose toxicity study in rats and using a safety factor of 100. In this study, the dietary concentrations of diazinon were converted to units of milligrams per kilogram body weight per day using a default conversion factor; the present Meeting considers this less reliable than the conversion using feed consumption data.

The Meeting reaffirmed the ARfD of 0.03 mg/kg bw established by the 2006 JMPR. This ARfD was based on the NOAEL of 2.5 mg/kg bw identified in studies of acute (neuro)toxicity in rats, and using a safety factor of 100. This ARfD was supported by the NOAEL of 0.21 mg/kg bw, the highest dose tested, identified in the study in which a limited number of male volunteers were given a single dose of diazinon, with application of a safety factor of 10.

Levels relevant to risk assessment of diazinon

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of carcinogenicity[a,b]	Toxicity	200 ppm, equal to 31 mg/kg bw per day	300 ppm, equal to 46 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 46 mg/kg bw per day[c]	–
Rat	Acute (neuro)toxicity studies[d,e]	Toxicity	2.5 mg/kg bw	25 mg/kg bw

Species	Study	Effect	NOAEL	LOAEL
	(acetylcholinesterase inhibition)			
	Four-week or 3-month studies of (neuro)toxicity[a,e]	Toxicity	5 ppm, equal to 0.3 mg/kg bw per day[f]	10 ppm, equivalent to 1 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,e]	Toxicity	0.1 mg/kg bw per day[f]	1.5 mg/kg bw per day
		Carcinogenicity	800 ppm, equivalent to 40 mg/kg bw per day[c]	–
	Two-generation studies of reproductive toxicity[a,b,e]	Reproductive toxicity	100 ppm, equal to 7.48 mg/kg bw per day	500 ppm, equal to 32.85 mg/kg bw per day
		Parental toxicity	10 ppm, equal to 0.77 mg/kg bw per day	100 ppm, equal to 7.48 mg/kg bw per day
		Offspring toxicity	10 ppm, equal to 0.77 mg/kg bw per day	100 ppm, equal to 7.48 mg/kg bw per day
	Developmental toxicity study[b,d]	Maternal toxicity	20 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	20 mg/kg bw per day	100 mg/kg bw per day
Rabbit	Developmental toxicity studies[b,d,e]	Maternal toxicity	25 mg/kg bw per day	40 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	40 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[a,e]	Toxicity	0.3 mg/kg bw per day[f]	3 mg/kg bw per day
Rat, dog	Repeat-dose (neuro)toxicity studies[e]	Toxicity	5 ppm, equal to 0.3 mg/kg bw per day	10 ppm, equivalent to 1 mg/kg bw per day
Human	Acute toxicity study[d]	Toxicity	0.21 mg/kg bw[c]	–
	Four/five-week studies of toxicity[d,e]	Toxicity	0.03 mg/kg bw per day[c]	–

[a] Dietary administration.

[b] Acetylcholinesterase activity not measured.

[c] Highest dose tested.

[d] Gavage administration.

[e] Two or more studies combined.

[f] Included in the overall NOAEL for rats and dogs.

Estimate of acceptable daily intake (ADI)

0–0.003 mg/kg bw

Estimate of acute reference dose (ARfD)

0.03 mg/kg bw

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Nearly complete and rapid (~90% at 10 mg/kg bw within 24 h)
Dermal absorption	No data
Distribution	Widely distributed at low concentrations
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Predominantly in urine (86–93% at 10 mg/kg bw within 24 h)
Metabolism in animals	Rapidly degraded to diazoxon and subsequently mainly via oxidase/hydrolase-mediated cleavage of the ester bond, and further oxidation at the isopropyl substituent to yield hydroxy pyrimidinols
Toxicologically significant compounds in animals and plants	Parent compound and diazoxon
Acute toxicity	
Rat, LD_{50}, oral	300 to > 2 150 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	3.1 mg/L
Rabbit, dermal irritation	Mildly irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Sensitizing (Magnusson and Kligman maximization test)
Repeat-dose studies of (neuro)toxicity	
Target/critical effect	Acetylcholinesterase inhibition
Overall oral NOAEL	0.3 mg/kg bw per day (rat, dog)
Lowest relevant dermal NOAEL	3 mg/kg bw per day (21 days; rat)
Lowest relevant inhalation NOAEC	0.46 mg/m^3 (21 days; rat)
Long-term studies of carcinogenicity	
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	No evidence of genotoxicity by the oral route[a]
Reproductive toxicity	
Target/critical effect	Mortality, reduced parental body-weight gain, reduced viability of pups and pup weights, prolonged gestation duration, decrease in number of pregnancies, and reduced fertility and mating indices
Lowest relevant parental NOAEL	0.77 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	0.77 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	7.48 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Clinical signs, reduced maternal body weight and feed consumption, and reduced fetal weight
Lowest relevant maternal NOAEL	25 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	10 mg/kg bw per day (rabbit)
Neurotoxicity[b]	
Acute neurotoxicity NOAEL	2.5 mg/kg bw (acetylcholinesterase inhibition; rat)
Developmental neurotoxicity NOAEL	No data
Acute delayed neurotoxicity	No evidence (hens)
Human data	Acetylcholinesterase inhibition:
	Acute toxicity NOAEL: 0.21 mg/kg bw, highest dose tested
	Subchronic toxicity NOAEL: 0.03 mg/kg bw per day, highest dose tested (4/5 weeks)

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

[b] Ninety-day neurotoxicity study in rats is covered by the overall NOAEL for repeated-dose studies of (neuro)toxicity.

Summary

	Value	Study	Safety factor
ADI	0–0.003 mg/kg bw	Repeated-dose toxicity studies (rat, dog)	100
ARfD	0.03 mg/kg bw	Acute (neuro)toxicity studies (rat)	100

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GLYPHOSATE

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Explanation

Glyphosate is the International Organization for Standardization–approved common name for *N*-(phosphonomethyl)glycine (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service (CAS) number 1071-83-6. It is a broad-spectrum systemic herbicide.

Glyphosate was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for toxicology in 1986, 1997 (evaluation of the metabolite aminomethylphosphonic acid, or AMPA), 2004 and 2011 (evaluation of new plant metabolites in genetically modified maize and soya beans).

Glyphosate was last re-evaluated for toxicology within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR) in 2004. The compound was reviewed by the present Meeting following the recommendation of an electronic task force of the World Health Organization (WHO) Core Assessment Group on Pesticides 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 considered toxicological data in addition to new published or unpublished toxicological studies and published epidemiological studies on cancer outcomes. The evaluation of the biochemical aspects and systemic toxicity of glyphosate was based on previous JMPR evaluations, updated as necessary with additional information. The particular focus of the current Meeting was on genotoxicity, carcinogenicity, reproductive and developmental toxicity and epidemiological studies on cancer outcomes. The scope was restricted to the active ingredient.

All critical unpublished studies contained statements of compliance with good laboratory practice (GLP), unless otherwise specified. The studies on human volunteers were conducted in accordance with the principles expressed in the Declaration of Helsinki or equivalent ethical standards.

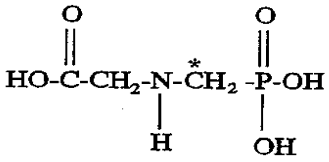
Evaluation for acceptable intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of glyphosate was studied in rats following a single oral low dose, a single oral high dose and a single oral low daily dose repeated for 14 days followed by a radioactive dose. In addition, absorption and excretion of glyphosate was studied via intravenous and intraperitoneal administration in rats and intramuscular administration in Rhesus monkeys.

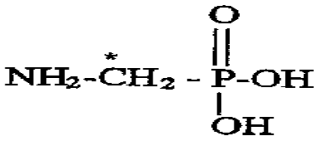
Fig. 1 shows the structure of radiolabelled glyphosate

Fig. 1. Structure of glyphosate – ^{14}C-labelled at the methylene carbon at C1 or C2-glycine carbon



*** Denotes position of ^{14}C label.**

Fig. 2. Structure of aminomethylphosphonic acid (AMPA)



*** Denotes position of ^{14}C label.**

1.1 Absorption, distribution and excretion

(a) Oral route

The excretion and residue levels found by various studies following a single oral dose or repeated oral administration of glyphosate in rats and rabbits are shown in the Table 1.

Table 1. Total elimination and residues of administered radioactivity after single or repeated oral administration of ^{14}C-labelled glyphosate

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
6.7 mg/kg bw Single dose 120 hours	Rat	14–16	35–43	81–85	49–55	0.14–0.65	0.83–1.02	Colvin & Miller[a] (1973a)

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
10 mg/kg bw Single dose 24/48 hours	Rat	17.9/34.0	12.8/12.5	59.3/60.5	80.3/91.2	ND	ND	Davies, (1996a)
10 mg/kg bw Single dose 72 hours	Rat	13	10.6	88.5	88.7	0.59	0.49	Davies, (1996d)
10 mg/kg bw Single dose 7 days	Rat	28.6	22.5	62.4	69.4	0.44	0.31	Ridley & Mirly, (1988)
10 mg/kg bw Repeated dosing 72 hours	Rat	10.6	10.7	86.6	90.7	0.46	0.41	Davies[b] (1996c)
10 mg/kg bw Repeated dosing 7 days	Rat	30.9	23.1	61.0	70.9	0.54	0.35	Ridley & Mirly[b] (1988)
30 mg/kg bw Single dose 168 hours	Rat	29.04	30.71	58.84	56.53	0.62	0.64	Powles, (1992a)
30 mg/kg bw Repeated dosing 168 hours	Rat	34.28	34.63	49.64	46.73	0.96	0.83	Powles, (1992b)
1000 mg/kg bw Single dose 72 hours	Rat	16.7	17.5	89.6	84.5	0.52	0.58	Davies (1996b)
1 000 mg/kg bw Single dose 168 hours	Rat	30.55	22.41	53.27	60.37	0.47	0.40	Powles (1992b)
1 000 mg/kg bw Single dose 7 days	Rat	17.8	14.3	68.9	69.4	0.28	0.24	Ridley & Mirly (1988)
10 mg/kg bw Single dose 168 hours	Rat	22.5	19.4	74.6	84.3	0.33	0.27	McEwen[c] (1995)
10 mg/kg bw Single dose 168 hours	Rat	30.3	29.5	74.7	74.2	0.31	0.39	McEwen[c] (1995)
1 mg/kg bw Single dose 168 hours	Rat	18.4	27.2	72.6	62.4	0.8	1.0	Knowles & Mookherjee (1996[c])
100 mg/kg bw Single dose 168 hours	Rat	39.4	43.1	41.2	42.4	0.8	1.0	Knowles & Mookherjee (1996[c])

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
5.7–8.8 mg/kg bw Single dose 120 hours	Rabbit	7–11	ND	80–97	ND	0.1–1.2	ND	Colvin & Miller[a] (1973c)

bw: body weight; ND: not determined; no. number

[a] Glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon.

[b] Groups of male and female rats were given 14 consecutive daily oral doses of 10 mg/kg bw of unlabelled glyphosate followed by a single oral dose 10 mg/kg bw of [^{14}C]glyphosate.

[c] Residual activity in carcass only.

The excretion and residue levels found by various studies following single intraperitoneal, intravenous or intramuscular administration in rats and Rhesus monkeys are shown in Table 2.

Table 2. Residues of administered ^{14}C-labelled glyphosate[a] after single dose administration

Dose / Means of administration / Length of observation	Species	Percentage of administered dose (%)						Reference
		Total excretion via urine		Total faecal excretion		Total tissue and residual carcass residues		
		Males	Females	Males	Females	Males	Females	
6.7 mg/kg bw Intraperitoneal 120 hours	Rat	82–90	ND	6–14	ND	< 1	ND	Colvin & Miller[a] (1973a)
10 mg/kg bw Intravenous 7 days	Rat	79.0	74.5	4.65	8.3	1.27	1.09	Ridley & Mirly (1988)
30 mg/kg bw Intravenous 168 hours	Rat	85.98	84.18	3.42	1.48	1.35	1.09	Powles (1992b)
4 mg Intramuscular 7 days	Monkey	89.9	ND	ND	ND	ND	ND	Maibach (1983)

bw: body weight; ND: not determined

[a] Glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon.

Rats

In a pre-GLP study, aqueous solutions of glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon and at the C2-glycine carbon were administered to Wistar rats by gavage. The radiochemical purity of the labelled materials used were 95% and higher for ^{14}C-methylene glyphosate, ^{14}C-C1-glycine glyphosate and ^{14}C-C2-glycine glyphosate. For the first series of experiments, eight male and four female rats were fasted for four hours and then administered, by gavage, aqueous solutions of [^{14}C]glyphosate at a dose level of 6.7 mg/kg body weight (bw). Two male rats and one female rat were administered ^{14}C-methylene glyphosate, three male rats and one female rat were administered ^{14}C-C1-glycine glyphosate, and three male rats and two female rats were administered ^{14}C-C2-glycine glyphosate. In a second series of experiments, three treatment groups of

three male rats each were dosed separately, via intraperitoneal injection, with ^{14}C-methylene glyphosate (2.33 mg/kg bw), ^{14}C-C1-glycine glyphosate (2.91 mg/kg bw) and ^{14}C-C2-glycine glyphosate (3.63 mg/kg bw). In a third series of experiments designed to determine the gross distribution of plant-derived metabolites of glyphosate, aqueous extracts of soybeans grown in hydroponic solutions of [^{14}C]glyphosate were administered orally to rats. The extracts were obtained from soybean plants, which had been cultured for 4 weeks in separate hydroponic media containing the three forms of [^{14}C]glyphosate. Treatment groups composed of three male rats each for each type of radiolabelled material were dosed separately with the aqueous extracts of the roots of soybeans. A fourth treatment group of three male rats was also dosed with the aqueous extract of the aerial portion of soybean plants grown in hydroponic media containing ^{14}C-methylene glyphosate.

Approximately 94–98% of the [^{14}C]glyphosate orally administered to male rats was excreted in urine and faeces within 48 hours of administration. Approximately 15% of the dose was excreted in the urine within 120 hours of administration, with most of the remainder excreted in the faeces (81%–85%). Of the [^{14}C]glyphosate absorbed through the gut, only very small amounts were catabolized. The percentage of administered radioactivity recovered as expired $^{14}CO_2$ was 0.5%. Tissue retention 120 hours post-administration was less than 1% of the dose for the three ^{14}C-labelled forms of glyphosate.

The percentage of radioactivity excreted by female rats after oral administration of [^{14}C]glyphosate was 82–84% at 48 hours and 91–93% 120 hours. Between 34% and 40% of the administered radioactivity was excreted in the urine within 120 hours, with most of the remainder excreted in the faeces (49–55%). The levels of exhaled $^{14}CO_2$ were also slightly higher for female than male rats, as were carcass retentions. For female rats, the percentage of administered radioactivity recovered as expired $^{14}CO_2$ was 0.72%. Tissue retention at 120 hours was approximately 1% for the three ^{14}C-labelled forms of glyphosate. For both sexes, the order of retention of radioactivity in tissues 120 days post-administration was similar, although the female tissues contained higher concentrations. The highest concentrations of radioactivity were found in the liver, kidney and gut, but in all cases these were 0.20 parts per million (ppm) or less on a fresh-weight basis.

About 74–78% of the dose of [^{14}C]glyphosate administered to male rats via intraperitoneal injection was excreted in the urine within 12 hours. At 96 hours post-administration, total urinary excretion ranged from 81–90% of the administered dose. Faecal excretion ranged from 6–14% of the administered radioactivity within 96 hours and strongly suggests that [^{14}C]glyphosate is also eliminated via the bile. The percentage of radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that following oral administration, but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater in female than in male rats after oral administration, but in all cases was 1% or less of the administered dose.

When extracts of soybeans grown in hydroponic solutions of [^{14}C]glyphosate were orally administered to male rats, 96–99% of the administered radioactivity was excreted in the faeces and urine within 120 hours. The exception were the rats dosed with extracts of soybean roots from plants treated with ^{14}C-C2-glycine glyphosate, for which only 76% of the administered dose was found in the excreta. The relatively high tissue retention (5.19% and 1.86% of the administered dose) and $^{14}CO_2$ expiration (3.67% and 3.49% of the administered dose) by rats administered extracts of roots from plants treated with ^{14}C-C2-glycine glyphosate and the extracts of the aerial portion of plants treated with [^{14}C]methylene glyphosate was attributed to the metabolism of natural plant products since the radioactivity in these extracts was due to 30% and 10% natural products, respectively (Colvin & Miller, 1973a).

In a pre-GLP study, the accumulation and depletion of glyphosate was investigated by the daily administration of feed containing 0, 1, 10 and 100 ppm of [^{14}C]glyphosate to Wistar rats (15/sex per dose) for 14 days, followed by a 10-day depuration period on a control ration. Tissue residues were measured after 2, 6, 10 and 14 days on dosed feed and 1, 3, 6 and 10 days after withdrawal from the dosed feed. The excretion of ingested [^{14}C]glyphosate in faeces and urine were determined daily.

Body and organ weights indicated that the continuous administration of feed containing 1, 10 and 100 ppm of glyphosate for 14 days had no detrimental effect on the growth or relative organ size of rats. Of the [^{14}C]glyphosate ingested, 8.3–10.5% of the daily intake was excreted in the urine. The combined urinary and faecal excretion of radioactivity was approximately equal to the total intake of [^{14}C]glyphosate after 6 days, indicating that a plateau had been reached. By day 4 of dosing, radioactivity in the urine plus faeces exceeded 90% of the cumulative intake, and by the end of the 14-day dosing period the combined excretion of radioactivity was 96, 115 and 93% of the cumulative intake of the 1, 10 and 100 ppm dosing levels, respectively. Since the amount of radioactivity excreted was directly proportional to the intake, the elimination kinetic of [^{14}C]glyphosate could be described as a first-order process, precluding the potential of unlimited accumulation. Most tissues reached maximum [^{14}C]glyphosate residue levels during the dosing period in 10 days or less. There was a modest cumulative effect in the body as a result of chronic [^{14}C]glyphosate administration, but the effect was not localized in a single tissue type or organ system. The order of decreasing tissue propensity for [^{14}C]glyphosate, on a fresh-weight basis, was kidney, spleen, fat, liver, ovaries, heart, muscle, brain and testes. On a dry-weight base the order was spleen, kidney, ovaries, heart, liver, testes, fat, brain and muscle. Accumulation of [^{14}C]glyphosate in muscle tissue was very low on either a fresh- or dry-weight basis, indicating a very low propensity for accumulation. The residues in the tissues were reversibly bound and began to deplete as soon as the dosed feed was withdrawn (Colvin & Miller, 1973b).

Seven different test groups of Sprague Dawley (Crl:CD[SD]BR) rats, each with an equal number of males and females, were dosed with [^{14}C]glyphosate labelled in the methylene position between the nitrogen and phosphorous atoms (radiochemical purity ≥ 98%). Single oral doses (10 and 1000 mg/kg bw) were administered by gastric intubation, and intravenous doses (10 mg/kg bw) were injected into the lateral tail vein. Another group of five male and five female rats was treated with unlabelled glyphosate as 14 consecutive oral doses at 10 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 10 mg/kg bw. Blood, urine and faeces were sampled at various time points. At the end the study, the animals were terminated and different tissues as well as the carcass analysed for radioactivity.

The distribution of radioactivity in the excreta and the tissue samples are summarized in Table 3.

Table 3. Recovery of radioactivity as a percentage of the administered ^{14}C-labelled glyphosate dose

	Per cent of administered radioactive dose (%)							
	Single IV dose 10 mg/kg bw		**Single oral dose 10 mg/kg bw**		**Repeated oral dose 10 mg/kg bw**		**Single oral dose 1 000 mg/kg bw**	
Excreta/tissue	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Urine	79.0	74.5	28.6	22.5	30.9	23.1	17.8	14.3
Faeces	4.65	8.30	62.4	69.4	61.0	70.9	68.9	69.4
Organs/tissues	0.09	0.05	0.05	0.02	0.05	0.03	0.04	0.03
Residual carcass	1.18	1.04	0.40	0.29	0.50	0.32	0.25	0.21
Gastrointestinal contents	0.04	0.04	0.02	0.01	0.01	0.01	0.03	0.04
Cage wash	0.89	1.30	1.30	1.96	0.82	1.96	3.86	8.00
Total recovery[a]	86.0	85.3	92.8	94.2	93.3	96.3	90.9	92.1

bw: body weight; IV: intravenous

[a] Total recovery is the mean of individual animal data.

Source: Ridley & Mirly (1988)

The major route of elimination of an oral dose of ^{14}C-labelled glyphosate at 10 mg/kg body bw was faeces. After a 7-day elimination period, the faeces contained 62.4% and 69.4% of the administered dose for males and females, respectively. The majority of the remaining radioactivity, 28.6% of the dose for the males and 22.5% of the dose for females, was excreted in the urine. More of the administered dose remained in the organs, tissues and residual carcasses of the males than of the females, although the overall amount of retained radioactivity was very low (< 0.5% of the administered dose). The tissue with the highest concentrations of radioactivity was bone, with 0.552 ppm and 0.313 ppm found for the males and females, respectively.

For the test group orally dosed at 1000 mg/kg bw, 68.9% and 69.4% of the administered dose was excreted in the faeces and 17.8% and 14.3% was excreted in the urine of the male and female rats, respectively. Very low levels (< 0.4%) of the administered dose remained in the gastrointestinal contents, residual carcasses, organs and tissues 7 days after dosing. The tissues showing more than 1.0 ppm of radioactivity were the liver, kidney, spleen, lung, stomach, small intestines, bone and residual carcass. Bone retained the greatest amount of radioactivity, 30.6 ppm and 19.7 ppm for the males and females, respectively.

For the test group treated with 14 daily doses of non-labelled glyphosate at 10 mg/kg prior to receiving a single oral dose of labelled glyphosate at 10 mg/kg bw, males excreted 61.0% and 30.9% and females 70.9% and 23.1% of the dose in the faeces and urine, respectively. Very low levels (< 0.7%) of the administered dose remained in the gastrointestinal contents, residual carcasses, organs and tissues 7 days after dosing. Again, bone was the tissue with the highest concentration of radioactivity, containing 0.748 and 0.462 ppm glyphosate equivalents for male and female rats, respectively.

The half-lives of the α and β elimination phases were 5.9–6.2 hours and 79–106 hours, respectively, following a single oral dose of 10 mg/kg bw. In the 1000 mg/kg bw dosed group, the α phase was comparable to 10 mg/kg bw group, but the β phase was found to be 181–337 hours. Comparison of the area under the curves of plots of radioactivity levels in the blood versus time for the two groups indicated that the orally administered glyphosate was 30–35% absorbed. These values are in good agreement with the absorption values of 30–36% found by dividing the per cent urinary excretion of administered radioactivity for the group dosed orally at 10 mg/kg bw by the per cent urinary excretion of administered radioactivity from the group dosed intravenously at 10 mg/kg bw. The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated after a single oral dose at 10 or 1000 mg/kg bw (Ridley & Mirly, 1988).

In a preliminary study of absorption and distribution, male Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material = 98.6%; radiochemical purity = 94.3–97.4%) as a single oral dose at 30 mg/kg bw in 0.9% saline by gavage. Blood samples were taken from the tail vein of three animals periodically between 0.5 and 48 hours after dosing. Additional animals were terminated 4, 10 and 24 hours after dosing, and the tissue distribution of radioactivity was investigated by whole-body autoradiography.

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations (C_{max}) reached within 4 hours were 1.769, 1.137 and 0.705 µg eq/mL. Thereafter, plasma levels decayed exponentially to non-detectable levels 12 hours post dose. The elimination half-lives were 6.196 hours and 12.35 hours for two animals. A value could not be obtained for the third animal. The concentration of radioactivity was highest after 10 hours, with the highest concentrations in bone, bone marrow, cartilage, parts of the gastrointestinal tract, kidney, urinary tract and nasal mucosa. The highest concentrations within bone were associated with the epiphyses. Lower concentrations were found in a number of other tissues. Twenty-four hours after dosing, tissue concentrations of radioactivity were negligible in all tissues except bone, bone marrow, parts of the gastrointestinal tract, bladder and kidney cortex (Powles, 1992a).

In a study of absorption, distribution and excretion, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material = 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw. A group of five male and five female rats was administered unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by [^{14}C]glyphosate as a single oral dose at 30 mg/kg bw. The animals were housed individually in metabolism cages from which urine, faeces and expired air were collected at regular intervals. The rats were terminated after 90% of the dose had been eliminated or 7 days after dosing, whichever was sooner. At necropsy, a blood sample was drawn and selected tissues removed.

Following administration of the single intravenous dose of 30 mg/kg, more than 84% of the radioactivity was eliminated in urine, mostly within 8 hours. Faecal elimination accounted for less than 3.5% of the administered radioactivity and only a very small proportion was eliminated in exhaled air; less than 1.4% remained in tissues and the residual carcass after termination. In contrast, faeces were the major route of elimination when [^{14}C]glyphosate was administered orally. Approximately 56–59% of the oral dose of 30 mg/kg was excreted in faeces, mostly within 12–36 hours. Urinary elimination of the oral dose was slower than for the intravenous dose, with 29–31% eliminated, mostly within 36 hours of dosing. Excretion was unaffected by administering unlabelled glyphosate for 14 days prior to dosing with [^{14}C]glyphosate, and the routes and rates of excretion of a high dose of [^{14}C]glyphosate (1000 mg/kg) were essentially identical to that of the low dose. There was no significant sex difference in the elimination of glyphosate for any dose regimen. Irrespective of the dose, route or frequency of duration, less than 1.4% of a dose was retained in tissues. The highest concentration of radioactivity was in bone and lower concentrations were in bone marrow, kidneys, liver, lungs and the residual carcass (Powles, 1992b).

In a study of absorption, distribution, excretion and metabolism, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material 98.9%; radiochemical purity > 98%) as a single dose at 10 or 600 mg/kg bw by gavage in water. For the excretion study, urine and faeces (5/sex) were collected at selected intervals for 168 hours. Animals were terminated at 168 hours post dosing and the radioactivity in blood and selected tissues analysed. For the plasma concentration study, blood samples (total nine per sex per dose) were drawn at selected intervals up to 168 hours. For the tissue distribution study, 12 rats (six male, six female) were administered single oral doses of either 10 or 600 mg/kg bw per day by gavage. The animals were divided into two groups of six (three per sex) and terminated by cervical dislocation 6 and 18 hours (the low-dose study) or 3 and 9 hours (for the high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC).

During the 7-day observation period, up to about 23% and 30% of the radioactivity of the low dose was excreted in the urine of low- and high-dose animals, respectively. At both doses, about three quarters of the radioactivity was detected in the faeces within 7 days (75% for males and 84% for females, 10 mg/kg bw; 75% and 74%, 600 mg/kg bw; Table 4) (McEwen, 1995).

Table 4. Radioactivity in rat excreta and tissue over 168 hours after a single dose of ^{14}C-labelled glyphosate

	Percentage of administered radioactive dose (%)			
	10 mg/kg bw		**600 mg/kg bw**	
Excretion intervals (h)	**Males**	**Females**	**Males**	**Females**
Urine				
0–6	2.63	3.25	11.55	9.08

	Percentage of administered radioactive dose (%)			
	10 mg/kg bw		600 mg/kg bw	
Excretion intervals (h)	**Males**	**Females**	**Males**	**Females**
6–24	15.85	12.69	13.85	13.36
24–48	2.82	2.41	2.33	4.40
48–72	0.54	0.44	0.59	1.07
72–96	0.24	0.19	0.30	0.40
96–120	0.15	0.13	0.21	0.24
120–144	0.09	0.07	0.17	0.17
144–168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal (urine plus cage wash)	22.51	19.37	30.26	29.50
Faeces				
0–24	60.28	74.59	58.94	46.28
24–48	11.72	7.56	13.41	22.87
48–72	1.18	1.34	1.36	3.83
72–96	0.29	0.36	0.35	0.47
96–120	0.17	0.27	0.36	0.23
120–144	0.35	0.08	0.08	0.12
144–168	0.64	0.10	0.15	0.35
Subtotal faeces	74.63	84.30	74.65	74.15
Residual carcass	0.33	0.27	0.31	0.39
Total	97.47	103.94	105.22	104.04

bw: body weight

Source: McEwen (1995)

After a single dose of 10 mg/kg bw, peak mean concentrations of radioactivity in plasma occurred at 6 and 2 hours in males (0.22 µg eq/mL) and females (0.28 µg eq/mL) (Table 5). After a single oral dose of 600 mg/kg bw, peak mean concentrations of radioactivity in plasma occurred at 3 hours in both males (26 µg eq/mL) and females (29 µg eq/mL). The area under the concentration versus time–curve (AUC_t) was calculated at 400 and 355 µg eq/mL*hour in males and females, respectively. These values were around 120 times higher than the AUCt obtained in the low-dose group.

Table 5. Pharmacokinetic parameters of total rat plasma radioactivity following single oral doses of ^{14}C-labelled glyphosate

	Measures per administered dose			
	10 mg/kg bw		600 mg/kg bw	
Parameter	**Males**	**Females**	**Males**	**Females**
C_{max} (µg eq/mL)	0.2219	0.2789	25.97	28.84
T_{max} (hour)	6.00	2.00	3.00	3.00
AUC_t (µg eq/mL*hour)	3.20	3.70	399.90	355.30
AUC (µg eq/mL*hour)	3.80	4.20	419.00	–[a]

Terminal rate constant (per hour)	0.0840	0.0887	0.1174	–[a]
Terminal half-life (hour)	8.30	7.80	5.90	–[a]
Absorption rate constant (per hour)	0.2963	0.4239	0.2845	0.4477

AUC: area under the plasma concentration–time curve; AUC_t: area under the curve calculated up to the last detectable sample (calculations done up to 24 hours); bw: body weight; C_{max}: maximum concentration; eq: equivalent; T_{max}: time to reach the maximum concentration

[a] Could not be calculated accurately as the values were at or close to the limit of reliable measurement.

Source: McEwen (1995)

There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract, the stomach, muscles and the kidneys, the organs of excretion contained concentrations of radioactivity higher than the plasma (Table 6). High levels of radioactivity were detected in the content of stomach and the gastrointestinal tract. The radioactivity in most tissues had decreased to around the limit of detection 7 days after dosing.

Table 6. Radioactivity in male and female rat tissue over 168 hours after a single oral dose of 10 mg/kg bw ^{14}C-labelled glyphosate

	Proportion of administered dose over time (%)					
	Male[a]			**Females[a]**		
Tissue	**6 hours**	**18 hours**	**168 hours**	**6 hours**	**18 hours**	**168 hours**
Bone[b]	0.12	0.10	0.02	0.10	0.09	0.03
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
Gastrointestinal tract contents	31.56	4.89	0.01	34.54	14.30	0.01
Kidneys	0.79	0.36	< 0.01	0.67	0.26	< 0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	< 0.03
Stomach	3.47	0.60	0.60	2.56	0.62	< 0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Plasma	0.12	0.03	< 0.01	0.13	0.03	< 0.01
Whole blood	0.20	0.04	< 0.03	0.15	0.05	< 0.03

bw: body weight

Results expressed as mean percentage (%) of applied dose, except bone, which is expressed as percentage (%) of applied dose/g.

[a] $N = 5$

[b] $n = 3$

Source: McEwen (1995)

A major component of urine or the [14C]phosphonomethyl-labelled glyphosate-treated animals was unchanged glyphosate, accounting for 18–27% of both the administered doses. A minor component, accounting for 0.1–0.3% of the administered dose, was shown to co-chromatograph (using normal phase TLC and reverse phase HPLC) with aminomethylphosphonic acid.

Unchanged glyphosate was the major component of the faecal extract of the [^{14}C]phosphonomethyl-labelled glyphosate-treated animals, accounting for 65–78% of both the administered doses. Two minor metabolites accounted for 0.3–1.6% of the administered dose; one of these was shown to co-chromatograph with aminomethylphosphonic acid (McEwen, 1995).

In a series of experiments that compared the faecal and urinary excretion of ^{14}C-labelled glyphosate, five male and five female Alpk:AP_fSD rats were each given a single oral dose of 10 mg/kg bw and 1000 mg [^{14}C]phosphonomethyl-labelled glyphosate (radiochemical purity > 98%) in deionized water. Excretion was measured over 72 hours, after which the animals were terminated and the radioactivity in blood and selected tissues including residual carcasses analysed.

Excretion of radioactivity was rapid for both sexes and most of the administered dose was eliminated, principally in faeces, within 24 hours. Males excreted 13.0% and 88.5% of the lower dose and 16.7% and 89.6% of the higher dose in urine and faeces, respectively. Females excreted 10.6% and 88.7% of the lower dose and 17.5% and 84.5% of the higher dose in urine and faeces, respectively.

At termination, radioactivity in the tissues accounted for only 0.6% and 0.5% of the lower dose in males and females, respectfully. The highest concentrations were in bone (0.5 and 0.4 µg eq/g of the lower dose and 50 and 45 µg eq/g of the higher dose for males and females, respectively). All other tissue concentrations were 0.07 µg/g or less for the lower dose and 7 µg eq/g or less for the higher dose. No marked sex difference was seen in the tissue distribution of radioactivity (Davies, 1996a,b).

A similar experiment was conducted using five male and five female Alpk:AP_fSD rats pre-treated with 10 mg/kg bw of unlabelled glyphosate (purity 99.2%) for 14 days before being given the single oral dose of 10 mg/kg bw of [^{14}C]phosphonomethyl-labelled glyphosate (radiochemical purity > 98%) in deionized water. Once again, excretion was measured over 72 hours. The animals were then terminated and the radioactivity in blood and selected tissues including residual carcass analysed.

Excretion of radioactivity was rapid in both sexes and most of the administered dose was eliminated, principally in faeces, within 24 hours. Males excreted 10.6% and 86.6% and females 10.7% and 90.7% of the administered dose in urine and faeces, respectively.

At termination, tissue concentrations of radioactivity accounted for 0.5% of the administered dose in both sexes. The amount in the tissue and contents of the intestinal tract were 0.12% of the administered dose in both sexes. The highest concentrations were in bone (0.36 and 0.35 µg eq/g in males and females, respectively). All other tissue concentrations were 0.07 µg eq/g or lower. No marked sex difference was seen in the distribution of radioactivity in the tissues. Comparison of these results with those obtained when [^{14}C]glyphosate was administered without pretreatment shows that pre-dosing has no significant effect on either the routes or rates of elimination of a single dose of the radiolabelled test material (Davies, 1996c).

Two male and two female Alpk:APfSD rats were each given a single oral dose of 10 mg/kg bw [^{14}C]phosphonomethyl-labelled glyphosate (radiochemical purity > 96%) in deionized water. Excretion was measured throughout the study. At intervals of 24 and 48 hours after dosing, one rat of each sex was terminated and rapidly frozen for whole-body autoradiography.

Within 24 hours of dosing, male rats excreted 22.3% and 55.5% and female rats 11.9% and 83.8% of the administered dose in the urine and faeces, respectively. Within 48 hours of dosing, the remaining male rats excreted 34.0% and 60.5% and the female rats 12.5% and 91.2% of the administered dose in the urine and faeces, respectively.

The whole-body autoradiography showed no marked differences in the distribution of radioactivity between male and female rats. The high levels of radioactivity in the gastrointestinal tract were consistent with faeces being the predominant route of elimination; accordingly, these levels had declined markedly by 48 hours. The greatest intensity of tissue radiolabelling at both 24 and 48 hours was in bone. Some radioactivity was in the kidney after 24 hours but had declined by 48 hours. No significant levels of radioactivity were apparent in other tissues (Davies, 1996d).

In a study of absorption, distribution, excretion and metabolism, groups of five male and five female Sprague Dawley (Crl:CD BR) rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (two batches of unlabelled test material, purity 95.3% and 96.0%; radiochemical purity > 99%) as a single gavage dose of 1 or 100 mg/kg bw in water. For the excretion study, urine and faeces (5/sex per dose) were collected at selected times for 168 hours and samples pooled and analysed directly by TLC or HPLC. At 168 hours, the animals were terminated and radioactivity in blood and selected tissues analysed. For the pharmacokinetic study, blood was drawn (5/sex per dose) at selected intervals up to 72 hours after dosing. For the tissue distribution study, 12 male and 12 female rats were administered a single daily gavage dose of either 10 or 100 mg/kg bw. The treated animals were divided into four groups (three per sex) and terminated at 4, 12, 24 and 72 hours after dosing. For the biliary excretion study, seven male and seven female cannulated rats were administered a single gavage dose of 1 mg/kg bw. Urine, faeces and bile were collected periodically up to 48 hours after dosing.

Following a single gavage dose of 1 mg/kg bw, the major route of elimination was the faeces with 72.62% recovered in males and 62.40% in females, mostly within 24 hours of dosing (63.93% in males and 49.69% in females), suggesting this proportion of the dose was not systemically absorbed. During the 7-day observation period, 18.44% (male) and 27.15% (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose. The remainder of the radioactivity was recovered in the cage wash (6.48% in males and 7.71% in females), cage debris (0.03% in males and 0.58% in females) and carcass (0.75% in males and 0.98% in females).

Following the single gavage dose of 100 mg/kg bw, elimination of radioactivity in the urine (39.42% in males and 43.07% in females) was quantitatively more significant than in to the low-dose group. Faecal elimination accounted for 41.23% in males and 42.37% in females. The remainder of the radioactivity was recovered in the cage wash (13.85% in males and 11.96% in females), cage debris (0.98% in male and 0.10% in female) and carcass (0.84% in male and 0.98% in female). Renal elimination was essentially complete in 48 hours.

In the cannulated rats dosed with 1 mg/kg bw by gavage, the majority of the administered dose was recovered in faeces (55.33% in male and 60.97% in female) in 48 hours. Renal elimination accounted for 27.45% in males and 24.21% in females. The remainder of the radioactivity was recovered in the cage wash (6.57% in male and 6.77% in female), cage debris (0.26% in male and 0.15% in female) and carcass (4.99% in male and 3.82% in female).

The mean terminal elimination half-lives were 10.86 hours and 8.07 hours with corresponding area under the plasma concentration–time curve (AUC) of 0.319 and 0.340 µg eq/mL*hour in males and females, respectively (Table 7). As the elimination half-lives could not be calculated for several high-dose animals, mean $AUC_{0–24}$ (0.257 and 0.338 µg eq/mL*hour in males and females) were calculated to compare the results of both groups. Following a single oral dose of 100 mg/kg bw, mean $AUC_{0–24}$ were 58.2 and 50.7 µg eq/mL*hour in males and females, respectively.

Table 7. Kinetic parameters in male and female rat plasma after a single oral dose of ^{14}C-labelled glyphosate

	Measures per administered dose			
	1 mg/kg bw		100 mg/kg bw	
Kinetic parameters	**Males**	**Females**	**Males**	**Females**
C_{max} (µg eq/mL)	0.016	0.037	8.909	7.634
T_{max} (hour)	3.900	8.000	3.600	4.000
$AUC_{0–24}$ (µg eq/mL*hour)	0.257	0.338	58.200	50.700
AUC (µg eq/mL*hour)	0.319	0.340	–	–

Terminal half-life (hour)	10.860	8.065	–	–

AUC: area under the plasma concentration–time curve; $AUC_{0–24}$: area under the plasma concentration–time curve from time 0 to 24 hours; bw: body weight; C_{max}: maximum concentration; eq: equivalent; T_{max}: time to reach the maximum concentration

Source: Knowles & Mookherjee (1996)

At 1 mg/kg bw, radioactivity was detected in all tissues 4 hours post dose, indicating rapid absorption and distribution in the body. Apart from the gastrointestinal tract (and contents) and the carcass, the kidneys was the only tissue with any notable amounts throughout the observation period. At 72 hours, post-dose concentrations had decreased or plateaued to less than 2% of the administered dose in all tissues in both males and females, with the carcass containing most of the remaining radioactivity. At 100 mg/kg bw, all the tissues were exposed to radiolabelled material 4 hours post dose. Again, only the gastrointestinal tract, carcass and kidneys contained significant amounts of radioactivity. After 72 hours, concentrations had decreased or plateaued to less than 2% of the dose in all tissues in both sex, with the carcass containing most of the remaining radioactivity.

In conclusion, following oral administration of glyphosate at 1 mg/kg bw and 100 mg/kg bw, the absorption, distribution, metabolism and excretion was independent of dose level and sex. Metabolism of glyphosate was very low with more than 90% of the administered dose eliminated unchanged in the urine and faeces. Elimination was essentially completed by 48 hours, and the majority of the radioactivity was recovered in faeces (Knowles & Mookherjee, 1996).

Rabbits

In a pre-GLP study, glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon and at the C2-glycine carbon was dissolved in isotonic saline and administered by gavage to male New Zealand White rabbits fasted for 3 hours. In two replicate experiments, three rabbits were administered ^{14}C-methylene glyphosate, two were administered ^{14}C-C1-glycine glyphosate and two were administered ^{14}C-C2-glycine glyphosate. All the doses were within a range of 5.7–8.8 mg/kg bw.

Approximately, 80–97% of the oral dose of [^{14}C]glyphosate was excreted in the faeces and 7–11% in the urine over 120 hours. Less than 1% of the dose was exhaled. Approximately 1.2%, 0.7% and 0.1% of the dose was retained in the tissues (excluding gastrointestinal tract contents) for ^{14}C-C2-glycine, ^{14}C-C1-glycine and ^{14}C-methylene glyphosate, respectively. The radioactivity in the tissues differed between ^{14}C-C2-glycine and ^{14}C-C1-glycine by 4 or 5 times, but the ranking was similar: the liver had the highest concentrations followed by the kidney, the spleen, the heart, skeletal muscle and gonads, in that order. Only ^{14}C-C2-glycine radioactivity was incorporated in the fat (Colvin & Miller, 1973c).

(b) Intraperitoneal route

In the previously described Colvin & Miller (1973a) study, three treatment groups each with three male Wistar rats were dosed via intraperitoneal injection with ^{14}C-methylene glyphosate (2.33 mg/kg bw), ^{14}C-C1-glycine glyphosate (2.91 mg/kg bw) and ^{14}C-C2-glycine glyphosate (3.63 mg/kg bw). Within 12 hours, 74–78% of the ^{14}C-glyphosate was excreted in the urine. At 96 hours post-administration, total urinary excretion was 81–90% of the administered radioactivity and faecal excretion was 6–14% of the administered radioactivity, indicating that [^{14}C]glyphosate is also eliminated via the bile. The percentage of radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that observed following oral administration (Section 1.1 (a)), but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater than after oral administration, but was in all cases less than or equal or 1% of the administered dose (Colvin & Miller, 1973a).

[^{14}C]glyphosate with a radiochemical purity of 98% was administered by intraperitoneal injection to nine male and nine female Sprague Dawley rats at a dose level of 1150 mg/kg bw. The rats were subsequently housed in metabolism cages, and blood samples were collected from three to six rats at approximately 0.25, 0.50, 1, 2, 4, 6 and 10 hours. At approximately 0.5, 4 and 10 hours after dosing, three animals of each sex were terminated and the femoral bone marrow isolated. The plasma and bone marrow samples were analysed for radioactivity by liquid scintillation counting.

Peak levels of radioactivity were observed in plasma and bone marrow about 0.5 hours after dosing. When expressed as glyphosate acid equivalents, the peak values for bone marrow and plasma in males and females combined were approximately 340 and 1940 ppm, respectively. The radioactivity in plasma decreased rapidly but remained more constant in bone marrow over the 10 hours of the experiment. The analysis of the first-order elimination rates indicated a half-life of elimination from the plasma of approximately 1 hour for both males and females. Elimination from bone marrow was slower with a half-life of 4.2 hours for females and 7.6 hours for males (Ridley, 1983).

(c) Intravenous route

In a previously described study by Ridley & Mirly (1988) (Section 1.1 (a)), groups of male and female Sprague Dawley rats (Crl:CD(SD)BR) were injected with a single intravenous dose of 10 mg/kg bw [^{14}C]glyphosate into the lateral tail vein. Urine and faeces were collected at intervals for 7 days, and the animals were terminated and tissues and carcass analysed for radioactivity.

The majority of the dose – 79.0% in males and 74.5% in females – was excreted in the urine. Faecal excretion was 4.65% and 8.30% of the administered dose, respectively, which suggests that glyphosate is eliminated via the bile. Very little (< 0.1%) of the administered dose was found in the tissues and organs. An intravenous dose resulted in significantly higher levels of radioactivity in the residual carcasses than those found following oral dosing, with the highest concentrations in bone: 1.48 ppm glyphosate equivalents in males and 1.59 ppm glyphosate equivalents in females (Ridley & Mirly, 1988).

In the previously described absorption, distribution and excretion study by Powles (1992b) (section 1.1(a)), groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material, 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw.

Following administration of the 30 mg/kg bw intravenous dose, more than 84% of the radioactivity was eliminated in urine, mostly within 8 hours. Faecal elimination accounted for less than 3.5% of the administered radioactivity. Only a very small proportion of the radioactivity was eliminated in exhaled air and less than 1.4% remained in the tissues and the residual carcass after the animals were terminated. In contrast, faeces were the major route of elimination when [^{14}C]glyphosate was given orally (Powles, 1992b).

(d) Intramuscular route

In a two-phase excretion study, [^{14}C]glyphosate was mixed with isopropylamine and unlabelled glyphosate isopropylamine salt and dissolved in water to make a solution of 4 mg glyphosate/mL. One millilitre of this solution was injected into the thigh muscle of each of four male Rhesus monkeys. Urine samples were collected at intervals for up to 7 days.

During the 7-day collection period following intramuscular injection, 89.9% of the applied radioactivity was excreted in urine. The overall urinary elimination half-life was 19.7 hours. There were two distinct phases to the elimination kinetics, a rapid phase with a half-life of 6.9 hours (over the first 24 hours) and a slow phase with a half-life of 35.1 hours (Maibach, 1983).

(e) Dermal route

In vitro

The absorption of glyphosate acid (purity 95.93%) from a dried glyphosate wet cake preparation through abraded rabbit whole skin was measured in vitro over 24 hours. The dose was placed on the abraded skin at a nominal rate of 79.8 mg/cm^2 (48.3 mg glyphosate acid/cm^2), calculated as equivalent to the 5000 mg/kg bw per day dose administered to rabbits in an in vivo dermal toxicity study (Johnson, 1982). The diffusion cell was left unoccluded for 6 hours, and the surface of the skin was then decontaminated with a sponge wash. Physiological saline was used as the receptor fluid.

The total recovery of the individual cells was 87.3–98.2%, with an overall mean recovery of 93.3% of applied dose. The majority of the applied glyphosate acid (mean 87.9%) was washed off the skin at 6 hours, with a further 2.38% washed off at 24 hours. A small proportion (0.041%) of the dose applied was recovered from the epidermis, with 0.243% remaining in the dermis. The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 μg/cm^2, corresponding to 2.42% of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis, was 2.66%. The results of this in vitro study indicated that dermal absorption of glyphosate through abraded rabbit skin is slow (Hadfield, 2012a).

The penetration through human epidermis of glyphosate from a formulation concentrate was measured in vitro over 24 hours. The glyphosate formulation concentrate, containing a nominal 360 g/L of an isopropylamine salt of glyphosate at a 1:133 weight per volume (w/v) aqueous dilution was applied to the epidermal membranes at a rate of 10 μL/cm^2 and left unoccluded for 8 hours.

Penetration of glyphosate was fastest between 0 and 2 hours after application (0.914 μg/cm² per hour). The mean penetration rate slowed to 0.074 μg/cm² per hour between 2 and 24 hours. The mean amount penetrated over the entire 24-hour exposure period was 3.51 μg/cm^2, corresponding to 0.096% of the applied dose (Hadfield, 2012b).

The absorption and distribution of glyphosate from a 360 g/L soluble (liquid) concentrate (MON 79545) through human epidermis was measured in vitro. The doses were applied as the concentrate formulation (450 g/L of glyphosate) and as 1:15.6 volume per volume (v/v) and 1:188 v/v (nominally 28.8 and 2.4 g/L of glyphosate) aqueous spray dilutions of the formulation. ^{14}C-radiolabelled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to the epidermal membranes at a rate of 10 μL/cm^2 and left unoccluded for 24 hours.

The mean total amount of absorbed glyphosate in 24 hours was 0.573 μg/cm^2 (0.012% of applied dose) from the 450 g/L concentrate formulation. From the 1:15.6 v/v and 1:188 v/v aqueous dilutions, the mean total amounts of absorbed glyphosate in 24 hours were 0.379 and 0.021 μg/cm^2 (0.129% and 0.082% of applied dose), respectively (Ward, 2010a).

The absorption and distribution of glyphosate from a 360 g/L soluble (liquid) concentrate (MON 79351) through human epidermis was measured in vitro when doses were applied as the concentrate formulation (480 g/L of glyphosate) and as 1:16.7 v/v and 1:200 v/v (nominally 28.7 and 2.4 g/L) aqueous spray dilutions of the formulation. ^{14}C-radiolabelled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to the epidermal membranes at a rate of 10 μL/cm^2 and left unoccluded for 24 hours.

The mean total amount of absorbed glyphosate in 24 hours was 0.342 μg/cm^2 (0.0070% of applied dose) from the 480 g/L concentrate formulation. From the 1:16.7 v/v and 1:200 v/v aqueous

dilutions of the formulation, the mean total amounts of absorbed glyphosate in 24 hours were 0.0.553 and 0.015 μg/cm^2 (0.182% and 0.0488% of applied dose), respectively (Ward, 2010b).

The absorption and distribution of glyphosate from a 360 g/L soluble (liquid) concentrate was measured in vitro through human epidermis when it was applied as the concentrate formulation (360 g/L of glyphosate) and a 3:200 v/v aqueous spray strength dilution of the formulation. ^{14}C-radiolabelled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The actual concentrations achieved were 364 g/L and 6.70 g/L of glyphosate for the concentrate and the spray dilution, respectively. The doses were applied to the epidermal membranes at a rate of 5 μL/cm^2 and left unoccluded for 24 hours.

For the concentrate, the mean rate of absorption in 24 hours was 0.02 μg/cm^2 per hour. For the 3:200 v/v aqueous dilution, the mean rate of absorption in 24 hours was 0.001 μg/cm^2 per hour. For the concentrate, mild skin washing at 6 and 24 hours removed practically all of the applied dose from the surface of epidermal membrane. For the 3:200 v/v spray dilution skin washing at 6 and 24 hours removed 90.8% and 87.9% of the applied dose, respectively (Davies, 2003).

In vivo

In the dermal penetration phase of the Maibach (1983) study described above (section 1.1 (d)), 25 μL of [^{14}C]glyphosate solution containing 8.9 mg glyphosate was placed on the shaved abdomens (7.9 cm^2 area) of six male Rhesus monkeys. After 24 hours, each abdomen was swabbed twice with water, twice with acetone and again twice with water to remove any residual glyphosate. Urine samples were collected periodically for up to 7 days post application.

The washing procedure removed 14.2% of the applied ^{14}C label. A mean total of 1.8% of the applied dose of [^{14}C]glyphosate was recovered in the urine during the 7-day collection period. Glyphosate penetrated the monkey skin slowly as only 0.4% of the topically applied dose appeared in the urine after 24 hours. The urinary elimination half-life for topically applied glyphosate was 59 hours (Maibach 1983).

1.2 Biotransformation

Seven test groups, each with an equal number (between three and five) of male and female Sprague Dawley Crl:CD(SD)BR rats, were dosed with *N*-(phosphono[^{14}C]methyl)glycine glyphosate. The radiochemical purity was 98% or greater. Single oral doses were administered by gastric intubation whereas the intravenous doses were injected into the lateral tail vein. Comparison of the areas under the curves for radioactivity levels in whole blood after oral (mean dose for males: 10.2 mg/kg bw; for females: 10.6 mg/kg bw) and intravenous (mean dose for males: 10.7 mg/kg bw; for females: 11.0 mg/kg bw) administration of radiolabelled glyphosate indicated that absorption of the oral dose of glyphosate at the 10 mg/kg bw dose level was 30.4% for males and 35.4% for the females. Glyphosate was isolated as the predominant radioactive fraction in urine (overall recovery of 81.3%) and faeces (overall recovery of 99.2%), and was positively identified in each case by various analytical methods. The minimum glyphosate content as a per cent of either urine or faecal extract contained radioactivity in all of the individual rat excreta samples at 97.46%. HPLC analyses further indicated that glyphosate in the excreta accounted for 98.50–99.33% of the administered [^{14}C]glyphosate.

In groups orally treated with a mean dose of 9.41 mg/kg bw for males and 9.28 mg/kg bw for females and with a mean dose of 10.7 mg/kg bw for males and 10.3 mg/kg bw for females, there was evidence that glyphosate was metabolized to produce 0.2–0.3% and 0.4% AMPA, respectively. The remainder of the radioactivity in the excreta was due to low-level impurities in the dosing material or changes during storage of the excreta samples (Howe, Chott & McClanahan, 1988).

Urine and faeces samples from the previously described study by Powles (1992b) (Section 1.1 (c)) were analysed for identification of glyphosate metabolites. Briefly, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material: 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw. Another group of five male and five female rats were administered unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 30 mg/kg bw.

The recovery of radioactivity from urine and faecal samples was generally greater than 90%. For both dose groups only one major region of radioactivity was detected when extracts were analysed by either liquid chromatography or TLC and this co-chromatographed with a glyphosate standard. The identity of the major component as glyphosate was confirmed by comparing its Fourier transform infrared spectroscopy spectrum with a glyphosate standard. Small amounts of other components were detected but no radiolabelled metabolites were identified (Powles, 1992b).

Urine and faeces samples from the previously described McEwen (1995) study (section 1.1 (a)) were analysed for identification of glyphosate metabolites. Briefly, groups of five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material: 98.9%; radiochemical purity > 98%) as a single dose at 10 or 600 mg/kg bw by gavage in water. Urine and faeces were collected for 7 days and analysed for metabolites.

The major urinary component was unchanged glyphosate, accounting for 18–27% of the administered dose. Only 0.1–0.3% of the administered dose was shown to co-chromatograph, using normal phase TLC and reverse phase HPLC, to aminomethylphosphonic acid. Faecal extract contained 65–78% of administered dose as unchanged glyphosate. Two minor metabolites were in faecal extract, accounting for 0.3–1.6% of the administered dose; one of these two metabolites was shown to co-chromatograph with aminomethylphosphonic acid (McEwen, 1995).

The biotransformation of ^{14}C-labelled glyphosate was investigated in male and female rats administered either as a single 10 mg/kg dose or a single 10 mg/kg dose following repeated oral doses of 10 mg/kg unlabelled glyphosate or as a single 1000 mg/kg bw dose. The metabolites in excreta from the Davies (1996a,b,c) studies were identified (Section 1.1 (a)). In addition, a single oral dose of 1000 mg/kg of [^{14}C]glyphosate (97.8 radiochemical purity) was administered to male and female Alpk:AP$_f$SD rats fitted with a bile duct cannulae. The structural identification of metabolites isolated from urine, bile and faeces, collected over 48 hours (biliary study) or 72 hours, was characterized using various analytical methods.

Biliary excretion of radioactivity over 48 hours was negligible, 0.055% and 0.062% of the administered dose for male and female rats, respectively. The greater percentage of excreted dose was in faeces in both male (39.1%) and female rats (30.5%). Urinary excretion accounted for 20.8% of the administered dose in male rats and 16.3% of the administered dose in female rats. In cannulated rats, the excreted radioactivity (including cage wash) after 48 hours accounted for 62.5% and 52.0% of the administered dose in male and female rats, respectively.

The main urinary metabolite was unchanged glyphosate, which accounted for virtually the entire radioactivity present, with minor amounts of AMPA, which represented less than 1% of the dose in each study (see Table 8). Solvent extraction of faeces, collected from the various excretion and tissue distribution studies, resulted in the extraction of 53–79% of the radioactivity present. In each case the extracts contained a single peak, which corresponded to unchanged glyphosate (Macpherson, 1996).

Table 8. Quantification of glyphosate metabolites as percentages of single doses of ^{14}C-labelled glyphosate administered orally to rats

		Percentage of administered dose (%)					
		Low-dose study 10 mg/kg bw		Repeat dose study[a] 10 mg/kg bw		High-dose study 1000 mg/kg bw	
Sample	**Analyte**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Urine	Glyphosate	12.7	10.5	10.5	10.5	16.0	16.7
	AMPA	0.2	0.1	< 0.1	< 0.1	0.6	0.7
Faeces	Glyphosate	74.8	55.2	52.9	72.1	79.3	63.9
Total	Glyphosate	87.5	65.7	63.3	82.6	95.3	80.6
	AMPA	0.2	0.1	< 0.1	< 0.1	0.6	0.7

AMPA: aminomethylphosphonic acid; bw: body weight

[a] Following 14 repeated oral doses of 10 mg/kg bw unlabelled glyphosate.

Source: Macpherson, 1996

Urine and faeces samples from the previously described Knowles & Mookherjee (1996) study (Section 1.1 (a)) were analysed for identification of glyphosate metabolites. Briefly, five female Sprague Dawley (Crl:CD BR) rats were administered [^{14}C]phosphonomethyl-labelled glyphosate as a single dose at 1 or 100 mg/kg bw by gavage in water. For the excretion study, urine and faeces (5/sex per dose) were collected at selected times for 168 hours.

Metabolite profiles of pooled urine and faecal samples were investigated by HPLC. Only one major peak was detected in urine and faeces (> 90% of the total activity); this was subsequently identified as glyphosate. A minor component observed in the radiochromatograms had a similar retention time to AMPA; however, it could not be positively identified due to very low levels (Knowles & Mookherjee, 1996).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies of glyphosate (including skin and eye irritation and dermal sensitization studies) are summarized in Table 9.

Table 9. Summary of acute toxicity studies with glyphosate

Species	**Strain**	**Sex**	**Purity (%)**	**LD_{50} (mg/kg bw) / Result**	**Reference**
Oral					
Mouse	ICR	M + F	96.7	> 10 000	Shirasu & Takahashi (1975)
Mouse	NMRI	M + F	98.6	> 2 000	Dideriksen (1991)
Mouse	ICR(Crj:CD-1)	M + F	95.68	> 5 000 (M) > 5 000 (F) > 5 000 (combined)	Komura (1995a)
Mouse	ICR(Crj:CD-1)	M + F	62.34% glyphosate isopropylamine salt	> 5 000	Enami & Nakamura (1995)
Rat	Sprague Dawley	F	96.40 & 96.71	> 5 000	Komura (1995b)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rat	HanRcc: WIST	F	96.66	> 2 000	Simon (2009a)
Rat	CD/Crl:CD(SD)	F	97.52	> 2 000	Haferkorn (2009a)
Rat	Sprague Dawley	F	96.40 & 96.71	> 5 000	You (2009a)
Rat	CD/Crl:CD(SD)	F	95.23	> 2 000	Haferkorn (2010a)
Rat	CD/Crl:CD(SD)	F	97.3	> 2 000	Haferkorn (2010b)
Rat	Sprague Dawley derived	F	97.23	> 5 000	Merkel (2005a)
Rat	Wistar Hannover	F	98.05	> 2 000	Do Amaral Guimaraes (2008a), with addendum dated 2010
Rat	HanRcc: WIST(SPF)	F	95.1	> 2 000	Talvioja (2007a)
Rat	Sprague Dawley	M + F	97.76	> 5 000 (M) > 5 000 (F) > 5 000 (combined)	Reagan & Laveglia (1988a)
Rat	Wistar	M + F	99	5 600 (combined)	Heenehan, Rinehart & Braun (1979)
Rat	Sprague Dawley	M + F	85.5	> 5 000	Blaszcak (1988a)
Rat	Sprague Dawley	M + F	98.6	> 5 000	Cuthbert & Jackson (1989a)
Rat	Alpk:AP_fSD (Wistar derived)	M + F	95.6	> 5 000 (male) > 5 000 (female) > 5 000 (combined)	Doyle (1996a)
Rat	HanRcc:WIST(SPF)	F	96.1	> 5 000	Arcelin (2007a)
Rat	RjHan:WI	F	96.3	> 5 000	Tavaszi (2011a)
Rat	Wistar	M + F	99	5 600	Heenehan (1979a)
Rat	Sprague Dawley derived	M + F	62% glyphosate isopropylamine salt	> 5 000	Moore (1999)
Acute dermal					
Rat	Sprague Dawley	M + F	Not reported	> 2 000	Cuthbert & Jackson (1989b)
Rat	Sprague Dawley	M + F	96.40 & 96.71	> 5 050	You (2009b)
Rat	SD(Crj:CD)	M + F	95.68	> 2 000	Komura (1995c)
Rat	HanRcc: WIST(SPF)	M + F	96.66	> 2 000	Simon (2009b)
Rat	CD/Crl:CD(SD)	M + F	97.52	> 2 000	Haferkorn (2009b)
Rat	CD/Crl:CD(SD)	M + F	95.23	> 2 000	Haferkorn (2010c)
Rat	CD/Crl:CD(SD)	M + F	96.6	> 2 000	Haferkorn (2010d)
Rat	Sprague Dawley	M + F	97.23	> 5 000	Merkel (2005b)
Rat	Wistar Hannover	M + F	98.05	> 2 000	Do Amaral Guimaraes (2008b)
Rat	HanRcc: WIST(SPF)	M + F	95.1	> 2 000	Talvioja (2007b)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rat	Alpk:AP_fSD (Wistar derived)	M + F	95.6	> 2 000	Doyle (1996b)
Rat	HanRcc: WIST(SPF)	M + F	96.1	> 5 000	Arcelin (2007b)
Rat	RjHan (WI) Wistar	M + F	96.3	> 5 000	Zelenak (2011a)
Rabbit	New Zealand White	M + F	85.5	> 5 000	Blaszcak (1988b)
Rabbit	New Zealand White	M + F	97.76	> 5 000	Reagan (1988a)
Rabbit	New Zealand White	M + F	99	> 5 000	Heenehan (1979b)
Inhalation (nose only)					
Rat	CD/Crl:CD(SD)	M + F	96.6	> 5.18	Haferkorn (2010e)
Rat	F344/DuCrj(SPF)	M + F	97.56	> 5.48	Koichi (1995)
Rat	HsdRcc Han	M + F	96.66	> 5.04	Griffiths (2009)
Rat	CD/Crl:CD(SD)	M + F	97.52	> 5.12	Haferkorn (2009c)
Rat	CD/Crl:CD(SD)	M + F	95.23	> 5.02	Haferkorn (2010f)
Rat	Sprague Dawley	M + F	96.40 & 96.71	> 2.24	Carter (2009)
Rat	Sprague Dawley	M + F	97.23	> 2.04	Merkel (2005c)
Rat	Not reported	M + F	98.05	> 5.21	Dallago (2008)
Rat	HanRcc: WIST(SPF)	M + F	95.1	> 3.252	Decker (2007)
Rat	Alpk:AP_fSD (Wistar derived)	M + F	95.6	> 4.43	Rattray (1996)
Rat	Wistar RjHan (WI)	M + F	96.9	> 5.04	Nagy (2011)
Rat	Sprague Dawley	M + F	62% glyphosate isopropylamine	> 2.08	Wnorowski (1999)
Rat	Hsd:Sprague Dawley	M + F	47.2% glyphosate acid equivalent	> 5.27	Bonnette (2004)
Primary dermal irritation					
Rabbit	New Zealand White	M + F	95.1	Non-irritating	Talvioja (2007c)
Rabbit	Himalayan	M	95.23	Non-irritating	Leuschner (2009a)
Rabbit	New Zealand White	F	97.56	Non-irritating	Hideo (1995a)
Rabbit	Himalayan	M	97.52	Non-irritating	Leuschner (2009c)
Rabbit	Himalayan	M	96.6	Non-irritating	Leuschner (2010a)
Rabbit	New Zealand White	M + F	96.71	Non-irritating	You (2009c)
Rabbit	New Zealand White	M	97.23	Non-irritating	Merkel (2005d)
Rabbit	New Zealand White	F	98.05	Non-irritating	Canabrava Frossard de Faria (2008a)
Rabbit	New Zealand White	M + F	97.76	Non-irritating	Reagan & Laveglia (1988b)
Rabbit	New Zealand White	M + F	99	Slightly irritating	Heenehan (1979c)
Rabbit	New Zealand White	F	95.6	Non-irritating	Doyle (1996c)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rabbit	New Zealand White	M + F	96.1	Non-irritating	Arcelin (2007c)
Rabbit	New Zealand White	M	96.3	Mildly irritating	Zelenak (2011b)
Rabbit	New Zealand White	M + F	85.5	Slightly irritating	Blaszcak (1988c)
Eye irritation					
Rabbit	New Zealand White	M + F	95.1	Mildly irritating	Talvioja (2007d)
Rabbit	Himalayan	M	95.23	Moderately irritating	Leuschner (2009b)
Rabbit	New Zealand White	F	97.56	Severely irritating	Hideo (1995b)
None	n/a	–	Not stated	pH of a 1% solution in water was 1.93. Not tested because pH < 2 indicates corrosive properties	Simon (2009c) [a]
Rabbit	Himalayan	M	97.52	Mildly irritating	Leuschner (2009d)
Rabbit	Himalayan	M	96.6	Mildly irritating	Leuschner (2010b)
Rabbit	New Zealand White	M + F	96.40 & 96.71	Moderately irritating	You (2009d)
Rabbit	New Zealand White	M	97.23	Moderately irritating	Merkel (2005e)
Rabbit	New Zealand White	M + F	98.05	Severely irritating	Canabrava Frossard de Faria (2008b)
Rabbit	New Zealand White	Not reported	97.76	Severely irritating	Reagan & Laveglia (1988c)
Rabbit	New Zealand White	F	95.6	Mildly irritating	Johnson (1997)
Rabbit	New Zealand White	M + F	96.1	Mildly irritating	Arcelin (2007d)
Rabbit	New Zealand White	M	96.3	Severely irritating	Tavaszi (2011b)
Rabbit	New Zealand White	M + F	85.5	Moderately irritating	Blaszcak (1988d)
Rabbit	New Zealand White	M + F	46.6	Non-irritating	Blaszcak (1998e)
Rabbit	New Zealand White	M + F	57.8% glyphosate potassium (47.13% glyphosate acid equivalent)	Mildly irritating	Bonnette (2001)
Rabbit	New Zealand White	M + F	Not reported (MON 0139)	Non-irritating	Branch (1981)
Rabbit	New Zealand White	Not specified	90.8% (MON 8722)	Mildly irritating.	Busch (1987a)
Rabbit	New Zealand White	Not specified	70.7% (MON 8750)	Mildly irritating	Busch (1987b)
Rabbit	New Zealand White	Not specified	99	Moderately irritating	Heenehan (1979d)
Rabbit	New Zealand White	Not specified	97.76	Severely irritating	Reagan (1988b)

F: female; LD_{50}: median lethal dose; M: male

[a] According to Simon (2009c): "A 1% w/w solution of glyphosate technical in purified water was found to have a pH of 1.93. According to Council Regulation (EC) No. 440/2008, B.5. and OECD Guidelines 405, a test item is not required to be tested if the pH value is less than 2, because it is assumed that the test item has corrosive properties… Therefore, no eye irritation with glyphosate technical will be performed"

(a) Oral toxicity

Mice

Groups of 10 ICR mice of each sex were administered a single dose of glyphosate (purity 96.7%) at 1000, 5000 or 10 000 mg/kg bw orally by gavage and were observed for 14 days before termination.

Decreased locomotor activity was observed in all the mice at doses of 5000 mg/kg bw and higher. Two of high-dose males and one of the high-dose females died; the others recovered fully within 2 days. No abnormalities were found during necropsy.

The acute oral median lethal dose (LD_{50}) of glyphosate (96.7%) in mice was greater than 10 000 mg/kg bw (Shirasu & Takahashi, 1975).

Groups of five male and five female Bom:NMRI mice were administered a single dose of glyphosate (purity 98.6%) at 2000 mg/kg bw by gavage.

All the animals survived until the scheduled termination (day 14). Toxicological signs included piloerection and sedation in all mice on day 1. No macroscopic abnormalities were observed at necropsy.

The acute oral LD_{50} of glyphosate (98.6%) in mice was over 2000 mg/kg bw (Dideriksen, 1991).

Five male and five female ICR(Crj:CD-1) mice were orally dosed with 5000 mg/kg bw glyphosate (purity 95.68%). The test material was administered as a 25% suspension in 0.5% carboxymethylcellulose (CMC) sodium solution at 20 mL/kg bw.

Signs of toxicity observed at 1 and/or 3 hours after administration included decreased spontaneous activity in one female and one male; another male was sedate and had a hunched posture. One male lost a slight amount of weight on days 0–7 after dosing, but all the mice gained weight over the 14-day observation period. There were no observed abnormalities at necropsy.

The acute oral LD_{50} of technical (95.68%) glyphosate in mice was greater than 5000 mg/kg bw (Komura, 1995a).

Five male and five female ICR(Crj:CD-1) mice were dosed with a formulation (described as a light viscous solution with a specific gravity of 1.23) containing 62.34% glyphosate isopropylamine salt. The test material was administered undiluted.

None of the mice died and there were no signs of toxicity. There was a slight retardation in mean body-weight gain in the males from day 0–7 compared with their controls (5000 mg/kg bw: 32.8–35.1 g; controls: 32.6–37.3 g). No gross pathological abnormalities were observed at gross necropsy.

The mouse acute oral LD_{50} of a formulation containing 62.34% glyphosate isopropylamine salt was greater than 5000 mg/kg bw (Enami & Nakamura, 1995).

Rats

In an acute oral toxicity study, five male and five female Sprague Dawley (Crj:CD) rats were orally dosed with 5000 mg/kg bw glyphosate (purity 95.68%). The test material was administered as a 25% suspension in 0.5% CMC sodium solution at 20 mL/kg bw.

There were no mortalities, but spontaneous motor activity was decreased in five male and three females, and one male had salivation. All the rats gained weight on days 0–7 and 7–14 after dosing. No abnormalities were seen at necropsy.

The acute oral LD_{50} of technical (95.68%) glyphosate in male and female rats was greater than 5000 mg/kg bw (Komura, 1995b).

Three female albino Sprague Dawley rats were administered 5000 mg/kg bw glyphosate (purity, 96.40% and 96.71%) by gavage. The test material was mixed with deionized water and administered as a 40% suspension at 12.5 mL/kg bw.

There were no mortalities. One rat showed slight to moderate signs of salivation, piloerection, diarrhoea, polyuria and decrease in activity, with recovery by day 8. The other two rats showed no indications of toxicity. All the rats gained weight days on days 0–7 and 7–14 after dosing. There were no observed abnormalities at necropsy.

The acute oral LD_{50} of technical (96.40% and 96.71%) glyphosate in female rats was greater than 5000 mg/kg bw (You, 2009a).

Two groups of three female HanRcc:WIST rats were orally dosed with 2000 mg/kg bw technical glyphosate (purity 96.66%). The test material was administered as a 20% suspension in purified water at a dose volume of 10 mL/kg.

All the rats survived. There were no signs of toxicity. Body-weight gain was normal and no macroscopic lesions were observed at necropsy.

The acute oral LD_{50} of technical (96.66%) glyphosate in rats was greater than 2000 mg/kg bw (Simon, 2009a).

Two groups of three female CD/Crl:CD(SD) rats were orally dosed with 2000 mg/kg bw technical glyphosate at purities of 97.52%, 95.23% and 97.3%. The test material was administered as a 20% suspension in 0.8% aqueous hydroxypropylmethylcellulose gel at a dose volume of 10 mL/kg.

All the rats survived. There were no signs of toxicity in the case of any of the test material purity. Body-weight gain was normal, and no pathological findings were noted at necropsy.

The acute oral LD_{50} of technical glyphosate (97.52%, 95.23% and 97.3%) in female rats was greater than 2000 mg/kg bw (Haferkorn, 2009a, 2010a,b).

Three female Sprague Dawley–derived albino rats were orally dosed with 5000 mg/kg bw technical glyphosate (purity 97.23%). The test material was administered as a 50% w/v suspension in distilled water (specific gravity: 1.252 g/mL).

All the rats survived. Clinical signs exhibited by all the rats were diarrhoea, anogenital and facial staining and/or reduced faecal volume, with recovery by day 4. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no gross abnormalities at necropsy. The acute oral LD_{50} of technical (97.23%) glyphosate in female rats was greater than 5000 mg/kg bw (Merkel, 2005a).

Two groups of three female Wistar Hannover rats were orally dosed with 2000 mg/kg bw technical glyphosate (purity 98.05%). The test material was mixed with deionized water to form a dosing mixture containing 200 mg/mL glyphosate technical.

All the rats survived. There were no signs of toxicity, all gained weight on days 0–7 and 7–14 after dosing, and there were no specific signs at necropsy.

The acute oral LD_{50} of technical (98.05%) glyphosate in female rats was greater than 2000 mg/kg bw (Do Amaral Guimaraes, 2008a, with an addendum dated 2010).

Two groups of three female HanRcc:WIST(SPF) rats were administrated 2000 mg/kg bw technical glyphosate (purity 95.1%) by gavage. The test material was diluted in polyethylene glycol (PEG 300) to 0.2 g/mL and administered at a dosing volume of 10 mL/kg.

All the rats survived. All showed piloerection at 1–3 or 2–3 hours after dosing. No other clinical signs were observed. All gained weight on days 1–8 and 8–15 after dosing. There were no macroscopic signs at necropsy.

The acute oral LD_{50} of technical (95.1%) glyphosate in female rats was greater than 2000 mg/kg bw (Talvioja, 2007a).

Five male and five female Sprague Dawley rats were orally dosed with 5000 mg/kg bw of technical glyphosate (purity 97.76%). The test material was administered as a 50% w/v aqueous suspension.

All the rats survived. All had diarrhoea, with recovery by day 4. In addition, three of the male and two of the female rats had wet abdomens (“apparent urinary incontinence”) and one male and one female had hair loss on the abdomen at termination. All gained weight on days 1–8 and 8–15 after dosing. No internal abnormalities were observed at necropsy.

The acute oral LD_{50} of technical (97.76%) glyphosate in rats was greater than 5000 mg/kg bw (Reagan & Laveglia, 1988a).

Groups of five male and five female Wistar albino rats were dosed with 2.5, 3.5 5.0, 7.0 or 9.9 g/kg of technical glyphosate (purity 99%) administered as a 25% solution) in distilled water.

At 2.5 g/kg one of the five males died; at 3.5 g/kg one of the males died; at 5.0 g/kg three females died; at 7.0 g/kg all the males and three females died; at 9.9 g/kg all the animals died. Signs of toxicity included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and faecal staining of the abdomen. The rats that died at 2.5 g/kg (day 5) and 3.5 g/kg (day 8) had considerable weight loss. At 7 and 9.9 g/kg, all the deaths occurred on day 1, except for one 9.9 g/kg male, which died on day 12. At necropsy, the male that died on day 5 after dosing at 2.5 g/kg had urinary and faecal staining of the abdomen, bright red lungs, stomach containing dark red fluid, upper intestines containing dark grey fluid, lower intestines distended with air and containing yellow fluid. The male that died on day 8 after dosing at 3.5 g/kg had white lungs. Almost all the surviving rats at 2.5 and 3.5 g/kg had red spots on the lungs, and mottled or purple livers. Surprisingly, most of the surviving rats at 5.0 g/kg had no visible abnormalities.

The oral LD_{50} (combined sexes) of technical glyphosate in rats was calculated to be 5.6 g/kg (95% confidence limits: 4.9–6.3 g/kg) (Heenehan, Rinehart & Braun, 1979).

Groups of five male and five female fasted CD Sprague Dawley–derived rats were administered glyphosate (purity 85.5%) as a single dose at 5000 mg/kg bw orally by gavage and observed for 14 days before termination.

All the animals survived until termination. One of the females exhibited weight loss on day 7 after dosing but gained weight on days 7–14. Toxicological signs included wet rales, faecal staining, urinary staining and soft stool. Some animals had decreased feed consumption after dosing, which continued in one animal through day 2. No gross abnormalities were found at necropsy (day 14).

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw (Blaszcak, 1988a).

Groups of five male and five female fasted Sprague Dawley rats were administered a single dose of glyphosate (purity 98.6%) at 5000 mg/kg bw orally by gavage and observed for 14 days before termination.

All the rats survived until termination. Toxicological signs included piloerection, reduced activity and ataxia through day 9. No gross abnormalities were found during necropsy.

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw (Cuthbert & Jackson, 1989a).

Five male and five female Alpk:AP_fSD (Wistar-derived) rats were dosed at 5000 mg/kg bw with technical glyphosate (purity 95.6%) administered as a 0.5 g/mL suspension in deionized water.

None of the rats died and there were no signs of toxicity. All gained weight days 1–8 and 8–15 after dosing. At necropsy, two of the males and two of the females had mottled or red areas on the lungs and one male had red areas on the thymus.

The acute oral LD_{50} of technical (95.6%) glyphosate in rats was greater than 5000 mg/kg bw (Doyle, 1996a).

Three female HanRcc:WIST(SPF) rats were dosed at 5000 mg/kg bw with technical glyphosate (purity 96.1%) administered as a 0.5 g/mL suspension in purified water.

None of the rats died. All had slightly ruffled fur (persisting in one rat through day 3) and all had hunched posture from 1–5 or 2–5 hours after dosing. All gained weight on days 1–8 and 8–15 after dosing. There were no macroscopic findings at gross necropsy.

The acute oral LD_{50} of technical (purity 96.1%) glyphosate in female rats was greater than 5000 mg/kg bw (Arcelin, 2007a).

Three female RjHan:WI rats were dosed at 5000 mg/kg bw with technical glyphosate (purity 96.3%), administered as a 0.5 g/mL suspension in 0.5% CMC.

None died and there were no signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. At necropsy, no abnormalities were noted.

The acute oral LD_{50} in female rats was greater than 5000 mg/kg bw (Tavaszi, 2011a).

Groups of five male and five female Wistar albino rats were orally dosed with glyphosate technical (purity 99%) at 2.5, 3.5, 5.0, 7.0 or 9.9 g/kg bw. The test material was administered as a 25% w/v solution in distilled water.

Of the 10 rats in each dose group, one died at 2.5 g/kg, one at 3.5 g/kg, three at 5.0 g/kg, eight at 7.0 g/kg and all 10 at 9.9 g/kg. Signs of toxicity included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and faecal staining of the abdomen.

The acute oral LD_{50} in rats was 5.6 g/kg (95% confidence limits: 4.9–6.3 g/kg) (Heenehan, 1979a).

In an acute oral toxicity study five male and five female Sprague Dawley–derived albino rats were orally dosed with a formulation (described as a clear viscous amber liquid with a specific gravity of 1.214 g/mL) containing 62% isopropylamine glyphosate.

There were no deaths. There were no signs of toxicity in the males; four of the females had anogenital staining, and one of these four had diarrhoea and another, soft faeces. All the rats had fully recovered by day 3. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no gross abnormalities at necropsy.

The rat acute oral LD_{50} of a formulation containing 62% isopropylamine glyphosate was greater than 5000 mg/kg bw (Moore, 1999).

(b) Acute dermal toxicity

Rats

In an acute dermal toxicity study, five male and five female Sprague Dawley rats were dermally dosed with 2 000 mg/kg glyphosate technical (purity not reported), moistened with an unspecified amount of water before application, for 24 hours.

There were no deaths. Clinical signs during exposure consisted of piloerection and reduced activity. All the rats gained weight on days 0–7 after dosing and all, except a female that lost 30 g, gained weight on days 7–14 after dosing. No abnormalities were detected at necropsy.

The rat dermal LD_{50} of technical (purity not reported) glyphosate was greater than 2000 mg/kg bw (Cuthbert & Jackson, 1989b).

Five male and five female Sprague Dawley albino rats were dermally dosed with 5050 mg/kg glyphosate technical (two analyses: 96.40 and 96.71% purity), for 24 hours. The test material was moistened with deionized water at 0.284 mL/g test material and placed on the skin.

There were no deaths and no clinical signs. All the rats gained weight on days 0–7 after dosing; except for one female that lost 3 g of weight, all gained or maintained weight on days 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat dermal LD_{50} of technical (two analyses: 96.40 and 96.71%) glyphosate was greater than 5050 mg/kg bw (You, 2009b).

Five male and five female Sprague Dawley (Crj:CD) rats were dermally exposed to technical glyphosate (purity 95.68%) at a concentration of 2000 mg/kg. Appropriate amounts of finely ground test material were applied to a shaved 4 × 5 cm area of skin on each rat. Each site was then covered with a filter paper moistened with 0.5 mL deionized water. A control group of five male and five female rats was similarly treated without the test material.

Following a 24-hour exposure, there were no deaths and no clinical signs. All the rats gained weight on days 0–7 and 7–14 after dosing, and the weight gains were similar in the glyphosate-treated rats and the controls. There were no abnormalities at necropsy.

The rat dermal LD_{50} of technical (95.68%) glyphosate was greater than 2000 mg/kg bw (Komura, 1995c).

In an acute dermal toxicity study, five male and five female HanRcc:WIST(SPF) rats were dermally exposed to 2000 mg technical glyphosate (purity 96.66%) over a 24-hour exposure. The test material was formulated in purified water at a concentration of 0.5 g/mL and applied at a volume dose of 4 mL/kg.

There were no deaths or any clinical signs. There was no dermal irritation in males. Dermal irritation (slight erythema, scaling, scabs) was seen in four females from day 4, persisting to day 12 at the latest. All the males gained weight on days 1–8 and 8–15 after dosing. Two females had slight (0.6

and 1.7 g) weight losses on days 1–8, but all had good weight gains on days 8–15. No macroscopic findings were observed at necropsy.

The rat dermal LD_{50} of technical (purity 96.66%) glyphosate was greater than 2000 mg/kg bw (Simon, 2009b).

In a series of acute dermal toxicity studies using 2000 mg technical glyphosate (purity 97.52%, 95.23% or 96.6%), five male and five female CD/Crl:CD(SD) rats were dermally exposed over 24-hour periods (Haferkorn, 2009b). In each study, the test material was suspended (0.2 g/mL) in *aqua ad iniectabilia*. This suspension was applied to eight layers of gauze, which was placed on a 5 × 6 cm patch of intact skin site. The gauze was covered with a plastic sheet secured with adhesive plaster.

There were no deaths. There were no signs of toxicity. All the rats gained weight on days 0–8 and 8–15 after dosing. No skin irritation was observed. No pathological changes were observed at necropsy.

The rat dermal LD_{50} of technical glyphosate (purity 97.52%, 95.23% and 96.6%) was greater than 2000 mg/kg bw (Haferkorn, 2009b, 2010c,d).

Five male and five female Sprague Dawley–derived albino rats were dermally exposed to 5000 mg technical glyphosate (purity 97.23%) for 24 hours. The test material was mixed with distilled water to form a dry paste (70% w/w mixture in distilled water). An appropriate amount of this paste was applied to a 2 × 3 inch (about 5.1 × 7.6 cm) 4-ply gauze pad which was placed on the skin. The gauze pad and trunk of the rat were then wrapped with Durapore tape.

There were no deaths and no signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no abnormalities at necropsy.

The rat dermal LD_{50} of technical (97.23%) glyphosate was greater than 5000 mg/kg bw (Merkel, 2005b).

Five male and five female Wistar Hannover rats were dermally exposed to 2000 mg technical glyphosate (purity 98.05%) for 24 hours. The test material was placed on a porous gauze dressing moistened with deionized water. The gauze dressing was held on the skin with a non-irritating tape, and the test site and trunk of the animal covered with adhesive tape.

There were no deaths and no signs of toxicity. All the rats gained weight on days 0–7 after dosing and on days 7–14, with the exception of two females (one lost 2 g, the other maintained weight). There were no specific findings at necropsy.

The rat dermal LD_{50} of technical (980.5 g/kg) glyphosate was greater than 2000 mg/kg bw (Do Amaral Guimaraes, 2008b).

Five male and five female HanRcc:WIST(SPF) rats were dermally exposed to 2000 mg technical glyphosate (purity 95.1%) for 24 hours. The test material was diluted in PEG 300 to a concentration of 0.33 g/mL, and 6 mL/kg of this dilution was applied to intact, shaved skin and covered with a semi-occlusive dressing that was wrapped around the abdomen and fixed with an elastic adhesive bandage.

There were no deaths and no clinical signs were observed. All the rats gained weight on days 1–8 and 8–15 after dosing except for one female that maintained weight on days 8–15. There were no macroscopic findings at necropsy.

The rat dermal LD_{50} of technical (95.1%) glyphosate was greater than 2000 mg/kg bw (Talvioja, 2007b).

Five male and five female Alpk:AP_fSD (Wistar-derived) rats were dermally dosed with 2000 mg technical glyphosate acid (purity 95.6%) for 24 hours. The appropriate amount of test material was weighed out onto a plastic weighing boat and moistened to a dry paste with 0.6–0.8 mL deionized water before being applied onto approximately half of a 10 × 5 cm clipped area of skin. The amount of test material applied per unit area of exposed skin was about 20.0–21.9 mg/cm^2 for males and 16.2–17.3 mg/cm^2 for females. The paste was covered by a 4-ply gauze patch (about 7 × 7 cm) kept in contact with the skin for 24 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film held in place by an adhesive bandage (about 25 × 7 cm) secured by two pieces of PVC tape (about 2.5 × 20 cm).

None of the animals died and there were no significant signs of systemic toxicity. Some rats showed signs of urinary incontinence, but this is common in dermal toxicity studies because of bandaging and is not considered toxicologically significant. The skin of all rats was stained cream by the test material for up to 8 days, but there were practically no signs of skin irritation. One male had slight erythema on days 2–3 after dosing, and one female had small scabs on days 3–8 after dosing. All gained weight on days 1–8, and, with the exception of one female that lost 2 g, all gained weight on days 8–15 after dosing. At necropsy, the only finding was that one female had red mottled lungs, which was reported as common in rats of this age and strain and not considered treatment related.

The rat acute dermal LD_{50} of technical (95.6%) glyphosate acid was greater than 2000 mg/kg bw (Doyle, 1996b).

Five male and five female HanRcc:WIST(SPF) rats were dermally exposed to 5000 mg technical glyphosate acid (purity 96.1%) for 24 hours. The appropriate amount of test material was weighed out onto a plastic weighing boat and moistened to a dry paste with 0.5–0.6 mL purified water. The dry paste was applied evenly on an intact 8 cm^2 area of clipped skin which was covered with tape.

There were no deaths and no clinical signs were observed. All the rats gained weight on days 1–8 and 8–15. There were no macroscopic findings at necropsy.

The rat dermal LD_{50} of technical (95.6%) glyphosate acid was greater than 5000 mg/kg bw (Arcelin, 2007b).

Five male and five female Rj:Han (WI) Wistar rats were dermally exposed to 5000 mg technical glyphosate (purity 96.3%) for 24 hours. Sufficient water to moisten the test material was used to ensure good contact with the skin. The test material suspension was applied uniformly at the dermal site. Gauze pads were placed over the site, and these were covered with a hypoallergenic plaster. The entire trunk of the rat was then wrapped with semi-occlusive plastic wrap for 24 hours.

There were no deaths and no clinical signs were observed. There was no treatment-related dermal irritation. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no macroscopic observations at necropsy.

The rat dermal LD_{50} of technical (96.3%) glyphosate acid was greater than 5000 mg/kg bw (Zelenak, 2011a).

Rabbits

In an acute dermal toxicity study, five male and five female New Zealand White rabbits were dermally exposed to 5000 mg/kg bw glyphosate (purity 85.5%) for 24 hours. The test material was

applied dry to a strip of 8-ply gauze and then moistened with about 15 mL 0.9% saline. The gauze strip was then placed on the skin.

All the rabbits survived the 14-day observation period, with little or no change in body weights. No clinical signs were observed. There was no dermal irritation. Nothing remarkable was observed at gross necropsy.

The rabbit dermal LD_{50} of glyphosate (85.5%) was greater than 5000 mg/kg bw (Blaszcak, 1988b).

Five male and five female New Zealand White rabbits were dermally exposed to 5000 mg/kg glyphosate (purity 97.76%) for a 24-hour occluded exposure. The test material was moistened with 0.9% saline (about 1 mL/g of test material). An appropriate amount of this mixture was then applied to each application site.

One female rabbit died at 14 days, but this death was attributed to mucoid enteropathy and not to exposure to the test material. Other signs were anorexia, diarrhoea and soft stools. Most rabbits gained slight amounts of weight in the 14-day observation period. At necropsy, one male rabbit had a white caseous substance adhering to the lungs but this was not ascribed to exposure to the test material; otherwise, there was nothing remarkable.

The rabbit dermal LD_{50} of glyphosate (97.76%) was greater than 5000 mg/kg (Reagan, 1988a).

In an acute dermal toxicity study, two male and two female New Zealand White rabbits were dermally exposed (on abraded skin) to 5000 mg glyphosate technical (99%)/kg for a 24-hour occluded exposure. The test material was applied as a 25% w/v solution in physiological saline.

All the rabbits survived. All had a clear nasal discharge, which had cleared by day 6. One male lost weight over the 14-day observation period. At 24 hours, there was well-defined erythema in two rabbits and very slight erythema in the two others; two had very slight oedema. At necropsy, there were no internal or external abnormalities.

The rabbit dermal LD_{50} of glyphosate technical was greater than 5000 mg/kg (Heenehan, 1979b).

(c) Exposure by inhalation

In an acute inhalation toxicity study, five male and five female CD/Crl:CD(SD) rats were exposed (nose only) for 4 hours to a mean concentration (HPLC-determined) of 5.18 mg/L (5.05 mg/L as measured gravimetrically) with glyphosate technical (purity 96.6%).

There were no mortalities. All the rats exhibited tremors and dyspnoea, which remained for 3 hours after exposure (last observation on day 1); these effects were no longer present on test day 2 (the day following exposure). All the rats gained weight on days 0–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation median lethal concentration (LC_{50}) of glyphosate (purity 96.6%) was greater than 5.18 mg/L (Haferkorn, 2010e).

In an acute inhalation toxicity study, five male and five female F344/DuCrj(SPF) rats were exposed (whole body) for 4 hours to a mean concentration (determined analytically) of 5.48 mg/L glyphosate technical (purity 97.56%).

There were no deaths. All the rats' fur in the perioral and periocular regions was wet and stained red with sticky material, which disappeared by day 4 in males and by day 5 in females. All the rats gained weight on days 0–7 and 7–14 after dosing. No abnormalities were detected at necropsy.

The rat inhalation LC_{50} of technical (97.56%) glyphosate was greater than 5.48 mg/L (Koichi, 1995).

In an acute inhalation toxicity study, five male and five female HsdRccHan rats were exposed (nose only) to a mean concentration (gravimetrically determined) of 5.04 mg/L glyphosate technical (purity 96.66%).

There were no deaths. All the rats showed an increased respiratory rate, hunched posture, piloerection and wet fur; these signs were still present 1 hour after exposure but were gone the following day. All the rats gained weight on days 0–7 after dosing, and all gained or maintained weight on days 7–14 after dosing. There were no macroscopic observations at necropsy.

The rat inhalation LC_{50} of technical (96.66%) glyphosate was greater than 5.04 mg/L (Griffiths, 2009).

In an acute inhalation toxicity study of glyphosate technical (purity 97.52%), five male and five female CD/Crl:CD(SD) rats were exposed (nose only) to 5.12 mg/L (determined by HPLC).

There were no deaths. All the rats had slight dyspnoea and ataxia which were still present at 1 hour but not at 3 hours. All the rats gained weight on days 0–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (97.52%) glyphosate was greater than 5.12 mg/L (Haferkorn, 2009c).

In an acute inhalation toxicity study, five male and five female CD/Crl:CD(SD) rats were exposed (nose only) for 4 hours to a mean concentration (HPLC-determined) of 5.02 mg/L (4.99 mg/L measured gravimetrically) glyphosate technical (purity 95.23%).

There were no deaths. All rats showed slight ataxia, slight tremors and slight dyspnoea which were still present in all the animals at 3 hours (last observation on day 1) after exposure; these signs were no longer present on test day 2 (the day following exposure). All the rats gained weight on days 0–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (95.23%) glyphosate was greater than 5.02 mg/L (Haferkorn, 2010f).

In an acute inhalation toxicity study, five male and five female Sprague Dawley rats were exposed (nose only) for 4 hours to a mean concentration of 2.24 mg/L (nominal concentration: 7.89 mg/L) glyphosate (two batches: purity 96.40% and 96.71%).

There were no deaths. All the rats showed piloerection and activity decrease from 4.5 hours after exposure began until day 4. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate (two analyses: 96.40% and 96.71%) was greater than 2.24 mg/L (Carter, 2009).

In an acute inhalation toxicity study, five male and five female Sprague Dawley rats were exposed (nose only) for 4 hours to a gravimetrically determined mean concentration of 2.04 mg/L (nominal concentration: 8.99 mg/L) glyphosate technical acid (purity 97.23%).

There were no deaths or signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate acid technical (97.23%) was greater than 2.04 mg/L (Merkel, 2005c).

In an acute inhalation toxicity study, five male and five female rats (strain not reported: "healthy young adults supplied by BIOAGRI'S rearing house") were exposed (nose only) for 4 hours to a gravimetrically determined mean concentration of 5.211 mg/L glyphosate acid technical (purity 98.05%).

There were no deaths or signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate acid technical (purity 98.05%) was greater than 5.211 mg/L (Dallago, 2008).

In an acute inhalation toxicity study, five male and five female HanRcc:WIST(SPF) rats were exposed (nose only) for 4 hours to a gravimetrically determined concentration of 3.252 mg/L (nominal: 6.304 mg/L) technical (purity 95.1%) glyphosate.

There were no deaths. Two males had salivation and rales following exposure, and another male had rales only. Two females had rales. All signs were gone two days after exposure. All gained weight on days 1–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (95.1%) glyphosate was greater than 3.252 mg/L (Decker, 2007).

In an acute inhalation toxicity study, five male and five female Alpk:AP_fSD (Wistar derived) rats were exposed (nose only) for 4 hours to a particulate concentration of 4.43 mg/L glyphosate acid (purity 95.6%); the chemical concentration was 4.27 mg/L. Two males and one female exposed to 4.43 mg/L were found dead and one female was terminated in extremis; these events took place on days 5, 6 or 9 after dosing. Clinical signs seen in all rats included decreased activity, irregular breathing, hunched posture and piloerection. Signs observed in some rats included splayed gait, reduced stability, signs of urinary incontinence, gasping and vocalization. Hunched posture persisted in some females until day 13 after dosing. All the surviving males and females lost weight on days 1–8, but gained weight days on 8–15 after dosing.

The two males found dead had dark lungs, probably as a result of agonal congestion; the lungs of the decedent females were normal and the report states that the dark lungs in the males were probably the result of agonal congestion.

Because of the high mortality at 4.43 mg/L, a second group of five male and five female rats was exposed to a particulate concentration of 2.47 mg/L glyphosate acid (the chemical concentration was measured to be 2.43 mg/L). No mortality occurred in this group. Clinical signs seen in all rats included hunched posture, piloerection and salivation. All the males and four of the females had abnormal respiratory noise, which was still present in one male on day 15 after dosing. All the rats gained weight on days 1–8 and 8–15 after dosing. At necropsy one female had dark lungs and another had a few red spots on the lung. These were probably incidental observations,

The rat inhalation LC_{50} of glyphosate acid (95.6%) was greater than 4.43 mg/L, although mortality (in 4/10 rats) occurred at this concentration. No mortality occurred at 2.47 mg/L, although there were signs of toxicity (Rattray, 1996).

In an acute inhalation toxicity study, five male and five female Wistar RjHan (WI) rats were exposed (nose only) for 4 hours to a gravimetrically determined concentration of 5.04 mg/L (nominal: 7.71 mg/L) glyphosate technical (purity 96.9%). The percentage of aerosol that was less than 4 µm (considered the inhalable portion) was 54.4%.

One male was found dead on day 4. All the rats had laboured and noisy respiration, respiratory rate increase, gasping, sneezing, decreased activity and looked thin. All the surviving rats recovered by day 3; the male that died had slight noisy respiration, slight laboured respiration and a wasted appearance on day 3 (this animal had lost 47 g from day 0–3 after dosing). Specific cause of death was not determined. All the survivors gained weight on days 0–7 after dosing except for one male which lost 9 g; all gained weight on days 7–14. At necropsy, the male decedent had dark/red discolouration of the lungs and thymus. No observations were noted for the surviving rats.

The rat inhalation LC_{50} of glyphosate technical (96.9%) was greater than 5.04 mg/L, with one rat dying following exposure to this concentration (Nagy, 2011).

In an acute inhalation toxicity study of NUP5a99 (described as a clear viscous liquid containing 62% isopropylamine glyphosate and 31% other ingredients), five male and five female Sprague Dawley–derived albino rats were exposed (whole body) for 4 hours to a gravimetrically determined concentration of 2.08 mg/L (nominal value: 18.38 mg/L).

There were no deaths. In-chamber clinical observations included ocular and nasal discharge, hunched posture and hypoactivity, but the rats recovered quickly on removal from the chamber and the only finding 1 hour post-exposure was test material on the fur. All the rats gained weight on days 0–7 and 7–14 post dosing. There were no gross abnormalities at necropsy.

The inhalation LC_{50} of NUP5a99 glyphosate MUP (62% isopropylamine glyphosate) was greater than 2.08 mg/L (Wnorowski, 1999).

In an acute inhalation toxicity study of MON 78623 (47.2% glyphosate acid equivalent; 57.8% potassium salt of glyphosate), two groups of five male and five female Hsd:Sprague Dawley rats were exposed for 4 hours to either 2.21 or 5.27 mg/L glyphosate equivalent.

There were no deaths at either 2.21 or 5.27 mg/L. At 2.21 mg/L, breathing was congested and there was dark material around the eyes and/or nose, both of which cleared by day 8 after dosing. At 5.27 mg/L, the rats exhibited congested breathing, with reduced faecal output in two females on day 1. All signs of toxicity had cleared by day 3 after dosing. At 2.21 mg/L, all the rats gained weight on days 0–7 and 7–14 after dosing. At 5.27 mg/L all the males gained weight on days 0–7 and 7–14 after dosing, while two females (the ones with reduced faecal output on day 1) lost 2 and 6 g on days 0–7; another female lost 6 g on days 7–14; otherwise females gained weight on days 0–7 and 7–14 after dosing. At both 2.21 and 5.27 mg/L, none of the tissues showed any abnormalities at necropsy.

The inhalation LC_{50} of MON 78623 (47.2% glyphosate acid equivalent; 57.8% potassium salt of glyphosate) was greater than 5.27 mg/L (Bonnette, 2004).

(d) Dermal irritation

The results of studies of primary dermal irritation with glyphosate are summarized in Table 9.

In a dermal irritation study, three male and three female New Zealand White rabbits were dermally exposed for 4 hours to 0.5 g glyphosate technical (NUP 05068; purity 95.1%) mixed in about 0.5 mL purified water and applied to a 4 × 4 cm gauze patch that was placed on the skin. The patch was covered with a semi-occlusive dressing that was wrapped around the abdomen and anchored with tape.

All irritation scores were zero. The primary dermal irritation index (PDII) was zero. A 4-hour semi-occluded exposure to glyphosate technical (95.1%) over a skin area of about 16 cm^2 (rather than the usual 6 cm^2) resulted in no dermal irritation (Talvioja, 2007c).

In three separate dermal irritation studies, three male Himalayan rabbits per study were dermally exposed for 4 hours with 1000 or 2000 mg of glyphosate technical (purity 95.23%) (Leuschner, 2009a), glyphosate technical (purity 97.52%) (Leuschner, 2009c) or glyphosate technical (purity 96.6%) (Leuschner, 2010a) mixed with 0.5 (for 1000 g) or 1.0 mL (for 2000 g) *aqua ad iniectabilia*. This paste (750 mg, containing 500 mg glyphosate) was applied to a 6 cm^2 area of skin on each of the rabbits. The paste was covered with a gauze patch held in place with non-irritating hypoallergenic) tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour dermal exposure to glyphosate technical (purity 95.23%, 97.52% or 96.6%) resulted in no dermal irritation (Leuschner, 2009a,c, 2010a).

In a dermal irritation study, six female New Zealand White rabbits were dermally exposed for 4 hours to glyphosate technical (HR-001; purity 97.56%). The test material was finely ground in a mortar and 0.5 g put on a 2.5 × 2.5 cm area on each rabbit. A 2.5 × 2.5 cm gauze patch moistened with 0.5 mL water was then placed over the test material and held in place with a polyethylene sheet and non-irritating occlusive tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour exposure to HR-001 (97.56% active glyphosate) resulted in no dermal irritation (Hideo, 1995a).

In a dermal irritation study, one male and two female New Zealand White rabbits were dermally exposed for 4 hours to 500 mg glyphosate technical (purity 96.71%) moistened with 0.2 mL deionized water. This mixture was applied to each test site and covered with a 2.5 × 2.5 cm gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each rabbit was loosely wrapped with a semi-permeable orthopaedic stockinette secured at both edges with strips of tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour exposure to glyphosate technical grade (96.71%) resulted in no dermal irritation (You, 2009c).

In a dermal irritation study, three male New Zealand White rabbits were dermally exposed for 4 hours to a 70% w/w mixture of glyphosate acid technical (97.23% active) in distilled water. Some of this paste (0.71 g) was placed on 1 × 1 inch (2.54 × 2.54 cm) 4-ply gauze pads which were applied to a 6 cm^2 area of intact skin on each rabbit. The pad and entire trunk of each rabbit were then wrapped with semi-occlusive 3-inch Micropore tape.

At 1 hour after exposure, one site scored 1 for erythema using the Draize scoring method; all other scores were zero. All scores were zero at 24, 48 and 72 hrs. The PDII was 0.08. A 4-hour exposure to glyphosate acid technical (97.23%) resulted in very slight dermal irritation (Merkel, 2005d).

In a dermal irritation study, three female New Zealand White rabbits were dermally exposed for 4 hours to glyphosate technical (purity 98.0%). A moistened gauze pad with 0.5 g test material was placed on a 6 cm^2 area of skin and held in place with an adhesive non-irritating tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was zero. A 4-hour dermal exposure to glyphosate technical (purity 98.05%) resulted in no dermal irritation (Canabrava Frossard de Faria, 2008a).

In a dermal irritation study, three male and three female New Zealand White rabbits were dermally exposed for 4 hours to 0.5 g glyphosate (purity 97.76%) moistened with 0.5 mL physiological saline and applied to two intact test sites per rabbit. The test sites were semi-occluded with a 1 × 1 inch (2.54 × 2.54 cm) gauze patch held in place with Micropore tape.

All irritation scores at 0.5, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour dermal exposure to glyphosate (purity 97.76%) resulted in no dermal irritation (Reagan & Laveglia, 1988b).

In a dermal irritation study, three male and three female New Zealand White rabbits were dermally treated for 24 hours with 0.5 mL glyphosate technical (purity 99%) as a 25% w/v solution in distilled water applied to four sites (two intact, two abraded) on each of six albino rabbits.

At 24 hours, one rabbit scored 1 for erythema at an intact site using the Draize scoring method and 1 for erythema and 1 for oedema at an abraded site. Another rabbit scored 1 for erythema at an abraded site. All other scores at 24 hours were zero. All scores for irritation at 72 hours after dosing were zero (Heenehan, 1979c).

In a dermal irritation study, 500 mg of glyphosate acid (purity 95.6%) was moistened with 0.5 mL of distilled water to form a dry paste that was applied to a 2.5 × 2.5 cm test site on the left flank of each of six female New Zealand White rabbits. The treated area was covered with an 8-ply 2.5 × 2.5 cm surgical gauze pad that was secured by two strips of surgical tape. This was covered by impermeable rubber sheeting that was wrapped once around the trunk of the animal and secured with adhesive polyethylene tape. Exposure was for 4 hours.

No irritation was observed at 30 minutes to 1 hour or 1, 2 or 3 days after dosing. All irritation scores were zero. The PDII was 0.00 (Doyle, 1996c).

In a dermal irritation study, 0.5 g of glyphosate technical (96.1% glyphosate acid) was moistened with about 0.5 mL purified water and placed on a 2.5 × 2.5 cm 8-ply gauze surgical patch that was applied to intact skin on the left flank of each of three male and three female New Zealand White rabbits. Each patch was covered with a semi-permeable dressing that was wrapped around the abdomen and held in place with tape. Exposure was for 4 hours.

No irritation was observed a 1, 24, 48 or 72 hours after dosing. All irritation scores were zero. The PDII was 0.00 (Arcelin, 2007c).

In a dermal irritation study, 0.5 g glyphosate technical (purity 96.3%) was dampened with water, and placed on a 2.5 × 2.5 cm surgical gauze pad that was kept on the skin of three male New Zealand White rabbits with hypoallergenic plaster for 4 hours. The entire trunk was wrapped with plastic wrap held in place with an elastic stocking.

One rabbit had grade 1 erythema at 1 and 24 hours after dosing. All other irritation scores were zero. The PDII was 0.17 (Zelenak, 2011b).

In a dermal irritation study, 0.5 g glyphosate wet cake (purity 85.5%) was moistened with 0.5 mL 0.9% saline and applied to the skin of six rabbits (two applications per rabbit). The applications were covered with 2.5 × 2.5 cm gauze squares for 4 hours of occluded exposure.

Five of the six rabbits showed grade 1 erythema at one or both sites at 0.5, 24 and/or 48 hours after dosing. All scores were zero at 72 hours. The PDII was 0.31 (Blaszcak, 1988c).

(e) Ocular irritation

The results of studies of primary eye irritation with glyphosate are summarized in Table 9.

In an eye irritation study, 0.1 g glyphosate technical (purity 95.1%) was instilled into the conjunctival sac of the left eye of each of three male and three female New Zealand White rabbits.

There was no iridial irritation (all irritation scores were zero). Corneal opacity along with positive conjunctival irritation (grade 2–3 redness and/or grade 2–3 chemosis) was in all the treated eyes at 1, 24 and 48 hours after dosing and in 2/3 treated eyes (with grade 2 redness) at 72 hours after dosing. On day 7 all scores for corneal opacity were zero; three eyes scored 1 (not considered a positive irritation effect) for conjunctival redness. All scores were zero on days 10 and 14.

Glyphosate technical (purity 95.1%) was considered to have caused significant but reversible damage to the rabbit eye (Talvioja, 2007d).

In eye irritation studies, 100 mg glyphosate technical (purity 95.23%, 97.52% or 96.6%) were instilled into the conjunctival sac of the right eye of each of three male Himalayan rabbits for each strength. An hour after instillation, the eyes were rinsed with 20 mL sodium chloride solution.

At purity 95.23%, corneal opacity (maximum score 1) was in all three eyes at 24, 48 and 72 hours after dosing; in two of the three eyes on day 4 after dosing; and in one of the three eyes on days 5, 6 and 7 after dosing; by day 8, clearing was complete. The maximum score for iritis was 1, which was observed in all three eyes at 24 hours, in two eyes at 48 hours, in one eye at 72 hours and in none of the eyes on day 4 and subsequently. The maximum score for conjunctival redness was 1, as was the maximum score for chemosis. All scores for conjunctival effects were zero by day 5. A fluorescein test at 24 hours showed corneal staining of between half and three quarters of the surface of two eyes, and in one quarter to half of the surface of one eye. A fluorescein test on day 7 showed corneal staining in one eye (up to one quarter of the surface).

At purity 97.52%, fluorescein testing at 24 hours showed corneal staining in two of the three eyes. At 24 and 48 hours, two eyes had corneal opacity and one of these still had corneal opacity at 72 hours. All the eyes had completely cleared (all eye irritation scores were zero) by day 4.

At purity 96.6%, all three eyes had corneal opacity at 24, 48 and 72 hours. At 4 days, two eyes had corneal opacity and one of these also had corneal opacity on day 5. All eyes had completely cleared (all eye irritation scores were zero) by day 7.

The three reports each concluded that "glyphosate TC was non-irritating to eyes, hence, no labelling is required" (Leuschner, 2009b, 2009d, 2010b).

In an eye irritation study of HR-001 (purity 97.56%), 0.1 g of the test material was placed in the conjunctival sac of the left eye of each of 12 female New Zealand White rabbits. Six rabbits (group A) did not receive an eyewash; three rabbits (group B) had their eyes washed out 30 seconds after instillation; and three rabbits (group C) had their eyes washed out 2 minutes after instillation.

All six rabbits in group A had corneal opacity through day 4. On day 7, five had corneal opacity. On day 21, three still had corneal opacity while the remaining three had completely cleared. In group B, all three rabbits had corneal opacity at 24 and 48 hours, but their eyes had completely

cleared (all scores were zero) by day 7. In group C, one rabbit was positive for corneal opacity at 24 hours, and none of the rabbits had corneal opacity at 48 hours. One group C rabbit was positive for conjunctival effects at 72 hours; the other two rabbits had completely cleared (all eye irritation scores were zero). None of the group C rabbit eyes was positive for irritation on day 4.

The report concluded that the test material had severely irritating potential for the eye mucosa of rabbits and that irrigation at 30 seconds or 2 minutes after application was effective for reduction of eye irritation and for recovery (Hideo, 1995b).

In an eye irritation study of glyphosate technical grade (two analyses: 96.40 and 96.71%), 0.1 mL (93.2 mg) was placed into the conjunctival sac of the right eye of each of two male and one female New Zealand White rabbits.

Of the three eyes, two still had corneal opacity at 24, 48 and 72 hours and at day 4. One eye had corneal opacity on day 7. All eyes had cleared by day 10.

The test material was rated as "moderately irritating and assigned to [United States Environmental Protection Agency; USEPA] Toxicity Category II" (You, 2009d).

In an eye irritation study of glyphosate acid technical (purity 97.23%), the test material was ground to a powder with a mortar and pestle and 0.1 mL (0.06 g) was instilled into the conjunctival sac of the right eye of three male New Zealand White rabbits. The pH of a 1% solution was reported as 2.5.

All three eyes were positive for corneal opacity through day 7, and for iritis and conjunctivitis through day 4 (one eye was also positive for conjunctival redness on day 7). All eyes had cleared (all irritation scores were zero) by day 10. According to the report,

> The Maximum Mean Total Score of Glyphosate Technical is 40.3. Based on the classification system used the test substance is considered severely irritating to the eye. The classification was raised from moderately to severely [irritating] because all three animals had scores greater than 10 on day 7 of the study (Merkel, 2005e).

In an eye irritation study, 0.1 g of glyphosate technical (purity 980.5 g/kg) was instilled in an eye of each of male and female New Zealand White rabbits. Because of the severity of the effects only two eyes were tested. The pH of a 1% solution is reported as 2.2.

In one rabbit there was corneal opacity, iritis and conjunctival effects through day 4 with clearing by day 7. In the other rabbit there was corneal opacity at 1, 24, 48 and 72 hours and at 7, 14 and 21 days after dosing. The eye was also positive for conjunctival irritation on day 14 after dosing (Canabrava Frossard de Faria, 2008b).

In an eye irritation study, 0.1 g glyphosate (purity 97.76%) was instilled in the conjunctival sac of one eye of each of six New Zealand White rabbits (sex not reported). The eyes were not washed out until 24 hours after instillation of the test material.

Corneal opacity and conjunctival irritation with blistering was observed in all the rabbits. One rabbit (which still had corneal opacity on day 14) was found dead at 20 days after instillation; the death was considered unrelated to exposure to the test material. Of the five surviving rabbits, three still had corneal opacity on day 21.

Because the glyphosate (97.76%) was severely irritating to the eye, it was assigned to USEPA Toxicity Category I for this exposure route (Reagan & Laveglia, 1988c).

In an eye irritation study of glyphosate acid (purity 95.6%), 100 mg was applied into the conjunctival sac of one female New Zealand White rabbit. This application caused moderate pain in this first rabbit so the other five animals were pre-treated with a local anaesthetic. Nevertheless, "between one quarter and one half of the test material was displaced from the eye of each animal immediately after dosing", according to the report (Johnson, 1997).

Corneal, iridial and conjunctival effects were seen in all rabbits for up to 4 days post dosing. Corneal opacity was seen in five of the six rabbits on day 4, but had cleared in all of them by day 7. All scores were 0 on day 7 except for one rabbit which had grade 1 (not considered positive for irritation) conjunctival redness, which had cleared by day 8.

Glyphosate acid (purity 95.6%) was classified as a mild irritant (class 5 on a 1–8 Draize scoring method) to the rabbit eye (Johnson, 1997).

In an eye irritation study, 0.1 g of glyphosate technical (purity 96.1%) was instilled into the conjunctival sac of the left eye of each of three New Zealand White rabbits. The pH of the test material was reported as 2.12.

There was no corneal opacity or iritis. All three rabbit eyes were positive for conjunctival irritation at 1 hour, and two were positive for these effects at 24, 48 and 72 hours after dosing. All scores were 0 by day 7.

The report concluded that "…the test item did not induce significant or irreversible damage to the rabbit eye" (Arcelin, 2007d).

In an eye irritation study, 0.1 g of glyphosate technical (purity 96.3%) was instilled into the conjunctival sac of the left eye of one male New Zealand White rabbit. The pH of the test material was reported as 1.99.

An Initial Pain Reaction score of 3 (on a scale of 0–5) was observed. Irritation effects were scored at 1 and 24 hours after instillation. According to the report (Tavaszi, 2011b):

> Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit at 1 and 24 hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, and a few black points on the conjunctiva and dry surface of the eye were noted at one hour after the treatment. Fluorescein staining was positive at the 24 hour observation. Based on the symptoms, no further animals were dosed and the study was terminated after the 24 hour observation…
>
> Glyphosate Technical was classified as corrosive to the eye. (Tavaszi, 2011b).

In an eye irritation study of glyphosate wet cake (purity 85.5%), 0.1 mL (68.9 mg) was instilled into the lower conjunctival sac of the right eye of six New Zealand White rabbits. The eyes were not washed out until 24 hours after instillation.

All the rabbits showed positive irritation effects (corneal opacity and/or grade 2 chemosis and/or redness and/or iritis) at 1–48 hours after dosing, and two rabbits showed positive irritation effects at 72 hours. None of the eyes was positive for irritation on day 7. The report concluded that "Glyphosate Wet Cake produced moderate to severe but reversible ocular irritation in all animals… Five had iritis and corneal opacities" (Blaszcak, 1988d).

In an eye irritation study of MON 77945 (described as an amber liquid, pH 4.59, containing 46.6% glyphosate acid), 0.1 mL was instilled into one eye of each of six rabbits.

There were no positive irritation effects (one eye scored 1 for conjunctival redness at 1 hour, all other scores were zero). The report concluded that "under conditions of this study, MON 77945 produced very mild, transient ocular irritation" (Blaszcak, 1998e).

In an eye irritation study of MON 78623 (described as an amber liquid with 57.8% potassium salt of glyphosate; 47.13% glyphosate acid equivalent), 0.1 mL was instilled into an eye of each of three rabbits. Two rabbits vocalized following instillation.

There was no corneal opacity. At 1 hour, all eyes scored 1 for iritis, two for conjunctival redness and two for conjunctival swelling. At 24 hours, one eye scored 1 for iritis. All scores were zero at 48 hours. The report concluded that, "based on [European Economic Community] labelling criteria, MON 78623 is classified as a non-irritant to the ocular tissue of the rabbit" (Bonnette, 2001).

In an eye irritation study of MON 0139 (described as an amber liquid, with no information on pH or the active ingredient) 0.1 mL was instilled into an eye of each of nine rabbits. Six eyes were unwashed; three were washed out with physiological saline about 20 seconds after instillation.

All irritation scores were zero. No signs of irritation were observed in any rabbit eye (Branch, 1981).

In an eye irritation study of MON 8722 (described as a white powder, 90.8% purity, which was ground with a mortar and pestle prior to dosing), 0.1 g was instilled into an eye of each of six rabbits.

There was no corneal opacity or iritis. At 1 hour, conjunctival irritation (grade 2 redness and/or chemosis) was seen in five of the six eyes. At 24 and 48 hours, some of the eyes scored 1 for conjunctival redness. At 72 hours, all scores were zero (Busch, 1987a).

In an eye irritation study of MON 8750 (described as a white powder, 70.7% purity, which was ground with a mortar and pestle prior to dosing), 0.1 g (0.1 mL) was instilled into an eye of each of six rabbits.

There was no corneal opacity or iritis. At 1 hour, conjunctival irritation (grade 2 redness and/or chemosis) was seen in five of the six eyes. At 24 hours, one eye scored 1 for conjunctival redness (not considered a positive irritation effect). At 48 hours all scores were zero (Busch, 1987b).

In an eye irritation study, 0.1 mL of a 25% w/v solution of glyphosate technical (purity 99%), in distilled water was instilled into the conjunctival sac of an eye of each of nine rabbits. Six eyes were unwashed, while the other three were washed out for 1 minute with lukewarm water starting 20 seconds after instillation.

One unwashed eye and two washed eyes showed corneal opacity, with clearing by day 4. All scores were zero by day 7. In this study, glyphosate (purity 99%) was moderately irritating to the eye (Heenehan, 1979d).

In an eye irritation study, 0.1 g glyphosate (purity 97.76%) was instilled into the conjunctival sac of one eye of each of six rabbits. Corneal opacity and conjunctival irritation were noted in all rabbits at 24, 48 and 72 hours and on day 7.

One rabbit was found dead at 20 days; however, the death was considered unrelated to exposure. On day 21, three of the remaining five rabbits still showed corneal opacity. In this study, glyphosate (97.76%) was severely irritating to the eye (Reagan, 1988b).

(f) Dermal sensitization

Results of studies of skin sensitization with glyphosate are shown in Table 10.

Table 10. Results of skin sensitization studies with glyphosate

Species	Strain	Sex	Route	Purity (%)	Results	Reference
Mouse	CBA/Ca	F	LLNA	96.1	Negative	Betts (2007)
Mouse	CBA/J Rj	F	LLNA	96.3	Negative	Török-Bathó (2011)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	95.1	Negative	Talvioja (2007e)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	97.52	Negative	Haferkorn (2009d)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	Two analyses: 95.23 & 96.4	Negative	Haferkorn (2010g)
Guinea pig	Hartley	F	Magnusson–Kligman Maximization	97.56	Negative	Hideo (1995c)
Guinea Pig	Hartley	M	Magnusson–Kligman Maximization	96.66	Negative	Simon (2009d)
Guinea pig	Dunkin Hartley	M	Magnusson–Kligman Maximization	Two analyses: 97.52 & 98.8	Negative	Haferkorn (2010h)
Guinea pig	Short-haired Hartley albino	M + F	Buehler	Two analyses: 96.4 & 95.71	Negative	You (2009e)
Guinea pig	Hartley albino	M + F	Buehler	97.23	Negative	Merkel (2005f)
Guinea pig	Hartley	M	Buehler	98.05	Negative	Lima Dallago (2008)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	95.7	Negative	Richeux (2006)
Guinea pig	Albino Crl (HA) BR	F	Magnusson–Kligman Maximization	95.6	Negative	Doyle (1996d)
Mouse	CBA/Ca	F	LLNA	96.1	Negative	Betts (2007)
Mouse	CBA/J Rj	F	LLNA	96.3	Negative	Török-Bathó (2011)

F: female; LLNA: local lymph node assay; M: male

Mouse

In a local lymph node assay, about 25 µL of a 10, 25 or 45% w/v preparation of glyphosate technical (96.1% glyphosate acid) in dimethyl sulfoxide (DMSO) was applied to the dorsal surface of each ear of groups of four female CBA/Ca mice. A vehicle control group was similarly treated with DMSO alone. The procedure was repeated daily for 3 consecutive days.

Three days after the third application, all the animals were injected in the tail vein with about 250 µL of phosphate buffered saline containing 20 µCurie (µCi; 74×10^{10} Bq) [methyl-^{3}H]thymidine. The mice were terminated after about 5 hours. The drained auricular lymph nodes were removed from

each animal and, together with the nodes from the other animals in that group, placed in a container of phosphate buffered saline.

Single cell suspensions were prepared by straining the lymph nodes from a single group through a 200-mesh stainless steel gauze. The cell suspensions were washed three times by centrifugation with about 10 mL phosphate buffered saline. Approximately 3 mL of 5% w/v trichloroacetic acid was added and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were resuspended in approximately 1 mL of trichloroacetic acid, and the suspensions transferred to scintillation vials; 10 mL of scintillant was added prior to ß-scintillation counting.

The following disintegrations per minute were obtained: 0% (DMSO alone): 3912; 10%: 2394 (Stimulus Index or SI of 0.61 relative to vehicle control); 25%: 3292 (SI: 0.84); 45%: 508 (SI: 1.04). The following disintegrations per minute were obtained from the positive control (α-hexylcinnamaldehyde in 4 parts acetone and 1 part olive oil): 0%; (vehicle alone): 5939; 5%: 10 111 (SI: 1.70); 10%: 13 747 (SI: 2.31); and 25%: 38 015 (SI: 6.40, positive response > 3).

The study concluded that glyphosate technical material is not a skin sensitizer under these test conditions (Betts, 2007).

A local lymph node assay of glyphosate technical (96.3%) used groups of four female CBA/J Rj mice with each mouse topically dosed on the dorsal surface of each ear with 25 µL of 10%, 25% or 50% w/v preparation of glyphosate technical in propylene glycol, propylene glycol alone or 25% *α*-hexylcinnamaldehyde in propylene glycol. The procedure was repeated daily for three consecutive days.

Three days after the third application, all the animals were injected in the tail vein with about 250 µL of phosphate buffered saline containing 20 µCi (74×10^{10} Bq) [methyl-^{3}H]thymidine. The mice were terminated about 5 hours later. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in that group, placed in a Petri dish containing 1–2 mL phosphate buffered saline.

Single cell suspensions of pooled lymph node cells were prepared and collected in tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer. The cell strainer was washed with phosphate buffered saline. Pooled lymph node cells were pelleted in a centrifuge at about 190 g for 10 minutes at 4 °C. Afterwards, the centrifugation supernatants were discarded. The pellets were gently resuspended and 10 mL phosphate buffered saline added to the tubes. The washing step was repeated twice. This was repeated for each group of pooled lymph nodes. After the final washing, suspensions were centrifuged and most of the supernatant was removed except for a small volume (< 0.5 mL) above each pellet. Each pellet was resuspended in 3 mL of 5% trichloroacetic acid. After an 18-hour incubation with 5% trichloroacetic acid at 2–8 °C, the precipitate was recovered by centrifugation at 190 g for 10 minutes. The supernatants were removed and the pellets resuspended in 1 mL of 5% trichloroacetic acid solution and dispersed using an ultrasonic water-bath. Each precipitate was transferred to a scintillation vial with 10 mL of scintillation liquid and thoroughly mixed prior to ß-scintillation counting.

The following disintegrations per minute were obtained after accounting for the background: 0% (propylene glycol alone): 681; 10%: 794 (Stimulus Index or SI of 1.2 relative to vehicle control); 25%: 678 (SI: 1.0); 50%: 683 (SI: 1.0); positive control (*α*-hexylcinnamaldehyde in propylene glycol): 25%: 8302 (12.2 SI, positive response > 3).

The study concluded that under the conditions of this local lymph node assay, glyphosate technical had no skin sensitization potential (i.e. it was a non-sensitizer) (Török-Bathó, 2011).

Guinea pigs

In a Magnusson–Kligman maximization test with female Dunkin Hartley guinea pigs, intradermal induction treatments were with a 3% dilution of glyphosate technical (95.1%) in PEG 300 and in an emulsion of Freund's Complete Adjuvant/physiological saline. Epidermal induction (1 week after the intradermal induction) was for 48 hours under occlusion with the test material at 50% in PEG 300. Two weeks later, the five control and 10 test guinea pigs were challenged. Patches (3 × 3 cm) of filter paper were saturated with about 0.2 mL of the test material at the highest tested non-irritating concentration of 25% in PEG 300 (applied to the left flank) and about 0.2 mL PEG 300 alone (applied to the right flank) for 24 hours. The application sites were scored at 24 and 48 hours after exposure ended.

All challenge irritation scores (for the 10 test and five control animals) were zero. A positive control assay with *α*-hexylcinnamaldehyde gave appropriate results. Based on these findings, glyphosate technical does not have to be classified and labelled as a skin sensitizer (Talvioja, 2007e).

In a Magnusson–Kligman maximization test with female Dunkin Hartley guinea pigs, intradermal induction treatments were with a 0.01% concentration of glyphosate technical (two analyses: 95.23% and 96.4%) in *aqua ad iniectabilia*. The day before topical induction, the application site was treated with 0.5 mL sodium lauryl sulfate 10% in Vaseline. Topical induction (1 week after the intradermal induction) was 2 mL of a 50% concentration of glyphosate technical in *aqua ad iniectabilia* applied for 48 hours. The challenge, 2 weeks after the intradermal induction, was 2 mL of the test material placed on a filter paper on the left flank of each guinea pig; a filter paper with 2 mL vehicle was placed on the right flank as a control. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and five control guinea pigs) were zero. A positive control assay with benzocaine gave the expected results. Glyphosate technical (purity 95.23% and 96.4%) was determined to be not sensitizing to guinea pigs (Haferkorn, 2010f).

In a Magnusson–Kligman maximization test with female Hartley guinea pigs, a 5% suspension of glyphosate technical (purity 97.56%) in paraffin oil was intradermally injected. Six days later, the treatment site was treated with 10% sodium lauryl sulfate in white petrolatum; the topical induction (the following day) was with 0.4 g of the test material preparation (25% test material in white petrolatum) on a 2 × 4 cm piece of filter paper for 48 hours. The challenge application (2 weeks after the topical induction) was 25% test material in white petrolatum for 24 hours, with scoring at 24 and 48 hours following the end of this exposure.

None of the 20 induced guinea pigs and none of the 10 negative control guinea pigs showed any signs of irritation at the application site following challenge. A positive control assay with 2,4-dinitrochlorobenzene gave appropriate results.

The study concluded that glyphosate technical (purity 97.56%) had no dermal sensitization potential in guinea pigs (Hideo, 1995c).

In a Magnusson–Kligman maximization test with male Hartley guinea pigs, a 10% w/v dilution of glyphosate technical (purity 96.66%) in purified water and Freund's Complete Adjuvant was intradermally injected. Seven days later the application site was treated with the test material at 50% in purified water (about 0.3 mL applied on a 2 × 4 cm filter paper) with 48-hour exposure. Two weeks later, the guinea pigs were treated with the test material at 15% in purified water (about 0.2 mL applied on a 3 × 3 cm filter paper) for 24 hours, with scoring at 24 and 48 hours following the end of this exposure.

None of the 10 induced guinea pigs and none of the five control guinea pigs showed any signs of irritation at the application site following challenge. A positive control assay with α-hexylcinnamaldehyde gave the appropriate results.

Based on the results of this study, there is no sensitization potential of glyphosate technical (purity 96.66%) in the guinea pig (Simon, 2009d).

In a Magnusson–Kligman maximization test with male Dunkin Hartley guinea pigs, intradermal inductions were with a 0.5% concentration of glyphosate technical (purity 97.52% and 98.8%) in *aqua ad iniectabilia*. The day before the topical induction, the application site was treated with 0.5 mL sodium lauryl sulfate 10% in Vaseline. Topical induction (1 week after the intradermal induction) was 2 mL of a 50% concentration of glyphosate technical in *aqua ad iniectabilia* for 48 hours. The challenge was two weeks after the intradermal induction. Filter paper with 2 mL of the test material was placed on the left flank; as a control, a filter paper with 2 mL of the vehicle was placed the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and five control guinea pigs) were zero. A positive control assay with benzocaine gave the expected results. Glyphosate technical (purity 97.52% and 98.8%) was found to be not sensitizing to guinea pigs (Haferkorn, 2009d).

In a dermal sensitization (Magnusson–Kligman maximization test with male Dunkin Hartley guinea pigs), intradermal induction was with a 0.5% concentration of glyphosate technical (purity 96.6% and 97.3%) in *aqua ad iniectabilia*. The day before the topical induction, the application site was treated with 0.5 mL sodium lauryl sulfate 10% in Vaseline. The topical induction (1 week after the intradermal induction) was 2 mL of a 50% concentration of the test material in *aqua ad iniectabilia* for 48 hours. The challenge was two weeks later: filter paper with 2 mL of the test material was applied to the left flank for 48 hours, with filter paper with 2 mL of the vehicle applied to the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and five control guinea pigs) were zero. A positive control assay with benzocaine gave the expected results. Glyphosate technical (purity 96.6% and 97.3%) was found to be not sensitizing to guinea pigs (Haferkorn, 2010g,h).

In a Buehler method dermal sensitization study with glyphosate technical (purity 96.40% and 96.71%), 15 male and 15 female short-haired Hartley albino guinea pigs were divided into two groups: group I (five males and five females) and group II (10 males and 10 females). For each induction treatment, 400 mg of the test material was placed on a four-ply 2.5 × 2.5 cm gauze pad and moistened with 2 mL deionized water. Each gauze pads was secured with non-irritating adhesive tape, which in turn was covered with a strip of clear polyethylene film. Exposures lasted for at least 6 hours and took place on days 1, 8 and 15. Group I animals were untreated during this period. After a 2-week rest period, all animals (groups I and II) were challenged at a previously unexposed site with 400 mg test material moistened with 2 mL deionized water.

All challenge irritation scores (for the 20 induced and 10 control guinea pigs) were zero. A positive control assay with α-hexylcinnamaldehyde gave the expected results. Glyphosate technical (purity 96.40% and 96.71%) did not elicit a sensitizing reaction in guinea pigs (You, 2009e).

In a Buehler method dermal sensitization study, a group of 20 male and 20 female Hartley albino guinea pigs were exposed once a week to 0.4 g of 70% w/w glyphosate acid technical (purity 97.23%) in distilled water. The mixture was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber, which was secured in place and wrapped with non-allergenic

adhesive tape. After each 6-hour exposure, the chambers were removed and any residual test material gently cleansed off. Twenty-seven days after the first induction dose, 0.4 g of a 70% w/w mixture of the test material in distilled water was applied to a naive site on the right side of each guinea pig. These sites were evaluated and scored approximately 24 and 48 hours after the challenge application. A group of 10 controls was similarly treated with the vehicle alone.

There were no positive irritation scores (defined as > 0.5). A positive control assay with *α*-hexylcinnamaldehyde gave the expected results. Glyphosate technical (97.23%) is not considered a contact sensitizer (Merkel, 2005f).

In a dermal sensitization study (Buehler method), a group of 20 male Hartley guinea pigs were treated three times with once-a-week 6-hour exposures to 1.0 mL of a 50% w/v solution of glyphosate technical (purity 98.05%) in a DMSO vehicle. The solution was applied in a cotton lint patch which covered approximately 6 cm^2 of the left flank. A group of 10 control guinea pigs was similarly treated with 1.0 mL DMSO. Two weeks after the last induction treatment, both induced and control guinea pigs were exposed for 4 hours to 1.0 mL of a 50% w/v solution of test material in DMSO on the right flank.

One of the 20 induced guinea pigs had a score of 1 (positive response) at 24 and 48 hours following challenge. All of the other induced and control animals scored zero.

The study concluded that the epidermal application of glyphosate technical (purity 98.05%) with DMSO as vehicle does not cause skin sensitization in guinea pigs according to the Buehler test method (Lima Dallago, 2008).

In a dermal sensitization (Magnusson–Kligman maximization test) study with glyphosate technical (purity 95.7%), the hair was clipped from an area approximately 4 × 6 cm on the shoulder region of each of a group of 20 female Dunkin Hartley guinea pigs on day 0. A row of three injections (0.1 mL each) was made on each side of the spine. The injections were: a) 1:1 Freund's Complete Adjuvant in isotonic sodium chloride; b) a 0.195% (v/v) formulation of the test material in isotonic sodium chloride; c) a 0.195% (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. On day 6, the scapular region was treated with 10% sodium lauryl sulfate (10% in petroleum jelly). On day 7 the same area used for the intradermal injections was treated with a 60% w/w mixture of the test material in distilled water, with 48-hour occluded exposure. The challenge was approximately 2 weeks later. One site was treated with 60% w/w mixture of the test material in distilled water; a second site was treated with a 30% w/w mixture of the test material in distilled water. The sites were scored for irritation at 24 and 48 hours following exposure.

A group of 10 control guinea pigs was similarly treated using the vehicle only during the induction period.

All 18 induced guinea pigs (2 had died during the study) scored zero at 24 and 48 hours following challenge, as did all 10 controls. The study reported that glyphosate technical (95.7) produced a 0% (0/18) sensitization rate and was classified as a non-sensitizer to guinea pig skin under the conditions of the test (Richeux, 2006).

In a dermal sensitization (Magnusson–Kligman maximization test) with glyphosate acid (purity 95.6%), a group of albino Crl (HA) BR guinea pigs each had the hair clipped from an area about 5 × 5 cm on the scapular region. A row of three injections (0.05–0.1 mL each) was made on each side of the spine. The injections were: a) 1:1 Freund's Complete Adjuvant in deionized water; b) a 0.1% (w/v) preparation of the test material in deionized water; c) a 0.% (w/v) preparation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus deionized water. On day 6 the application site was clipped and 0.5 mL of a 10% preparation of sodium lauryl sulfate in paraffin wax

applied. On day 7 the test area was treated with a topical application of the test material (75% w/v) in deionized water. The preparation (0.2–0.3 mL) was put on a 4 × 2 cm piece of filter paper held in place with surgical tape. The filter paper was covered by a strip of adhesive tape secured using self-adhesive PVC tape. This occlusive dressing was kept in place for about 2 days. Ten control animals were similarly treated with deionized water. Challenge (for both the induced animals and their controls) was at approximately 21 days. An area about 15 × 15 cm on both flanks of the test and control animals was clipped free of hair. An occlusive dressing was prepared using two pieces of approximately 1 × 1.75 cm filter paper stitched to a piece of rubber sheeting (about 12 × 5 cm). A 75% w/v preparation of the test material in deionized water (0.05–0.1 mL) was applied to a piece of filter paper and a 30% w/v preparation in deionized water (0.05–0.1 mL) applied to the second. These were covered with strips of adhesive bandage (about 25–40 cm × 7.5 cm) and secured with a self-adhesive PVC tape. Exposure was for about 24 hours. The sites were scored for irritation at 24 and 48 hours following the end of exposure.

Exposure to the 75% w/v preparation resulted in mild and scattered redness (score of 1) in three of the 20 induced and one of the 10 control animals at 24 hours only, with all scores zero at 48 hours. Because the redness was observed at similar incidences in both induced and control guinea pigs and because it occurred only at 24 hours, it was considered to be due to skin irritation rather than the test material. All sites exposed to the 30% w/v preparation scored zero at both 24 and 48 hours. A positive control assay with α-hexylcinnamaldehyde demonstrated the sensitivity of the test system.

The study concluded that glyphosate acid is not a skin sensitizer under the test conditions (Doyle, 1996d).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 13-week oral toxicity study, groups of 15 male and 15 female CD-1 mice were fed diets containing glyphosate (purity 98.7%) at dietary concentrations of 0, 5000, 10 000 or 50 000 ppm (0, 944, 1870 and 9710 mg/kg bw per day for males and 0, 1530, 2740 and 14 800 mg/kg bw per day for the females, respectively).

There was no treatment-related mortality or clinical signs of toxicity, organ-weight change, macroscopic and histopathological findings. At study termination, body-weight gains of the males and females at 50 000 ppm were about 24% and 18% lower, respectively, than that of the control animals. Body-weight gains of both males and females at 5000 ppm and 10 000 ppm were comparable to those of the controls.

The no-observed-adverse-effect level (NOAEL) in the 13-week toxicity study in mice was 10 000 ppm (equal to 1870 mg/kg bw per day) based on reduced body weights at 50 000 ppm (equal to 9710 mg/kg bw per day) (Tierney & Rinehart, 1979).

In a 13-week oral toxicity study, groups of 10 male and 10 female CD-1 mice were fed diets containing glyphosate (purity 99.5%) at a concentration that was adjusted weekly to give doses of 200, 1000 or 4500 mg/kg bw per day. The animals were observed daily for symptoms of ill health and mortality. Body weights and feed consumption were recorded weekly, and water consumption was monitored throughout the study. Ophthalmoscopic examinations were performed during week 12. Blood samples were collected from the orbital sinus for haematology (seven parameters) and from the dorsal aorta at necropsy for clinical chemistry analysis (16 parameters). However, the small sample volumes precluded analysis of total protein, albumin and cholesterol. All the animals were terminated and necropsied, 13 organs were isolated and weighed, and about 35 separate tissues were fixed for microscopy. All tissues from animals in the highest dose group and in the control group and the

kidneys, liver and lungs of animals at the lowest (200 mg/kg) and intermediate (1000 mg/kg) doses underwent a full histopathological examination.

No treatment-related mortalities, clinical signs, haematological or biochemical findings and no organ-weight changes were observed. Gross or histopathological examination did not show any effects of glyphosate administration.

Taking into account the limited range of clinical chemistry parameters evaluated, the NOAEL in the 13-week toxicity study in mice was 4500 mg/kg bw per day, the highest dose tested in this study (Perry et al., 1991a).

In a 13-week oral toxicity study, groups of 10 male and 10 female B6C3F1 mice were fed diets containing glyphosate (purity 99%) at concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm (equal to 0, 507, 1065, 2273, 4776 and 10 780 mg/kg bw per day for males and 0, 753, 1411, 2707, 5846 and 11 977 mg/kg bw per day for females). All tissues from the highest-dose and control animals were examined microscopically. The salivary glands were also examined in all groups receiving lower doses.

Reduced body-weight gain was observed at 25 000 and 50 000 ppm in both males and females. There were no differences in feed consumption between control and treated mice. The only significant gross finding in the study was a "dark" salivary gland in a male at the highest dose; no other gross abnormalities were observed at necropsy. Histological changes were observed only in the parotid salivary gland (Table 11). The cytoplasmic alterations consisted of a diffuse increase in the basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared to be enlarged and had fewer ducts. No histological changes were observed in the submandibular and sublingual glands.

Table 11. Incidence and severity of cytoplasmic alteration of the parotid and submandibular salivary glands (combined) in mice administered glyphosate for 13 weeks

	No. of cases per dietary concentration of glyphosate					
	0 ppm	**3 125 ppm**	**6 250 ppm**	**12 500 ppm**	**25 000 ppm**	**50 000 ppm**
Males	0/10	010	5/10 (1.0)	9/10 (1.6)	10/10 (2.8)	10/10 (4.0)
Females	0/10	0/10	2/10 (1.0)	9/10 (1.3)	10/10 (2.4)	10/10 (3.1)

no.: number; ppm: parts per million

Results presented as number of mice showing cytoplasmic alterations / total number of mice in the group, with average severity score in parentheses. Severity score is based on a scale of 1 = minimal, 2 = mild, 3 = moderate or 4 = marked.

Source: Chan & Mahler (1992)

The NOAEL in the 13-week toxicity study in mice was 3125 ppm (equal to 507 mg/kg bw per day) based on parotid salivary gland lesions at 6250 ppm (equal to 1065 mg/kg bw per day) (Chan & Mahler, 1992).

In a 13-week oral toxicity study, groups of 12 male and 12 female ICR(Crj:CD-1)SPF mice were administered glyphosate (purity 97.56%) at dietary concentrations of 0, 5000, 10 000 or 50 000 ppm (equal to a mean daily glyphosate intake of 0, 600, 1221 and 6295 mg/kg bw per day for males and 0, 765, 1486 and 7435 mg/kg bw per day for females).

There were no treatment-related clinical signs, mortality or ophthalmological and haematological findings. At 50 000 ppm, mean body weights of the males were 91% that of the controls from week 2 to the end of the treatment; body weights of females were comparable to that of the controls. Similarly, feed consumption was slightly decreased in males at the highest dose. At

50 000 ppm, feed efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment.

At 50 000 ppm, females showed a significant treatment-related increase in creatine phosphokinase ($P < 0.01$). Other statistically significant ($P < 0.01$) changes in clinical chemistry were observed in high-dose male and female mice; however, these changes were minor and not associated with any histological findings and not considered adverse. In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

At 50 000 ppm, males and females showed significant ($P < 0.01$) increases in both absolute and relative caecum weights (238% and 263%, respectively, for males, and 187% and 195%, respectively, for females) (Table 12).

Table 12. Caecum weights of mice administered glyphosate for mice 13 weeks

	Absolute and relative weight per dietary concentration of glyphosate			
	0 ppm	**5 000 ppm**	**10 000 ppm**	**50 000 ppm**
Males				
Absolute weight ± SD (mg) [a]	624 ± 86	609 ± 116	718 ± 177	1 484 ± 359
Relative weight ± SD (%)	1.45 ± 0.19	1.38 ± 0.26	1.61 ± 0.33	3.82 ± 1.15**
Females				
Absolute weight ± SD (mg) [a]	497 ± 96	474 ± 115	604 ± 123	958 ± 163**
Relative weight ± SD (%)	1.43 ± 0.26	1.37 ± 0.30	1.67 ± 0.42	2.79 ± 0.53**

ppm: parts per million; SD: standard deviation; **: $P < 0.01$

Relative weight expressed as (organ weight / body weight) × 100.

[a] At 50 000 ppm, both males and females showed significant increases in absolute weights (238% for males and 187% females).

Source: Kuwahara (1995)

At 50 000 ppm, males and females showed a significant increase in incidence of distension of the caecum (12/12 males and 10/12 females, in contrast to none in the control group). In addition, at this dose males showed significant increases in incidence of cystitis (4/12 compared to none in the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distension of the caecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the caecum.

The NOAEL in the 13-week toxicity study in mice was 10 000 ppm (equal to 1221 mg/kg bw per day) based on the decrease in body weights in males, increase in absolute and relative caecum weights in both sexes and increased incidence of distension of the caecum in both sexes at 50 000 ppm (equal to 6295 mg/kg bw per day) (Kuwahara, 1995).

Rats

In a 4-week range-finding study of oral toxicity, groups of five male and five female Sprague Dawley rats were fed diets containing glyphosate (purity 97.7%) at concentrations of 0, 30 000, 40 000 or 50 000 ppm (equivalent to approximately 1500, 2000 and 2500 mg/kg bw per day).

No animals died during the study. The only clinical signs of toxicity were soft stools and/or diarrhoea, which occurred in both sexes at all doses with diarrhoea being the predominant sign in animals at the highest dose during the last 3 weeks of the study. Slightly reduced body-weight gains were noted in both sexes at all the doses, although significant reductions consistently occurred only in males and females at the highest dose (9.6% and 9.0%, respectively, after 4 weeks). Daily feed

consumption was reduced for males at the intermediate and highest dose during the first week of the study. Feed intake for treated females was comparable to that of controls throughout the study. The only clinical signs of toxicity were soft stools and/or diarrhoea, which occurred in both sexes at all doses with diarrhoea being the predominant sign in animals at the highest dose during the last 3 weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions.

Because of the frequent occurrence of soft stools and/or diarrhoea at all doses, no NOAEL could be derived from this 4-week dietary toxicity study in rats (Reyna & Thake, 1989).

In a 4-week oral toxicity study, groups of five male and five female Sprague Dawley rats were fed diets containing glyphosate (purity 99.5%) at a concentration that was adjusted weekly to give doses of 0, 50, 250, 1000 or 2500 mg/kg bw per day. All the animals were terminated and necropsied, and the livers, hearts, kidneys, spleens and adrenals of control and highest-dose animals processed and examined histopathologically. Examination was subsequently extended to include the kidneys from all females in all the groups.

Soft faeces were noted in three males in the highest-dose group during weeks 3 to 4, but not in any other group. No treatment-related effects were observed on mortality, clinical signs of toxicity, body weights, feed and water consumption or haematological parameters. In males, equivocal increases in plasma alanine transaminase [alanine aminotransferase] and alkaline phosphatase activities were observed at 250, 1000 or 2500 mg/kg bw. In females, plasma alanine transaminase activity was significantly increased at the highest dose, as was total bilirubin. In addition, increased plasma concentrations of phosphate were noted in males at 1000 or 2500 mg/kg bw. There were neither notable intergroup differences in organ weights nor gross pathological findings. However, an increase in the incidence of very mild to slight nephrocalcinosis was observed in female rats at 250 mg/kg bw and higher doses (Table 13).

Table 13. Nephrocalcinosis in rats administered glyphosate for 4 weeks

	No. per dietary concentration of glyphosate									
	Males					**Females**				
	0 mg/kg bw per day	**50 mg/kg bw per day**	**250 mg/kg bw per day**	**1 000 mg/kg bw per day**	**2 500 mg/kg bw per day**	**0 mg/kg bw per day**	**50 mg/kg bw per day**	**250 mg/kg bw per day**	**1 000 mg/kg bw per day**	**2 500 mg/kg bw per day**
No. of cases	0	NI	NI	NI	NI	0	0	2	2	4
No. of very mild/minimal cases	0	NI	NI	NI	NI	0	0	1	1	2
No. of mild/slight cases	0	NI	NI	NI	NI	0	0	1	1	2

bw: body weight; NI: not investigated; no.: number

Source: Atkinson et al. (1989)

The NOAEL in the 4-week dietary toxicity study in rats was 50 mg/kg bw per day for slight nephrocalcinosis in female rats at 250 mg/kg bw per day (Atkinson et al., 1989). This finding was not confirmed in a separate study by Perry et al., 1991b.

In a 90-day oral toxicity study, groups of 12 male and 12 female Sprague Dawley rats were fed diets containing glyphosate (purity 95.2%) at concentrations of 0, 1000, 5000 or 20 000 ppm (calculated mean intakes equal to 0, 63, 317 and 1267 mg/kg bw per day for males and 0, 84, 404 and 1623 mg/kg bw per day for females). Clinical signs, body weight, feed consumption, haematology

and clinical chemistry parameters were monitored routinely. Gross examinations were performed for all groups, and the kidneys, liver and testes weighed after termination. A standard range of tissues from control and highest-dose animals was microscopically examined as well as the kidneys, livers and lungs from animals at all doses.

No treatment-related effects were observed at up to the highest dose. However, parotid salivary glands were not included in the histopathological examination.

The NOAEL in the 90-day dietary toxicity study in rats was 20 000 ppm (equal to 1267 mg/kg bw per day), the highest dose tested (Stout & Johnson, 1987).

In a 13-week oral toxicity study, groups of 10 male and 10 female Sprague Dawley rats were fed diets containing glyphosate (purity 98.6%) at concentrations that were adjusted weekly to doses of 0, 30, 300 or 1000 mg/kg bw per day. All tissues from control and highest-dose animals, in addition to the kidneys, liver, lungs and parotid salivary glands of all the test animals, underwent a full histopathological examination.

There were no mortalities, clinical signs or changes in body or organ weights, feed and water consumption, haematological parameters and ophthalmoscopic and macroscopic findings. Females at the highest dose showed slight but statistically significant increases in concentrations of glucose (11%; $P < 0.05$), total protein (9%; $P < 0.001$), albumin (9%; $P < 0.05$) and creatinine (8%; $P < 0.01$) compared with those in the control group. Urinalysis revealed a reduction in pH in males at the highest dose.

In contrast to results from a 4-week study in rats conducted at the same testing facility (Atkinson et al., 1989), the incidence of nephrocalcinosis in this 13-week study was evenly distributed in dose groups and sexes and was not dose dependant; it is therefore clearly not treatment related.

An increase in the incidence of cellular alterations (deep basophilic staining and enlargement of cytoplasm) was observed in the parotid salivary glands of both sexes in all treated groups. In addition, the severity (graded as very mild, mild, moderate, severe and very severe) of these findings showed a dose-related increase, but only reached statistical significance in males at the highest dose (Table 14), suggesting these changes are of equivocal toxicological significance.

Table 14. Cytoplasmic alteration of the parotid salivary gland in rats administered glyphosate in the diet for 13 weeks

	No. per dietary concentration of glyphosate							
	Males				**Females**			
	0 mg/kg bw per day	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Severity [a]								
Very mild	3	7	6	0	2	7	7	1
Mild	0	0	3	2	0	1	2	4
Moderate	0	0	1	3	0	0	0	3
Severe	0	0	0	5*	0	0	0	1
Total incidence	3	7	10**	10**	2	8*	9**	9**

bw: body weight; no.: number; *: $P < 0.05$; **: $P < 0.01$

[a] Severity graded as very mild, mild, moderate, severe and very severe.

Source: Perry et al. (1991b)

The NOAEL in this 90-day toxicity study in rats was 300 mg/kg bw per day based on the more pronounced severity of cellular alterations in the parotid salivary gland at 1000 mg/kg bw per day (Perry et al., 1991b).

In a 13-week oral toxicity study, groups of 10 male and 10 female F344/N rats were fed diets containing glyphosate (purity 99%) at concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm. Ten more animals of each sex were included at each dietary concentration for evaluation of haematological and clinical pathology parameters. The calculated mean intakes were equal to 0, 205, 410, 811, 1678 and 3393 mg/kg bw per day, respectively, for males and 0, 213, 421, 844, 1690 and 3393 mg/kg bw per day, respectively, for females. All tissues from the control and highest-dose animals were examined microscopically. Salivary glands were also examined for the animals at all lower doses.

Diarrhoea was seen in males at the highest dose and in all females for the first 50 days of the study. Weight gain was reduced in males at 50 000 and 25 000 ppm, and the final mean body weight was approximately 18% and 6% less than that of controls, respectively. Small increases in several erythrocyte parameters were noted in males at 12 500 ppm and higher doses. These changes were unremarkable and generally consistent with a mild dehydration. Plasma alkaline phosphatase and alanine transaminase activities were slightly increased in males at 6250 ppm and greater and in females at 12 500 ppm and greater. In the absence of histopathological findings in the liver, these increases are considered not toxicologically significant.

No treatment-related gross abnormalities or organ-weight changes were observed at necropsy. Histopathological changes were observed only in the parotid and submandibular glands of both male and female rats. The study authors combined the findings for these two glands (Table 15). The findings for each gland individually or for individual animals were not reported. No histological alterations were observed in the sublingual gland. The changes were described as cytoplasmic alterations and consisted of basophilic changes and hypertrophy of the acinar cells. Considering the 16-fold difference between the lowest dose of 3125 ppm and the highest dose of 50 000 ppm, the incidence response curve appears to be relatively flat and the degree of change is slight, progressing from only minimal to moderate, suggesting that any changes are of equivocal toxicological significance.

Table 15. Cytoplasmic alterations of the parotid and submandibular salivary glands (combined) in rats administered glyphosate for 13 weeks

	Incidence per dietary concentration of glyphosate					
	0 ppm	**3 125 ppm**	**6 250 ppm**	**12 500 ppm**	**25 000 ppm**	**50 000 ppm**
Males	0/10	6/10 (1.0)	10/10 (1.0)	10/10 (1.8)	10/10 (2.7)	10/10 (2.9)
Females	0/10	8/10 (1.0)	10/10 (1.0)	10/10 (2.1)	10/10 (2.4)	10/10 (1.0)

ppm: parts per million

Results presented as number of rats showing cytoplasmic alterations / total number of rats in the group, with average severity score in parentheses. The severity score is based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Source: Chan & Mahler (1992)

The NOAEL in the 13-week dietary toxicity study in rats was 6250 ppm (equal to 410 mg/kg bw per day) based on the more pronounced cellular alterations in the salivary glands at 12 500 ppm and above (Chan & Mahler, 1992).

In a 90-day range-finding study, groups of 10 Sprague Dawley rats per sex were administered daily doses of glyphosate technical (purity 97.5%) at concentrations of 0, 2000, 6000 and 20 000 ppm

(equal 0, 125.2, 371.9 and 1262.1 mg/kg bw per day for males and 0, 156.3, 481.2 and 1686.5 mg/kg bw per day for females) in the diet. Blood was collected pretreatment and at termination to measure selected haematological and clinical chemistry parameters. At necropsy, selected organs were weighed. Histopathological examination was conducted on all tissues taken at necropsy.

Diets were homogeneously distributed and stable for at least 10 days. Analytical concentrations were within 10% of the nominal concentrations. No treatment-related effects was observed on mortality, body weights, body-weight gains, feed consumption, urine analysis, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights and macroscopic and microscopic examinations. The only obvious treatment-related clinical observations was diarrhoea seen in all 10 males and nine females in the 20 000 ppm treatment group.

The NOAEL in the 90-day toxicity study in rats was 6000 ppm (equal to 371.9 mg/kg bw per day) based on diarrhoea at the lowest-observed-adverse-effect level (LOAEL) of 20 000 ppm (Parker, 1993).

In a 13-week feeding study, groups of 12 Sprague Dawley rats per sex were administered daily dietary doses of glyphosate (purity 95.3 %) at concentrations of 0, 3000, 10 000 and 30 000 ppm (equal to 0, 168.4, 569 and 1735 mg/kg bw per day for males and 0, 195.2, 637 and 1892 mg/kg bw per day for females) in the diet.

There were no treatment-related mortalities or clinical signs of toxicity. At 30 000 ppm, body weights were slightly lower (by about −5 to −10% in males and −5% in females) than those in the control. The overall feed consumption by males and females was comparable to the control. No treatment-related ocular effects or changes in haematological and clinical chemistry parameters were observed. At 30 000 ppm, urine pH in males and females was significantly lower ($P < 0.01$) than that in the control. Urine protein was significantly decreased ($P < 0.05$) in males and showed a decreasing trend in females. In addition, females showed a significantly ($P < 0.05$) higher urine volume, but males showed a decreasing trend in urine volume compared with the controls. At 10 000 ppm, urine, pH and protein in males were lower than those in the controls. In females, no statistically significant changes were observed in any parameter. No statistically significant changes were observed in either sex at 3000 ppm.

At 30 000 ppm, both sexes showed significant ($P < 0.01$) increases in absolute and relative weights of the caecum (with contents). In addition, females in this highest-dose group also showed significant ($P < 0.05$) increases in relative weights of the brain and liver. At 10 000 ppm, the absolute and relative weight of the caecum showed a statistically significant ($P < 0.01$) increase in males and increasing trend in females. At 3000 ppm, there were no treatment-related abnormalities in either sex (Table 16).

Table 16. Caecum weights of rats administered glyphosate for 13 weeks

	Absolute and relative weight per dietary concentration of glyphosate			
	0 ppm	**3 000 ppm**	**10 000 ppm**	**30 000 ppm**
Males				
Absolute weight ± SD (mg)	2 823 ± 794	3 187 ± 609	3 383 ± 1 081 (11%)	5 854 ± 2 053**
Relative weight ± SD (%)	0.55 ± 0.16	0.62 ± 0.13	0.64 ± 0.20 (11%)	1.22 ± 0.41**
Females				
Absolute weight in mg ± SD	2 367 ± 582	2 586 ± 462	3 546 ± 959*	5 268 ± 1 189**
Relative weight ± SD (%)	0.79 ± 0.17	0.84 ± 0.17	1.22 ± 0.32*	1.92 ± 0.41**

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

Relative weight = (organ weight/body weight) × 100.

Results expressed as absolute weight or relative weight and, in parentheses, this weight as a percentage of that of controls for males only.

Source: Kinoshita (1995)

At 30 000 ppm, 9 of the 12 males and 7 of the 12 females had statistically significantly distended caeca ($P = 0.01$). At 10 000 ppm, 3 of the 12 males showed distension of the caecum, but there were no macroscopic abnormalities in females. At 3000 ppm, there were no macroscopic abnormalities attributable to the treatment in either sex.

Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male at 10 000 ppm and one female at 30 000 ppm had renal lesions (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). However, these were considered of a genetic nature and not treatment related.

The NOAEL in this 90-day toxicity study in rats was 3000 ppm (equal to 168.4 mg/kg bw per day) based on increased caecum weight at 10 000 ppm and above (Kinoshita, 1995).

In a 90-day oral toxicity study, groups of 12 male and 12 female Alpk:AP Wistar-derived rats were fed diets containing glyphosate (purity 97.4%) at concentrations of 0, 1000, 5000 or 20 000 ppm (equal to mean intakes of 0, 81, 414 and 693 mg/kg bw per day for males and 90, 447 and 1821 mg/kg bw per day for females).

There were no mortalities. A low incidence of diarrhoea and light-coloured faeces was seen in both sexes at 20 000 ppm in the second week of the study. Males at the highest dose showed statistically significant reductions in body-weight gain and food utilization efficiency compared with controls. There was some evidence for a reduction in platelet count in males and females at 5000 and 20 000 ppm. A marginal dose-related increase in prothrombin time was observed in males at all doses. The differences, however, were small and considered not of haematological significance. Plasma alkaline phosphatase and alanine transaminase activities were increased in both sexes at 20 000 ppm and, to a lesser extent, in males at 5000 ppm. In addition, plasma aspartate aminotransferase activity was increased in females at the highest dose at this early time point, but not at study termination. The changes in clinical chemistry parameters were small, often lacking a clear dose–response relationship, and therefore not considered biologically relevant. There were no treatment-related effects on urine biochemistry and organ weights.

The only notable histopathological finding was a uterine leiomyosarcoma in a female at 5000 ppm. Although these are rare, finding such a tumour in an animal at the intermediate dose was considered incidental to treatment.

The NOAEL in the 90-day toxicity study in rats was 5000 ppm (equal to 414 mg/kg bw per day) based on the reduced growth in males at 20 000 ppm (Botham, 1996).

In a 90-day feeding study, groups of 10 Sprague Dawley rats per sex were administered daily doses of glyphosate (purity 95.3%) at concentrations of 0, 1000, 10 000 or 50 000 ppm (equal to 0, 79, 730 and 3706 mg/kg bw per day for males and 0, 90, 844 and 4188 mg/kg bw per day for females) in the diet.

There were no deaths. Animals of both sexes treated with 50 000 ppm had soft faeces and diarrhoea throughout the study period from day 4. Both sexes at 50 000 ppm showed a reduction in body-weight gain over the first 4 weeks of treatment. Body-weight development was unaffected at the other doses. Both males and females at 50 000 ppm showed a reduction in dietary intake and feed efficiency over the first 4 weeks of treatment compared with controls. Water consumption, measured ocular parameters or haematological parameters for either sex were unaffected. Both males and females at 10 000 or 50 000 ppm showed a statistically significant ($P < 0.05$ at 10 000 ppm and $P < 0.01$ at 50 000 ppm) reduction in plasma calcium concentration and an increase in alkaline phosphatase compared with controls. A statistically significant ($P < 0.05$) increase in inorganic phosphorus and reduction in plasma creatinine were also evident in males and females at 50 000 ppm, while females at this dose level showed statistically significant ($P < 0.01$) reductions in total plasma protein and albumin compared with controls. There were no other treatment-related effects. Both males and females at 50 000 ppm showed statistically significant increases in relative liver and kidney weights compared with controls (Table 17).

Table 17. Group mean relative organ-weights of rats administered glyphosate for 90 days

Dietary concentration of glyphosate (ppm)	Mean relative organ weight (%)			
	Liver		Kidney	
	Male	Female	Male	Female
0	2.974 9 ± 0.2629	2.973 4 ± 0.1558	0.586 1 ± 0.0575	0.651 6 ± 0.0523
1 000	2.886 8 ± 0.2552	2.909 3 ± 0.2146	0.590 1 ± 0.0804	0.6257 ± 0.0375
10 000	2.885 3 ± 0.3758	2.980 1 ± 0.1556	0.607 0 ± 0.0552	0.645 4 ± 0.0532
50 000	3.243 3 ± 0.2452*	3.198 9 ± 0.2098*	0.6963 ± 0.0436**	0.718 0 ± 0.0707*

ppm: parts per million; *: $P < 0.05$; **: $P < 0.001$

Results expressed as mean organ-weight as a percentage of mean body-weight, ± standard deviation.

Source: Coles et al. (1996)

At 50 000 ppm all animals had enlarged and fluid-filled caeca while one female had gaseous distension of the stomach at the final termination. There were no treatment-related macroscopic abnormalities at 10 000 or 1000 ppm.

Treatment-related changes were observed in the caeca. Atrophy, characterized by flattening of the intestinal mucosa, was observed in five rats of both sexes at 50 000 ppm ($P < 0.05$ for male rats) and for one male and two female rats at 10 000 ppm. The etiology of this change is uncertain and may represent no more than atrophy of the mucosa resulting from caecal distension. There were no other treatment-related changes.

The NOAEL in this 90-day toxicity study in rats was 1000 ppm (equal to 79 mg/kg bw per day) based on the reduced plasma calcium concentration and increased alkaline phosphatase concentrations at 10 000 ppm (Coles et al., 1996).

Dogs

In a 7-day oral toxicity study, one male and one female beagle dog were fed gelatin capsules containing glyphosate (purity 99.5%) at increasing daily doses of 100, 300 or 1000 mg/kg bw per day. A second pair of dogs were administered gelatin capsules containing glyphosate at a dose of 1000 mg/kg bw per day for 14 consecutive days.

In the first pair of dogs, no treatment-related clinical signs or effects on body weight, body-weight gain, feed consumption and haematological parameters were observed. There was a slight increase in plasma alanine transaminase activity in the male dog, and cholesterol concentrations were slightly reduced in both the male and female. At termination, there were no treatment-attributable lesions.

In the second pair of dogs, no treatment-related clinical signs or effects on body weight, body-weight gain, feed consumption and haematological parameters were observed. Plasma alanine transaminase activity was slightly increased in the male dog which also had loose faeces throughout the study. No treatment-attributable lesions were found at termination (Goburdhun & Oshodi, 1989).

Glyphosate technical (purity 94.61%) was continuously fed in the basal diet to groups of four males and four females beagle dogs for at least 90 days. Dietary concentrations were 0, 1600, 8000 and 40 000 ppm (equal to 0, 39.7, 198 and 1015 mg/kg bw per day for males and 0, 39.8, 201 and 1014 mg/kg bw per day for females).

There were no treatment-related effects on mortality, clinical signs, body weight, feed consumption, test material intake, ocular changes or macroscopic findings.

Although statistically significant changes in haematology parameters and in some clinical chemistry parameters were observed in both sexes, these were not dose dependent. At 40 000 ppm, three females showed a decrease in urine pH at week 13, although these differences were not statistically significant. Although a statistically significant increase was noted in the relative weight of the adrenals in females at 1600 ppm, the change was considered incidental due to the lack of dose dependency. There were no histopathological changes related to the treatment in the treated groups of either sex. One female in the 40 000 ppm group showed cutaneous histiocytoma which is a nonspecific lesion in young dogs.

The NOAEL in this 90-day toxicity study in dogs was 40 000 ppm, equal to 1015 mg/kg bw per day, the highest dose tested **(**Yoshida, 1996**)**.

Glyphosate acid (purity 99.1%) was administered at doses of 0, 2000, 10 000 or 50 000 ppm (equal to 0, 68, 323, 1680 mg/kg bw per day for males and 0, 68, 334, 1750 mg/kg bw per day for females) via the diet for 90 days to one control and three treatment groups each with four male and four female beagle dogs.

There was neither any mortality nor any treatment-related clinical signs of toxicity. The body-weight gain of males at the highest dose showed a slight depression throughout the study, but the differences were not statistically significant. Females at 50 000 ppm showed occasionally statistically significant slight depressions in body-weight gains throughout the study. No treatment-related ophthalmological and haematological findings or differences in urine clinical chemistry parameters and urinary sediment examinations were observed. Changes in clinical chemistry parameters were small and therefore not considered biologically relevant. Kidney weights of males at 10 000 or 50 000 ppm were slightly but not dose dependently increased. There was also a small increase in liver weight at these doses, but in male dogs only. No macroscopic or microscopic findings were observed.

The NOAEL in this 90-day toxicity study in dogs was 10 000 ppm (equal to 323 mg/kg bw per day) based on the decreased body-weight gains in female dogs at 50 000 ppm (Hodge, 1996).

In a 90-day feeding study, groups of four beagle dogs per sex were administered glyphosate technical (purity > 95%) at daily doses of 0, 200, 2000 and 10 000 ppm in the diet (corresponding to 0, 5.3, 53.5 and 252.6 mg/kg bw per day).

All the animals survived until scheduled necropsy. Neither clinical signs of toxicity nor treatment-related effects on body weights, urine analysis, organ weights, gross pathology or histopathology were observed.

A significant increase in clotting time and gamma-glutamyltransferase activity was observed in both sexes at the 45-day interim bleed; however, in the absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to reflect a systemic error rather than a real effect. Total bilirubin was higher; however, in the absence of a histopathological correlate on the liver, the effect was not considered adverse.

The NOAEL in this 90-day toxicity study in dogs was 10 000 ppm (equal to 252.6 mg/kg bw per day), the highest dose tested (Prakash, 1999).

In a 13-week oral toxicity study, groups of four beagle dogs per sex were administered glyphosate (purity 95.7%) in daily doses of 0, 30, 300 and 1000 mg/kg bw by capsule.

One male and one female at 1000 mg/kg bw per day were euthanized in extremis; one male that vomited once in week 7 (before dosing) and had liquid faeces frequently in weeks 8 and 9 was euthanized on day 61. One female was euthanized on day 72; this animal had frequent liquid or soft faeces from week 4, was seen to vomit in week 10, and was dehydrated from week 9.

No treatment-related clinical signs were noted in the control animals or those at 30 or 300 mg/kg bw per day. The following treatment-related clinical signs were reported in animals at 1000 mg/kg bw per day (excluding those terminated in extremis, which are discussed separately): liquid or soft faeces on several occasions in all animals; vomiting in two of the three surviving females within 30 minutes or 3–5 hours after treatment; thin appearance in one of the three surviving males and all the females; dehydration in one of the three males and two of the three females; pale ears and mouth in one of the three females.

At 30 or 300 mg/kg bw per day, there were no histopathological changes or changes in the mean body-weight gain. At 1000 mg/kg bw per day, mean body-weight gain in males was slight (+4% vs +31% in controls) while females lost weight (−7% vs +14% in controls) from day 1. This effect on body weight was considered treatment-related. Feed consumption was reduced to 25–75% of the amount given. Neither ophthalmological findings nor treatment-related effects on haematological and clinical chemistry parameters were observed in any of the treated groups. Urinalysis showed a decrease in mean specific gravity in one of the three remaining males and all three remaining females at the highest dose in week 11. Mean absolute and relative prostate weights were reduced by 68% and 56%, respectively, but there were no other treatment-related effects on organ weights. All the macroscopic changes noted in surviving animals at termination were considered normal variations, except for the reduced uterus size.

The treatment-related changes in surviving animals at 1000 mg/kg bw per day consisted of increased number of adipocytes in the sternum of two of the three males and the three females, prostate atrophy in two of the three males and uterine atrophy in two of the three females.

The NOAEL in this 90-day toxicity study in dogs was 300 mg/kg bw per day for mortality and decreased body-weight gains at 1000 mg/kg bw per day (Gaou, 2007). This study found very pronounced toxic effects, results which differ considerably from what was seen in other studies in dogs or other species.

In a 52-week oral toxicity study, groups of six male and six female beagle dogs were fed gelatin capsules containing glyphosate (purity 96.13%) at a dose of 0, 20, 100 or 500 mg/kg bw per day once daily.

All the dogs survived. There were no treatment-related effects on body or organ weights or feed consumption and no clinical signs of toxicity, ocular abnormalities or changes in haematological or urinary parameters or macroscopic and histological findings.

The NOAEL in this 1-year toxicity study in dogs was 500 mg/kg bw per day, the highest dose tested (Reyna & Ruecker, 1985).

In a 1-year oral toxicity study, groups of four male and four female beagle dogs were administered gelatin capsules containing glyphosate (purity 98.6–99.5%) at concentrations of 0, 30, 300 or 1000 mg/kg bw per day once daily for 52 weeks.

There were no mortalities throughout the test period. Changes in faecal consistency (soft/loose/liquid) were recorded frequently for the highest-dose animals, starting 4 to 6 hours after dosing; these were also noted on occasion in a few animals at 300 mg/kg bw and were considered to be treatment related. Feed consumption was maximal or near maximal for all test groups. Mean body-weight gain showed a non-statistically significant reduction in males at all doses (approximately 83%, 75% and 75% that of the control group for the lowest, intermediate and highest doses, respectively) and in females at the highest dose (81% that of the control group). Ophthalmoscopic and laboratory examinations revealed no treatment-related abnormalities. Plasma glyphosate concentrations, which remained constant throughout the study, suggested that absorption was dose related; mean values detected were 0.36, 1.82 and 6.08 μg/mL for the lowest, intermediate and highest doses, respectively. At necropsy, no abnormal gross findings and no significant intergroup organ-weight differences attributable to treatment with glyphosate were noted. In males, absolute and relative weights of the liver were slightly but nonsignificantly increased (4%, 8% and 10% above that of the control group for absolute weights, and 10%, 17% and 19% above that of the control group for relative weights for the groups for the lowest, intermediate and highest doses, respectively). There were no significant histopathological findings at any dose.

The NOAEL in this 52-week study in dogs was 300 mg/kg bw per day based on the changes in faecal consistency (Goburdhun, 1991).

In a 52-week oral toxicity study, groups of four male and four female beagle dogs were fed diets containing glyphosate (purity 95.6%) at concentrations of 0, 3000, 15 000 or 30 000 ppm (equal to 0, 91, 440 and 907 mg/kg bw per day for males and 0, 92, 448 and 926 mg/kg bw per day for females) for 1 year. Selected organs were weighed and specified tissues taken from all groups for histopathological examination.

There were no mortalities during the study. There was no effect on feed consumption; only three dogs left small amounts of feed intermittently during the study. Body weight was slightly reduced in females at 30 000 ppm, with a maximum reduction of 11% (compared with that of controls) in week 51. These dogs showed a gradual reduction in growth rate which was consistently significant from week 23 onwards. A similar change in body-weight gain in females at the lowest dose, although occasionally reaching statistical significance, was not regarded as treatment related since a dose–response relationship was lacking. There was no effect on body weight in males at any dose tested. There were no toxicologically significant effects on any of the haematological and clinical chemistry parameters measured or any of the clinical chemical parameters measured in urine. No adverse effects of glyphosate were seen at necropsy, and there were no treatment-related effects on organ weights. No histopathological changes attributable to administration of glyphosate were found.

The NOAEL in this 1-tear toxicity study was 15 000 ppm (equal to 448 mg/kg bw per day) based on the reduced body weights at 30 000 ppm in female dogs (Brammer, 1996).

In an 12-month oral toxicity study, groups of four male and four female beagle dogs were administered glyphosate technical (purity 94.61%) in the diet at concentrations of 0, 1600, 8000 or 50 000 ppm (equal to 0, 34.1, 182 and 1203 mg/kg bw per day for males and 0, 37.1, 184 and 1259 mg/kg bw per day for females, respectively) for 1 year. A detailed histopathological examination was performed on all sampled tissues of all dogs, except for the femur, larynx, oviducts, tongue, ureter and vagina.

There were no deaths in any dose groups of either sex. No treatment-related effects were observed during periodic clinical and eye examinations, in urine analysis, weight change and macroscopic and histopathological findings. At 50 000 ppm, three of the four males and four of the four females had loose stools. The animals in the 8000 and 1600 ppm groups did not show any clinical signs. At the end of the study, mean body weights at 50 000 were reduced by 6% in males and 11% in females compared to the controls, but these reductions were not statistically significant. Feed consumption was unaffected.

Males showed no significant changes in any haematological parameters. Females at 50 000 ppm had significantly decreased haematocrit, haemoglobin concentrations and erythrocyte count. However, these changes were small and often lacked a dose–response, and so were not considered biologically relevant.

Females at 50 000 ppm showed significant changes in clinical chemistry parameters. However, these changes were within biological variability ranges and therefore not considered adverse.

The NOAEL in this 12-month toxicity study was 8000 ppm (equal to 182 mg/kg bw per day based on the loose stools in both sexes and decreased body weights in females at 50 000 ppm (Nakashima, 1997).

In a 12-month oral toxicity study, groups of four beagle dogs per sex were administered 0, 30, 100 and 300 mg/kg bw per day glyphosate technical (purity 97.5%) daily in gelatin capsules. Dose formulations were prepared weekly by adding the required amount to the capsules.

No deaths occurred in any group. At the highest dose, all males and females had soft stools, diarrhoea or mucous faeces and, rarely, bloody stools or faeces visibly containing the test material as well as vomiting. At 100 mg/kg bw per day, changes were similar to those observed at 300 mg/kg but at lower frequencies. A histopathological examination of a mid-dose male with bloody faeces continually from day 346 onward showed an ulcer by intussusception. Changes observed at 30 mg/kg bw per day were comparable to those observed in untreated animals. A significant decrease in body weight compared to that of the control group was recorded from week 24 in females at 300 mg/kg ($P < 0.01$) and from week 27 in females at 30 mg/kg ($P < 0.05$) largely continually until the end of the administration period. There were no treatment-related effects in females at 100 mg/kg. There were no treatment-related changes in feed consumption, urine analysis, haematology, blood biochemistry, ophthalmoscopy, organ weights, necropsy or histopathology.

The NOAEL was 30 mg/kg bw per day based on the changes in faecal consistency in male and female dogs and reduced body weights in females at the LOAEL of 100 mg/kg bw per day group (Teramoto, 1998).

In a 1-year oral toxicity study, groups of four beagle dogs per sex were administered glyphosate technical (purity 95.7%) at daily doses of 0, 30, 125 and 500 mg/kg bw per day in gelatin capsules for 52 consecutive weeks.

No mortalities occurred during treatment. There were no treatment-related effects on clinical signs, body weight, feed consumptions, haematology and clinical chemistry parameters, ophthalmoscopic findings, organ weights, macroscopic or microscopic findings.

The NOAEL in this 1-year toxicity study in dogs was 500 mg/kg bw per day, the highest dose tested (Haag, 2008).

(b) Dermal application

Rats

In a 21-day dermal toxicity study, groups of five male and five female Alpk:AP_fSD rats were exposed to glyphosate (purity 95.6%) at 0, 250, 500 or 1000 mg/kg bw per day. The test material was moistened with deionized water and the resultant paste spread on the previously clipped back of each of the animals on a gauze patch that was covered with occlusive dressing. The application site was rinsed after 6 hours of exposure. A total of 15 six-hour applications were made over 21 days.

No treatment-related effects were noted on mortality, body or organ weights, body-weight gains, feed consumption, haematology, clinical chemistry parameters, macroscopic findings and histopathological findings at any doses.

The systemic toxicity NOAEL in this 21-day dermal toxicity study in rats was 1000 mg/kg bw per day, the highest dose tested (Pinto, 1996).

Rabbits

In a 21-day GLP-compliant dermal toxicity study, groups of 10 male and 10 female New Zealand White rabbits were exposed to glyphosate (purity not reported) at 0, 100, 1000 or 5000 mg/kg bw per day. The test material was moistened with physiological saline and applied onto the skin, which was then covered with a gauze patch secured with a tape. The material was applied on intact skin (5/sex per dose) and abraded skin (5/sex per dose) for 6 hours per day, 5 days per week, for 3 weeks. Physiological saline only was applied onto the control group.

There were no deaths and no clear effects on clinical condition. Slight dermal irritation was noted in both intact and abraded skin at 5000 mg/kg bw per day but not at milder doses or the control. No treatment-related effects were observed on body weights, body-weight gains, feed consumption, haematology and clinical chemistry parameters at any doses. At termination, no treatment-related macroscopic lesions were observed at the application site or in any other tissues or organs from all test groups. No treatment-related variations in organ-weight or histopathological findings were noted.

The systemic toxicity NOAEL in the 21-day dermal toxicity study in rabbits, was 5000 mg/kg bw per day, the highest dose tested (Johnson, 1982).

In a 28-day dermal toxicity study, groups of five male and five female New Zealand White rabbits were exposed to glyphosate (purity 99.6%) at 0, 500, 1000 or 2000 mg/kg bw per day. The test material was homogenized in water, placed on a gauze pad and then applied to the clipped area of rabbit skin. The pad was covered with a sheet of polyethylene material secured with tape. The test material covered approximately 10% of the body surface area.

No treatment-related effects were noted on mortality, body weights, body-weight gains, feed consumption, haematology, clinical chemistry parameters, macroscopic findings, organ weights and histopathological findings at any dose. Very slight erythema was observed in 2000 mg/kg bw per day dose group.

The systemic toxicity NOAEL in the 28-day dermal toxicity study in rabbits was 2000 mg/kg bw per day, the highest dose tested (Tornai, 1994).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an unpublished non-GLP carcinogenicity study, glyphosate (purity 99.7%) was administered in the diet to groups of 50 male and 50 female CD-1 mice per dose at concentrations of 0, 1000, 5000 or 30 000 ppm (equal to 0, 157, 814, 4841 mg/kg bw per day, respectively, for males and 0, 190, 955, and 5874 mg/kg bw per day, respectively, for females) for 24 months. Cage-side and detailed clinical observations were conducted and body weight and feed intake monitored throughout the study. Water consumption was measured during months 12 and 24. Erythrocyte, as well as total white blood cell counts and differentials, were conducted at months 12, 18 and 24. Tissues and organs were collected from all mice whether they died during the study or were terminated. Microscopic analyses were conducted on all collected tissues.

Analysis of treated diets demonstrated that glyphosate homogeneously mixed with rodent diet remained stable for the 1-week feeding period used in this study. Glyphosate test concentrations averaged approximately 95% of the target concentrations throughout the study. No treatment-related physical or behavioural signs of toxicity or mortality were observed. Yellow staining of the anogenital area, scabbing on the ears, alopecia, excessive lacrimation, displacement of the pupils and ocular opacities seen in all groups of male and female mice were not dose related; all occurred at low incidences. Body weights for both males and females at 30 000 ppm were consistently less than the controls throughout the study. Although the decreases were slight (1%–11%), several were statistically significant. Other statistically significant decreases were noted in the mid- and low-dose animals; however, these were sporadic and did not reflect a recognizable dose–response relationship. Although sporadic statistically significant effects were noted for feed consumption in treated male and female mice, none were dose or treatment related. Also, no treatment-related effects were observed for water consumption. No biologically or toxicologically relevant effects were noted on total erythrocyte or white blood cell counts, haemoglobin, haematocrit or platelet counts. No treatment-related changes were observed in absolute or relative organ weights. Several statistically significant changes in organ/body weight ratios were observed, but these were attributed to the statistically significant decreases in terminal (fasted) body weights rather than to specific organ effects. There were no dose–response relationships or any correlated gross or microscopic observations in any of the organs.

No remarkable treatment-related effects were noted at necropsy. Statistically significant positive trends were observed for central lobular hepatocyte hypertrophy, centrilobular hepatocyte necrosis (Table 18) and chronic interstitial nephritis in males, and for proximal tubule epithelial basophilia and hypertrophy in females. Statistically significant increases in the incidence of lesions were observed for centrilobular hepatocyte necrosis in high-dose males and proximal tubule epithelial basophilia and hypertrophy in high-dose females. While the incidences and/or dose–response trends of these individual microscopic kidney lesions were found to be statistically significant, they were considered part of a spectrum of lesions which, as a whole, constitute spontaneous renal disease.

Table 18. Hepatocellular lesions in mice administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Lesion		**0 ppm**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Centrilobular hypertrophy	M	9/49 [a]	5/50	3/50	17/50
	F	0/49	5/50	1/49	1/49
Centrilobular necrosis	M	0/49[b]	2/50	2/50	10/50 [a,b]

F: female; M: male; ppm: parts per million

Results presented as number of mice showing hypertrophy or necrosis / number of mice examined.

[a] Statistically significant linear trend ($P \leq 0.01$) using the Cochran–Armitage test.

[b] Statistically significant increase compared to control ($P \leq 0.01$) using the Chi squared test.

Source: Knezevich & Hogan (1983)

Neoplastic outcomes were of the type common in mice of this age and strain. Of the tumour types observed, bronchiolar-alveoli tumours of the lungs, hepatocellular neoplasms and tumours of the lymphoreticular system, none were dose related and all were seen in all treatment groups (Table 19). Lymphoreticular tumours were more frequently observed in female mice, but the incidences were low and did not approach statistical significance (nonsignificant trend and pair wise comparison). With the possible exception of kidney tumours (renal tubular adenomas) in males, all tumour types were considered spurious and unrelated to treatment (see Table 19).

Table 19. Neoplasia in male and female mice treated with glyphosate for 24 months

	Incidence per dietary concentration of glyphosate							
	Males				**Females**			
Site / Neoplasia	**0 ppm [a]**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**	**0 ppm [a]**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Lung								
Bronchiolar alveolar adenoma	5/48	9/50	9/50	9/50	10/49	9/50	10/49	1/50
Bronchiolar alveolar adenocarcinoma	4/48	3/50	2/50	1/50	1/49	3/50	4/49	4/50
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/48	4/50	3/50	1/50	–	–	–	–
Liver								
Hepatocellular adenocarcinoma	5/49	6/50	6/50	4/50	1/49	2/50	1/49	0/49
Hepatocellular carcinoma	0/49	0/50	0/50	2/50	2/49	1/50	0/49	4/49
Lymph node (mediastinal)								
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/45	2/49	1/41	2/49	–	–	–	–
Kidney								
Renal tubular adenoma	0/49	0/49	1/50	3/50	–	–	–	–
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49	3/49	2/50	2/50	–	–	–	–
Total lymphoreticular neoplasms (sum of lymphoblastic lymphosarcoma, composite lymphosarcoma and histiocytic sarcoma)	2/48	6/49	4/50	2/49	5/50	6/48	6/49	10/49

ppm: parts per million; PWG: Pathology Working Group

Results presented as number of neoplasm-bearing animals / number of animals examined.

[a] Incidence of effect in controls from the study report prior to PWG re-evaluation.

Source: (Knezevich and Hogan, 1983)

At the request of the USEPA, the Pathology Working Group (PWG) examined all sections of the kidneys from this study as well as additional renal sections. The PWG evaluation included a renal tubule adenoma in one control male mouse that was identified during a re-evaluation of the original renal section. The PWG noted that because differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and because both lesions are derived from the same cell type, it appropriate to combine the incidences for statistical analysis. Statistical analyses performed by the PWG are presented in Table 20. The PWG concluded that these lesions are not treatment-related based on the following considerations: 1) renal tubular-cell tumours are spontaneous lesions for which there is a paucity of historical control data for this mouse stock; 2) there was no statistical significance

in a pairwise comparison of treated groups with the controls and there was no evidence of a significant linear trend; 3) multiple renal tumours were not found in any animal; and 4) treatment-related nephrotoxic lesions, including pre-neoplastic changes, were not present in male mice in this study. In addition, there was no increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/regeneration, hyperplasia or hypertrophy). Although the incidence of tubular adenomas exceeded the testing laboratory's historical control range (0–3.3%), the increase at the high dose was not statistically significant compared to the concurrent controls. However, the re-analysis of the tumour indicated that kidney adenomas and kidney adenoma/carcinoma combined showed statistically significant positive trend.

Table 20. Results of re-examination of incidence of renal tumours in male mice treated with glyphosate for 24 months

	Incidence of renal tumours per dietary concentration of glyphosate			
Tumour type	**0 ppm**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Adenomas	1/49 (2%)	0/49 (0%)	0/50 (0%)	1/45 (2%)
	P = 0.442 2	*P* = 1.000 0	*P* = 1.000 00	*P* = 0.757 6
Carcinomas	0/49 (0%)	0/49 (0%)	1/50 (2%)	2/50 (4%)
	P = 0.063 5	*P* = 1.000 0	*P* = 0.505 1	*P* = 0.252 5
Combined	1/49 (2%)	0/49 (0%)	1/50 (2%)	3/50 (6%)
	P = 0.064 8	*P* = 1.000 0	*P* = 0.757 6	*P* = 0.316 3

ppm: parts per million

Results presented as the number of tumour-bearing animals / number of animals examined, with the resulting percentage in parentheses.

P values determined using the Cochran–Armitage test and Fisher Exact test.

Source: Knezevich & Hogan (1983)

The NOAEL for the systemic toxicity in the two-stage carcinogenicity study in mice was 5000 ppm (equal to 814 mg/kg bw per day) based on the slightly reduced body weights, increased centrilobular hepatocellular necrosis in high-dose males and proximal tubular epithelial basophilia in high-dose females seen at the systemic LOAEL of 30 000 ppm; equal to 4841 mg/kg bw per day for males and 5874 mg/kg bw per day for females (Knezevich & Hogan, 1983).

The present Meeting concluded that there is some indication, by trend test but not pairwise comparison, of induction of kidney adenomas in male mice.

In a 22-month carcinogenicity study, trimethylsulfonium carboxymethylamino-methylphosphonate (Company code SC-0224; glyphosate trimethylsulfonium; purity 56.17%) was administered in the diet to groups of 80 ICR(Crl:CD-1)BR mice per sex per dose at concentrations of 100, 1000 or 8000 ppm for 22 months (mean test material intake 11.7, 118 and 991 mg/kg bw per day for male mice and 16.0, 159 and 1341 mg/kg bw per day for female mice, respectively). One control group of 60 male and female mice were fed the basal diet only. An additional control group of 80 male and female mice were fed the basal diet plus 1% propylene glycol vehicle. Interim terminations of different numbers of mice occurred at 6, 12 and 18 months. The number of mice scheduled for the full 22-month study was 50/sex per dose. Blood samples were drawn from 10 fasted male and female mice per dose at 6, 12, 18 and 22 months for haematology and clinical chemistry measurements. At the same time points, brain cholinesterase concentrations from left and right sides of the brains of five mice/sex per dose were measured; urine analysis for 10 fasted mice/sex per dose was performed; and ophthalmoscopic examinations of all the mice were conducted. Macroscopic examinations of all the animals and histopathological examinations of selected tissues from all the animals were conducted. Selected organs were weighed.

The mean body weights of the highest-dose male mice were decreased by 3–11% and that of the highest-dose female mice were decreased by 4–17% during most of the study. Feed consumption was also slightly decreased in male and female mice at 8000 ppm. Survival of male mice was not affected by the treatment and the survival of female mice was apparently increased. There were no treatment-related effects on clinical signs, urine analysis, haematology and clinical chemistry parameters or ophthalmoscopic parameters at 6, 12, 18 or 22 months. Similarly, there were no treatment-related effects on organ weights (absolute or relative to body weight) and palpable masses. Analysis of the brain, erythrocytes and serum cholinesterase activity did not reveal any toxicologically significant differences. In female mice, the increased incidence of non-neoplastic epithelial hyperplasia of the duodenum at 8000 ppm was considered treatment related: the per cent response of hyperplasia in females was 10, 13, 16, 15 and 24% at 0, 100, 1000 and 8000 ppm, respectively. Male mice exhibited a treatment-related increased incidence of white matter degeneration in the lumbar region of the spinal cord at 8000 ppm. Increased white masses in male mice were 2%, 3%, 4% and 8% at 0, 100, 1000 and 8000 ppm, respectively. There were no treatment-related neoplastic lesions in male and female mice. In addition, there was no decrease in latency.

The systemic toxicity NOAEL in the 22-month carcinogenicity study in mice was 1000 ppm (equal to 118 mg/kg bw per day) based on the decreased body weights and feed consumption in both sexes and increased incidence of white matter degeneration in the lumbar region of the spinal cord in male mice and epithelial hyperplasia of the duodenum in female mice at 8000 ppm. There were no treatment-related neoplastic lesions in male and female mice (Pavkov & Turnier, 1987).

Groups of 25 male and 25 female Balb/c inbred albino mice (source not specified; 5–8 weeks old at the start of treatment) per dose were administered glyphosate technical (batch and purity not given) for 80 weeks at dietary levels of 0, 75, 150 and 300 ppm. The actual mean daily compound intake was not calculated.

Survival was not affected by treatment, and there were no overt clinical signs of toxicity. Body weight in high-dose male animals tended to decrease towards the end of treatment. In females, a similar trend was obvious from the beginning of the study up to week 21 at the highest and the mid-dose level; during the last 20 weeks, mean body weight was reduced again but only in females at the highest dose. Feed consumption was markedly diminished in high-dose males from week 9 onwards and in high-dose females from week 6. Haematology and clinical chemistry assessments showed no treatment-related changes after 9 months or after 18 months. Mean organ weights were not affected. Gross and histopathological examination did not provide evidence of treatment-related lesions. The incidence of neoplasia was not increased. The total number of tumours was considerably low in all groups.

The NOAEL for chronic toxicity in the 80-week study in mice was 150 ppm for body weight and feed consumption changes. When the usual conversion factor of 10 is applied, this value would correspond to a daily intake of 15 mg/kg bw. A no-observed-effect level could not be established because a weak effect on body weight in mid-dose females cannot be completely excluded. In contrast, the study author concluded that toxicological effects did not occur up to the highest dietary level of 300 ppm although the reduction in body weight and feed consumption was mentioned in the study report. It should be noticed that body weight and feed intake were not affected at much higher doses in the other available long-term studies in mice. Thus, it is not likely that these effects were actually related to treatment (Bhide, 1988).

The draft assessment report concluded that the study is unacceptable for a reliable assessment of carcinogenicity because the number of animals used was too small. In addition, the highest dose level of 300 ppm is considered too low. However, the study provides supplementary information about chronic toxicity.

In an 18-month non-GLP carcinogenicity study, glyphosate (purity unknown[7]) was administered to groups of 50 male and female CFLP/LATI mice (bred in a facility in Godollo, Hungary; 26–30 days old at study initiation) at dietary levels of 0, 100 or 300 ppm. The actual daily intake was not calculated. The administration period was 18 months.

The mortality rate was high in all study groups: only 11, 14 and 23 males and 14, 16 and 14 females survived to the scheduled termination and pathological examination in the control, low- and high-dose groups. Because clinical signs of toxicity were lacking and the mortality rates were not dose dependant, a treatment-related effect on survival is not likely. Body weight and feed consumption were not affected. Gross and histopathological examination did not reveal treatment-related changes. The overall tumour rate was high in all study groups including the controls. However, no significant difference in tumour incidence was observed between the groups.

There was no clear evidence of adverse effects of glyphosate administration up to the highest tested dose of 300 ppm (about 30 mg/kg bw per day), considered the no-observed-effect level in this study. However, the scientific value of this experiment is limited (Vereczkey & Csanyi, 1982, revised 1992).

The draft assessment report concluded that no conclusion could be reached due to the low quality of the report. The study is unacceptable as a reliable assessment of carcinogenicity because the number of animals surviving to scheduled termination and pathological examination was too small. In addition, the highest dose of 300 ppm was insufficient for evaluating carcinogenicity since no evidence of toxicity was obtained at that dose level. However, the study can be considered a source of supplementary information with regard to chronic toxicity.

In an unpublished carcinogenicity study, glyphosate (purity 97.5–100.2%) was administered to groups of 50 CD-1 mice/sex per dose in the diet at concentrations of 0, 100, 300 or 1000 mg/kg bw per day for 104 weeks. The dietary concentrations were adjusted weekly for the first 13 weeks and every 4 weeks thereafter. No interim terminations were conducted. Mortality, body weight, body-weight gain and feed consumption were monitored throughout the study. White blood cell differential counts were conducted during weeks 52, 77 and 102. Organs were weighed and tissues collected for microscopic analyses following pre-terminal deaths or at scheduled termination.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage acceptable. There were no unscheduled deaths attributable to the administration of glyphosate. No treatment-related clinical signs of toxicity or biologically relevant or toxicologically significant effects on body weight or body-weight gain were observed during the study. Although statistically significant effects were noted, none were treatment related although test groups' responses were typically higher than those in the corresponding control mice. No treatment-related effects were noted on feed or water consumption. Ophthalmoscopic examinations, urine analysis and clinical chemistry parameters were not evaluated. Intergroup differences in differential blood counts in either sex at any of the time points tested were unremarkable. The absolute and relative to body thymus weights of male mice in the 300 and 1000 mg/kg bw per day groups were statistically significantly increased, but the increase in thymus weights was slight and lacked a dose–response. No histological correlates were found. In addition, no increase in absolute or relative thymus weights were found in female mice. The incidence of lung masses was slightly increased in high-dose male mice (control: 10/50; low dose: 13/50; mid dose: 12/50; and high dose: 18/50); however, histopathology failed to reveal adverse lung findings. No increase in lung masses was found in female mice. The occurrence of mineral deposits in the brain was significantly increased in males at the highest dose compared with the control group (13/50 vs 4/49). It should be noted that this is a common finding in this strain of mice at this age.

[7] The relevant supplement was not submitted to the Meeting Rapporteur and the manufacturer's name was not provided.

There were no statistically significant increases in the incidence of any tumours, benign and malignant, in either sex; however, the number of animals with multiple tumour types was slightly increased in the high-dose group of both sexes (males: 16/50; females: 11/50) compared to the control (males: 11/50; females: 6/50). This led to a slight increase in the total number of tumours in the high-dose group of both sexes (males: 60; females: 43) compared to the control (males: 49; females: 36).

Haemangiosarcoma in the vascular system was evident in 4/50 high-dose males, 2/50 low-dose females and 1/50 high-dose females compared to 0/50 controls. Of the high-dose mice, one had tumours in the liver and spleen; one had a tumour in the liver only; one had tumours in the liver, spleen and prostate; and one had a tumour in the spleen only. The incidence of haemangiosarcoma in males was positive in Exact trend test and nonsignificant in pairwise comparison (Table 21). In female mice, incidence of haemangiosarcoma did not achieve statistical significance.

Table 21. Haemangiosarcomas in male mice administered glyphosate for 104 weeks

	Measure per dietary dose of glyphosate			
	0 mg/kg bw per day	**100 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Haemangiosarcomas	0/47 (0%)	0/46 (0%)	0/50 (0%)	4/45 (9%)
	P = 0.002 96**	P = 1.000 00	P = 1.000 00	P = 0.053 32

bw: body weight; **: significance of trend ($P < 0.01$) denoted at control, using Fisher Exact test and Exact Trend test.

Results presented as number of tumour-bearing animals / number of animals examined less those that died before week 52, with the resulting percentage in parentheses.

Source: Atkinson et al., 1993a

Histiocytic sarcoma in the lymphoreticular/haematopoietic tissue was evident in 2/50 low- and high-dose males and 3/50 low- and intermediate-dose females and 1/50 high-dose female (none were evident in the respective controls). Due to a lack of dose relationship and statistical significance, these changes are not considered treatment related. Other tumours seen were considered typical for mice of this age and strain.

The NOAEL for systemic toxicity in the 104-week carcinogenicity study in mice was 1000 mg/kg bw per day, the highest dose tested (Atkinson et al., 1993a).

In an 18-month carcinogenicity study, glyphosate (two lots of HR-001, purity 97.56% and 94.61%) was fed in the diet to groups of 50 male and 50 female ICR(Crj:CD-1)(SPF) mice at 0, 1600, 8000 or 40 000 ppm (equal to 0, 165, 838.1 or 4348 mg/kg bw per day for males and 0, 153.2, 786.8 or 4116 mg/kg bw per day for females) for 18 months. During treatment, all animals were observed for clinical signs and changes in body weight, and feed consumption was measured. At week 21, urine analysis was carried out on 20 males from all groups. Differential leukocyte counts were determined in blood smears from 10 males and 10 females from all groups at week 52 and after 78 weeks of treatment and also in animals terminated in extremis during the treatment, as possible. At final necropsy after 78 weeks of treatment, organ weights of 10 males and 10 females were analysed to determine differential leukocyte counts. All animals of both sexes were necropsied and their histopathology examined.

At 1600 ppm, there were no treatment-related changes in either sex in any parameters. At 8000 ppm, retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9 to 24. No treatment-related changes were seen in males. At 40 000 ppm, the incidence of pale skin increased in males. In addition, loose stools were found in all the cages from week 21 in males and week 20 in females. Retarded growth was persistently observed during treatment, with statistically significant differences in weight from week 16 to 36 in males and from week 6 to the end of the treatment in females. These changes were associated with depressed feed

consumption and feed efficiency. At necropsy, the increased incidences of distension of the caecum were noted in males and females in all the animals examined, which were consistent to increases in absolute and relative weights of the caecum. However, no histopathological abnormalities were recorded in the caecum. In males, a significant increase was noted for the overall incidence of anal prolapse that corresponded with erosion/ulcer of the anus.

The incidence of lymphoma was increased in the high-dose males but lacked a clear dose–response (see Table 22). It was significant by trend test and not by pairwise comparison. In female mice, the increased incidences of lymphoma were not statistically significant (trend test and pairwise comparison). The overall incidences of lymphomas observed were well below the historical control range of 0–18% (Baldrick & Reeve, 2007). Kidney adenomas and carcinomas in male mice were slightly increased at the high dose of 40 000 ppm. The statistical significance was achieved by the trend test and not by pairwise comparison. The incidences of kidney tumours in males exceeded the historical control range. Incidence of haemangiosarcomas was statistically significantly increased in the mid and high dose according to the trend test but not in a pairwise comparison.

Table 22. Selected neoplastic findings in male and female mice administered glyphosate for 18 months

	Incidence per dietary concentration of glyphosate			
Neoplastic findings	**0 ppm**	**1 600 ppm**	**8 000 ppm**	**40 000 ppm**
Males				
Lymphoma	2/50	2/50	0/50	6/50
Kidney (adenoma/carcinoma)	0/50	0/50	0/50	2/50
Haemangiosarcoma (various organs)	1/50	0/50	0/50	0/50
Females				
Lymphoma	6/50	4/50	8/50	7/50
Kidney (adenoma/carcinoma)	0/50	0/50	0/50	0/50
Haemangiosarcoma (various organs)	0/50	0/50	3/50	5/50

No.: number; ppm: parts per million

Results presented as number of tumour-bearing animals / number of animals examined.

Source: Sugimoto (1997)

Based on these results, the NOAEL was 1600 ppm (153.2 mg/kg bw per day) and the LOAEL was 8000 ppm (838.1 mg/kg bw per day) for females based upon retarded growth with statistically significant decreases in weight at week 6 and weeks 9 to 24 (Sugimoto, 1997).

In a 78-week carcinogenicity study, glyphosate (purity 97.5%) was fed to groups of 50 male and 50 female Crj:CD-1 mice per dose at dietary concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 67.6, 685 and 7470 mg/kg bw per day for males and 0, 93.2, 909 and 8690 mg/kg bw per day for females) for 78 weeks. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were conducted and body weight and feed intake monitored throughout the study. Differential white blood cell counts were performed at week 52, and haematological parameters evaluated at the end of the treatment. Gross pathological examinations were conducted at termination and on euthanized moribund and pre-terminally dead mice. Selected organs (brain, liver, both kidneys, both adrenal glands and both testes) were weighed. The tissue samples from control and high-dose animals and animals that died or were terminated in extremis were histopathologically examined.

Prepared diets were stable at room temperature for 4 months and the test material was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations ranged from 80–110% of the nominal concentrations. At 50 000 ppm, all the mice had loose stools throughout the treatment period, although some showed improvement as treatment continued. In the same group, nine males and eight females had treatment-related anus prolapse at week 10 or later. Other clinical signs and incidences were similar in both control and treated groups. A statistically significant difference in mortality rate in males was noted between the 50 000 ppm group and the control group at week 26 or later. Mortality in mid- and low-dose males and females at all doses was unaffected. At 50 000 ppm, body-weight gain significantly decreased or appeared to decrease throughout the treatment in males and at week 24 or later in females. No effects of treatment were observed in treated males and females in the mid and low dose at any time compared to controls. In both males and females at 50 000 ppm, feed consumption decreased compared with controls; the change was considered treatment related. No treatment-related changes were observed in haematology parameters. In the females at 50 000 ppm, the relative weights of kidneys (total) significantly increased. These changes were considered treatment related, though no corresponding histopathological findings were observed. In addition, decreases in the absolute weights of liver and right and left kidneys and significant increases in the relative weights of brain, left kidney, left adrenal gland, and right and left testes in males, and a decrease in the absolute weight of brain in females were noted at 50 000 ppm. The changes in the adrenal and brain were not considered adverse since they were not accompanied with histopathological findings. Macroscopic examination revealed luminal dilation of the large intestine, which may be associated with loose stool, in most of the terminated males and females at 50 000 ppm. Treatment-related non-neoplastic lesions were found in the kidneys in males and the rectums in males and females at 50 000 ppm. The renal findings included significant increases in tubular epithelial cell hypertrophy, tubular dilation, degeneration/necrosis and an increasing tendency in basophilic tubules proliferation (based on data from all animals). The rectal findings included significant increases in anus prolapse-associated erosion and luminal dilation (Table 23).

Table 23. Non-neoplastic lesions in mice administered glyphosate for 78 weeks

	Incidence per dietary concentration of glyphosate							
	Male				**Female**			
Non-neoplastic lesion	**0 ppm**	**500 ppm**	**5 000 ppm**	**50 000 ppm**	**0 ppm**	**500 ppm**	**5 000 ppm**	**50 000 ppm**
Kidney								
Tubular dilation	4/50	7/50	4/50	20**/50	8/50	12/50	5/50	8/50
Tubular epithelial cell hypertrophy	13/50	10/50	13/50	25*/50	13/50	17/50	14/50	13/50
Basophilic tubules	21/50	16/50	17/50	28/50	14/50	14/50	10/50	13/50
Tubular degeneration/necrosis	9/50	6/50	5/50	15/50	5/50	8/50	8/50	7/50
Rectum								
Luminal dilation	0/48	0/12	0/7	6*/46	0/44	0/11	0/10	6*/44
Erosion	0/48	0/12	0/7	3/46	0/44	0/11	0/10	6*/44

ppm: parts per million; *: $P < 0.05$, **: $P < 0.01$ (Fisher Exact test).

Results presented as number of tumour-bearing animals / number of animals examined.

Source: Takahashi (1999a)

Incidences of lymphomas in female mice were 3/50, 1/50, 4/50 and 6/50 in the control, 500, 5000 and 50 000 ppm dose group, respectively. The increased incidences of lymphoma at high doses were statistically significant in the trend test but not in a pairwise comparison. Renal cell adenoma was observed in three males and renal cell carcinoma in one male at 50 000 ppm; renal cell adenoma

was also observed in one male at 5000 ppm and none in any of the females (based on data from all animals). The incidence of other tumour types in glyphosate-treated groups and controls were similar.

These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel, Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. The haematoxylin-and-eosin–stained kidney sections prepared in the original study had faded and could not be evaluated; the paraffin-embedded blocks of 50 males from each group which had been stored for each observation period were sectioned and stained by haematoxylin and eosin for microscopic re-examination. The data from the re-examination and the original data are shown in Table 24.

Table 24. Renal tumours in male mice administered glyphosate for 78 weeks

Dietary concentration of glyphosate (ppm)	Findings	No. of cases		Incidence[a]
		Original study	Re-examination	
50 000	Renal cell adenoma	3	1	1/50 (2%)
	Renal cell carcinoma	1	1	1/50 (2%)
5 000	Renal cell adenoma	1	1	1/50 (2%)
500	Renal cell adenoma	0	1	1/50 (2%)

no.: number; ppm: parts per million

[a] Results presented as number of tumour-bearing animals / number of animals examined, with the resulting percentage in parentheses.

Source: Nippon Experimental Medical Research Institute (2012)

Upon re-examination (using Fisher Exact probability test, $P > 0.05$), the incidence of renal tumours in each treatment group no longer significantly differed from that in the control group. The historical control data for the Takahashi (1999a) study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13%) in males and 0/725 (0%) in females and for renal cell adenoma were 3/564 (0.53%) in males and 0/564 (0%) in females (Chandra & Frith, 1994; Baldrick & Reeve, 2007). The re-examination report also provides reference data: 0/55, 0/55, 1/55, 0/55 and 0/55 (0–1.8%) in males and 0/55 for all doses (0%) in females for renal cell carcinoma; and 0/55, 1/55, 1/55, 1/55, 0/55 (0–1.8%) in males and 0/55, 0/55, 0/55, 0/55, 1/55 (0–1.8%) in females for renal cell adenoma. The results of the re-examination revealed that the incidence of tubular epithelial cell hypertrophy in each treatment group did not significantly differ from that in the control group. In addition, the tubular epithelial cell hypertrophy was localized. These findings indicate no association between the tubular epithelial cell hypertrophy and the development of renal tumours.

In conclusion, the renal cell tumours observed in this study are not relevant for human risk assessment because (1) the incidence of renal tumours in males at 50 000 ppm did not significantly differ from that in the control group up on re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the highest dose (50 000 ppm) used in this study far exceeded the limit dose for mice (7000 ppm) specified by the Organisation for Economic Co-operation and Development (OECD) and USEPA.

The NOAEL in the 78-week carcinogenicity study in mice was 5000 ppm (equal to 685 mg/kg bw per day) for loose stools, decreased body-weight gain, decreased feed consumption and increased incidences of rectal and renal non-neoplastic lesions observed in male and female mice at the LOAEL of 50 000 ppm (equal to 7470 mg/kg bw per day), the highest dose tested (Takahashi, 1999a).

In an 18-month carcinogenicity study, glyphosate (purity > 95%) was fed to groups of HsdOla:MF1 Swiss Albino mice (50/sex per dose) in the diet at concentrations of 0, 100, 1000 or

10 000 ppm (equal to 0, 14.5, 149.7 and 1453 mg/kg bw per day for males and 0, 15.0, 151.2 and 1466.8 mg/kg bw per day for females) for 18 months. The stability, homogeneity and dietary concentrations were measured periodically. All the prepared diets were stable for 30 days. The test material was homogenously distributed; mean prepared dietary admixture concentrations were within 10% of the nominal concentration for all diet samples.

A detailed veterinary examination of all mice was conducted before and after grouping and monthly thereafter. Clinical signs of toxicity, appearance, behaviour and neurological changes and mortality of all mice were checked daily. Ophthalmological examinations of all mice occurred prior to the start of treatment and at 6, 12 and 18 months. Mortality, body weight, body-weight gain and feed consumption were monitored throughout the study. White blood cell differential counts were conducted at 9 months and at scheduled termination of all surviving animals and those terminated in extremis. All the animals that died or were terminated in extremis were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy. All the surviving mice were terminated at scheduled termination. A gross pathological examination was performed on all mice. Adrenals, kidneys, liver and gall bladder, ovaries and testes from 10 mice per sex per dose were weighed, and selected tissues from control and high-dose animals and those animals that died or were terminated in extremis histopathologically examined.

There were no treatment-related effects on clinical signs, body weights, body-weight gains, feed consumption, ophthalmoscopic examination or absolute and relative organ weights. The survival percentage was slightly decreased at the highest dose, but the decrease was not statistically significant and the mortality at 10 000 ppm remained within the historical control range. There were no significant treatment-related changes in the white blood cell counts for either sex at 9 or 18 months.

In mice found dead or terminated moribund, cystic glands of the stomach were significantly increased in high-dose males and for both sexes combined, but did not show dose dependency and were considered incidental. Observations at lower doses or findings that were not dose dependent included increased haematopoiesis in femurs of high-dose males and mid- and high-dose combined sex groups; increased cell debris in tubules of epididymides in mid-dose males; increased incidence of subcapsular cell hyperplasia in the adrenals of low-dose males; decreased incidence of kidney nephropathy in mid-dose females; and decreased incidence of lymphocyte infiltration of epididymides in mid-dose males. At termination, cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose–response relationship. Degenerative heart changes were higher in high-dose males and females, and significantly higher when sexes were combined, but the incidences were similar to the historical controls and the severity was not dose dependant. In mandibular lymph nodes, lymphoid hyperplasia was significantly increased in low- and mid-dose males and when sexes were combined, whereas the incidence was significantly lower in high-dose females. In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level when sexes were combined. Extramedullary haematopoiesis in the spleen was significantly increased in females and when the sexes were combined at the low-dose level. In the absence of any dose relation, these findings, as well as several statistically nonsignificant changes, were considered incidental.

The number of malignant lymphoma (Table 25) was slightly elevated in the high-dose group compared to controls. However, this haemolymphoreticular system tumour is one of the most common, accounting for the highest percentage of spontaneous tumours in mice, and the observed incidence is considered incidental and not treatment related. A statistically significant increase in malignant lymphoma was noted in both the male and female high-dose groups. Although malignant lymphoma are common in mice, accounting for 54.6% of all tumours in this study, that the higher incidence in the high-dose groups is treatment related cannot be excluded.

Table 25. Malignant lymphoma in glyphosate-treated mice

			Measure per dietary concentration of glyphosate							
			Males				Females			
	M	F	0 ppm	100 ppm	1 000 ppm	10 000 ppm	0 ppm	100 ppm	1 000 ppm	10 000 ppm
Dead and moribund mice										
No. examined	75	77	22	20	22	27	16	16	20	20
No. affected	20	49	9	12	13	13	9	10	13	12
Incidence (%) [a]	26.7	63.6	41.0	60.0*	59.0*	48.0	56.0	63.0	65.0	60.0
Terminated mice										
No. examined	175	173	28	30	28	23	34	34	30	30
No. affected	26	50	1	3	3	6*	9	10	6	13
Incidence (%) [a]	14.9	28.9	3.6	10.0	10.7	26.1*	26.5	29.4	20.0	43.3*
Mean percentage	14.9	28.8	–	–	–	–	–	–	–	–
Range of percentage	8–24	2–43	–	–	–	–	–	–	–	–
All fates										
No. examined	250	250	50	50	50	50	50	50	50	50
No. affected	46	99	10	15	16	19*	18	20	19	25
Incidence (%) [a]	18.4	39.6	20.0	30.0	32.0	38.0*	36.0	40.0	38.0	50.0*
Mean percentage	18.4	41.6	–	–	–	–	–	–	–	–
Range percentage	6–30	14–58	–	–	–	–	–	–	–	–

F: females; M: males; –: not examined/not determined; *: significant increase compared with historical controls (no *P* value provided)

[a] Incidence expressed as number of animals affected as a percentage of the number examined.

Source: Kumar (2001)

The increased incidences of kidney tumours at high doses (0/50, 0/50, 1/50 and 2/50 at 0, 100, 1000 and 10 000 ppm, respectively) were statistically significant in the trend test but not in a pairwise comparison. No historical control data were available.

The NOAEL for systemic toxicity in the 18-month carcinogenicity study in mice was 1000 ppm (equal to 149.7 mg/kg bw per day) for increased mortality at 10 000 ppm. Glyphosate was not carcinogenic in mice at doses up to 10 000 ppm, the highest dose tested (Kumar, 2001).

In a carcinogenicity study, glyphosate (purity 95.7%) was fed in the diet to groups of 51 male and 51 female CD-1 mice per dose at concentrations of 0, 500, 1500 and 5000 ppm (equal to 0, 71.4, 234.2 and 810 mg/kg bw per day for males and 0, 97.9, 299.5 and 1081.2 mg/kg bw per day for females) for 79 weeks. An additional 12 mice per sex, designated as veterinary controls, were housed and maintained alongside the treated animals. Ten animals per sex from each group were set aside for an interim termination (toxicity assessment) at week 39. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were conducted, and body weight and feed intake monitored throughout the study. Water consumption was observed daily. Blood smear samples were collected after 12 months and at termination from all animals and from mice terminated in extremis. Differential white blood cell counts were performed on all control and high-dose animals and on the animals terminated in extremis. Gross pathological examinations were conducted at termination and on moribund and pre-terminally dead mice. Selected

organs of 10 mice per sex per dose were weighed. Histopathological examination was performed on all sampled tissues from control and high-dose animals and on animals that died or were terminated in extremis.

Analyses indicated that the dose preparations were homogeneous and stable for at least six weeks and that the mean prepared dietary admixture concentrations were within 5% of the nominal concentration for all doses except one low-dose sample, which was over 10% of the nominal concentration.

There were no treatment-related effects on the number of mortalities observed and no significant differences in mortality rates during the study. No significant treatment-related clinical observations were reported. Similarly, no treatment-related effects on body weights, body-weight gains, absolute or relative organ weights, and feed and water consumption were observed. There were no significant differences in proportion of white blood cell populations of either sex at both 12 and 18 months, no trends in the proportion of palpable masses and no treatment-related macroscopic findings observed for any of the mice. There appears to be a dose-related increase in malignant lymphomas in the male mice only (0/51, 1/50, 2/51 and 5/51 at 0, 500, 1500 and 5000 ppm, respectively). The increased incidences at high doses were statistically significant in the trend test and not in a pairwise comparison; they are attributed to an unusually low incidence in the controls[8] (and presumably also for the low-dose treated mice). The observed increase appears to be well within the historical range and thus not biologically significant.

The NOAEL for carcinogenicity and systemic toxicity was 5000 ppm (equal to 810 mg/kg bw per day) in the 79-week study in mice, the highest dose tested (Wood et al., 2009a).

Roundup Original (glyphosate 41%, polyoxyethyleneamine [POEA] approximately equal to 15%) was evaluated in Swiss mice for tumour promotion via topical administration using a two-stage cancer model. In this study, a known tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and tumour initiator, 7,12-dimethylbenz[a]anthracene, were used. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (> twofold) on glyphosate, 7,12-dimethylbenz[a]anthracene and TPA application compared with the untreated control. Among them, nine proteins (translation elongation factor eEF-1 α chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3 and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. The study authors concluded that this glyphosate formulation has tumour-promoting potential in skin and that its mechanism seemed similar to that of TPA (George et al., 2010).

Rats

In a non-GLP combined chronic toxicity and carcinogenicity study, groups of Sprague Dawley rats (50/sex per dose) were fed diets containing glyphosate (purity 98.7%) at concentrations of 0, 30, 100 or 300 ppm for the first week. Concentrations were subsequently adjusted so actual doses of 0, 3.05, 10.30 and 31.49 mg/kg bw per day in males and 0, 3.37, 11.22, and 34.02 mg/kg bw per day in females were maintained for approximately 26 months. The diets were periodically analysed for stability, homogeneity and dietary concentrations. All the rats were observed twice daily for mortality and toxic signs. Body weights and feed consumption were determined at pretest, weekly for 14 weeks and biweekly thereafter. Water consumption was determined for 10 rats/sex per group for two separate 3-day periods at 18 and 24 months. Blood and urine samples were collected at 4, 8, 12, 18 and 24 months from 10 rats/sex per group. Selected haematological and clinical chemistry

[8] The historical control value for lymphomas in CD-1 mice from the testing facility, Harlan laboratory, in 2000–2010 ranged from 0–32% with a mean of 7.51% (letter from Wood E to Bond A, Regulatory Affairs Manager, Nufarm UK, Ltd., titled 'Historical incidences of malignant lymphoma in CD-1 mouse').

parameters were evaluated. Complete necropsies were performed on all rats that died or were terminated during or at the end of the study. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, testes/ovaries, pituitary, spleen and thyroid. The tissues were preserved for histopathology.

There was no significant difference in survival rate between the control and treated groups of both sexes, and survival was approximately 80–90% through month 20 of the study for all groups. No treatment-related clinical observations were reported in any of the treated groups. Although statistically significant differences in mean feed consumption were occasionally noted, these differences occurred sporadically and were not dose dependant. Water consumption of the treated and control groups were similar at the 18- and 24-month intervals. During the intermediate months, mean body weights of the treated animals were slightly lower than that of the controls. Maximum body-weight reductions for males ranged from 6% in the high-dose group to 2–3% in the low-dose group. For females these differences were statistically significant only during months 20 and 21 and were not dose related. From month 24 until study termination the mean body weights of all treated groups were comparable to the controls. Haematology, blood biochemistry and urine analysis parameters deviated occasionally and some of them differed significantly from controls, but these differences were not dose related and not consistent over time or between sexes. No statistically significant differences were noted in the absolute and relative organ weights of the treated groups compared to the controls. The few intergroup differences were neither dose related nor consistent. Lesions consisting primarily of inflammatory and structural changes that are common in rats of this strain in lifetime studies were similar in incidence and severity to control groups for both sexes. The most frequently observed changes occurred in the lungs and the kidneys, and were associated with chronic respiratory disease and chronic progressive nephropathy. Both these sites of lesions (lungs and kidneys) are a common age-related disease in this strain of rats.

A variety of neoplasms were found in both control and treated animals, the most common being common spontaneous neoplasms in the pituitary glands and in the mammary glands. Female rats showed an increased incidence of spleen and liver lymphoma combined (positive trend: 0/50, 0/50, 1/50 and 2/50 at 0, 30, 100 and 300 ppm respectively); however, the pair wise comparison was nonsignificant. Similarly, pancreatic islet cell tumours were observed in male rats with no clear dose–response relationship. The incidence of all tumour-bearing animals in the treated groups and the controls were similar (19–23% combined adenomas and carcinomas for males and 36–42% for females) and did not exhibit a dose–response relationship.

The pancreatic islet cell tumours were observed in male rats with no clear dose–response relationship. The Meeting concluded that the pancreatic islet cell adenoma and carcinoma were incidental for several reasons: the tumours occurred in only one study in males only; other studies that used appreciably higher doses did not find any excess tumours; there was no dose–response relationship; and incidences in controls was unusually low; the Meeting also noted that there was a negative dose–response relationship in females.

Although the incidence of interstitial cell tumours in the testes was increased in the treated animals (12% at the highest dose at termination), this was not considered relevant to human risk assessment based on the following weight-of-evidence considerations: 1) a monotonic dose–response relationship was lacking; 2) pre-neoplastic lesions (i.e. interstitial cell hyperplasia) were absent; 3) the incidences were within the normal biological variation seen for this tumour type in this strain of rats; 4) the incidences in the concurrent controls (0%) was not representative of the normal background incidences noted in the historical control animals; and 5) no interstitial cell tumours were seen when tested at much higher doses in the same strain of rats in an another study of glyphosate (Stout & Ruecker, 1990); and 6) due to major differences between rodents and humans with respect to prevalence of different testicular tumour types, hormonal physiology and response and risk factors for Leydig cell tumours, chemical induction of Leydig cell tumours in rats is generally considered of limited relevance to humans (Alison, Capen & Prentice, 1994; Clegg et al., 1997; Cook et al., 1999).

The NOAEL for systemic toxicity in rats after 26 months of dietary exposure to glyphosate was 31.5 mg/kg bw per day, the highest dose tested. It was concluded that the glyphosate was not carcinogenic in rats (Lankas, 1981).

In a 24-month combined chronic toxicity and carcinogenicity study, groups of Sprague Dawley rats were fed daily dietary doses of 0 (group 0, with 60 rats/sex, was fed basal diet with no vehicle, and group 1, with 80 rats/sex, was fed the basal diet plus the propylene glycol vehicle), 100 (group 2, with 80 rats/sex), 500 (group 3, with 80 rats/sex) and 1000 (group 4: 90 rats/sex) ppm of active ingredient (0, 178, 890 and 1779 ppm technical glyphosate trimesium [trimethylsulfonium carboxymethylamino-methylphosphonate, company code SC-0224]. Average doses for the 2-year treatment period, based on the nominal concentrations of active ingredient, were 4.2, 21.2 and 41.8 mg/kg bw per day for males and 5.4, 27.0 and 55.7 mg/kg bw per day for females.

Interim terminations of between 10 and 20 rats took place at 6, 12 and 18 months. All the surviving rats in all groups were terminated at 24 months.

The only indication of toxicity was a significant reduction in growth in both sexes in group 4 (1000 ppm). The test material at the doses tested did not cause dose-related effects involving survival, histopathological changes or any indications of carcinogenicity. Although various common tumour types were found in both sexes, the majority were pituitary and mammary gland adenomas and adrenal pheochromocytomas, which occurred at comparable incidences in the controls.

The NOAEL for systemic toxicity was 500 ppm in rats (equal to 21.2 mg/kg bw per day) based on the significant reduction in growth at 1000 ppm in both sexes. There was no evidence of carcinogenicity of glyphosate trimesium in rats in this study (Pavkov & Wyand, 1987).

In a 2-year combined chronic toxicity and carcinogenicity study, groups of Sprague Dawley rats (60/sex per dose) were fed diets containing glyphosate (purity 96.5%) at dietary concentrations of 0, 2000, 8000 or 20 000 ppm for 24 months (equal to 0, 89, 362 or 940 mg/kg bw per day for males and 0, 113, 457 or 1183 mg/kg bw per day for females). All animals were observed twice daily for mortality and moribundity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and feed consumption were determined each week for the first 13 weeks and then every fourth week thereafter. Ophthalmic examinations were performed at pretest and just prior to termination. Haematology, blood biochemistry and urine analysis tests were conducted on 10 animals per sex per dose at months 6, 12 (the interim termination), 18 and 24 (study termination). Ten animals per sex per dose were terminated at month 12, and all the survivors at month 24. All animals were given a complete gross necropsy. Brain, kidneys, liver and testes with epididymides were weighed. Approximately 40 tissues were preserved and examined microscopically.

Analyses indicated that the neat test material was stable throughout the study, that the homogeneity of the diet mixtures was adequate, and that average glyphosate concentrations were 95% of target levels for all dose groups. There were no statistically significant differences in group survival rates. At the end of the study, the percentages of animals surviving at 0, 2000, 8000, and 20 000 ppm were 29%, 38%, 34% and 34% for males, respectively, and 44%, 44%, 34% and 36% for females. Various clinical signs were noted throughout the study, but they were typical of those frequently observed in chronic studies and appeared to be randomly distributed in all groups. Statistically significant reductions in body weight were noted in high-dose females from week 7 through approximately month 20. During this time, absolute body weights gradually decreased to 14% below the control value. Body-weight gain in high-dose females was also consistently reduced compared to the controls. At the point of maximum body-weight depression (20 months), cumulative body-weight gain was 23% less than control. Body-weight gain in all treated male groups was comparable to controls. No statistically significant decreases in feed consumption in either sex took place at any time in the study; significant increases were noted frequently in high-dose males.

The ophthalmic examination prior to study termination revealed a statistically significant difference ($P < 0.05$) in the incidence of cataractous lens changes between control and high-dose males (0/15 vs 5/20). This incidence (25%) was within the range (0–33%) observed in previous studies of untreated male CD rats at this laboratory (Monsanto Agricultural Company, St. Louis, MO, USA). The incidences of cataractous lens changes in low- and mid-dose males, as well as all treated female groups, were comparable to their respective controls. An examination by an independent pathologist from Monsanto (Dr Rubin) also showed a statistically significant increase ($P < 0.05$) in cataractous lens changes in high-dose male animals (8/19 vs 1/14 for controls) and concluded that a treatment-related occurrence of lens changes affected high-dose males. Further histopathological re-evaluation of eyes by Experimental Pathology Laboratories Incorporation revealed cataract and/or lens fibre degeneration (Table 26). Because the number of rats ophthalmologically examined and affected at termination was small, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes appears to be exacerbated by treatment in high-dose males.

Table 26. Cataract and lens fibre degeneration in male rats administered dietary glyphosate for 24 months

	Incidence per dietary concentrations of glyphosate			
	0 ppm	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Terminal kill	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

ppm: parts per million

Results presented as number of rats affected / number of rats examined.

Source: Strout & Ruecker (1990)

While there were various changes in haematology and serum chemistry parameters, these were not consistently noted at more than one time point; were small and within historical control ranges; and/or did not occur in a dose-related manner and so were considered either unrelated to treatment or toxicologically insignificant. There was a statistically significant increase in urine specific gravity in high-dose males at 6 months and statistically significant reductions in urine pH in high-dose males at months 6, 18 and 24 months; this may have been due to the excretion of glyphosate, which is an acid. Statistically significant increases in liver-to-body weight ratio at 12 months and absolute liver weight and liver-to-brain weight ratio at 24 months occurred in males at 20 000 ppm. There were no other statistically significant changes in organ weights. Gross abnormalities seen at necropsy were not glyphosate related.

Histopathological examination revealed an increase in the number of mid-dose females with inflammation of the stomach squamous mucosa, the only statistically significant occurrence of non-neoplastic lesions. Although the incidence (15%) of this lesion in mid-dose females was slightly outside the laboratory historical control range (0–13.3%), there was no dose-related trend across all groups of treated females and no significant difference in any male group, leading to the conclusion that the finding was not treatment related (Table 27).

Table 27. Inflammation of the stomach squamous mucosa in rats administered glyphosate for 24 months

	Incidence per dietary concentrations of glyphosate			
	0 ppm	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Males	2/58	3/58	5/59	7/59
Females	0/59	3/60	9/60**	6/59

ppm: parts per million; **: $P \leq 0.01$ (Fisher Exact test with Bonferroni inequality)

Results presented as number of rats with the inflammation / number of rats examined.

Source: Strout & Ruecker (1990)

The only statistically significant difference in neoplastic lesions between control and treated animals was an increase in the number of low-dose males (14%) with pancreatic islet cell adenomas (Table 28). The historical (1983–1989) control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of reported studies revealed a prevalence of 0–17% in control males with several values greater than or equal to 8%. The incidences of islet cell adenomas did not follow a clear dose-related trend in the treated male groups as indicated by the lack of statistical significance in the Peto trend test, meaning that the distribution of incidences in the four groups was most likely random. There was also considerable intergroup variability in the numbers of females with this tumour (5/60, 1/60, 4/60 and 0/59 in the control, low-, mid- and high-dose groups, respectively) and no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a control male, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support a conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to glyphosate administration.

Table 28. Incidence of pancreatic islet cell findings in rats administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Finding	**Sex**	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Hyperplasia	M	2/58 (3%)	0/57 (0%)	4/60 (7%)	2/59 (3%)
	F	4/60 (7%)	1/60 (2%)	1/60 (2%)	0/59 (0%)
Adenoma	M	1/58 (2%)	8/57** (14%)	5/60 (8%)	7/59*** (12%)
	F	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)
Carcinoma	M	1/58 (2%)	0/57 (0%)	0/60 (0%)	0/59 (0%)
	F	0/60 (0%)[a]	0/60 (0%)	0/60 (0%)	0/59 (0%)
Adenoma + carcinoma (combined)	M	2/58 (3%)	8/57*** (14%)	5/60 (8%)	7/59 (12%)
	F	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)

ppm: parts per million; **: $P < 0.01$ (Fisher Exact test with Bonferroni inequality); ***: noted to be statistically significant but not analysed in the original report

Results presented as number of rats affected / number of rats examined with the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

There was a statistically significant trend for hepatocellular adenomas in males only, but a significant trend was not seen for adenomas and carcinomas combined ($P > 0.05$) (Table 29). These tumours were not considered to treatment related since 1) their incidences were within the testing facility's historical control range (1–18%); 2) pre-neoplastic lesions (i.e. cell hyperplasia or pre-neoplastic foci) were absent; and 3) there was no evidence of progression to malignancy (adenoma to carcinoma).

An increased incidence of thyroid C-cell adenomas was observed at 8000 and 20 000 ppm in both sexes but this did not reach statistical significance compared to the control animals (Table 29). There was a statistically significant dose trend for C-cell adenomas and adenomas/carcinomas combined in females. The testing laboratory historical control range for C-cell adenomas was 1.8–10.6% for males and 3.3–10% for females; the range for C-cell carcinomas was 0–5.2% for males and 0–2.9% for females. These tumours are not considered relevant to human risk assessment because 1) the increased incidences in males were not statistically significant; 2) there was no evidence of progression from adenoma to carcinoma; 3) and there were no dose-related increases in the incidence or severity of pre-neoplastic lesions (hyperplasia); and 4) they occurred in only one study.

Table 29. Thyroid C-cell tumours in male and female rats administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Finding	**Sex**	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Adenoma	M	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
	F	2/57 (4%)*	2/60 (3%)	6/59 (10%)	6/55 (11%)
Carcinoma	M	0/54 (0%)	2/55 (4%)	0/58 (0%)	1/58 (2%)
	F	0/57 (0%)	0/60 (0%)	1/59 (2%)	0/55 (0%)
Adenoma + carcinoma (combined)	M	2/54 (4%)	6/55 (11%)	8/58 (14%)	8/58 (14%)
	F	2/57 (4%)*	2/60 (3%)	7/59 (12%)	6/55 (11%)

F: females; M: males; ppm: parts per million; *: $P < 0.05$ (Cochran–Armitage Trend Test)

Results presented as number of rats affected / number of animals examined, excluding those that died or were terminated prior to study week 55, and the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

The incidence of benign keratoacanthoma was increased in male rats, but as there was no dose–response relationship, it was not considered treatment related (Table 30).

Table 30. Skin keratoacanthoma in male rats administered glyphosate for 24 months

	Incidence per dietary concentration of glyphosate			
Finding	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Benign keratoacanthoma (dead and moribund animals)	0/36 (0%)	1/31 (3%)	2/33 (6%)	1/32 (3%)
Benign keratoacanthoma (terminal kill)	0/13 (0%)	2/19 (11%)	2/17 (12%)	2/17 (12%)

ppm: parts per million

Results presented as number of rats with skin keratoacanthoma / number of rats assessed, with the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

Lymphoma/lymphosarcoma was observed in multiple tissues in male and female rats; however, the incidences in treatment groups were lower than in the controls and no dose relationship was observed.

The NOAEL for toxicity in rats was 8000 ppm (equal to 362 mg/kg bw per day) for decreased body-weight gains in females and cataractous lens changes in males seen at the LOAEL of 20 000 ppm (Strout & Ruecker, 1990).

In a combined 2-year chronic toxicity/carcinogenicity study, glyphosate (two batches, purity 98.9 and 98.7%) was fed in the diet to 85 Sprague Dawley rats/sex per dose for 104 weeks in amounts adjusted to deliver 0, 10, 100, 300 and 1000 mg/kg bw per day to both sexes throughout the study. Out of each group of 85 rats, 35 were designated for the toxicity portion of the study while the remainder was designated for the oncogenicity portion of the study. The animals were inspected twice daily for signs of toxicity and mortality. All were clinically examined, including palpitation for tissue masses, prior to the start of the study and weekly thereafter. The animals were weighed and feed consumption measured weekly during weeks 1–13 and once monthly thereafter. Water consumption was inspected throughout the treatment period. An ophthalmoscopic examination was carried out on 20 males and 20 females from each dose group in the oncogenicity study before treatment started and on 20 males and 20 females from the control and high-dose oncogenicity groups at weeks 25 and 51. In addition, all control and high-dose oncogenicity and toxicity study rats were examined at week 102. Blood was collected from the retro-orbital sinus of fasted animals for haematology and clinical chemistry while the animals were under light ether anaesthesia. Samples were obtained from 10 animals/sex per group in the toxicity study at weeks 14, 25, 51, 78 and 102. Urine samples were obtained from 10 animals/sex per group at weeks 14, 26 and 53 in the oncogenicity study and from 10 animals/sex per group at weeks 14, 25, 51, 78 and 102 in the toxicity study. After 52 weeks, 15 males and 15 females from each toxicity study group were terminated and necropsied; all the remaining study animals were terminated and necropsied after 104 weeks. All premature decedents were also necropsied. Selected organs were weighed from all interim kill animals and 10 males and 10 females terminated at the end of the oncogenicity study. All collected tissues from all decedents prior to week 52, those terminated at 52 weeks, and the control and high-dose animals terminated at the end of the study were examined microscopically. Only the salivary glands were examined on the decedents after 52 weeks and the rats from the other dose groups at final termination.

Light-coloured faeces were observed during weeks 16–104 in both sexes at the high dose and in low-mid and high-mid females; however, this sign was not considered toxicologically significant. There were no statistically significant differences in survival rates between each group receiving glyphosate and the control group, in either sex. No treatment-related effect was observed in feed consumption, water consumption and haematology, ophthalmoscopic examinations and gross pathology data. High-dose males had statistically lower mean body weight ($P < 0.01$) by 5–11% from week 2 until week 104; at termination, mean body weight was 10% lower (−14% weight gain). High-dose females had statistically lower body weight ($P \leq 0.05$) by 5–12% from week 20 through week 80 (with several exceptions); at termination, mean body weight was 8% lower (−11% weight gain). Statistically significantly increased alkaline phosphatase activities (+46% to +72%) were observed in high-dose males throughout the study except for week 51 when the mean value was 31% higher than control. Similarly, elevated alkaline phosphatase activities were observed in females at the high dose (+34% to +53%) throughout the study and through most of the study at the high-mid dose (by +20% to +67%, though not always statistically significant). These changes in the alkaline phosphatase activity are considered of little toxicological significance. Urine analysis data showed reduced pH (5.5–6) in males at the high dose throughout the study.

The absolute liver weight was statistically significantly decreased in females at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight, the difference was statistically nonsignificant at all three doses. In males, the absolute liver weight was decreased significantly at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight the difference was also not statistically significant. The parotid salivary-gland weight was increased significantly in males at 100, 300 and 1000 mg/kg bw per day (56–111%) after 52 weeks, but not after 104 weeks; the combined weight of the sublingual and submaxillary salivary glands was significantly increased by 13% (22% after correcting for body weight) at 1000 mg/kg bw per day after 52 weeks. In females, the parotid gland was not affected but the sublingual and submaxillary combined weight was significantly higher by about 15%. The changes in salivary-gland weights were accompanied by increased incidence of mild to severe parotid salivary gland cell alterations and slight to moderate mandibular salivary gland cell alterations in both sexes at week 52 and week 104. The lesions were described as cells and/or acini that appeared larger and stained in a weakly basophilic manner without showing a tendency towards proliferative or degenerative changes

over time. In males, the increased incidence and severity of lesions in the parotid gland were significant ($P < 0.01$) at all doses at 52 weeks and at high-mid and high doses at 104 weeks. The increased incidence of lesions in the mandibular gland was significant at high-mid and high doses at 52 weeks and significant ($P < 0.001$) at all doses at 104 weeks. In females, the increased incidence of parotid lesions was significant ($P = 0.001$)at high-mid and high doses at 52 weeks and at all doses at 104 weeks. The increased incidence in the mandibular gland lesions was significant at the high dose at both 52 and 104 weeks. The incidence and/or severity of kidney nephropathy decreased in males at all doses at 52 weeks and at the high dose at 104 weeks. Urothelial hyperplasia was significantly decreased in females from the high-dose group at both the 52-week and 104-week intervals.

Although all groups had neoplastic lesions, none proved to be treatment related when histopathology data from treated groups were compared to that of controls at 104-week termination.

In conclusion, the liver and the salivary glands were identified as the main target organs of glyphosate-related toxicity in the long-term study. At 100 mg/kg bw per day, the changes in salivary glands were only minimal in terms of severity and not considered toxicologically significant. The NOAEL in the 104-week study was 100 mg/kg bw per day in rats based on the more pronounced cellular alteration of salivary glands at 300 mg/kg bw per day and greater. There was no treatment-related increase in tumour incidence at doses up to 1000 mg/kg bw per day (Atkinson et al., 1993b).

In a dietary toxicity study in rats, groups of 24 male and 24 female Alpk:AP$_f$SD (Wistar-derived) rats were fed diets containing glyphosate (purity 95.6%) at concentrations of 0, 2000, 8000 or 20 000 ppm (equal to 0, 141, 560 and 1409 mg/kg bw per day for males and 0, 167, 671 and 1664 mg/kg bw per day for females) for 1 year. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. The animals were monitored daily for mortality and clinical signs. Body weights and feed consumption were measured at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination, and the rats were terminated and necropsied. Blood and urine samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

None of the pre-terminal deaths during the study could be attributed to the administration of glyphosate. Apart from a small increase in the number of high-dose male and female animals that showed wet or dry urinary staining, no treatment-related clinical changes were seen. In addition, there were no treatment-related ophthalmological findings. Body weights of high-dose animals were lower than concurrent controls throughout the study; body weights of animals at 8000 ppm were slightly reduced (but not significantly in males and significantly only from week 46 in females). There was no effect on body weight in animals on 2000 ppm glyphosate. The changes in body weights in males and females were not considered biologically significant since the magnitude of change was small (less than 10%).

Feed consumption was lower and feed utilization was slightly less efficient at 20 000 ppm, the reductions being most marked at the start of the study. There was a trend for reduced feed intake for females at 8000 ppm, which correlates with the reduction in body-weight gain at this dose in the latter stages of the study.

Some statistically significant differences in haematological parameters were seen between treated and control animals, but the differences were small and inconsistent across the various time points, and were considered unrelated to the administration of glyphosate. Deviations in some clinical chemistry parameters, such as reductions in plasma concentrations of cholesterol and triglycerides or a dose-related increase in plasma alkaline phosphatase activity throughout the study as well as occasional increases in the activities of plasma aspartate aminotransferase, alanine transaminase and creatine kinase, were mostly confined to high- and intermediate-dose groups. In the absence of any histopathological findings these marginal changes are not considered toxicologically significant.

There was no evidence of any effect of glyphosate on urine parameters. At necropsy, there were no treatment-related gross pathological findings or consistent organ-weight changes. An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland

were seen in both sexes at 20 000 ppm. At 8000 ppm, examples of focal parotid basophilia were of minimal severity and the incidence was slightly above that in the control animals. No other microscopic findings could be ascribed to administration of glyphosate.

Similar numbers and types of neoplasms were diagnosed in the control group and in the 20 000 ppm group, but the study was too short to be able to reach any conclusions about carcinogenicity.

The NOAEL for the increased incidence of basophilia of parotid acinar cells in the 1-year toxicity study in rats was 8000 ppm (equal to 560 mg/kg bw per day) based on the increased incidence of basophilia of parotid acinar cells at 20 000 ppm (Milburn, 1996).

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 96.8 and 90.0%, two batches) was fed in the diet to 50 Wistar rats per sex per dose for up to 2 years at concentrations of 0, 100, 1000 or 10 000 ppm (equal to 0, 6.3, 59.4 and 595.2 mg/kg bw per day for males and 0, 8.6, 88.5 and 886 mg/kg bw per day for females). In addition, one vehicle control (acetone) group with 10 rats per sex and one high-dose group with 20 rats per sex were included for interim termination at the twelfth month to study non-neoplastic histopathological changes. Veterinary examinations took place before and after grouping and at the end of each month of experimental schedule. Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Feed consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently over 1 week in every 4 weeks until termination. Individual blood samples were collected from 20 rats/sex per group at 3, 6, 12, 18 and 24 months. At scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex per group underwent clinical chemistry analysis. Individual urine samples were collected from 10 rats/sex per group at 3, 6, 12, 18 and 24 months. Histopathological examination was carried out on all tissues collected at interim termination of control and high-dose groups; on all pre-terminally dead and moribund terminated rats in the low- and mid-dose groups; and on all lesions of the terminated rats from the low- and mid-dose groups. Selected organs from 10 rats/sex per dose were weighed. The stability of glyphosate was determined at 2000 and 20 000 ppm which demonstrated that prepared diets were fairly stable for 30 days at room temperature with a degradation of less than 7% of the pure compound. The analysis of diets indicated that the achieved concentrations were within acceptable range. There were no treatment-related effects on mortality, clinical observations, body weights, body-weight gains, feed consumption, urine analysis and haematology. The following significant ($P < 0.05$) dose-related changes in blood chemistry parameters were seen at the high dose: decrease in gamma-glutamyltransferase levels at 12 months in male rats; a decrease in albumin levels at 6 months in female rats; and increases in alkaline phosphatase levels at 6, 12 and 18 months in female rats. The increase in alkaline phosphatase in high-dose females were 235, 231, 194, and 249 (U/L) at 6, 12, 18 and 24 months, respectively, while the corresponding control values were 133, 141, 101, 254 for females at 6, 12, 18 and 24 months, respectively.

Neither treatment-related macroscopic findings nor changes in organ weights or relative organ weights were observed during the study period. None of the significant microscopic changes or increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) showed dose relationships, indicating that they were incidental and not related to the treatment with the glyphosate. At terminal kill, the incidence of cataracts in males at 0, 100, 1000 and 10 000 ppm was 3/20, 3/20, 1/18 and 6/29, respectively, while in females it was 1/24, 1/26, 5/33 and 4/21, respectively. The historical data on neoplasm incidence for the test species indicates that the incidences of the various tumours observed are within the normal range. The types of tumours seen were also comparable to the historical records. No statistically significant intergroup difference between the control and low-, mid- and high-dose treatment groups was recorded in terms of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sex-wise or for combined sex.

The NOAEL in this combined chronic toxicity/carcinogenicity study in rats was 10 000 ppm, the highest dose tested, equal to 595.2 mg/kg bw per day. There was no evidence of carcinogenicity of glyphosate at doses up to 10 000 ppm in rats at in this study (Suresh, 1996).

In a combined chronic toxicity and carcinogenicity study, groups of 50 Sprague Dawley rats per sex were fed daily dietary doses of 0, 3000, 15 000 and 25 000 ppm (equal to 0, 180, 920 and 1920 mg/kg bw per day for males and 0, 240, 1130 and 2540 mg/kg bw per day for females) glyphosate technical for 2 years. In addition, 20 rats/sex per dose were included for interim termination in week 52 as part of the chronic toxicity study to study non-neoplastic histopathological changes; the dose levels were the same except the highest dose was 30 000 ppm. Test diets were prepared weekly by mixing appropriate amounts of the test material with the basal diet. The stability and homogeneity of the test material in feed was determined in an in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

No treatment-related clinical signs or deaths were observed in the 52-week chronic toxicity study. In the 104-week carcinogenicity study, male animals of the high-dose group exhibited slight but statistically insignificant higher mortalities. No significant toxic signs were observed in treated or control groups. Significantly reduced body-weight gain that lasted throughout the study was observed in high-dose males. In all other groups, body-weight gain at termination was comparable to the control. No treatment-related effects on feed consumption for either sex or any group were noted during the study. The results show a higher intake for females compared to males for each dose level. The mean intake in the chronic toxicity study was 0.18, 0.92 and 1.92 g/kg bw per day (males) and 0.24, 1.13 and 2.54 g/kg bw per day (females) for 3000, 15 000 and 30 000 ppm, respectively. The mean intake in the carcinogenicity study was 0.15, 0.78 and 1.29 g/kg bw per day (males) and 0.21, 1.06 and 1.74 g/kg bw per day (females) for 3000, 15 000 and 25 000 ppm, respectively.

Ophthalmological examinations revealed no abnormalities. Haematological examination showed no treatment-attributable abnormalities. A significant increase in the alkaline phosphatase level was only seen at 25 000 ppm in the carcinogenicity study at study termination. Other significant changes observed in haematological and biochemical parameters were within the range of the historical control data, indicating that they were of no biological significance. Urine analysis did not reveal any treatment-attributable abnormalities. No treatment-related macroscopic findings were observed during the study period.

Significant and dose-dependent effects were found in high-dose males and females in the chronic toxicity study. In males, weights of kidneys, brain and testes were increased; in females, in addition to increased weights of kidneys and brain, liver weight was also increased.

Histopathological changes were found at all dose levels including the control, indicating that these are no treatment-related effects. There were no treatment-related neoplasms observed.

Based on mild effects on body-weight gain and the increased organ weights without histopathological changes, the NOAEL in rats after chronic exposure to glyphosate technical for 24 months was 15 000 ppm (920 mg/kg bw per day) (Bhide, 1997).

In a 2-year combined chronic toxicity and carcinogenicity study, groups of 50 Sprague Dawley rats/sex per group were fed daily dietary doses of HR-001 at concentrations of 0, 3000, 10 000 or 30 000 ppm (equal to 0, 104, 354 and 1127 mg/kg bw per day for males and 0, 115, 393 and 1247 mg/kg bw per day for females) for 24 months. In addition, 30 rats per sex per group were included for interim termination at 26, 52 and 78 weeks.

At 3000 ppm, males exhibited significant increases in incidence of decreased spontaneous motor activity, bradypnea and soiled fur (predominantly in external genital area and foreleg) and a significant decrease in incidence of tactile hair loss. Females at 3000 ppm showed significant increases in incidence of ptosis and tactile hair loss. At 10 000 ppm, the incidence of tactile hair loss

was significantly decreased in males and significantly increased in females compared to their respective controls.

At 30 000 ppm, neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period, with statistical significance most weeks. In all other groups, mortality was comparable to the control. Males had significant increases in incidence of bradypnea, palpable masses and soiled fur (at the external genital or perianal region) compared to controls. Palpable masses in the tail were present in 27 males, a high incidence compared to 11 for the controls; the incidences of masses in other locations were comparable to the controls. Males at 30 000 ppm also showed significant decreases in incidence of tactile hair loss, incidence of wounds and hair loss. In females, a significant increase in incidence of wet fur, mainly in the external genital area, was observed. In addition, loose stools were observed in all cages from week 24 in males and week 23 in females until the end of the treatment.

There was an increase in benign keratoacanthoma in males at 24 months that was statistically significant in trend wise comparison but not in pair wise comparison (Table 31). However, skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats (Chandra, Riley & Johnson, 1992). Adenomas of the kidney were observed in four males in the 30 000 ppm grouped compared to zero in the controls. The background incidence of this tumour in this strain of rat is reported to be 0.7% (0–2.9%), and the incidence of the tumour in the 30 000 ppm group was only slightly higher than this background incidence. Because there was no statistically significant difference in incidence between the control and the 30 000 ppm group, the slightly higher incidence was not considered due to the treatment with glyphosate.

Table 31. Skin keratoacanthoma in male rats administered HR-001 for 24 months

	Incidence per dietary concentration of HR-001			
Finding	**0 ppm**	**3 000 ppm**	**10 000 ppm**	**30 000 ppm**
Benign keratoacanthoma (dead and moribund animals)	2/32 (6%)	1/30 (3%)	0/32 (0%)	1/21 (5%)
Benign keratoacanthoma (terminal kill)	1/18 (6%)	2/20 (10%)	0/18 (0%)	6/29 (21%)

ppm: parts per million

Results presented as number of male rats with skin keratoacanthoma / number assessed, with resulting percentage in parentheses.

Source: Enomoto (1997)

The NOAEL for chronic toxicity was 3000 ppm (104 mg/kg bw per day) and the LOAEL 10 000 ppm (354 mg/kg bw per day) based on an increase in ptosis and of tactile hair loss in female rats in 24-month study. There was an increased incidence of multiple clinical signs at 30 000 ppm (Enomoto, 1997).

In a combined chronic toxicity and carcinogenicity study, groups of Fischer F344/DuCrlCrlj rats (50/sex per dose) were fed diets containing glyphosate (purity 97.5%) at concentrations of 0, 500, 4000 or 32 000 ppm (equal to 0, 25, 201 and 1750 mg/kg bw per day for males and 0, 29.7, 239 and 2000 mg/kg bw per day for females) for 104 weeks. An interim termination was conducted on 14 rats per sex per dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet determined. Clinical observations (including ophthalmoscopy), body weights, feed consumption, haematology and clinical biochemistry (blood and urine) were measured throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment of the study. At the end of the scheduled period the animals were terminated and necropsied. Blood samples were taken for

clinical pathology, selected organs weighed and specified tissues prepared for subsequent histopathological examination.

Prepared diets were stable at room temperature for 4 months and the test material was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations ranged from 80–110% of the nominal concentrations. All males and females at 32 000 ppm had diarrhoea or soft stools from immediately after the start of administration and throughout the administration period. Mortality was not affected. Statistically significantly reduced body weights were observed throughout the study in high-dose males (beginning week 1) and females (beginning week 2). Feed consumption in all dosed group decreased or increased (no statistical significance) at various intervals. The only treatment-related effects observed in urine analysis were increased urinary proteins in three high-dose females at week 104. These changes were thought to be related to the histological changes in the kidney. There were no remarkable changes in females at any other dose or other examination time or in males at any dose. Males and females at 32 000 ppm showed statistically significant decreases or tendencies towards decreases in erythrocyte count, haematocrit and haemoglobin concentration in weeks 26, 52 and 78, and males in this group also showed significant increases in platelet count and leukocyte count in week 52 and a significant increase in platelet count in week 78. At 4000 ppm, females showed a significant decrease in erythrocyte count in week 26 (94% of the control value) and males showed significant decreases in erythrocyte count (96% of the control value) and haematocrit (95% of the control value) in week 52. In males and females at 500 ppm, there were no significant differences compared to the controls at 0 ppm in any examination parameter. The historical control values for haematological parameters from the performing laboratory were not available, however, and the historical control data for Fisher Inbred Strain F344/ DuCrlCrlj were used to compare with study results. Throughout the study, except at week 104, the control group had higher erythrocyte counts and haematocrit values than the range reported in the literature for this strain of rats. This suggests that erythrocyte and haematology values for the control groups of the TAC study were unusually high, and that statistically significant decreases in test groups may not be toxicologically significant or relevant. Males and females at 32 000 ppm showed a tendency towards a decrease in albumin at each examination time, and the values were statistically significant in males and females in week 26 and in males in week 78 compared to controls. In addition, males in this group showed significant increases in gamma-glutamyltransferase, alkaline phosphatase and total bilirubin in week 52. Otherwise the following changes were not observed continuously or at 32 000 ppm and were therefore considered unrelated to administration of the test material: significant decreases in creatinine, alanine transaminase [serum glutamic pyruvic transaminase] and total bilirubin in males or females in week 26 at 32 000 ppm and significant increases in creatinine, total protein and albumin in females at 500 ppm. Ophthalmoscopic examination indicated treatment-related opacity in one high-dose female at week 104 but was considered incidental. At 32 000 ppm, a statistically significant increase in relative kidney weights was observed in males after the scheduled termination in week 79 and in males and females at the scheduled termination in weeks 105–106. Otherwise, the following changes were recorded, but were thought to be due to suppressed body-weight gain as there were no corresponding abnormalities in histopathological examination: significant increases in the relative weights of the brain and liver in males at the week 79 and week 105–106 scheduled terminations and females in the week 105–106 scheduled termination; a significant decrease in the absolute weight of the adrenal in high-dose males in the week 105–106 scheduled termination; and a significant decrease in the absolute weight of the brain in mid-high (4000 ppm) males in the week 79 scheduled termination. High-dose males and females showed an increase in luminal dilatation of the large intestine at necropsy at the week 79 termination, but there were no histological changes. Thymic involution increased in all females at 32 000 and 500 ppm. However, these effects were thought to be incidental since they are age-related changes.

Histopathological examination showed an increase in glomerulosclerosis in females at 4000 ppm and 32 000 ppm during the scheduled necropsy at week 105–106 and increases in eosinophilic granule/hyaline droplets in the tubular epithelium in the kidney in females at the week 79 necropsy and in males and females at the week 105–106 scheduled necropsy. Monsanto and TAC co-sponsored the PWG to re-evaluate the microscopic kidney findings, specifically glomerulosclerosis, chronic

nephropathy and hyaline droplet renal tubule degeneration in female rats. The PWG concluded (Hardisty, 2013) that the kidneys of male and female rats did not confirm the study pathologist's reported conclusions that the incidence of glomerulosclerosis and the presence of eosinophilic granules/hyaline droplets of renal tubule epithelium were treatment related. The PWG found no histological evidence of renal toxicity in the sections of kidneys examined. The only frequently observed finding in the kidneys of male and female rats was chronic progressive nephropathy which, however, was similar in incidence and severity in control and treated groups. No treatment-related tumours were observed.

In conclusion, the NOAEL for chronic toxicity of glyphosate in rats was 4000 ppm (equal to 201 mg/kg bw per day) based on the decrease in body weights, transient haematological effects, diarrhoea, urine parameters, clinical chemistry effects, increased kidney weight relative weight seen at 32 000 ppm, the highest dose tested, in this 104-week study. Glyphosate was not carcinogenic in rats at doses up to 32 000 ppm (Takahashi, 1999b).

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 97.6%) was fed to 64 Alpk:AP$_f$SD Wistar-derived rats per sex per dose in the diet for up to 2 years at concentrations of 0, 2000, 6000 or 20 000 ppm (equal to 0, 121, 361 and 1214 mg/kg bw per day for males and 0, 145, 437 and 1498 mg/kg bw per day for females). An interim termination was conducted on 12 rats per sex per dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in the diet determined. Clinical observations (including ophthalmoscopy), body weights, feed consumption, haematology and clinical biochemistry (blood and urine) were conducted throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment part of the study. At the end of the scheduled study period, the animals were terminated and necropsied. Cardiac blood samples were taken for clinical pathology, selected organs weighed and specified tissues taken for subsequent histopathological examination.

The mean achieved concentrations of glyphosate in each dietary preparation were within 10% of the nominal concentration, and the overall mean concentrations were within 1% of nominal. The diets were homogenously distributed and prepared diets were stable at room temperature for 45 days. Survival in control, low- and mid-dose males approached 25% by week 104 of the study (criteria for termination of the study) although survival in the high-dose group was significantly better. Survival in the females was similar across all groups and better than in the lower-dose males. Treatment-related increase in the incidence of red-brown staining of tray papers (particularly in males) and isolated observations of red/brown coloured urine were noted in three males and one female at 20 000 ppm. The body weights of the high-dose rats were statistically significantly lower than controls throughout the study; however, these differences were not considered toxicologically relevant since maximum decreased in body weights were approximately 5% and 8% for males and females, respectively. Feed consumption and feed utilization were statistically significantly lower in high-dose males and females. Ophthalmoscopic examination did not reveal any treatment-related effects, and no treatment-related observations were noted in the functional observational battery, grip strength measurements, motor activity, landing foot splay measurements and time to tail flick. Haematological parameters were not affected by the treatment. Statistically significant increases in alkaline phosphatase activity occurred at all doses in both sexes up to week 79. There was evidence at one or more time points of increases in plasma alanine transaminase and aspartate aminotransferase activities and total bilirubin, but statistical significance was reached only at 6000 and 20 000 ppm. In the absence of any histopathological findings these marginal changes are not considered toxicologically significant. Plasma triglycerides and cholesterol were consistently decreased for all or part of the study in males at 20 000 ppm. Plasma creatinine values were lower in all treated female groups at week 27 and in females at 6000 and 20 000 ppm at week 14, but in the absence of any effects later in the study, this is considered not toxicologically significant. Urinary pH was lower than that of controls in high-dose males throughout the study. An increase in the incidence and severity of blood/red blood cells was seen in males and, to a lesser extent, in females at 20 000 ppm. There were no consistent, dose-related effects on organ weights indicative of a toxicologically significant effect of glyphosate.

Macroscopic findings consisting of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes were seen in males at 6000 and 20 000 ppm. A minor increase in the incidence but not the severity of proliferative cholangitis in the liver was observed at interim and terminal kills in high-dose males. Moreover, an increased incidence of hepatitis and periodontal inflammation was observed in high-dose males. There were a number of changes in the kidneys of high-dose males and females, notably renal papillary necrosis, with or without papillary mineralization, and transitional cell hyperplasia; the incidence was greater in males than females. These findings are considered treatment related but are consistent with ingesting high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation. The decrease in the incidence of tubular degeneration of the testis in high-dose males is considered of no consequence (Table 32). The incidence of prostatitis was higher than the control groups in all treated males but it was within historical background levels in all treated groups; however, as the control value in this study was low, the relationship to treatment at the high-dose level cannot be entirely dismissed.

Table 32. Selected microscopic findings in rats administered glyphosate for 2 years

	No. per dietary concentration of glyphosate							
	Males				**Females**			
Organ / Finding	**0 ppm**	**2 000 ppm**	**6 000 ppm**	**20 000 ppm**	**0 ppm**	**2 000 ppm**	**6 000 ppm**	**20 000 ppm**
Liver: Proliferative cholangitis	56	57	55	64	55	58	59	61
Liver: Hepatitis	8	6	9	13	6	7	4	6
Kidney: Papillary necrosis	0	1	0	14	0	1	2	5
Kidney: Transitional cell hyperplasia	2	3	0	5	3	1	0	1
Prostate: Prostatitis	13	22	23	37	–	–	–	–
Testis: Unilateral tubular degeneration	18	13	18	5				
Periodontal inflammation	25	27	23	42	18	24	32	28

no. number; ppm: parts per million

Results presented as number of rats with the finding. *N* = 64 for male and for female rats.

Source: Brammer (2001)

In contrast to a previously described 1-year feeding study in rats (Milburn, 1996), microscopic changes were seen in the liver and kidneys of high-dose rats but not the salivary glands, even though the study was conducted on the same strain of the rats and in the same laboratory.

The incidence of hepatocellular adenomas in male rats at the high dose increased compared to the controls (0/52 at 0 ppm, 2/52 [4%] at 2000 ppm, 0/52 [0%] at 6000 ppm and 5/52 [10%] at 20 000). However, this increase was considered incidental rather than treatment related, for the following reasons: 1) the absence of a dose–response relationship; 2) the lack of progression to malignancy; 3) no evidence of pre-neoplastic lesions; 4) the incidences were within the range (0–11.5%) of historical controls for this strain (Wistar) of rats in 26 studies conducted between 1984 and 2003 at the testing laboratory; and 5) the 0% incidence in the concurrent controls is lower than the average background incidence for liver adenomas in male Wistar rats, which distorts the comparison.

In conclusion, the NOAEL for chronic toxicity of glyphosate in rats was 6000 ppm (equal to 361 mg/kg bw per day) based on kidney, prostate and liver toxicity seen at 20 000 ppm (equal to 1214 mg/kg bw per day) in this 2-year study. There was no evidence of carcinogenicity in rats at glyphosate doses up to 20 000 ppm (Brammer, 2001).

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 95.7%) was fed to Han Crl:WI (GLx/BRL/HAN) IGS BR Wistar rats (51/sex per dose) in the diet for up to 104 weeks at concentrations of 0, 1500, 5000 or 15 000 ppm (equal to mean achieved doses of 0, 95.0, 316.9 and 1229.7 mg/kg bw per day). To ensure that a dose of 1000 mg/kg bw per day overall was received, the highest dose was progressively increased to 24 000 ppm. In addition, three satellite groups with 15 rats per sex each were included for interim termination at the twelfth month to study non-neoplastic histopathological changes. A satellite control group with 12 rats per sex served as veterinary control; these animals were to be used for investigations should any health problems have developed with the study animals. As no such problems occurred, observations of these animals have not been included in the report.

The prepared diets were stable for at least 6 weeks and their achieved dietary concentrations were within acceptable ranges.

Clinical signs, functional observations, body-weight changes and feed and water consumption were monitored throughout the study. Clinical chemistry and haematological examinations were performed on 10 animals per sex from the satellite and main groups at 3, 6 and 12 months. More haematological and clinical chemistry investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. Urine analysis of 10 animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months was conducted. All survivors at study termination (main groups: 104 weeks; satellite groups: 52 weeks) were necropsied as were all pre-terminal decedents or those terminated in extremis. Selected organs of 10 animals/sex per group terminated at the end of the study and all the animals from satellite groups were weighed. Histopathological examination was initially carried out on all tissues collected from control and high-dose groups; all pre-terminally dead and moribund euthanized rats and on all lesions and palpable masses of the terminated rats from the low- and mid-dose groups. Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

No significant treatment-related effects were observed on mortality, clinical signs, behavioural assessments, functional performance tests (motor activity, grip strength values), sensory reactivity, body weights, body-weight gains, feed consumption, water consumption, palpable masses, ophthalmoscopic examinations, haematology, clinical chemistry, urine analysis, organ weights and macroscopic findings.

Adipose infiltration of bone marrow was seen in the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, generally greater effects were seen in male rats at 15 000 ppm and this attained statistical significance for terminal kill animals, indicating the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the effect of other pathological conditions upon marrow cellularity in ageing rats, the effect – although not altogether convincing – cannot be dismissed as a similar effect was not seen in male rats in the remaining treatment groups. A higher incidence of higher grades of severity of adipose infiltration was seen in premature decedents of both sexes at 5000 ppm and females only at 1500 ppm. However, the variable duration of exposure and significant background pathology for pre-terminal decedents further negates this as an effect of treatment upon marrow cellularity for female rats.

At the highest dose, differences in the site of mineral deposition in the kidneys were significant compared with controls. Pelvic mineralization was commonly seen in both sexes and was more prevalent in female rats; however, corticomedullary mineralization was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time the incidence of renal pelvic hyperplasia was reduced in in both sexes as a consequence of the decreased mineral deposition. The effects on pelvic and corticomedullary mineralization as well as hyperplasia of the pelvic/papillary epithelium were confined to high-dose animals and there was no indication of a similar effect at any other treatment level for either sex.

Treatment did not affect the development of neoplasia in any organ or tissue or the overall frequency of benign or malignant tumours.

In conclusion, the NOAEL in rats after chronic exposure to glyphosate technical for 24 months was 15 000 ppm (equal to mean achieved dose level of 1229.7 mg/kg bw per day), the highest dose tested. Glyphosate was not carcinogenic in rats at doses up to and including 15 000 ppm, the highest dose tested (Wood et al., 2009b).

In a published drinking water study, ammonium salt of glyphosate (13.85% solution) was administered to groups of 85 male and 85 female Wistar–RIZ rats in drinking water at concentrations of 0, 300, 900 or 2700 mg/L for 2 years. Examination of peripheral blood parameters and bone marrow smears did not reveal any harmful effects. In addition, there was no treatment-related effects on the blood or urine biochemical parameters evaluated. The study authors concluded that glyphosate has no effect on neoplastic pathogenesis (Chruscielska et al., 2000a). The study report lacks detailed information on the formulated product or detailed description of the methodology, histopathological examination and tumour description.

In a published study, the health effects of a Roundup-tolerant NK603 genetically modified maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 parts per billion [ppb] of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in groups of 10 male and 10 female rats/dose. This study was used to evaluate the long-term toxicity and was not a carcinogenicity evaluation. The test material is a formulated product and the study report lacked details of the results (Séralini et al., 2014).

2.4 Genotoxicity

Glyphosate and its formulation products have been extensively tested for genotoxic effects using a variety of end-points in a wide range of organisms. These tests have ranged from standard, validated tests in bacteria and mammalian model organisms to less common and non-validated tests in phylogenetically distant species such as plants, earthworms, clams, frogs, tropical fish and caimans. In these studies, the test materials were administered through a variety of routes including parenteral routes used for specialized studies but considered largely irrelevant for assessing risks resulting from low-level dietary exposures. The reviewed studies for glyphosate are briefly summarized in the text and tables below (genotoxicity studies on AMPA, *N*-acetyl-glyphosate, *N*-acetyl-AMPA and other formulation ingredients are in Section 2.7, 2.8 and 2.9). Summary tables of studies conducted in non-traditional or phylogenetically distant organisms are shown in Appendix 1. In addition, a number of studies were conducted of humans exposed occupationally or environmentally to glyphosate and/or its formulation products. Many of these involved co-exposures to many different pesticides and were considered uninformative; however, the few studies that considered glyphosate the major agent are summarized and briefly discussed below.

A much smaller number of studies have been conducted on the glyphosate metabolite, AMPA, as well as the plant metabolites, *N*-acetyl-glyphosate and *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo studies (Table 35) investigated the ability of these metabolites to induce micronuclei in the bone marrow erythrocytes of mice and have largely been negative although a modest positive response was reported by Manas (2009b) when AMPA was administered in male mice by intraperitoneal injection. Studies by other investigators using the more relevant oral route of administration did not show an increase in micronuclei in either male or female mice.

In the in vitro studies, increases in mutation in bacteria were not seen for AMPA or the acetylated metabolites. Both positive and negative results were reported in studies of chromosome aberrations and DNA damage for AMPA. AMPA was negative in two studies of unscheduled DNA

synthesis in isolated rat hepatocytes. Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

(a) In vitro studies

Bacteria

Glyphosate or Roundup was used in approximately 40 studies of mutagenicity in bacteria. Most were conducted with and without metabolic activation (using S9, 9000 × *g* supernatant fraction from induced male rat liver homogenate). The actual number of tests performed was well over 150 as multiple tester strains with and without S9 were used in most studies. Glyphosate or Roundup was found to be negative for genotoxic effects in almost all of these; weak positive results were reported in only one or two studies. Glyphosate was also reported to be negative in three assays measuring DNA repair (rec) in *Bacillus subtilis* and positive in one SOS-chromotest assay in *Escherichia coli*. Several studies reported that glyphosate could enhance DNA strand breaks or interfere with DNA strand break repair in cyanobacteria following exposure to ultraviolet-B radiation.

In the case of AMPA or the acetylated metabolites, no increases in mutation in bacteria were seen in the in vitro studies (Table 33).

Table 33. Summary of in vitro genotoxicity studies with glyphosate, glyphosate formulations, AMPA or their metabolites in bacteria

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Point mutations	*Salmonella typhimurium* TA98, 100, 1535, 1537	0.1–1 000 µg/plate	Glyphosate (98.4%)	No	Negative	Negative	Kier (1978)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538	0.005–50 µL/plate	Glyphosate trimesium SC-0224 (19.2%)	Yes	Negative	Negative	Majeska (1982)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	10– 5 000 µg/plate	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	1.6–5 000 µg/plate	Glyphosate trimesium ICIA 0224	Yes	Negative	Negative	Callander (1988a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	313–5 000 µg/plate	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Negative	Yanagimoto (1991)
Point mutations	*S. typhimurium* TA98, 100, 1535 and 1537	160–5000 µg/plate	Glyphosate (98.6%)	Yes	Negative	Negative	Jensen (1991a)
Point mutations	*S. typhimurium* TA97, 98, 100, 1535	33–10 000 µg/plate	Glyphosate (98.6%)	No	Negative	Negative	Chan & Mahler (1992)
Point mutations	*S. typhimurium* strains TA98, 100, 1535, 1537	50–5 000 µg/plate	Rodeo (40% glyphosate)	Yes	Negative	Negative	Kier et al. (1992)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2, WP2 uvrA	100–5 000 µg/plate	Glyphosate trimesium TMSC (95%)	Yes	Negative	Negative	Callander (1993)
Point mutations	*S. typhimurium* TA98, TA100	180–1 440 µg/plate	Roundup	No	Weak positive / equivocal	Weak positive / equivocal	Rank et al. (1993)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537	156–5 000 µg/plate	HR-001 (95.7%)	Yes	Negative	Negative	Akanuma (1995a)
Point mutations	*S. typhimurium* strains TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	50–5 000 µg/plate	Glyphosate (95.3%)	Yes	Negative	Negative	Thompson (1996)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2,WP2 uvrA	100–5 000 µg/plate	Glyphosate (95.6%)	Yes	Negative	Negative	Callander (1996)
Point mutations	*S. typhimurium* TA97a, 98, 100, 1535	1–5 000 µg/plate	Glifos (360 g/L glyphosate)	No	Negative	Negative	Vargas (1996)
Point mutations	*S. typhimurium* TA97a, 98, 100, 102	0.025–0.3 µg/plate	Glyphosate formulation Perzocyd 10, soluble liquid concentrate	No	Negative	Negative	Chruscielska et al. (2000b)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	10–5000 µg/plate	Glyphosate technical (97%)	Yes	Negative	Negative	Schreib (2012)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	648–5000 µg/plate	Glyphosate technical Helm (98%)	Yes	Negative	Negative	Riberri do Val (2007)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (95.1%)	Yes	Negative	Negative	Sokolowski (2007a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (97.7%)	Yes	Negative	Negative	Sokolowski (2007b)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (95%)	Yes	Negative	Negative	Sokolowski (2007c)
Point mutations	*S. typhimurium* TA97a, 98, 100, 102, 1535	1–1000 µg/plate	Glyphosate TC (98%)	Yes	Negative	Negative	Miyaji (2008)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–3160 µg/plate	Glyphosate TC (97.5%)	Yes	Negative	Negative	Flügge (2009a)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2, WP2 uvrA	3–5000 µg/plate	Glyphosate (96.3%)	Yes	Negative	Negative	Sokolowski (2009)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–5000 µg/plate	Glyphosate (> 96%)	Yes	Negative	Negative	Donath (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–3160 µg/plate	Glyphosate TC (95.2%)	Yes	Negative	Negative	Flügge (2010)
Point mutations	*S. typhimurium* A98, 100, 1535, 1537; *E. coli* WP2 uvrA	31.6–5000 µg/plate	Glyphosate (96%)	Yes	Negative	Negative	Schreib (2010)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (> 95%) spiked with glyphosine (0.63%)	Yes	Negative	Negative	Sokolowski (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–5000 µg/plate	Glyphosate (> 95.8%)	Yes	Negative	Negative	Wallner (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	10–2000 µg/plate	Glyphosate (> 95.4%)	Yes	Negative	Negative	Donath (2011a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate (98.8%)	Yes	Negative	Negative	Donath (2011b)
Point mutations	*S. typhimurium* TA98, 100, 1535 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate (97.8%)	Yes	Negative	Negative	Donath (2011c)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	1.5–5000 µg/plate	Glyphosate (85.8%)	Yes	Negative	Negative	Thompson (2014)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate technical (94.1%)	Yes	Negative	Negative	Schreib (2015)
DNA damage	*B. subtilis* Rec assay H17 and M45	20–2 000 µg/disk	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
DNA damage	*B. subtilis* Rec assay H17 and M45	15–240 µg/disc	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Negative	Yanagimoto (1992b)
DNA damage	*B. subtilis* Rec assay H17 and M45	7.5–240 µg/disk	Glyphosate (95.7%)	Yes	Negative	Negative	Akanuma (1995b)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
DNA damage	*E. coli* SOS chromotest	0.1–0.25 µg	Roundup	No	Positive	N/A	Raipulis et al. (2009)
Enhanced UV-induced DNA strand breaks	Cyanobacteria (*Scytonema javanicum*)	10 µmol/L	Glyphosate	No	Positive	Negative	Wang et al. (2012)
Delayed UV–B-induced DNA strand break repair	Cyanobacteria (*Anabaena* sp.)	10 µmol/L	Glyphosate	No	Positive	N/A	Chen et al. (2012)
Delayed UV-B-induced DNA strand break repair	Cyanobacteria (*Microcystis viridis*)	10 µmol/L	Glyphosate	No	Positive	N/A	Chen et al. (2012)
DNA damage	Acellular prophage superhelical PM2 DNA	75 mmol/L	Glyphosate (98.4%)	No	Negative	N/A	Lueken et al. (2004)
AMPA							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	200–5 000 µg/plate	AMPA (99.3%)	Yes	Negative	Negative	Akanuma (1996)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	1.6–5 000 µg/plate	AMPA (> 99%)	Yes	Negative	Negative	Callander (1988b)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537	310–5 000 µg/plate	AMPA (99.2%)	Yes	Negative	Negative	Jensen (1993a)
***N*-Acetyl-AMPA**							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	50–5 000 µg/plate	*N*-acetyl-AMPA (76%; IN-EY252)	Yes	Negative	Negative	Wagner & Klug (2007)
***N*-Acetyl-glyphosate**							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	100–5 000 µg/plate	*N*-acetyl-glyphosate sodium salt (84.3%)	Yes	Negative	Negative	Mecchi (2004)

AMPA, aminomethylphosphonic acid; GLP: good laboratory practice; N/A: not applicable; S9: 9000 × *g* supernatant fraction from induced male rat liver homogenate; −S9: without metabolic activation; +S9: with metabolic activation; UV: ultraviolet

Mammalian cells

Glyphosate and its formulation products were tested for various types of genetic damage in mammalian cells in vitro (Table 34). The results are summarized as follows. Of the four in vitro studies of gene mutation in mammalian cells induced by glyphosate or its formulation products, no increases were reported. In contrast, nine of 10 studies investigating DNA strand breaks induced by glyphosate or Roundup in mammalian cells reported positive results, 4 of 11 studies of chromosome aberrations reported positive results. For two of these (Lioi et al., 1998a,b), the effects were seen at much lower concentrations than the other studies reporting negative results. Two studies reported

negative results for polyploidy. One study of the glyphosate formulation product Herbazed (Amer et al., 2006) reported an induction of chromosome aberrations in mouse splenocytes in vitro (see further discussion of Herbazed below). Five of eight studies of micronuclei were positive, two were negative and one was equivocal; three of the positive studies required S9 whereas two did not. Of the eight studies of sister chromatid exchanges induced in peripheral blood lymphocytes, seven were positive; four were in human peripheral blood lymphocytes, two were in bovine peripheral blood lymphocytes, and one was in mouse splenocytes. Both in vitro studies of unscheduled DNA synthesis in rat hepatocytes were negative.

AMPA was negative in two studies of unscheduled DNA synthesis in isolated rat hepatocytes (Bakke, 1991; Nesslany, 2002). Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

Table 34. Summary of in vitro genotoxicity studies with glyphosate, AMPA, metabolites of AMPA and formulants in mammalian cells

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Glyphosate							
Gene mutation (*HPRT*)	CHO cells	2–25 mg/mL	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	0.094–5 mg/mL	Glyphosate trimesium ICIA 0224 (57.6%)	Yes	Negative	Negative	Cross (1988)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	0.52–5 mg/mL	Glyphosate (98.6%)	Yes	Negative	Negative	Jensen (1991b)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	44–1 500 µg/mL	Glyphosate (95.6%)	Yes	Negative	Negative	Clay (1996)
Chromosomal aberrations	Mouse splenocytes	0.1–50 mmol/L	Herbazed (glyphosate, 84%)	No	Positive	N/A	Amer et al. (2006)
Chromosomal aberrations	CHO cells	4–10 µL/mL	Glyphosate trimesium SC-0224 (55.6%)	Yes	Negative	Negative	Majeska (1985)
Chromosomal aberrations	Chinese hamster cells (CHL/IU)	37.5–1 200 µg/mL	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Positive	Yanagimoto (1992a)
Chromosomal aberrations	Chinese hamster lung cells	62.5–1 000 µg/mL	HR-001 (95.7%)	Yes	Negative	Negative	Matsumoto (1995)
Chromosomal aberrations	Human peripheral blood lymphocytes	33–562 µg/mL	Glyfosaat	Yes	Negative	Negative	Van de Waart (1995)
Chromosomal aberrations	Chinese hamster lung cells	39–1250 µg/mL	Glyphosate (technical grade; 95.3%)	Yes	Negative	Negative	Wright (1996)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Chromosomal aberrations	Bovine lymphocytes	17–170 µmol/L	Glyphosate	No	Positive	N/A	Lioi et al. (1998a)
Chromosomal aberrations	Human peripheral blood lymphocytes	100–1250 µg/mL	Glyphosate (95.6%)	Yes	Negative	Negative	Fox (1998)
Chromosomal aberrations	Human peripheral blood lymphocytes	5–51 µmol/L	Glyphosate (≤ 98%)	No	Positive	N/A	Lioi et al. (1998b)
Chromosomal aberrations	Human peripheral blood lymphocytes	100–4 000 µg/mL	TMS Chloride (95%) [Glyphosate trimesium]	Yes	Equivocal	Equivocal	Griffiths & Mackay (1993)
Chromosomal aberrations	Human peripheral blood lymphocytes	0.2–6 mmol/L	Glyphosate (analytical grade; 96%)	No	Negative	N/A	Manas et al. (2009a)
Micronucleus	CHO K1 cells	5–100 µg/mL	Glyphosate	No	Negative	Positive	Roustan et al. (2014)
Micronucleus	Bovine lymphocytes	28–560 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Equivocal	N/A	Piesova (2004)
Micronucleus	Bovine lymphocytes	28–560 µg/mL	Glyphosate isopropylamine salt mixture (62%)	No	Equivocal	Negative	Piesova (2005)
Micronucleus	Bovine lymphocytes	28–1 120 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Negative	N/A	Sivikova et al. (2006)
Micronucleus	Human peripheral blood lymphocytes	0.5–580 µg/mL	Glyphosate (technical grade; 98%)	No	Negative	Positive	Mladinic et al. (2009)
Micronucleus	Human epithelial cancer cell line TR146	10–20 mg/L	Glyphosate (95%)	No	Positive	N/A	Koller et al. (2012)
Micronucleus	Human epithelial cancer cell line TR146	10–20 mg/L	Roundup	No	Positive	N/A	Koller et al. (2012)
Micronucleus	CHO K1 cells	5–100 µg/mL	Glyphosate	No	Negative	Positive	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM5757	75 mmol/L	Glyphosate (98.4%)	No	Negative alone; positive in presence of H_2O_2	N/A	Lueken et al. (2004)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
DNA strand breaks (Comet assay)	Human fibrosarcoma cell line HT1080	4.5–6.5 nmol/L	Glyphosate (technical grade)	No	Positive	N/A	Lopez et al. (2005)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM38	4.5–6.5 nmol/L	Glyphosate (technical grade)	No	Positive	N/A	Lopez et al. (2005)
DNA strand breaks (Comet assay)	Human liver HepG2 cell line	1–10 ppm	Roundup (R400)	No	Positive	N/A	Gasnier et al. (2009)
DNA strand breaks (Comet assay)	Human Hep2 cell line	3–7.5 mmol/L	Glyphosate (analytical grade; 96%)	No	Positive	N/A	Manas et al. (2009a)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	0.5–580 µg/mL	Glyphosate (technical grade; 98%)	No	Positive	Positive	Mladinic et al. (2009)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	10–2 000 mg/L	Glyphosate (95%)	No	Positive	N/A	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	10–2 000 mg/L	Roundup	No	Positive	N/A	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	N/A	Alvarez-Moya et al. (2014)
DNA strand breaks	Mouse spermatogonia	60–180 mg/L	Glyphosate		Positive	N/A	Ming et al. (2014)
Sister chromatid exchange	Mouse splenocytes	0.1–50 mmol/L	Herbazed (glyphosate, 84%)	No	Positive	N/A	Amer et al. (2006)
Sister chromatid exchange	CHO cells	4–10 µL/mL	Glyphosate trimesium SC-0224 (55.6%)	Yes	Negative	Negative	Majeska (1985)
Sister chromatid exchange	Bovine lymphocytes	28–1 120 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Positive	N/A	Sivikova et al. (2006)
Sister chromatid exchange	Bovine lymphocytes	17–170 µmol/L	Glyphosate	No	Positive	N/A	Lioi et al. (1998a)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.25–25 mg/mL	Roundup	No	Positive	N/A	Vigfusson & Vyse (1980)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.33–6 µg/mL	Glyphosate (analytical grade; 99.9%)	No	Positive	N/A	Bolognesi et al. (1997a)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Sister chromatid exchange	Human peripheral blood lymphocytes	0.1–0.33 µg/mL	Roundup (30.4% glyphosate)	No	Positive	N/A	Bolognesi et al. (1997a)
Sister chromatid exchange	Human peripheral blood lymphocytes	5–51 µmol/L	Glyphosate (≥ 98%)	No	Positive	N/A	Lioi et al. (1998b)
Unscheduled DNA synthesis	Rat hepatocytes	0.000 012 5–0.125 mg/mL	Glyphosate (98%)	No	Negative	N/A	Li & Long (1988)
Unscheduled DNA synthesis	Rat hepatocytes	0.2–111.7 mmol/L	Glyphosate (≥ 98%)	Yes	Negative	N/A	Rossberger (1994)
AMPA							
Gene mutation	Mouse lymphoma cells (L5871Y)	0.31–5.0 mg/mL	99.2%	Yes	Negative	Negative	Jensen (1993b)
Chromosomal aberrations	Human peripheral lymphocytes	0.9–1.8 mmol/L	99%	No	Weak positive	N/A	Manas et al. (2009b)
Micronucleus	CHO K1 cells	0.005–0.1 µg/L	AMPA (purity unspecified)	N/S	Positive	Positive	Roustan et al. (2014)
Micronucleus	CHO K1 cells	5–100	Glyphosate + AMPA	N/S	Negative	Negative	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human Hep2 cell line	2.5–7.5 mmol/L	99%	No	Positive	N/A	Manas et al. (2009b)
Unscheduled DNA synthesis	Rat hepatocytes	5–2 500 µg/mL	94.4%	N/S	Negative	N/A	Bakke (1991)
Unscheduled DNA synthesis	Rat hepatocytes	0.078–10 mmol/L	99.9%	N/S	Negative	N/A	Neslany (2002)
***N*-Acetyl-AMPA**							
Chromosomal aberrations	Human peripheral blood lymphocytes	191–1 530 µg/mL	76%; IN-EY252	Yes	Negative	Negative	Gudi & Rao (2007)
Gene mutation (*HPRT*)	CHO cells	100–1 531 µg/mL (active ingredient, adjusted for purity)	72%; IN-EY252	Yes	Negative	Negative	Glatt (2007)
***N*-Acetyl-glyphosate**							
Gene mutation (*HPRT*)	CHO cells	250–2 091 µg/mL (active ingredient, adjusted for purity)	*N*-acetyl-glyphosate sodium salt (63%)	Yes	Negative	Negative	Glatt (2006)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Chromosomal aberrations	CHO cells	960–2 800 µg/mL	*N*-acetyl-glyphosate sodium salt (84.3%)	Yes	Negative	N/A	Murli (2004)

AMPA: aminomethylphosphonic acid; CHO: Chinese hamster ovary; GLP: good laboratory practice; HepG2: hepatocellular carcinoma; Hep2: epidermoid cancer; HPRT: hypoxanthine-guanine phosphoribosyltransferase; N/A: not applicable; N/S: not stated; ppm: parts per million; S9: 9000 × *g* supernatant fraction from male rat liver homogenate; −S9: without metabolic activation; +S9: with metabolic activation; TK: thymidine kinase

(b) In vivo studies

Mammalian studies

Oral route

Thirty-three in vivo genotoxicity studies assessed the effect of orally administered glyphosate or its formulation products on rodents (29 in mice and four in rats). The end-points investigated included chromosomal alterations, micronuclei, sister chromatid exchanges, unscheduled DNA synthesis and dominant lethal mutations (Table 35). Fourteen of the studies were conducted using glyphosate (≥ 90% pure) with the remainder involving formulation products or less pure forms of glyphosate. The results were negative for 29 of the 33 studies. The majority of the studies were of good or acceptable quality, and included sponsored GLP studies conducted in compliance with OECD Guideline 474.

The four positive studies are briefly described here. A twofold statistically significant increase in micronucleus frequency was reported by Suresh (1993a) in female (but not male) mice treated with two 5000 mg/kg doses of glyphosate. (The JMPR committee noted that this dose exceeds the limit dose of 2000 mg/kg recommended by the OECD [2014] and the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [2011]. The micronucleus frequencies in the concurrent control were also higher than normal, and historical control frequencies for the lab were not provided. In addition, a study published the following year by the same group using the same doses of glyphosate did not see an increase in glyphosate-induced chromosome aberrations.] The three other positive studies were described in one article, a study published by Amer et al. (2006). In this article, positive results in both bone marrow cells and spermatocytes were reported after the administration of seven or more doses of a glyphosate formulation product called Herbazed (other positive results from that study are presented below). In contrast, in a repeated-dose study conducted by the United States National Toxicology Program (Chan & Mahler, 1992), increases in micronuclei were not seen in bone marrow erythrocytes of male and female mice administered glyphosate in the diet for 13 weeks. In another repeated-dose study, increases in chromosome aberrations were not seen in rat bone marrow cells harvested after 5 days of treatment with glyphosate trimesium (Matheson, 1982). Amer et al. (2006) also reported an increase in sister chromatid exchanges in mouse bone marrow cells after a single Herbazed dose.

Intraperitoneal injection

The JMPR committee concluded that genotoxic effects in animals treated with glyphosate or its formulation products by intraperitoneal injection were of limited value in assessing risks due to low-level dietary exposure. The following description of results is presented for completeness.

Twenty-one studies of micronuclei and chromosomal alterations were performed in the bone marrow cells of rodents administered glyphosate or its formulation products by intraperitoneal injection. Positive results were reported in approximately one third of the studies and negative/equivocal results for the remaining two thirds. The positive studies were reported in articles by four groups (Bolognesi et al., 1997; Prasad et al., 2009, Manas et al., 2009a; Rodrigues et al.,

2011) and involved the administration of both glyphosate and its formulation products. The Rodrigues et al. (2011) and Prasad et al. (2009) studies reported increases in micronuclei at doses (≥ 0.75 mg/kg bw and ≥ 25 mg/kg bw of Roundup, respectively) that were considerably lower than those reported as negative by other investigators (e.g. Jensen, 1991c [5000 mg/kg bw] and Kier, Flowers & Huffman, 1992 [850–3400 mg/kg bw]). When positive results were seen and when a direct comparison could be made, the formulation product was more potent than glyphosate itself (Bolognesi et al., 1997). Positive results in mouse spermatocytes were also reported with administration of 50 mg/kg bw of the glyphosate formulation product Herbazed for 5 days or more (but not 1 or 3 days) (Amer et al., 2006).

Increases in DNA strand breaks in the liver and kidney of mice were reported for both glyphosate and Roundup by Bolognesi et al. (1997). Heydens et al. (2008) conducted a follow-up study using the same Roundup formulation and reported that significant toxicity occurred in the liver and kidney when dosing was by intraperitoneal injection. They postulated that the DNA damage reported by Bolognesi et al. (1997) was likely a secondary effect of toxicity.

Bolognesi and colleagues (Peluso et al., 1998) also reported an increase in DNA adducts in mouse liver and kidney by the sensitive but nonspecific ^{32}P-postlabelling method following intraperitoneal administration of Roundup, but not glyphosate. They attributed the adducts to an unknown component of the herbicide mixture. This same group of investigators reported that intraperitoneal administration of glyphosate and Roundup resulted in an increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG) DNA adducts in the liver (glyphosate) and kidney (Roundup). A follow-up study on Roundup by Heydens et al. (2008) was unable to replicate the 8-OHdG adduct results.

Table 35. Summary of in vivo genotoxicity studies with glyphosate, glyphosate formulation products and AMPA and their metabolites in mammalian species

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Glyphosate						
Oral administration						
Dominant lethal test	Mouse fetuses and resorptions	200–2 000 mg/kg	Glyphosate (98.7%)	Yes	Negative	Rodwell (1980)
Chromosomal aberrations	Mouse bone marrow cells	50–5 000 mg/kg on 2 days	Glyphosate (96.8%)	Yes	Negative in males and females	Suresh (1994)
Chromosomal aberrations	Mouse bone marrow cells	1 080 mg/kg bw	Roundup (> 90% purity)	No	Negative in males	Dimitrov et al. (2006)
Chromosomal aberrations	Mouse bone marrow cells	50 and 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse spermatocytes	50 and 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Rat bone marrow cells	21–188 mg/kg	Glyphosate trimesium SC-0224 (58.5%)	No	Negative in males at all time points up to 5 days of exposure	Majeska (1982b)
Micronucleus	Mouse bone marrow erythrocytes	400–1 100 mg/kg	Glyphosate trimesium SC-0224 (55.3%)	Yes	Negative in males and females	Majeska (1986)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	3–50 mg/kg in the diet	Glyphosate (98.6%)	No	Negative in males and females	Chan & Mahler (1992)
Micronucleus	Mouse bone marrow erythrocytes	50–5 000 mg/kg bw; administered twice	Glyphosate (96.8%)	Yes	Negative for males; weak positive / equivocal for females at highest dose	Suresh (1993a)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw	Glyphosate (95.6%)	Yes	Negative in males and females	Fox & Mackay (1996)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate potassium salt (49% glyphosate acid by analysis) [indicated 59.3% in text]	Yes	Negative in males	Jones (1999)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78634 (65.2% glyphosate)	Yes	Negative in males	Erexson (2003)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	AK-01 Technical (99.1%)	Yes	Negative in males	Inoue (2004)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate technical (97.73%)	Yes	Negative in males and females	Honarvar (2005)
Micronucleus	Mouse bone marrow erythrocytes	1 080 mg/kg bw	Roundup (> 90% purity)	No	Negative in males	Dimitrov et al. (2006)
Micronucleus	Mouse bone marrow erythrocytes	8–30 mg/kg bw	Glyphosate technical Helm (≥ 95%)	Yes	Negative / equivocal in males	Zoriki Hosomi (2007)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate (99.1%)	Yes	Negative in males	Honarvar (2008)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 79864 (38.7% glyphosate)	Yes	Negative in males	Xu (2008a)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76171 (31.1% glyphosate)	Yes	Negative in males	Xu (2008b)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76313 (30.9% glyphosate)	Yes	Negative in males	Xu (2008c)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate (A17035A) (280 g/L)	Yes	Negative in males	Negro Silva (2009)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 79991 (71.6% glyphosate)	Yes	Negative in males	Xu (2009a)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76138 (38.5% glyphosate)	Yes	Negative in males	Xu (2009b)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78910 (30.3% glyphosate)	Yes	Negative in males	Xu (2010) [amended version of Erexson (2006)]
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	TROP M (Glyphosate 480) (358.4 g/L glyphosate acid; 483.6 g/L glyphosate isopropylamine salt)	Yes	Negative in males and females	Flügge (2010)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate soluble liquid concentrate (A13013Z) (500 g/L)	Yes	Negative in males	Negro Silva (2011)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78239 (36.6% glyphosate)	Yes	Negative in males	Xu (2011) [amended version of Erexson (2003)]
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate (96.3%)	Yes	Negative in males	Roth (2012)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate TGAI (98.9%)	Yes	Negative in males	Patel (2012)
Micronucleus	Rat bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate technical grade (98.8%)	Yes	Negative in males and females	Flügge (2009b)
Micronucleus	Rat bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate 75.5 DF (69.1% glyphosate)	Yes	Negative in males and females	Flügge (2010)
Unscheduled DNA synthesis	Rat liver hepatocytes	150–600 mg/kg bw	Glyphosate trimesium ICIA0224 (57.6%)	Yes	Negative in males	Kennelly (1990)
Sister chromatid exchange	Mouse bone marrow cells	50–200 mg/kg bw	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Intraperitoneal administration						
Chromosomal aberrations	Rat bone marrow cells	1 000 mg/kg bw	Glyphosate (98%)	No	Negative in males and females	Li & Long (1988)
Chromosomal aberrations	Mouse bone marrow cells	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse spermatocytes	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse bone marrow cells	25 and 50 mg/kg bw	Roundup (> 41%)	No	Positive in males	Prasad et al. (2009)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw	Glyphosate (98.6%)	Yes	Negative in males and females	Jensen (1991c)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	850–3 400 mg/kg bw	Rodeo formulation (40%)	Yes	Negative in males and females	Kier, Flowers & Huffman (1992)
Micronucleus	Mouse bone marrow erythrocytes	100–200 mg/kg bw	Glyphosate isopropylamine salt	No	Negative in combined males and females	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	133 and 200 mg/kg bw as glyphosate isopropylamine salt	Roundup (480 g/L)	No	Negative in combined males and females	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	68–206 mg/kg bw	Glifos (360 g/L glyphosate)	No	Negative in males and females	Zaccaria (1996)
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	450 mg/kg bw; 135 mg/kg as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	188–563 mg/kg bw	Glyphosate technical Nufarm (95%)	Yes	Negative in combined males and females	Carvalho Marques (1999)
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate technical grade	No	Negative in males	Chruscielska et al. (2000b)
Micronucleus	Mouse bone marrow erythrocytes	90 mg/kg bw	Glyphosate formulation Perzocyd 10 soluble liquid concentrate	No	Negative in males	Chruscielska et al. (2000b)
Micronucleus	Mouse bone marrow erythrocytes	50–200 mg/kg bw	Glyphosate (Roundup 69)	No	Negative (sex not specified)	Nascimento & Grisolia (2000)
Micronucleus	Mouse bone marrow erythrocytes	1 008–3 024 mg/kg bw	Glifosato IPA Technico Nufar; glyphosate isopropylamine salt (613 g/kg salt equivalent)	Yes	Negative in males and females	Gava (2000)
Micronucleus	Mouse bone marrow erythrocytes	50–200 mg/kg bw	Roundup (480 g/L)	No	Negative in combined males and females	Grisolia (2002)
Micronucleus	Mouse bone marrow erythrocytes	150–600 mg/kg bw	Glyphosate technical grade (95.7%)	Yes	Negative/equivocal in males	Durward (2006)
Micronucleus	Mouse bone marrow erythrocytes	15.6–62.5 mg/kg bw	Glyphosate technical grade (98%)	Yes	Negative in males and females	Costa (2008)
Micronucleus	Mouse bone marrow erythrocytes	25 and 50 mg/kg bw	Roundup (> 41%)	No	Positive in males	Prasad et al. (2009)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	100–400 mg/kg bw	Glyphosate (analytical grade; 96%)	No	Positive in combined males and females	Manas et al. (2009a)
Micronucleus	Mouse bone marrow erythrocytes	0.148–1.28 mg/kg bw	Roundup	No	Positive (sex not specified)	Rodrigues et al. (2011)
DNA strand breaks	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
DNA strand breaks	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
DNA adducts by ^{32}P-postlabelling	Liver and kidney of mice	130 and 270 mg/kg	Glyphosate isopropylammonium salt	No	Negative in combined males and females	Peluso et al. (1998)
DNA adducts by ^{32}P-postlabelling	Liver and kidney of mice	400–600 mg/kg	Roundup (30.4%)	No	Positive in combined males and females	Peluso et al. (1998)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	600 and 900 mg/kg bw	Glyphosate formulation (30.4%)	No	Negative in males	Heydens et al. (2008)
AMPA						
Micronucleus	Mouse bone marrow erythrocytes	100–1 000 mg/kg bw IP	AMPA (94.4%)	Yes	Negative in males and females	Kier & Stegeman (1993)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw oral route	AMPA (99.2%)	Yes	Negative in males and females	Jensen (1993c)
Micronucleus	Mouse bone marrow erythrocytes	200–400 mg/kg bw IP	AMPA (99%)	No	Positive	Manas et al. (2009b)
***N*-acetyl-AMPA**						
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw (active ingredient, adjusted for purity) oral route	*N*-acetyl-AMPA (72%; IN-EY252)	Yes	Negative in males and females	Donner (2007)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
***N*-Acetyl-glyphosate**						
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw (active ingredient, adjusted for purity) oral route	*N*-acetyl-glyphosate (63%; IN-MCX20)	Yes	Negative in males and females	Donner (2006)
Other related chemicals						
Chromosomal aberrations	Mouse bone marrow cells	10 and 100 mg/kg bw	Series of α-aminophosphonic acids	No	Positive	Naydenova et al. (2007)

AMPA: aminomethylphosphonic acid; bw: body weight; GLP: Good laboratory practice; IP: intraperitoneal; N/S: not stated; 8-OHdG: 8-hydroxy-2'-deoxyguanosine

(c) Non-traditional tests or tests in phylogenetically distant organisms

The results of genotoxicity studies in phylogenetically distant organisms or using non-traditional and generally non-validated assays are presented in Appendix 1. Studies were performed both in vitro and in vivo with most of the tests measuring DNA strand breakage or micronucleus formation. Approximately two thirds of these studies reported positive results. Mixed positive and negative results were seen in mutation studies in *Drosophila*. The reason for the differences in response between these species and those seen in mammals orally administered glyphosate is not known. Surfactants and other components of the glyphosate formulation products have been reported to be toxic to fish and other species, and this may contribute to the observed differences in test results (Howe et al., 2004; Guilherme et al., 2012a; Navarro & Martinez, 2014). For example, the surfactant polyoxyethylene amine, a common component in glyphosate formulations, was shown to induce several indices of toxicity in the neotropical fish *Prochilodus lineatus* at all of the doses tested (Navarro & Martinez, 2014).

(d) Human biomonitoring studies

The association between exposure to glyphosate and increase in micronucleus frequencies in peripheral blood lymphocytes, as well as the persistence of any effects over time, was evaluated over several months in individuals living in three areas of Colombia where glyphosate formulations were aerially sprayed over illicit and legal crops (Bolognesi et al., 2009). Significant increases in micronucleus frequencies were observed several days after spraying, but these increases did not correlate with glyphosate spray rates. Over time, the induced micronucleus frequencies decreased among the people in one area, remained the same among those in another, and increased among those in the third. In addition, in all three communities, the micronucleus frequencies of individuals who reported being directly exposed to glyphosate did not differ from those who reported no glyphosate exposure.

The JMPR committee reviewed the studies and considered the results to be inconclusive or equivocal. It noted that the micronucleus frequencies in the reference population were unusually low and that the frequencies within the glyphosate-exposed communities fall well within the normal range for non-exposed individuals (Bonassi et al., 2001). The results were considered to be inadequate to reach a conclusion on the potential chromosome-damaging properties of glyphosate in humans.

Another study used the Comet assay to determine the frequency of DNA strand breakage in the peripheral blood lymphocytes of individuals living in an Ecuadorian community within 3 kilometres of where glyphosate was sprayed. The frequency of DNA strand breakage was reported to be significantly higher than that of individuals living in a community 80 kilometres away where

glyphosate was not used (Paz-y-Mino et al., 2007). The samples were collected from exposed individuals 2 weeks to 2 months after the spraying had occurred. In reviewing the study, the JMPR committee noted that the study had some major deficiencies; the blood samples of the two groups were collected and processed at different times, a key consideration for an assay that is highly prone to technical artefacts during sample preparation. In addition, the two populations were located at considerable distance from each other, the background frequencies of DNA breakage in these communities was not known, and the median DNA migration values were identical for 20 of the 21 subjects in the control population, a result that was considered to be highly unusual.

The JMPR committee concluded that the study was inconclusive as problems with study design severely limit the conclusions that can be drawn.

In a follow-up study by the same authors, the frequency of structural chromosomal aberrations in peripheral blood lymphocytes was measured in the study population that two years previously had been exposed to glyphosate; the frequencies were found to be normal (Paz-y-Mino, 2011). The study results were considered to be negative but minimally informative as many types of chromosome alterations do not persist for extended periods of time.

In another study, the levels of 8-OHdG, a lesion formed from oxidative damage to DNA, were measured in the peripheral blood lymphocytes of workers spraying glyphosate (Koureas et al., 2014). A modestly elevated but statistically nonsignificant increase was reported.

Summaries of these biomonitoring studies are shown in Table 36.

Table 36. Summary of human biomonitoring studies

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Structural chromosomal aberrations	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Colombia	Glyphosate-containing mixture	No	Negative	Paz-y-Mino et al. (2011)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Narino, Colombia	Herbicide mixtures containing glyphosate and adjuvant	No	Equivocal/inconclusive	Bolognesi et al. (2009)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Putumayo, Colombia	Herbicide mixtures containing glyphosate and adjuvant	No	Equivocal / inconclusive	Bolognesi et al. (2009)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Valle del Cuaca, Colombia	Roundup 47	No	Equivocal / inconclusive	Bolognesi et al. (2009)
DNA strand breaks/Comet	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Colombia	Roundup Ultra (44%)	No	Equivocal/ inconclusive	Paz-y-Mino et al. (2007)
DNA adducts (8-OHdG)	Human peripheral blood cells	Pesticide applicators	Glyphosate	No	Negative	Koureas et al. (2014)

8-OHdG: 8-hydroxy-2'-deoxyguanosine

(e) Mechanistic considerations

Neither glyphosate nor its metabolites possess the chemical structural motifs commonly associated with mutagenesis or carcinogenesis (Ashby et al., 1989; Kier and Kirkland, 2013). However, one study investigating the effects of a series of α-aminophosphonic acids with structural similarities to glyphosate, reported moderate clastogenic activity in the mouse bone marrow chromosome aberration test when administered by intraperitoneal injection (Naydenova et al., 2007). In contrast, glyphosate bioassay results in 620 assays screening biological activity including cytotoxicity are reported in PubChem (accessed 20 April 2016). Positive results were seen only in 21 of the 620 assay reports, the majority of which appear to be closely related to glyphosate's herbicidal mechanism of action in plants. The few other positives involved protein-ligand binding and inhibition of the metabolic enzyme CYP71B1. These results indicate that, at the concentrations tested and at the end-points examined, glyphosate had few off-target molecular or cellular effects.

Summary:

The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg bw by the oral route, the route most relevant to human dietary exposure, was not associated with an increase in chromosome alterations or other types of genetic damage. The majority of the in vivo studies were conducted in rodents, a model considered physiologically relevant for assessing genotoxic risks to humans. The genotoxic effects reported to occur in vitro or in phylogenetically distant organisms have not been observed in vivo in appropriately treated mammalian models.

When administered by intraperitoneal injection, mixed, largely negative, results have been reported in studies of chromosomal damage of glyphosate, its formulation products and metabolites. Mixed, and somewhat contradictory, results have been reported in the few studies (all conducted by intraperitoneal injection) that have investigated DNA adducts induced by glyphosate or Roundup. Results obtained by this route of administration are considered to have limited relevance when estimating risks from human dietary exposure.

The positive results reported by Amer et al. (2006) using both oral and intraperitoneal routes of administration appear anomalous, and may have been due to impurities or other components within the Herbazed formulation product.

Biomonitoring studies of DNA and chromosomal alterations in humans conducted in five to six communities by several investigators found equivocal associations between glyphosate exposure and genetic damage.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a non-GLP three-generation reproduction study, glyphosate (purity 100%) was fed in the diet to 12 male and 24 female CD rats at concentrations of 0, 3, 10 or 30 mg/kg bw per day starting 63 days prior to mating. Each male was mated with two females. The first litters (F_{1A}, F_{2A}, and F_{3A}) from each mating were raised to weaning and then terminated. Second matings (F_{1B} and F_{2B}) were selected to become parents for subsequent generations or to undergo complete gross necropsy (F_{3B}). Tissues were also evaluated microscopically (10/sex/group) from the control and high-dose parental animals for all generations and F_{3B} offspring.

Analytical results demonstrated that glyphosate was stable and homogenously distributed in the diet. Analysis of various batches showed an average of 98.0 (± 6.8)% of the target concentration. No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or

duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

No adverse effects were noted in the study. The NOAEL for parental, reproductive and offspring toxicity was 30 mg/kg bw per day, the highest dose tested (Schroeder & Hogan, 1981).

In a two-generation reproduction study, glyphosate (purity 97.67%) was administered to Sprague Dawley rats (30/sex per dose) in the diet at concentrations of 0, 2000, 10 000 or 30 000 ppm (equal to 0, 132, 666 and 1983 mg/kg bw per day for males and 0, 160, 777 and 2322 mg/kg bw per day for females). After approximately 11 weeks of treatment, pairs of animals within each dose group were mated on a 1:1 basis to produce the F_1 litters. At weaning, 30 of these F_1 generation rats (referred to as F_{1A} in study report) per sex per dose were similarly exposed (approximately 14 weeks) and mated twice to produce F_{2A} and F_{2B} generations. On day 4 postpartum, litters were standardized (four males and four females when possible). Offspring not selected for mating, F_{2A} and F_{2B} pups, and adult females which had littered were terminated on or after day 21 of lactation. Adult males were terminated after mating. Organs were retained from all parental animals and one pup per sex per litter from F_{2A} and F_{2B}. Tissues from control and high-dose animals were examined microscopically.

The stability and homogeneity of glyphosate in the diet were acceptable. Analytical concentrations were, on the average, 95–96.7% of target levels. No treatment-related adverse effects were observed on mortality, feed consumption, organ weights or histopathological changes for parental animals of either generation. The incidence of soft stools was increased for high-dose adult animals in both generations (Table 37). Reduced body weights were noted in parental animals of both generations at termination: body weights were approximately 8–10% lower than controls for the F_0 generation and 10–13% lower than controls in the F_1 generation (Table 38).

No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Compared to the controls, there was a slight reduction in average litter size for F_0 dams in the highest dose group; an even smaller difference was noted after the first F_1 mating. However, the slight reduction in average litter size was not statistically significant. The F_{1a} adults were re-mated to produce the F_{2b} generation. There was no dose-related decrease in litter size in this second mating. Since the reductions in litter size were neither statistically significant nor consistently observed in all generations, the relationship to treatment could not be conclusively established. Therefore, it was concluded that litter size and viability were not affected by treatment.

No adverse effects were noted for offspring body weights or development. Statistically significant differences in pup body weights compared to controls were observed at mid and high dose, but these differences were small and within biologically variability.

Table 37. Soft stools in two successive generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 ppm	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
F_0 – males				
No. of animals	0	0	0	30/30
No. of occurrences	0	0	0	457
F_0 – females				
No. of animals	0	0	0	22/30
No. of occurrences	0	0	0	116
F_1 – males				
No. of animals	0	0	1/30	30/30
No. of occurrences	0	0	1/30	698
F_1 – females				
No. of animals	0	0	0	29/30

	Incidence per dietary concentration of glyphosate			
	0 ppm	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
Number of occurrences	0	0	0	537

ppm: parts per million; F_0: parental generation; F_1: first filial generation; No.: number

Results presented as number of animals with soft stools / number of animals examined.

Source: Reyna (1990)

Table 38. Terminal body weights in two successive generations of parental rats administered glyphosate

	Weight per dietary concentration of glyphosate			
	0	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
F_0				
Males	549.6 ± 46.8	550.2 ± 80.7	540.0 ± 58.1	503.5 ± 45.7 (↓8%)
Females	296.3 ± 23.6	290.6 ± 19.5	290.7 ± 25.4	265.9 ± 15.4 (↓10%)
F_1				
Males	625.0 ± 53.1	632.1 ± 74.6	591.0 ± 70.1	543.4 ± 58.1 (↓13%)
Females	316.2 ± 37.4	313.7 ± 30.5	312.4 ± 26.7	284.7 ± 18.4 (↓10%)

ppm: parts per million; F_0: parental generation; F_1: first filial generation; no.: number; ↓: decrease

Results presented as mean weight in grams ± standard deviations, with per cent change relative to controls in parentheses for the high-dose group only.

Source: Reyna (1990)

The NOAEL for parental toxicity was 10 000 ppm (equal to 666 mg/kg bw per day) based on decreased body weights and increased incidence of soft stools in rats at 30 000 ppm. As there were no effects on reproductive parameters or offspring measurements, the NOAEL for reproductive and offspring toxicity was 30 000 ppm (equal to 1983 mg/kg bw per day (Reyna, 1990).

In a two-generation reproduction study, groups of 28 male and 28 female Crl:CD(SD)BR VAF/Plus rats (aged 6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity 99.2%) at concentrations of 0, 1000, 3000 or 10 000 ppm (equal to 0, 66.4, 196.8 and 668.1 mg/kg bw per day for males and 0, 75.3, 226.0 and 752.3 mg/kg bw per day for females) for 70 days before their first mating and until termination. Each generation was mated twice, changing partners for the second mating and avoiding sister/brother matings throughout. On postnatal day 4, litters were standardized (four males and four females, when possible). The remaining pups and those not selected for mating were terminated and underwent gross pathological examinations. Treatment was continued for parental animals until day 21 of weaning of the second litter when animals were terminated for organ weighing, gross pathological examination and histopathological examination. Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analysed when an effect was seen in a tissue at the highest dose.

No treatment-related adverse effects on mortality, clinical signs, body weights, feed consumption, feed efficiency or organ weights were observed for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

Treatment-related histopathological changes were found in the parotid salivary gland of both sexes and submaxillary salivary gland of females in both generations (Table 39). The changes were

described as hypertrophy of acinar cells with prominent granular cytoplasm (minimal severity). Increased incidence of the effects was observed at the highest dose tested.

Table 39. Cellular alterations in salivary glands of two successive generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate							
	Males				Females			
Site of cellular alteration	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**
F_0								
Parotid gland	2/27	2/28	3/28	12/26	0/28	2/27	5/28	17/28
Submaxillary gland	0/27	–	–	0/26	0/28	1/27	4/28	14/28
F_1								
Parotid gland	1/24	0/24	4/23	11/23	0/24	0	4/24	9/23
Submaxillary gland	0/24	–	–	0	0/24	0	0/24	3/23

ppm: parts per million; F_0: parental generation; F_1: first filial generation; –: not examined.

Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analysed when an effect was seen at the highest dose.

Results presented as number of animals with hypertrophy of acinar cells with prominent granular cytoplasm / number of animals examined.

Source: Brooker et al. (1992)

The NOAEL for parental toxicity was 3000 ppm (equal to 196.8 mg/kg bw per day, based on increased incidence of histopathological effects observed in the parotid (males and females) and submaxillary (females only) salivary glands in both generations of rats at 10 000 ppm (equal to 668.1 mg/kg bw per day). As there were no effects on reproductive parameters or offspring measurements, the NOAEL for reproductive and offspring toxicity of glyphosate in rats is 10 000 ppm (equal to 668.1 mg/kg bw per day) (Brooker et al., 1992).

In a two-generation reproduction study, glyphosate (purity 96.8%) was administered to Wistar (30 rats/sex per dose) in the diet at concentrations of 0, 100, 1000 or 10 000 ppm (equivalent to 0, 6.6, 66.0 and 660 mg/kg bw per day) for two successive generations with one litter per generation. The mean daily intake of glyphosate was not reported for all dietary levels; however, the low dose of 100 ppm corresponds to an average of 7.7 mg/kg bw per day according to the original study report. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. On day 4 postpartum, litters were standardized (four males and four females, if possible). At weaning, 30 males and 30 females from each dose group were selected to produce the F_1 generation; these rats were dosed for at least 10 weeks and paired within their dose group to produce F_2 litters. All parental animals, non-selected pups from F_1 and all pups from F_2 were necropsied. Only parental tissue was collected.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

As no adverse effects were noted in the study, the NOAEL for parental, reproductive and offspring toxicity in rats was 10 000 ppm (equivalent to 660 mg/kg bw per day), the highest dose tested (Suresh, 1993b).

In a two-generation reproduction study, glyphosate (purity 94.61%) was administered to 24 Crl:CD(SD) rats/sex per dose at concentrations of 0, 1200, 6000 and 30 000 ppm (equal to 0, 83.6, 417 and 2150 mg/kg bw per day for males and 0, 96.9, 485 and 2532 mg/kg bw per day for females) for two successive generations with one litter per generation. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. On day 4 postpartum, litters were standardized (four males and four females, if possible). At weaning, 24 males and 24 females from each dose group were selected to produce the F_1 generation. Unselected offspring were terminated and underwent gross necropsy. The offspring selected for the F_1 generation were dosed for at least 10 weeks and paired within dose group to produce F_2 litters. At weaning, parental animals and their offspring were terminated and examined macroscopically. Organs were taken from all parental animals for weights and histopathological examination. For offspring, the same organs were taken from one animal per sex per litter at random. The overall calculated mean daily intake of glyphosate was 0, 84, 417 and 2150 mg/kg bw per day for F_0 males; 0, 97, 485 and 2532 mg/kg bw per day for F_0 females; 0, 92, 458 and 2411 mg/kg bw per day for F_1 males; and 0, 105, 530 and 2760 mg/kg bw per day for F_1 females.

There were no treatment-related adverse effects on mortality, body weights, feed consumption, feed efficiency or histopathological changes for parental animals of either generation. The incidence of loose stools was increased for high-dose parental animals in both generations (Table 40). In addition, the incidences of caecum distension were increased in high-dose parental animals in both generations (Table 41). Although increases in liver and kidney weights were noted in the high-dose group, these changes were not considered adverse given the magnitude of the change and/or lack of corresponding histopathological changes in these organs.

Table 40. Loose stools in two generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate											
	Pre-mating				**Mating/gestation**				**Lactation/post-weaning**			
	0 ppm	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**
F_0												
M	0/24	0/24	0/24	3/24	0/23	0/24	0/24	2/24	N/A	N/A	N/A	N/A
F	0/24	0/24	0/24	1/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	6/24
F_1												
M	0/24	0/24	0/24	13/24	0/23	0/24	0/23	0/24	N/A	N/A	N/A	N/A
F	0/24	0/24	0/24	4/24	0/23	0/23	0/21	0/19	0/23	0/23	0/21	2/19

F: female; F_0: parental generation; F_1: first filial generation; M: male; N/A: not applicable; ppm: parts per million

Results presented as number of animals with loose stools / number of animals examined.

Source: Takahashi (1997)

Table 41. Incidence of caecum distension in three generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 ppm	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**
F_0				
Males	0/24	0/24	0/24	21/24
Females	0/24	0/24	0/24	24/24
F_1				
Males	0/24	0/24	0/24	19/24
Females	0/24	0/24	0/24	17/24
Pups	0/136	0/141	0/143	89/141
F_2				
Pups	0/182	0/183	0/164	111/149

ppm: parts per million; F_0: parental generation; F_1: first filial generation; F_2: second filial generation

Results presented as number of animals with caecum distension / number of animals examined.

Source: Takahashi (1997)

No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. Body weights of offspring at high doses were decreased in both generations, starting typically on postnatal day 14 (Table 42). Gross pathological examinations found an increased incidence of caecum distension in high-dose offspring of both generations.

Table 42. Mean body weights of two generations of offspring of rats administered glyphosate

	Mean body weights per dietary concentration of glyphosate							
	F_1 pups – male				**F_2 pups – male**			
PND	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**	**0 ppm**	**1 200 ppm**	**6 000 ppm**	**30 000 ppm**
0	6.7 ± 0.6	6.8 ± 0.5	6.7 ± 0.4	7.2 ± 0.7*	7.0 ± 0.5	6.9 ± 0.6	7.3 ± 0.7	7.1 ± 0.5
4	11.6 ± 1.2	11.6 ± 1.2	11.7 ± 1.0	11.6 ± 1.2	12.0 ± 1.2	12.1 ± 1.5	12.5 ± 1.5	12.5 ± 1.3
7	19.5 ± 1.7	19.1 ± 2.0	19.5 ± 1.6	19.3 ± 1.2	19.8 ± 1.5	20.0 ± 1.9	20.4 ± 2.2	20.6 ± 1.7
14	39.5 ± 3.2	39.4 ± 2.6	39.3 ± 2.6	36.6 ± 2.6**	40.1 ± 3.0	39.0 ± 2.8	38.7 ± 2.9	39.1 ± 2.8
21	63.9 ± 4.4	63.8 ± 4.1	62.4 ± 3.7	55.1 ± 3.5***	58.6 ± 5.1	59.4 ± 4.4	58.3 ± 4.3	53.1 ± 4.4**
	F_1 pups – female				**F_2 pups – female**			
0	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	6.8 ± 0.6*	6.6 ± 0.5	6.6 ± 0.7	6.8 ± 0.6	6.8 ± 0.6
4	11.1 ± 1.2	11.2 ± 1.1	11.3 ± 0.9	11.3 ± 1.2	11.6 ± 1.2	11.5 ± 1.6	12.0 ± 1.5	12.1 ± 1.1
7	18.6 ± 1.8	18.4 ± 1.9	18.8 ± 1.5	18.3 ± 1.6	18.9 ± 2.0	19.1 ± 2.1	19.6 ± 2.2	19.9 ± 1.4
14	38.4 ± 3.6	37.9 ± 2.6	38.2 ± 2.2	35.4 ± 2.6**	38.7 ± 3.5	38.0 ± 2.2	37.5 ± 2.9	38.1 ± 2.9
21	61.0 ± 4.8	60.6 ± 3.9	59.8 ± 3.1	53.2 ± 4.0***	56.4 ± 5.5	57.1 ± 4.4	56.2 ± 4.5	51.8 ± 4.2*

F_1: first filial generation; F_2: second filial generation; PND: postnatal day; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

Results presented are mean weights in grams ± standard deviation. Statistics from study report.

Source: Takahashi (1997)

The NOAEL for parental toxicity was 6000 ppm (equal to 417 mg/kg bw per day) based on increased incidence of loose stools and caecum distension in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day). As there were no effects on reproductive parameters the NOAEL for reproductive toxicity was 30 000 ppm (equal to 2150 mg/kg bw per day). The NOAEL for offspring toxicity was 6000 ppm (equal to 417 mg/kg bw per day) based on decreased pup body weights and increased incidence of caecum distension in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day) (Takahashi, 1997).

In a two-generation reproduction study, groups of 26 male and female Wistar-derived Alpk:AP$_f$SD rats (aged 5–6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity 97.6%) at concentrations of 0, 1000, 3000 or 10 000 ppm (equal to 0, 99.4, 292.6 and 984.7 mg/kg bw per day for males and 0, 104.4, 322.8 and 1054.3 mg/kg bw per day for females) for 10 weeks before their first mating and until termination. Each generation was mated twice avoiding sister/brother matings throughout. Males were terminated after completion of mating and females on or soon after day 29 of lactation, after which their organs were weighed and gross pathological and histopathological examinations conducted. The offspring not selected for mating were also terminated on day 29 postpartum, with one pup/sex per litter used for organ-weight determination and two pups /sex per litter given macroscopic examinations. All the remaining pups were terminated with no further examination.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment.

The body weights of F_{1A} pups were lower compared to the control group from day 8 onwards, but a similar effect was not seen in the F_{2A} pups. There was no treatment-related effect on total litter weight (Table 43).

Table 43. Mean body weights of two successive generations of offspring of rats administered glyphosate

	Mean body weights per dietary concentration of glyphosate (g)							
	Males				**Females**			
PND	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**	**0 ppm**	**1 000 ppm**	**3 000 ppm**	**10 000 ppm**
F_{1A}								
1	5.8	6.1	6.0	6.1	5.4	5.8	5.6	5.7
5	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
8	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
15	26.8	26.1	25.8	24.6*	26.1	25.2	24.5	23.8*
22	43.4	42.4	41.4	39.2*	41.9	40.3	39.4	37.7*
29	81.7	79.5	79.6	74.6*	77.1	74.0	74.1	69.9**
F_{2A}								
1	6.3	6.3	6.3	6.2	6.1	5.9	5.9	5.8
5	9.7	9.9	9.3	9.5	9.3	9.6	9.1	9.1
8	14.3	14.7	13.8	14.2	13.8	14.2	13.4	13.7
15	27.4	28.3	26.4	27.5	26.7	27.5	25.8	26.5
22	44.5	46.2	43.1	44.9	42.7	44.8	41.8	42.9

	Mean body weights per dietary concentration of glyphosate (g)							
	Males				Females			
PND	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm
29	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4

F_{1A}: first filial generation, first litter; F_{2A}: second filial generation, second litter; PND: postnatal day; ppm: parts per million; *: $P = 0.05$ (Student *t*-test, 2 sided); **: $P = 0.01$ (Student *t*-test, 2 sided)

Source: Moxon (2000)

As no adverse effects were noted in the study, the NOAEL for parental and reproductive toxicity was 10 000 ppm (equal to 984.7 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 3000 ppm (equal to 292.6 mg/kg bw per day) based on reduced pup weights in the F_{1A} generation seen at 10 000 ppm; equal to 984.7 mg/kg bw per day (Moxon, 2000).

In a two-generation reproduction study, glyphosate (purity 95.7%) was administered in the diet to 28 Crl:CD(SD) IGS BR rats per sex per dose at 0, 1500, 5000 or 15 000 ppm (equal to 0, 104, 351 and 1063 mg/kg bw per day in males and 0, 126, 423 and 1273 mg/kg bw per day in females) for two successive generations with one litter per generation. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. At weaning, 24 males and 24 females from each dose group were selected to produce the F_2 generation. Surviving adult females and males and unselected offspring were terminated on day 21 postpartum. All adult animals and offspring underwent macroscopic examinations and parental organs were weighed. A small subset of organs were taken from one male and one female offspring from the F_0 and F_1 pairings (where available). Tissues from control and high-dose F_0 and F_1 animals underwent histopathological examination. As there were indications of changes in the adrenal glands of F_1 animals, microscopic examination was extended to include all dose groups.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed or feed efficiency, organ weights or histopathological changes in parental animals of either generation. No adverse effects were observed on mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size, viability and offspring body weights were not affected by treatment. Complete preputial separation was delayed by 2.9 days in high-dose F_1 male pups (2.9 days) and body weights were increased by 10% at attainment. There were no treatment-related effects on the age or weight at attainment of vaginal opening.

As there were no effects for parental animals or on reproductive parameters, the NOAEL for parental and reproductive toxicity was 15 000 ppm (equal to 1063 mg/kg bw per day), the highest dose tested. The NOAEL for offspring was 5000 ppm (equal to 351 mg/kg bw per day), based on delayed age and increased weight at attainment of preputial separation at 15 000 ppm (equal to 1063 mg/kg bw per day) (Dhinsa, 2007).

(b) Developmental toxicity

Rats

In a pre-GLP developmental toxicity study, glyphosate (purity 98.7%) suspended in 0.5% aqueous Methocel was administered to 25 copulated CD female rats per dose by oral gavage at concentrations of 0, 300, 1000 or 3500 mg/kg bw per day from gestation day 6 through 19. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

Soft stools, diarrhoea, red nasal discharge, reduced activity and rales (abnormal respiratory noise) were noted in the highest dose group. By gestation day 17, six rats in this group had died. A

reduced mean body-weight gain due to a loss in mean maternal weight over the first three days of treatment was noted in the high-dose group. No significant differences between the 300 and 1000 mg/kg bw per day dosage groups and the control group were observed in terms of the mean number of viable fetuses, implantations, post-implantation losses, corpora lutea or mean fetal body weight. The mean number of total implantations, viable fetuses and mean fetal body weight were significantly decreased in the 3500 mg/kg bw per day dosage group compared to controls. In addition, the dams in the high-dose group had a significant increase in early resorptions, causing a slight increase in post-implantation losses.

At 3500 mg/kg bw per day, the number of litters with malformations was identical to that of the control group, but the number of fetuses with malformations was increased. However, since the number and type of malformations observed were similar to those observed in historical control data, it was concluded that they were not treatment related. There were an increased number of fetuses with unossified sternebrae in the high-dose group; although treatment related, this is considered a developmental variation rather than a teratogenic malformation. No malformations were observed in the 300 and 1000 mg/kg bw per day dosage groups.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day based on mortality, soft stools and reduced body-weight gain at 3500 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day based on the decreased mean number of total implantations, viable fetuses, mean fetal body weight, increased early resorptions and increased number of fetuses with unossified sternebrae at 3500 mg/kg bw per day (Tasker, Rodwell & Jessup, 1980a).

In a developmental toxicity study, glyphosate (purity 98.6%) suspended in a 1.0% aqueous solution of methylcellulose was administered to 25 mated Crl:CD(SD)BR VAF/Plus female rats per dose by oral gavage at concentrations of 0, 300, 1000 or 3500 mg/kg bw per day from gestation days 6 through 15. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

At the highest dose, clinical abnormalities included salivation, loose stools and rales. The latter was also observed in two animals at the intermediate dose on one occasion. There were two maternal mortalities at the highest dose following signs of respiratory distress. Body-weight gain was markedly reduced at the highest dose (by 16–81% of control values, gestation days 6–20) and marginally reduced at the intermediate dose (by 86–97% of control values, gestation days 6–20). Feed consumption was slightly decreased at the highest dose during the dosing period (75–94% of control values, gestation days 6–15), but was comparable with controls thereafter. Water intake was increased at the highest dose (139–205% of control values, gestation days 6–15). No treatment-related changes were observed at any dose at necropsy.

A total of 23, 23, 25 and 22 dams had live young on day 20 in the control group and at 300, 1000 and 3500 mg/kg bw per day, respectively. Treatment had no significant effect on embryonic losses, litter size or sex ratio, but the litter weights were reduced at the highest dose (90% of control values) and mean fetal weights were statistically significantly reduced at the highest dose (94% of control values; $P < 0.01$). The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher at the highest dose and slightly higher at the intermediate dose; the incidences based on fetuses were 1, 0, 3 and 28 and on litters were 1, 0, 2 and 11 at 0, 300, 1000 and 3500 mg/kg bw per day, respectively. In addition, reduced ossification was seen slightly more frequently at the highest and intermediate doses. The percentage of fetuses showing skeletal anomalies (variations) was significantly increased at the two higher doses, but the percentage of fetuses affected at the intermediate dose exceeded the historical background range (21.9–27.2%) only slightly (Table 44).

Table 44. Skeletal anomalies in fetuses and litters of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 mg/kg bw per day	**300 mg/kg bw per day**	**1000 mg/kg bw per day**	**3500 mg/kg bw per day**
Fetal anomolies[a]	19/155	36/143	46/166	55/142
Litter anomolies[b]	11/23	16/23	19/25	19/22
Fetal skeletal variations (%)[c]	11.7	22.6	28.4*	35.7**
Historical range	21.9–27.2			

bw: body weight; *: $P < 0.05$; **: $P < 0.01$

Kruskal–Wallis H-statistic followed, if significant, by intergroup comparison with control (distribution-free Williams' test).

[a] Results presented as number of fetuses with skeletal anomalies / total number of fetuses.

[b] Results presented as number of litters with skeletal anomalies / total number of litters.

[c] Results expressed as number of fetuses with skeletal variations (with malformed fetuses excluded) as a percentage of the total number of fetuses examined.

Source: Brooker et al. (1991a)

The NOAEL for maternal toxicity was 300 mg/kg per day based on clinical signs and reduced body-weight gain at 1000 mg/kg bw per day and higher. The NOAEL for developmental toxicity was 300 mg/kg per day based on an increased incidence of delayed ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day and higher (Brooker et al., 1991a).

In a developmental toxicity study, glyphosate (purity 95.68%) suspended in a 0.5% aqueous solution of sodium carboxymethylcellulose was administered to 24 copulated Crj:CD(SD) female rats/dose by oral gavage at concentrations of 0, 30, 300 or 1000 mg/kg bw per day from gestation day 6 through 15. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in mortality, body weight, feed consumption or macroscopic findings in dams. An increased incidence of slightly loose stools was observed during the dosing period in 20 of the 22 pregnant females at 1000 mg/kg bw per day. Of these 20 animals, 9 still displayed the effect on the day after the last dosing.

There were no effects on number, growth or survival of fetuses. Any external, visceral or skeletal abnormalities were considered secondary to maternal toxicity; furthermore, the effects were also seen in the control group, incidences of the effects were low and/or there was no dose–response relationship for the effect.

The NOAEL for maternal toxicity was 300 mg/kg bw per day based on the increased incidence of slightly loose stools observed in dams at 1000 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 1000 mg/kg bw per day (Hatakenaka, 1995).

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionized water was administered to 24 time-mated female Alpk:APfSD (Wistar-derived) rats/dose by oral gavage at 0, 250, 500 or 1000 mg/kg bw per day from gestation day 7 through 16. On gestation day 22, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All the fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

One control animal was terminated on day 7 due to incorrect dosing. There were no treatment-related changes in clinical observations, body weight, feed consumption or macroscopic findings for dams.

There were no effects on number, growth or survival of fetuses and no treatment-related external, visceral or skeletal abnormalities.

As there were no maternal or developmental effects, the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day (Moxon, 1996a).

Rabbits

In a developmental toxicity study, glyphosate (purity 98.7%) suspended in a 0.5% aqueous Methocel solution was administered to 16 Dutch Belted female rabbits per dose by oral gavage at concentrations of 0, 75, 175 or 350 mg/kg bw per day from gestation day 6 through 27. On gestation day 28, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. This study was conducted prior to GLP.

Incidence of mortality was increased in the high-dose group. The number of spontaneous deaths in the control, low-, mid- and high-dose groups was 0/16, 1/16, 2/16 and 10/17, respectively. A slight increase in the incidence of soft stools and diarrhoea was noted in the medium–high-dose group (individual data not reported). At 350 mg/kg bw per day, soft stool and/or diarrhoea were observed in each animal at least once during treatment. An increased incidence of nasal discharge was also noted in the high-dose group (individual data not reported). There were no treatment-related changes in body weight or macroscopic findings for dams.

Due to the increased mortality at the high dose, the number of animals (6 pregnant females) available for evaluation of developmental effects was insufficient. The numbers of pregnant dams were also low for the other doses (12, 15 and 11 in the control, low- and mid-dose groups, respectively). limiting the evaluation of developmental effects in this study. The available data for the control, low- and mid-dose groups indicate no treatment-related adverse effects on the number, growth or survival of fetuses. Any external, visceral or skeletal abnormalities were not considered treatment related.

The NOAEL for maternal toxicity was 175 mg/kg bw per day based on increased incidence of clinical signs (soft stools and diarrhoea) and mortality at 350 mg/kg bw per day in rabbits. Individual data were not provided for the clinical signs at 175 mg/kg bw per day, and the increase in incidence was only slight at this dose. Due to the low number of pregnant dams, developmental effects could not be evaluated; however, the available data indicate no evidence of developmental effects (Tasker, Rodwell & Jessup, 1980b).

In a developmental toxicity study, glyphosate (purity 95%) suspended in a 0.1% aqueous gum acacia solution was administered to 15 New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 125, 250 and 500 mg/kg bw per day, respectively, from gestation day 6 through 18. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in mortality, feed consumption or macroscopic findings for dams. Two abortions were noted in the high-dose group. A slight decrease in body-weight gain was also noted at 500 mg/kg bw per day.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. The mean number of viable implants per litter was lower at the high dose than the other treatment groups and controls and accordingly the mean number of non-viable implants per litter was higher

than the other treatments groups; however, when taking into account the variability for these measurements, the changes were not considered adverse.

Incidences of external, visceral or skeletal variations/malformations in fetuses in the low- and mid-dose groups did not differ from those of the control group (Table 45). At 500 mg/kg bw per day, incidences of variations/malformations were higher than in the control group, but in many cases the increase was minimal or similar to the 125 and 250 mg/kg bw per day dose groups when evaluated on a litter basis. These increases in incidences of variations/malformation were observed in the presence of severe maternal toxicity. The occurrences of a variety of low-incidence fetal effects (malformations) were slightly increased at higher dose levels. These increases are considered secondary to maternal toxicity.

Table 45. Malformations and variations in fetuses and litters of rabbit administered glyphosate

	Incidence per dietary concentration of glyphosate			
Malformations / variations	**0 mg/kg bw per day**	**125 mg/kg bw per day**	**250 mg/kg bw per day**	**500 mg/kg bw per day**
Number of litters examined	13	14	14	12
Number of fetuses examined	109	113	120	78
Malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	2 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	2 (2)	6 (4)
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle smaller than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal fetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)
Cervical central 1–3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extrathoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra – 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)

Malformations / variations	Incidence per dietary concentration of glyphosate			
	0 mg/kg bw per day	125 mg/kg bw per day	250 mg/kg bw per day	500 mg/kg bw per day
Number of litters examined	13	14	14	12
Number of fetuses examined	109	113	120	78
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	3 (2)	2 (1)	2 (2)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

bw: body weight

Results presented as number of fetuses with malformations and variations and, in parentheses, the number of litters with malformations and variations.

Source: Bhide & Patil (1989)

The NOAEL for maternal toxicity was 250 mg/kg bw per day based on abortions observed at 500 mg/kg bw per day in rabbits. The NOAEL for developmental toxicity was 250 mg/kg bw per day based on increased incidence of variations/malformations observed at 500 mg/kg bw per day in rabbits. It should be noted that individual data, uterine weights, maternal necropsy results and statistical analyses were not provided for this study; therefore, the NOAEL and LOAEL values are based on the available data (Bhide & Patil, 1989).

In a developmental toxicity study, glyphosate acid (purity 98.6%) suspended in a 1% aqueous methylcellulose solution was administered to 19, 19, 16 or 20 New Zealand White rabbits per dose by oral gavage at concentrations of 0, 50, 150 or 450 mg/kg bw per day, respectively, from gestation day 7 through 19. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in body weight, feed consumption or macroscopic findings for dams. One high-dose animal was found dead on day 20 following signs of abortion on day 19 and soft/liquid faeces, a reduction in feed intake and body-weight loss from the start of treatment. The incidence of soft/liquid faeces was increased at the high dose (13/20 animals).

There were no treatment-related adverse effects on the number, growth or survival of fetuses. At termination, 18, 12, 15 and 13 pregnant females were available for evaluation in the control, low, mid and high doses, so evaluation of developmental effects is limited at the low and high doses. Embryo/fetal death and post-implantation loss were increased in all treatment groups; however, there was no dose–response and the values were within or slightly above the historical control range.

Any external, visceral or skeletal abnormalities were not considered treatment related. There was a slightly increased incidence of cardiac malformation (interventricular septal defect) at the high dose (4/13 pregnant animals); however, it was barely outside of the historical control range from studies conducted during the same period, and the number of litters to evaluate this dose was reduced. Furthermore, this effect was considered secondary to the maternal toxicity observed at 450 mg/kg bw per day.

The NOAEL for maternal toxicity was 150 mg/kg bw per day based on clinical signs (soft/liquid faeces) at 450 mg/kg bw per day in rabbits. The NOAEL for developmental toxicity was 150 mg/kg bw per day based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw per day (Brooker et al., 1991b).

In a developmental toxicity study, glyphosate acid (purity 96.8%) suspended in a 0.5% aqueous CMC solution was administered to 26, 17, 16 and 16 presumed-mated New Zealand White rabbits per dose by oral gavage at concentrations of 0, 20, 100 or 500 mg/kg bw per day, respectively, from gestation day 6 through 18. On gestation day 28, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All the fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in body weight, feed consumption or macroscopic findings for dams. There were two, zero, four and eight deaths in the control, low-, mid- and high-dose groups, respectively; the deaths in the control group were definitively attributed to gavage error. An increased incidence of soft stool/liquid faeces was observed at the high dose (12/15 animals). Other clinical signs at the high dose included rales, weakness, dyspnoea and ocular discharge; however, the incidence of these effects was low and some effects may indicate gavage error. At necropsy, various findings were noted in the lungs and trachea in mid- and high-dose animals, which also suggests possible gavage errors and/or issues with animal husbandry.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. However, the number of pregnant females available for evaluation in the control and the low-, mid- and high-dose groups was 20, 13, 12 and 6, respectively, limiting the study of developmental effects. Total litter loss was recorded for one female in the high-dose group. Any external, visceral or skeletal abnormalities were not considered treatment related. Major visceral malformations primarily affected the heart, but occurred in single incidences and/or showed no dose–response relationship except for the dilated heart; however, interpreting the dose–response relationship is difficult given the limited number of litters available, especially at the high dose. In addition, this effect was considered secondary to the maternal toxicity observed at 500 mg/kg bw per day.

Based on the uncertainties regarding gavage errors and mortalities across doses in this study and the reduced number of pregnant females, the study is considered unacceptable (Suresh, 1993c).

In a developmental toxicity study, glyphosate (purity 97.56%) suspended in a 0.5% aqueous solution of sodium carboxymethylcellulose was administered to 18 artificially inseminated Japanese white rabbits (Kbl:JW) per dose by oral gavage at concentrations of 0, 10, 100 or 300 mg/kg bw per day from gestation day 6 through 18. On gestation day 27, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in body weight, feed consumption or macroscopic findings for dams. One dam died on gestation 20 without showing any clinical signs, and the cause of death was undetermined. An increased incidence of loose stools was observed during the dosing period in four of the 17 remaining pregnant females In the high-dose group; two continued to display this effect during the post-dosing period and one aborted on gestation day 26.

There were no effects on number, growth or survival of fetuses. All observations of external or visceral malformations were sporadic in nature and not considered treatment related. Skeletal malformations and variations were also not considered treatment-related since these effects were also seen in the control group, incidences of the effects were low and/or there was no dose–response relationship for the effect.

The NOAEL for maternal toxicity was 100 mg/kg bw per day based on the increased incidence of loose stools observed in dams at 300 mg/kg bw per day. There were no developmental effects; therefore, the NOAEL for developmental toxicity is 300 mg/kg bw per day (Hojo, 1995).

In a developmental toxicity study, glyphosate (purity 95.3%) suspended in a 1% CMC was administered to 18 mated New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 50, 200 or 400 mg/kg bw per day from gestation day 7 through 19. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea,

implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in body weight, feed consumption or macroscopic findings for dams. One high-dose female was found dead prior to dosing on day 19 and another was terminated in extremis on day 20; one death also occurred in the control group and in the mid-dose group. An increased incidence of diarrhoea was observed at the high dose in 10 of the 16 surviving pregnant females. All other clinical observations were isolated or a dose–response relationship was not observed.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. The increases in late fetal deaths and post-implantation loss noted at the high doses were not considered adverse once the variability in the measurements were taken into consideration. In addition, the increase can mainly be attributed to one animal with nine late-death fetuses. No treatment-related external, visceral or skeletal abnormalities were observed.

The NOAEL for maternal toxicity was 200 mg/kg bw per day based on increased incidence of diarrhoea in dams at 400 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 400 mg/kg bw per day (Coles & Doleman, 1996).

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionized water was given to 20 time-mated New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 100, 175 or 300 mg/kg bw per day from gestation day 8 through 20. On gestation day 30, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in mortality, body weight, feed consumption or macroscopic findings for dams. There was a significant increase in the incidence of either diarrhoea or decreased faecal output at the mid and high doses (no statistical significance was provided) (Table 46). The incidence of staining in the genital area was also increased at the high dose.

Table 46. Clinical signs in pregnant rabbits administered glyphosate by gavage

	No. per dietary concentration of glyphosate			
Clinical sign	**0 mg/kg bw per day**	**100 mg/kg bw per day**	**175 mg/kg bw per day**	**300 mg/kg bw per day**
Few faeces in tray	3	3	9	9
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11

bw: body weight; no. number

Source: Moxon (1996b)

There were no treatment-related adverse effects on the number, growth or survival of fetuses. Although mean fetal weight was reduced at the high dose, this was not considered adverse once the variability in the measurements was taken into account. In addition, the decrease could be attributed to two litters with lower weights. Any external, visceral or skeletal abnormalities were not considered treatment related.

The NOAEL for maternal toxicity was 100 mg/kg bw per day based on increased incidence of clinical signs (decreased faecal output or signs of diarrhoea) in rabbits at 175 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 300 mg/kg bw per day (Moxon, 1996b).

2.6 Special studies

(a) Neurotoxicity

Cell cultures

In a non-guideline experiment, a cell culture model was used to determine if chronic exposure to organophosphate pesticides can alter the sensitivity of nerve cells to subsequent acute exposure to organophosphates or other compounds. NB2a neuroblastoma cells were grown in the presence of diazinon at a concentration of 25μmol/L for 8 weeks. The organophosphate was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides, including glyphosate (purity > 99%). The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the median inhibitory concentration (IC_{50}) for each organophosphate or formulation calculated. The IC_{50} values in cells pre-exposed to diazinon were compared with the equivalent values in cells not pre-exposed to diazinon. The IC_{50} for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20% and 90% after pretreatment with diazinon.

According to the study authors, the data support the view that long-term exposure to an organophosphate may reduce the threshold for toxicity of some environmental agents (Axelrad, Howard & McLean, 2003).

Rats

In an acute neurotoxicity study, groups of fasted (24 hours), approximately 42-day-old Alpk:APfSD rats (10/sex per dose) were given a single oral dose of glyphosate (purity 95.6%) in deionized water at concentrations of 0, 500, 1000 or 2000 mg/kg bw. They were then observed for 2 weeks. Neurobehavioural assessment (functional observational battery and motor activity testing) was performed in all animals in week −1 (pre-dosing), on day 1 (approximately 6 hours after dosing), day 8 and day 15. At study termination, five animals/sex per dose were euthanized and perfused. Of the perfused animals, the control and highest dose groups were used for neuropathological examinations with brain and peripheral nervous system tissues undergoing histopathological evaluation.

Administration of a single dose of glyphosate produced treatment-related clinical signs of general toxicity at 2000 mg/kg bw. On day 1, approximately 6 hours after dosing, three high-dose females were observed with decreased activity, subdued behaviour, hunched posture and/or hypothermia. Diarrhoea was also seen in another female at this dose. Full recovery was established by day 2. These clinical signs do not reflect signs of neurotoxicity and were mostly likely associated with the excessively high dose of glyphosate. No treatment-related effects were observed on mortality, body weight or brain weight. Similarly, neuropathological and histopathological examinations showed no treatment-related effects, and functional observational battery and motor activity tests revealed no treatment-related effects. Although overall motor activity at 2000 mg/kg bw for both sexes on day 1 was lower than that of controls, these differences were not statistically significant or dose dependent.

The NOAEL for neurotoxicity in rats was 2000 mg/kg bw. The NOAEL for systemic toxicity was 1000 mg/kg bw based on clinical signs of general toxicity (decreased activity, subdued behaviour, hunched posture, hypothermia and diarrhoea) and lethality at 2000 mg/kg bw. The LOAEL for systemic toxicity in rats was 1000 mg/kg bw (Horner, 1996a).

In a subacute neurotoxicity study, glyphosate (purity 95.6%) was administered to 12 Alpk:APfSD rats per sex per group in the diet at concentrations of 0, 2000, 8000 or 20 000 ppm (equal to 0, 155.5, 617.1 and 1546.5 mg/kg bw per day for males and 0, 166.3, 672.1 and 1630.6 mg/kg bw per day for females) for 13 weeks. Neurobehavioural assessment (functional observational battery and motor activity testing) was performed in all animals at weeks −1, 1, 5, 9 and 14. At study termination, six animals/sex per group were euthanized and perfused. Of these, the control and highest

dose groups were used for neuropathological examinations and brain and peripheral nervous system tissues histopathologically evaluated.

Overall mean body weight (92.8% of the controls; $P < 0.05$) and feed utilization ($P < 0.01$) were reduced in high-dose males with no treatment-related effect on feed consumption. Group mean body-weight was also lower than the controls in males at 8000 ppm from weeks 6–14 (not statistically significantly). No treatment-related effects on mortality, clinical signs or brain weight were observed. Functional observational battery and locomotor activity testing revealed no treatment-related effects. Neuropathological and histopathological examinations of the peripheral and nervous system did not yield any treatment-related effects from glyphosate administration.

The NOAEL for neurotoxicity in rats was 20 000 ppm, equal to 1547 mg/kg bw per day. The NOAEL for systemic toxicity was 20 000 ppm, equal to 1546.5 mg/kg bw per day (Horner, 1996b).

Hens

In an acute delayed neurotoxicity study, 20 hens (hybrid brown laying strain – Lohmann Brown) were given a single oral dose of glyphosate (purity 95.6%) of 2000 mg/kg bw. In addition, 12 negative control hens were dosed with distilled water and 12 positive control hens with 1000 mg/kg bw of triorthocresyl phosphate (TOCP). This was followed by an observation period of 21/22 days. The hens were examined for any clinical signs twice daily and for ataxia daily, and weighed weekly. Brain acetylcholinesterase, brain neuropathy target esterase (NTE) and lumbar spine NTE measurements were made on three hens, 48 hours after dosing. At the end of the observation period, six hens from each treatment group were selected for termination and macroscopic and histopathological examination. After perfusion through the heart with fixative, the selected tissues were processed and examined histopathologically.

No treatment-related mortality was observed in the study. There was no evidence of clinical ataxia in any of the negative controls or in any of the hens dosed with glyphosate. Of the 12 hens dosed with TOCP (positive controls), five developed clinical ataxia, starting between days 11 and 21. There was no effect on body weights for hens dosed with glyphosate, but TOCP-dosed hens showed an overall weight loss. Acetylcholinesterase was reduced by 6% in glyphosate-treated hens and 19% in TOCP-treated hens. There was no effect on NTE levels in brain or spinal cord for the glyphosate-treated hens, but compared to the negative controls, brain NTE levels were reduced by 84% and spinal cord NTE levels by 78% in the positive controls. No macroscopic abnormalities were seen in any of the hens examined. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment-related changes in glyphosate-treated hens. Hens dosed with TOCP showed significant axonal degeneration in spinal cord, peripheral nerve and cerebellum, demonstrating the validity of the test system.

In conclusion, oral administration of a single dose of 2000 mg/kg bw of glyphosate produced no clinical signs of delayed neurotoxicity, no significant reduction in acetyl cholinesterase and no histopathological findings in hens. The NOAEL for acute delayed neurotoxicity of glyphosate in hens was 2000 mg/kg bw (Johnson, 1996).

(b) Immunotoxicity

In an unpublished immunotoxicity study, glyphosate (purity 85.2%) was administered to female B6C3F1/Crl mice (10/dose) in the diet at dose levels of 0, 500, 1500 or 5000 ppm (equal to 0, 150.1, 449.1 and 1447.5 mg/kg bw per day, respectively) for 28 days. The positive control group (10 females) was administered 50 mg/kg bw per day of cyclophosphamide (10 mL/kg at a concentration of 5 mg/mL) by intraperitoneal injection from study days 24–27. On day 24, all the animals in all the groups received a single intravenous dose of 7.5 × 107 sheep red blood cells (SRBC) in 0.2 mL of Earle's Balanced Salt Solution with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. At termination, the spleen and thymus were removed and weighed. The T-cell–dependent antibody response to SRBC was measured with antibody-forming cell (AFC) assay.

There were no pre-terminal deaths, no treatment-related clinical signs and no treatment-related effects on feed and water consumption, mean body weights, organ weights and macroscopic findings in all treated groups. The body weights of the positive control group treated with cyclophosphamide did not differ significantly from those of the vehicle control group, but the absolute and relative spleen and thymus weights decreased statistically significantly ($P < 0.01$).

The systemic NOAEL was 5000 ppm (equal to 1448 mg/kg bw per day), the highest dose tested.

No statistically significant differences were observed in anti-SRBC antibody-forming cell responses for specific activity (AFC/106 spleen cells) and total spleen activity (AFC/spleen) in treated groups compared to the vehicle control group. The positive control group had a statistically significant ($P < 0.05$) decrease in spleen cell numbers, mean specific activity and mean total spleen activity. This confirmed the ability of the test system to detect immunosuppressive effects and confirmed the validity of the study design. Natural killer cell activity was not evaluated in this study.

The NOAEL for immunotoxicity was 5000 ppm (equal to 1448 mg/kg bw per day), the highest dose tested (Haas, 2012).

In a published study, female CD-1 mice were exposed to Tordon 202C (2,4-dichlorophenoxyacetic acid [2,4-D] and picloram) or Roundup in drinking water for 26 days at concentrations from 0–0.42% or 0–1.05%, respectively. Glyphosate isopropylammonium salt was administered in distilled drinking water at concentrations of 0%, 0.35%, 0.70% or 1.05% (approximately equal to 335, 670 and 1000 mg/kg bw). The mice were inoculated with SRBC to produce a T-lymphocyte macrophage-dependent antibody response on day 21 of the herbicide exposure period. Roundup exposure did not alter weight gain or water consumption. Antibody production was also unaffected by Roundup dosing, suggesting that Roundup is unlikely to cause immune dysfunction under normal conditions of application (Blakley, 1997).

The role of glyphosate in developing asthma and rhinitis among farmers was evaluated in a published study. The aim of this study was to explore the mechanisms of glyphosate-induced pulmonary pathology by utilizing murine models and real environmental samples. C57BL/6, TLR4_/_, and IL-13_/_ mice inhaled extracts of glyphosate-rich air samples collected on farms during spraying of herbicides or inhaled different doses of glyphosate and ovalbumin. The cellular response, humoral response and lung function of exposed mice were evaluated. Inhalation exposure to glyphosate-rich air samples as well as glyphosate alone increased eosinophil and neutrophil counts, mast cell degranulation and production of the cytokines interleukin-33 (IL-33), thymic stromal lymphopoietin, interleukin-13 (IL-13) and interleukin-5 (IL-5). In contrast, in vivo systemic interleukin-4 (IL-4) production was not increased. Co-administration of ovalbumin with glyphosate did not substantially change the inflammatory immune response. However, deficiency in IL-13 resulted in diminished inflammatory response, but did not have a significant effect on airway resistance upon methacholine challenge after 7 or 21 days of glyphosate exposure. Glyphosate-rich farm air samples as well as glyphosate alone were found to induce pulmonary IL-13–dependent inflammation and promote Th2-type cytokines, but not IL-4 for glyphosate alone (Kumar et al., 2014).

(c) Effects on the salivary gland

Groups of 24 male Alpk:AP$_f$SD (Wistar-derived; AP), Sprague Dawley (Charles River; CD) and Fischer 344 (F344) rats were fed diets containing 0 or 20 000 ppm glyphosate acid for 28 consecutive days. Eight animals from each group were terminated of day 29, and the remaining rats retained without treatment for an additional 4 (eight rats/group) or 13 weeks (eight rats/group).

Dietary exposure to 20 000 ppm glyphosate acid resulted in significant reductions in body weight and minor reductions in feed consumption in AP and CD rats, but not in F344 rats. Salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the 4-week dietary exposure period. Microscopic examination of the salivary glands showed that the most pronounced effected occurred in the F344 strain, where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells occurred in the AP and CD strains.

After four weeks on the control diet, the salivary glands of the F344 strain had significantly recovered, while AP and CD rats were indistinguishable from their corresponding controls.

After 13 weeks on the control diet, slightly more glyphosate-treated F344 rats showed minor focal changes in the salivary glands compared to their controls, and group mean salivary-gland weights were increased slightly (Allen, 1996).

In a study of the mechanism of induction of salivary gland lesions performed by the National Toxicology Program, two groups of four male F344/N rats were fed diets containing glyphosate (purity 99%) at a concentration of 50 000 ppm (the highest dose used in a short-term study on toxicity) and given a continuous subcutaneous infusion of propanolol (a β-blocker; 1.2 mg/kg bw per day) or a vehicle (water). Three additional groups of four male rats were fed a control diet and given a continuous subcutaneous infusion of isoproterenol (a β-adrenergic agonist; 1.0 mg/kg bw per day), isoproterenol plus propanolol, or a vehicle (water). After 14 days of treatment, the animals were terminated, and the parotid and submandibular/sublingual glands were removed, weighed and processed for electron and light microscopy.

All the rats survived to the end of the study. Rats subcutaneously infused with isoproterenol were hypoactive and had increased respiratory rates on day 1, but behaved normally by the following day. While there was no effect on feed consumption in any group, there was a significant decrease in body-weight gains in the groups fed glyphosate (6.3 g and 6.0 g, compared with 16.0 g in controls). Both glyphosate and isoproterenol produced increased salivary-gland weights, with the parotid gland being more affected (280% or 154% of weights in the control group for glyphosate or isoproterenol, respectively). When both compounds were given along with propanolol, parotid weights were 194% of those of the controls for glyphosate but only 109% of those of the controls for isoproterenol. In the parotid and in the submandibular gland, increased weights were associated with cytoplasmic changes of acinar cells (basophilic change, fine vacuolation, swelling, loss of the normal positive periodic acid–Schiff reactivity of the secretory granules). The study authors concluded that the salivary gland effects induced by glyphosate were mediated through an adrenergic mechanism (Chan & Mahler, 1992).

The hypothesis that glyphosate produced the changes to the salivary gland via β-adrenergic activity was questioned in a recent review paper (Williams, Kroes & Munro, 2000). The authors emphasized that if glyphosate was a β-agonist, it would stimulate β-receptors in other effector organs and produce a characteristic set of cardiocirculatory effects, such as increased heart rate and cardiac output as well as decreased blood pressure and peripheral resistance; none of these effects were noted in other toxicological studies. Similarly, it is known that isoproterenol and other β-agonists cause myocardial necrosis and enlargement of heart ventricles after prolonged treatment. Glyphosate did not produce any effects in heart tissue, even after long-term exposure at very high doses, further supporting for the argument that glyphosate does not act as a β-agonist. The authors concluded that glyphosate has no significant β-adrenergic activity and could not produce salivary-gland changes via β-agonist activity. They proposed a number of other potential mechanisms for salivary gland alteration, including non-chemical modes of action. For example, salivary gland secretion has been shown to be affected by the texture and moistness of feed, and salivary gland enlargement has been caused by malnutrition. Glyphosate could be acting by just such a non-chemical mechanism. Because glyphosate is a strong organic acid, dietary administration at relatively high concentrations may cause

mild oral irritation leading to increased salivary gland size and flow. In the long-term exposure studies with glyphosate, there were several changes to the salivary glands. These changes were most pronounced in the parotid gland, responsible for secreting serous fluid in response to stimuli such as acidic materials; absent in the sublingual gland that releases mucous fluid in response to other stimuli; and observed to an intermediate degree in the submandibular gland that contains a mixture of mucous and serous secreting cells. This pattern of observations was considered consistent with the hypothesis that the changes are a biological response to the acidic nature of glyphosate. These alterations are not known to represent any pathological condition and were not considered toxicologically significant or adverse (Williams, Kroes & Munro, 2000).

A 2-month exploratory study evaluated the effects of a low pH diet on the parotid salivary glands of rats. Five groups, each with 10 male Crl:CD (SD) rats, were dosed for 56 consecutive days. Group 4 animals were fed a low pH diet containing 14 000 ppm citric acid, and group 5 a high pH diet with 21 400 ppm trisodium citrate dihydrate and a citrate ion concentrate equivalent to group 4's. Group 2, the controls, were fed the basal diet. Group 3 were administered citric acid in deionized water by gavage at 791–1316 mg/kg bw per day, with the dosing calculated to maintain citric acid dose levels approximately equal to group 4's. Group 1 were gavaged with deionized water.

Treatment-related effects consisted of statistically significant higher parotid salivary-gland weights in group 4, compared to the group 2 controls. The higher parotid salivary-gland weights seen in groups 3 (gavaged citric acid) and 5 (fed a trisodium citrate dihydrate diet) were not statistically significant.

The report states that with the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are likely adaptive responses to the low pH diet causing local irritation in the oral cavity rather than adverse effects (Haas, 2010).

A 4-month study examined the effects of glyphosate acid on the salivary glands of different rat strains (AP, CD and F344) after feeding diets containing 20 000 ppm glyphosate acid to male rats for 28 consecutive days and monitoring recovery over 4 or 13 weeks.

Differences in terms of systemic toxicity (changes in body weight and feed consumption) were minor, but marked differences were seen in the severity of effect on the parotid salivary gland. Significant reductions in body weight with minor reductions in feed consumption were seen in AP and CD rats but not in F344 rats. In contrast, salivary-gland weight was unaffected in CD rats but was increased in both AP and F344 rats. Microscopic examination showed the most pronounced effect to be in F344 rats where cytoplasmic basophilia were diffuse and parotid acinar cells enlarged. Similar but lesser effects involving only small foci of cells occurred in the AP and CD strains.

Complete recovery was seen in AP and CD rats following the 4-week recovery period. Although significant recovery of salivary-gland change was observed in F344 rats, it may not have been complete after a 13-week recovery period (Wood, 1996).

In an in vivo study, five male and five female Sprague Dawley (CD) rats were dosed with glyphosate technical (purity 95.3%) at a dose level of 5000 mg/kg bw with similar sized control groups receiving the vehicle only. Approximately 1 hour after dosing, control and treated animals were examined for either haematological, electrocardiographic or behavioural/functional changes. There were no differences in response between treated and control animals.

Ex vivo studies evaluated the effect of saturated solutions of glyphosate technical on isolated guinea pig ileum and isolated rat gastrocnemius muscle. Glyphosate technical caused a contractile response in isolated guinea pig ileum similar to that seen with acetylcholine; the effect was negated when the ileum was pre-incubated with atropine sulfate (Wood, 1996).

(d) Gastrointestinal tract irritation

In a study comparing the irritant effects on the stomach and ileum of a glyphosate formulation containing isopropylamine salt (41%) and surfactant (15%) with hydrochloric acid, a Teflon-coated catheter was inserted into intestinal duct of beagle dogs to administer each ration of the test solutions. Each sample was left in the stomach and intestine for 30 minutes and then the tissues were washed with physiological saline and examined. Based on the histopathological findings, the study concluded that the mucosal damage in the stomach and intestine caused by glyphosate formulation was mild, equivalent to that caused by 0.25 eq/L hydrochloric acid. The intestine was more severely damaged than the stomach in every case (Mizuyama, 1987).

(e) Endocrine disruption

For the USA pesticide regulatory risk assessment, the USEPA Endocrine Disruptor Screening Program (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen-, androgen- or thyroid-signalling pathways. This interaction includes agonism and antagonism at estrogen and androgen receptors as well as at the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–thyroid axes, and altered steroidogenesis. In determining whether glyphosate interacts with estrogen-, androgen- or thyroid-signalling hormone pathways, the number and type of effects induced, the magnitude of responses and the pattern of responses observed across studies, taxa and sexes were considered. In addition, the conditions under which effects occur were considered, and in particular, whether endocrine-related responses occurred at doses that also resulted in systemic or overt toxicity.

This evaluation re-examines the data evaluated by the EDSP Tier 1 Assay Weight-of-Evidence Review Committee of the Office of Pesticide Programs as well as the Office of Science Coordination and Policy weight-of-evidence analysis of the potential interaction of glyphosate with the estrogen, androgen or thyroid hormone pathways, conducted on September 17, 2014, and concurs with the overarching conclusions.

For the estrogen pathway, there was no evidence of potential interaction of glyphosate with the estrogen pathway in the EDSP Tier 1 in vitro assays (i.e. estrogen-receptor binding assay, estrogen-receptor transactivation assay, aromatase and steroidogenesis assays). While glyphosate has been reported to show estrogen-receptor agonism in vitro with estrogen-dependent human breast cancer cells (Thongprakaisang et al., 2013), there were confounding issues with this study, and other in vitro estrogen receptor studies with glyphosate have not demonstrated an interaction (e.g. Kojima et al., 2004).

In addition, glyphosate was negative in the Tier 1 in vivo mammalian assays (i.e. uterotrophic or female pubertal assays). In the fish short-term reproduction assay (FSTRA), the non-treatment-responsive decrease (only significant at mid-treatment) in vitellogenin (VTG) was seen in isolation in the absence of any treatment-related effects in the other estrogen-related end-points such as gonado-somatic index, gonadal staging, fecundity and fertilization. In addition, there was no notable gonadal histopathology. In the open literature, glyphosate did not increase plasma VTG in juvenile rainbow trout (Xie et al., 2005). There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 US Toxicological Data Requirement mammalian or wildlife studies (only decreases in offspring body weight were reported in one avian reproduction study). Therefore, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate.

Tier 1 in vitro assays showed no evidence of glyphosate interacting with the androgen pathway via androgen-receptor binding, and glyphosate was negative in an androgen-receptor transactivation assay (Kojima et al., 2004; Kojima, Takeuchi & Nagai, 2010). However, evidence for the aromatase and steroidogenesis assays is conflicting: these were negative for glyphosate alone in the USEPA evaluation and a murine in vitro model (Forgacs et al., 2012), but positive for the coformulants in another laboratory (Benachour et al., 2007; Defarge et al., 2016), with mechanistic underpinning via both the regulatory steroidogenic acute regulatory protein (StAR) and the P450scc cleavage enzyme first shown by Walsh et al. (2000).

The in vivo Tier 1 FSTRA and mammalian assays (i.e. Hershberger) and male pubertal assays were negative in the absence of overt toxicity. The only treatment-related effects observed in the Part 158 mammalian studies in the absence of overt toxicity were decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial separation at 1234 mg/kg bw per day in the post-1998 two-generation reproduction study in rats (the EDSP Tier 2 study). Both effects were observed at a dose that was above the limit dose (1000 mg/kg bw per day) for those studies. No androgen-related effects were seen in the wildlife Part 158 studies (decreases in offspring body weight observed in one avian reproduction study).

For the thyroid pathway, there was no convincing evidence of potential interaction of glyphosate. There were no treatment-related effects on thyroid hormones (thyroxine [T4] and thyroid-stimulating hormone [TSH]), thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity; nor were there any thyroid-related effects observed in the female pubertal assay. In the amphibian metamorphosis assay, there were no developmental effects or alterations in thyroid histopathology. No thyroid-related effects were noted in any of the Part 158 studies.

There is little information about any endocrine-mediated effects of glyphosate, for example, in relation to retinoids, vitamin D receptors, metabolic syndrome, obesogens, glucocorticoids, etc., which is a major data gap. In nonmammalian models, two endocrine-relevant pathways have been reported: retinoic-acid dysfunction was observed in tadpoles exposed to glyphosate formulation, whereas inhibition of cortisol response in fish by selected pesticides was notable in an academic (non-industry funded) report because glyphosate did *not* present a stress response inhibition, unlike most of the other test pesticides (Koakoski et al., 2014). Mechanistic information on the induction of receptors such as aryl hydrocarbon receptor (Takeuchi et al., 2008; Kojima, Takeuchi & Nagai, 2010), peroxisome proliferator-activated receptors (Vainio et al., 1983; Takeuchi et al., 2008; Kojima, Takeuchi & Nagai, 2010) and pregnane X receptor (PXR) (Kojima, Takeuchi & Nagai, 2010) are all negative. While glyphosate was not included in the recent Toxcast screens due to solubility issues, some of the coformulants were, with positive results noted for FD&C Blue No. 1 in some of the endocrine end-points.

Adverse endocrine effects due to glyphosate poisoning in humans have not been reported by poison centres (Bradberry, Proudfoot & Vale, 2004; Kamijo, Takai & Sakamoto,. 2016).

(f) EDSP studies

In vitro assays

Androgen-receptor binding

In an in vitro androgen-receptor competitive binding assay, the binding of a single concentration (1 nmol/L) of [^{3}H]-R1881 (reference androgen) in the presence of increasing concentrations (10^{-10} to 10^{-3} mol/L) of glyphosate (purity 95.93%) was measured. Sprague Dawley rat ventral prostate cytosol was the source of the androgen receptor for the study. Low-salt TEGD buffer (which consists of tris hydrochloride or tris base, ethylenediaminetetraacetic acid, glycerol and dithiothreitol) was used as the vehicle. Altogether three runs were performed, each including dexamethasone as a weak positive control and R1881 as the ligand reference standard.

The saturation binding curves showed a dissociation constant (K_d) for [^{3}H]-R1881 of 0.613 (± 0.041) nmol/L and an estimated maximum amount of binding (B_{max}) of 0.817 (± 0.049) fmol per 100 µg protein for the batch of prostate cytosol used in the study. In the competitive binding runs, the estimated mean log IC_{50} for R1881 (strong positive control) was 9.0 mol/L and for the weak positive control (dexamethasone) was −4.6 mol/L; the mean relative binding affinity for the weak positive control, dexamethasone, was 0.004%. At glyphosate concentrations of 10^{-10} to 10^{-3} mol/L, specific binding of [^{3}H]-R1881 was 92.4–101.3% with the exception of one concentration (10^{-9} mol/L) in run 1, which had an average binding of 66.5%. Review of the data indicated that this value was a result of a single replicate with a specific binding of 7.5%. Excluding this value yielded a mean specific

binding of 96.0%, which concurs with the other runs. Since the specific binding was greater than 75% at all concentrations of glyphosate in all runs, no IC_{50} or relative binding affinity values were estimated. Based on the results from the three runs, glyphosate does not competitively bind to the androgen receptor (Willoughby, 2012a).

Estrogen-receptor binding

In an estrogen-receptor binding assay, the binding of a single concentration of [^{3}H]-17β-estradiol (1 nmol/L) in the presence of increasing concentrations (10^{-10} to 10^{-3} mol/L) of glyphosate (purity 95.93%) was measured. TEGD buffer was used as the solvent vehicle for glyphosate. A total of three runs was performed, each including 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control and 17β-estradiol as the natural ligand reference chemical.

The K_d for [^{3}H]-17β-estradiol was 0.331 (± 0.061) nmol/L and the estimated B_{max} was 74.55 (± 3.03) fmol per 100 µg protein for the prepared rat uterine cytosol. The K_d for each run was within the expected range of 0.03–1.5 nmol/L. In the competitive binding experiment, the estimated mean log IC_{50} for 17β-estradiol was −9.0 mol/L and for 19-norethindrone was −5.5 mol/L. The mean relative binding affinity was 0.032% for 19-norethindrone, compared to the natural ligand. Glyphosate was tested over a concentration range (10^{-10} to 10^{-3} mol/L) that fully defined the top of the curve. Across all runs, the lowest average per cent radiolabelled estradiol binding in the presence of glyphosate was greater than 81% (i.e. showed less than 25% displacement) at concentrations up to 10^{-3} mol/L. Based on the results from the three runs, glyphosate does not competitively bind to the estrogen receptor (Willoughby, 2012b).

Estrogen receptor transcriptional activation

In an estrogen receptor transcriptional activation (ERTA) assay, hERα-HeLa-9903 cells cultured in vitro were exposed to glyphosate (purity 85.14%) at logarithmically increasing concentrations from 10^{-10} to 10^{-3} mol/L in cell culture media for 24 hours in three independent runs. The experiments were performed using 96-well plates, and each glyphosate concentration was tested in six wells/plate in each run. The solvent vehicle was the culture media for glyphosate and DMSO (0.1%) for the reference chemicals. Cells were exposed to the test agent for 24 (± 2) hours to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor was measured using a luciferase assay.

Glyphosate was tested up to the limit dose, with no precipitation or cytotoxicity observed at any tested concentration. At concentrations up to 10^{-3} mol/L, the relative transcriptional activation of glyphosate was less than or equal to 2.4%. Glyphosate was only able to reach a maximum of 0.8–2.4% of the positive control, 1 nmol/L 17β-estradiol, when tested up to the highest concentration. Because the RPC_{max} (maximum level of response induced by a test chemical, expressed as a percentage of the response induced by the positive control) was less than the PC_{10} (concentration of a test chemical at which the response is 10% of the response induced by the positive control in both assay runs), glyphosate was considered negative for estrogen receptor transcriptional activation in this test system (Willoughby, 2012c).

Aromatase

Glyphosate (purity 95.93%) was evaluated for its potential to inhibit aromatase activity by incubating with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H(*N*)]-androstene-4-ene-3,17-dione; [^{3}H]ASDN) at log concentrations of 10^{-10} to 10^{-3} mol/L glyphosate. The solvent vehicle was 0.1 mol/L phosphate buffer for glyphosate, ethanol for ASDN and DMSO for 4-hydroxyandrostenedione (4-OH ASDN), with a final assay volume of less than or equal to 1% DMSO. Aromatase activity was determined by measuring the amount of tritiated water produced at

the end of a 15-minute incubation for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting. Each run included a full activity control, a background activity control, a positive control series (10^{-10} to 10^{-5} mol/L) with a known inhibitor (4-OH ASDN) and the test chemical series (10^{-10} to 10^{-3} mol/L) with three repetitions per concentration.

Aromatase activity in the full activity controls was 0.676 (± 0.072) nmol·mg-protein^{-1}·min^{-1}. The response of each full activity control within a run was between 90% and 110% of the average full activity. Activity in the background controls ranged from 0.23% to 0.38% and averaged 0.30% of the full activity control. For the positive control substance (4-OH ASDN), the estimated log IC_{50} averaged −7.29 mol/L and the Hill slope was −0.96. For glyphosate, aromatase activity averaged 0.673 (± 0.066) nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration of 10^{-10} mol/L and 0.741 (± 0.100) nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration of 10^{-3} mol/L. The average aromatase activity was greater than or equal to 99.67% of the control at all tested glyphosate concentrations for all runs. The results indicate that glyphosate does not inhibit aromatase activity (Wilga, 2012).

Steroidogenesis

The purpose of this study was to validate the use of a standardized steroidogenesis assay as detailed in OECD Guideline for the Testing of Chemicals: Draft Proposal for a New Guideline 4XX – The H295R Steroidogenesis Assay. In this validation study, 28 chemicals were selected as a screen for potential effects of endocrine-disrupting chemicals on the production of testosterone and 17β-estradiol. These chemicals were selected based on their known or suspected endocrine activity, or lack thereof, and included inhibitors and inducers of different potencies as well as positive and negative controls. In this steroidogenesis assay, H295R cells cultured in vitro in 24-well plates were incubated with glyphosate (purity and lot no. not provided) at seven concentrations between 0.0001 and 100 µmol/L for 48 hours in triplicate for three independent experiments. A quality control plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentrations; positive controls included the known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production. Testosterone and 17β-estradiol levels were measured using radioimmunoassays or enzyme-linked immunosorbent assay (ELISA); responses of the quality control plates measured by these assays were confirmed by liquid chromatography–mass spectrometry. In this validation study, the laboratories demonstrated that glyphosate does not affect testosterone or estradiol levels via this assay (Hecker et al., 2011).

In vivo assay

Hershberger assay

To screen for potential anti-androgenic activity, glyphosate in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six 54- or 55-day old, castrated male Sprague Dawley rats at concentrations of 0 (vehicle), 100, 300 or 1000 mg/kg bw per day with a daily dose of reference androgen testosterone propionate at 0.2 mg/kg bw per day by subcutaneous injection. The anti-androgenic positive control group consisted of six castrated rats exposed to 0.2 mg/kg bw per day testosterone propionate by subcutaneous injection and 3 mg/kg bw per day flutamide via oral gavage. Testosterone propionate alone was used as the anti-androgenic negative control. For both components of the assay, body weights were determined daily. The animals were dosed for 10 consecutive days and terminated approximately 24 hours after the final dose. At necropsy, the five androgen-dependent tissues were collected and weighed.

In the androgen-agonist assay, there were no treatment-related effects on body weights, overall body-weight gains or the weights of accessory sex organs for any glyphosate dose group. Animals in the positive testosterone propionate control group had increased ($P < 0.01$) accessory sex organ weights as follows: 437% in seminal vesicles; 728% in the ventral prostate; 200% in levator

ani-bulbocavernosus; 361% in the Cowper gland; and 45% in the glans penis. The performance criteria indicated that this assay was performing as expected.

In the anti-androgen assay, there were no treatment-related effects on body weights, overall body-weight gains or the weights of accessory sex organs for any glyphosate dose group. Animals dosed with testosterone propionate plus flutamide (positive control) had decreased ($P < 0.01$) accessory sex organ weights as follows: 76% in seminal vesicles; 80% in ventral prostate; 63% in the levator ani-bulbocavernosus; 70% in the Cowper glands; and 29% in glans penis. The performance criteria indicated that this assay was performing as expected.

Statistically significant changes were not seen in two or more of the five androgen sensitive tissue weights. Glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay (Stump, 2012a).

Uterotrophic assay

In a uterotrophic assay conducted to screen for potential estrogenic activity, glyphosate (purity 85.14%) in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six ovariectomized female Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300 or 1000 (limit dose) mg/kg bw per day on postnatal days 66/67 to 68/69. The positive control group was treated with a daily dose of 17α-ethynyl estradiol at 3 µg/kg per day by oral gavage. Body weights were determined daily. All the animals were terminated and necropsied approximately 24 hours after the final dose was administered on postnatal day 69/70 to determine wet and blotted uterine weights.

All the animals survived until scheduled termination and no treatment-related clinical findings were observed in glyphosate-dosed animals. Body weights, body-weight gains and uterine weights in the glyphosate groups were comparable to the vehicle control. As expected, absolute wet and blotted uterus weights were increased by 758% and 256%, respectively, in the positive control (17α-ethynyl estradiol) group.

The conclusion reached was that glyphosate was negative in the uterotrophic assay (Stump, 2012b).

Male pubertal assay

In a male pubertal assay, 15 Crl:CD(SD) male rats per dose group were treated daily via oral gavage (5 mL/kg) with glyphosate (purity 95.93%) in 0.5% methylcellulose at 0, 100, 300 or 1000 mg/kg bw per day (limit dose) from postnatal day 23–53. The animals were examined for preputial separation daily beginning on postnatal day 30, and age and weight at day of attainment were recorded. Following termination on postnatal day 53, blood was taken for total thyroxine, testosterone, TSH and clinical chemistry analysis. The hormones were analysed by radioimmunoassay or chemiluminescence.

Treatment-related clinical findings were limited to rales approximately 4 hours post dosing in 9/15 rats at 300 mg/kg bw per day and 14/15 rats at 1000 mg/kg bw per day. This finding persisted in the daily examinations in seven high-dose males throughout the study. On postnatal day 53, final body weights in the 300 and 1000 mg/kg bw per day groups were decreased ($P < 0.05$) by 7–10%. A treatment-related delay in the mean age of attainment of complete preputial separation was noted at 1000 mg/kg bw per day (48.0 days) compared to controls (45.9 days). However, it was determined that this delay at this dose was a result of the treatment-related decrease in body weight, rather than a direct anti-androgenic effect. No treatment-related effects on organ weights were observed at any dose. No treatment-related effects on T4, TSH or testosterone levels were observed at any dose. At 1000 mg/kg bw per day, there was a slight increase in the number of animals with thyroid colloid area grade 4 (five treated vs one control) and grade 5 (one treated vs zero controls). There were no treatment-related effects on follicular cell height at any dose compared to controls; nor were there any treatment-related findings in the testes, epididymides or kidneys.

In conclusion, glyphosate did not affect maturation and did not produce any thyroid toxicity at doses up to 1000 mg/kg bw per day (Stump, 2012c).

Female pubertal assay

In a female pubertal assay, 15 Crl:CD(SD) Sprague Dawley rats/dose group were treated daily via oral gavage with glyphosate (purity 95.93%) in 0.5% methylcellulose at doses of 0, 100, 300 or 1000 mg/kg bw per day (limit dose) from postnatal day 22–42. The animals were examined daily for vaginal opening beginning on postnatal day 22, and age and weight at day of attainment were recorded. Following termination on postnatal day 42, blood was collected for clinical chemistry analyses, including electrochemiluminescent immunoassay (to analyse total thyroxine) and a magnetic [125I]rTSH gamma counter immunoassay (to analyse TSH).

One animal in the control group was terminated in extremis on postnatal day 27 due to impairment of the right forelimb (due to possible mechanical injury). There were no treatment-related differences in age of attainment of vaginal opening, body weights at vaginal opening, final body weights or body-weight gains in the treated groups relative to controls. One control female and one at 300 mg/kg bw per day failed to attain vaginal opening. There were no statistically significant differences in mean age at first vaginal estrus, mean cycle length or per cent cycling. The cycle status at necropsy was similar across all groups. Serum total thyroxine and TSH concentrations were not affected by treatment, and no adverse treatment-related effects on any clinical chemistry parameter were observed at any dose. There were no treatment-related microscopic findings in the thyroid, ovaries, uterus or kidneys at any dose.

In conclusion, glyphosate did delay the maturation and no treatment-related effects were seen in thyroid toxicity (Stump, 2012d).

Additional literature reports

The published literature was reviewed and is included with the EDSP data, in the summary Table 47.

Estrogen pathway

With in vitro studies of estrogen receptor activation, Thongprakaisang et al. (2013) reported estrogen receptor agonism by glyphosate at concentrations from 10^{-12} to 10^{-6} mol/L in estrogen-dependent human breast cancer cells, but did not test the estrogen receptor α antagonism as recommended by the test developers (Evans, Gray & Wilson, 2012). In contrast, other studies reported negative results in reporter gene–transfected Chinese hamster ovary (CHO) cells (Kojima et al., 2004; Kojima, Takeuchi & Nagai, 2010) or that glyphosate formulations reduced the transcription of estrogen receptor α and estrogen receptor β in HepG2 cells transiently transfected with the reporter gene ERE, but the glyphosate parent did not (Gasnier et al., 2009).

In an in vivo rainbow trout VTG assay, glyphosate did not increase plasma VTG in juvenile rainbow trout, and plasma VTG levels in glyphosate plus surfactant–treated trout were only marginally greater than the controls, with no trend and no significance (Xie et al., 2005).

In conclusion, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate. The one positive in vitro study has not been reproduced by another laboratory.

Androgen pathway

The Séralini laboratory conducted several androgen pathway–related assays in equine testes utilizing principally non-validated in vitro assays as well as ex vivo assays. These suggested effects of glyphosate for anti-androgenicity and inhibition of aromatase activity (Richard et al., 2005; Benachour et al., 2007; Gasnier et al., 2009; Defarge et al., 2016). Studies in other laboratories did not report this (Kojima et al., 2004; Kojima, Takeuchi & Nagai, 2010) and particularly those that used the EDSP battery of validated tests for the androgen receptor–mediated and steroidogenesis.

The differences observed in the in vitro studies with positive results and those with negative results may be due to confounding by the glucocorticoid receptor interference in the cell line used in the non-validated assays; the MDA-MB453-kb2 cell line has a high glucocorticoid-receptor content in addition to androgen-receptor content.

Additional steroidogenic mechanisms of interest include a noted effect upon the post transcriptional expression of the StAR in mouse testicular Leydig cells (Walsh et al., 2000) This was also reported for P450scc, the enzyme responsible for the conversion of cholesterol to pregnenolone and for initiating the synthesis of all steroid hormones cells (Walsh et al., 2000). However, another in vitro Leydig cell model reported no effect of glyphosate on basal or recombinant human chorionic gonadotrophin (rhCG) (Forgacs et al., 2012).

In conclusion, there is no convincing evidence of a potential interaction between glyphosate and the androgen receptor pathway. Decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial separation (at 1234 mg/kg bw per day in the two-generation reproduction study in rats) were observed at a dose that was above the limit dose (1000 mg/kg bw per day), and therefore of low physiological relevance.

However there is plausible but equivocal evidence that glyphosate and glyphosate coformulants affect the steroidogenesis pathway, via P450scc and StAR. This requires further investigation.

Thyroid pathway

No relevant in vitro or mammalian in vivo reports on the effect of glyphosate on the thyroid pathway were identified in the literature, and the EDSP data had no evidence.

A handful of reports describe the effect of glyphosate on the negative metamorphosis of frog and tadpole species, including a 2014 report that identified alterations in genes encoding thyroid hormone receptor beta in brain, glucocorticoid receptor in tail and deiodinase enzyme in brain and tail (Lanctot et al., 2014), suggesting that glyphosate formulations have the potential to alter mRNA profiles during metamorphosis.

Other endocrine-related pathways

Following studies conducted in *Xenopus laevis* and chicken embryos, the retinoic acid–signalling pathway has been proposed as a mechanistic pathway that is adversely affected by glyphosate (Paganelli et al., 2010). In this study, a 1/5000 dilution of glyphosate induced reproducible skeletal and craniofacial malformations. Developmental toxicity studies in the rabbit (Section 2.5b, Rabbits) identified nonsignificant skeletal malformations, with the lowest NOAEL for developmental toxicity 250 mg/kg bw per day (Bhide & Patil, 1989). The NOAEL and LOAEL for this study are based on the available data (Bhide & Patil, 1989) as individual data were not provided. A subsequent study NOAEL of 300 mg/kg bw per day was based on delayed ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day (Brooker et al., 1991a). However, these effects were secondary to the observed severe maternal toxicity. Nevertheless, the retinoic-acid pathway constitutes a data gap that requires further research.

Other receptor-mediated pathways reported in the literature, including aryl hydrocarbon receptors and peroxisome proliferator-activated receptors, were negative.

Cortisol stress pathways

A study of the stress response of *Rhamdia quelen* fingerlings with acute exposure to a glyphosate formulation (360 g/L) at 45, 90, 135 and 180 days did not demonstrate impairment of cortisol release but did exert negative effects on growth and survival parameters (Koakoski et al., 2014).

Table 47. Summary of information supporting EDSP data in relation to glyphosate and endocrine end-points

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Estrogen pathway						
EDSP Tier 1 data 2014/2015: In vitro: ER binding; TG 455 ER STTA and Hela Assay In vivo: mammalian assays, i.e. uterotrophic and female pubertal assays and mammalian toxicity studies	Glyphosate Purity: 85.1–95.93% Concentration range: 10^{-10} to 10^{-3} mol/L	USEPA validated assays In vitro assays are well-characterized and OECD TGs ER STTA: uses HeLa cell line which has ER α not ER β. ER α perturbation is more strongly associated with adverse outcomes	There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies, however decreases in offspring body weight were observed in one avian reproduction study	High	Negative	USEPA (2015)
In vitro: ER agonism in estrogen-dependent T47D human breast cancer cells	Glyphosate Purity > 98%) Accustandard Concentration range: 10^{-12} to 10^{-6} mol/L	Validated assay	Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, and not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} mol/L in estrogen withdrawal condition, which was reported to be confirmed by the inhibitory effect of the ER antagonist ICI 182780. The T47D cell line contains both ERα and ERβ. While the use of ICI 182780 can exclude the possibility of dioxin-like interference of coformulant contaminant 1,4-dioxane with AhR interactions affecting the ER, this study is confounded because it was not tested with an ERα-specific antagonist, such as methylpiperidino pyrazole (CAS No. 289726-02-9). This would determine the relative activities of each ER (Evans, Gray & Wilson, 2012) The luciferase reporter system was then also used with combinations of genistein, an isoflavone in soy. Phytoestrogens such as genistein are known to overstimulate luciferase, and also are stronger ligands for ERβ. Non-receptor–mediated luminescence signals have	Low	Positive	Thongprakaisang et al. (2013)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			been reported at phytoestrogen concentrations higher than 1 μmol/L due to the over-activation of the luciferase reporter gene (Kuiper et al., 1998; Escande et al., 2006). While the dose–response curve indicates that true activation of the ER system occurs at lower concentrations, luciferase expression obtained at high concentrations of phytoestrogens or similar compounds suspected of producing phytoestrogen-like over-activation of the luciferase reporter gene needs to be examined carefully in stably transfected ERTA assay systems. (See Annex 2 of OECD TG 455)			
In vitro: hERα and hERβ (ant)agonism in reporter gene–transfected CHO cells	Glyphosate (> 95–100%); whether this is a formulation is not specified in the paper. Concentrations for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.		Concentrations of pesticides that tested negative, which included glyphosate, are not reported; only the results of those that tested positive are provided. Concentration of positively testing chemicals ranged from 10^{-6} to 10^{-12} mol/L Concentration of the test chemicals showing 20% of the agonistic activity of 10^{-10} mol/L 17β-estradiol, and is given as REC 20(mol/L)	Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hERα and hERβ transient transfection into human hepatocarcinoma HepG2 cells	Glyphosate formulations and glyphosate parent chemical. Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line	Formulations reduced transcription of *ERα* and *ERβ* in HepG2 cells transiently transfected with ERE, but glyphosate parent did not	Med	Parent-negative Formulations-positive	Gasnier et al. (2009)
In vivo: FSTRA		In this validated assay, the non-treatment-responsive decrease (only significant at mid-treatment) in VTG was seen in isolation in the absence of any treatment-related effects in the other estrogen-related end-points such as gonado-somatic		Med	Negative	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
		index, gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology				
In vivo: Rainbow trout VTG assay	Glyphosate and glyphosate plus surfactants; measured concentration of glyphosate 0.11 mg/L for 7 days	VTG induction in fish is a standard measure for estrogenicity in environmental regulatory toxicology that also considers the relevance to humans (e.g. USEPA FIFRA SAP 2009a,b, 2012). Glyphosate did not increase plasma VTG in levels in juvenile rainbow trout, glyphosates plus surfactants were only marginally greater than the controls, no trend, no significance		Med	Negative	Xie et al. (2005)
Overall conclusion: No convincing evidence of a potential interaction with the estrogen pathway. The one in vitro study that is positive has not been reproduced by another laboratory.						
Androgen pathway						
EDSP Tier 1 data 2014/2015: In vitro: negative; both for androgen-receptor binding assay and the aromatase assay In vivo mammalian assays: Hershberger and male pubertal assays	Glyphosate	Standardized and validated assays	Androgen-receptor binding assay is not a validated OECD TG but other validated androgen receptor assays not available in 2014/2015 Aromatase assay: highest soluble test concentration of glyphosate was 10^{-3} mol/L The in vivo Tier 1 FSTRA and mammalian assays (i.e. Hershberger and male pubertal assays) were negative in the absence of overt toxicity. The only treatment-related effects observed in the Part 158 mammalian studies in the absence of overt toxicity were decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial	High	Negative, but sperm count and delay in preputial separation effects seen at very high doses, > 1 000 mg/kg bw per day	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			separation at 1 234 mg/kg bw per day in the post-1998 two-generation reproduction study in rats (the EDSP Tier 2 study). Both effects were observed at a dose that was above the limit dose (1 000 mg/kg bw per day) for those studies. No androgen-related effects were seen in the wildlife Part 158 studies (decreases in offspring body weight observed in one avian reproduction study)			
In vitro: hAR transactivation assay in CHO cells	Glyphosate (> 95–100%) formulation not specified in the paper Concentrations given for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.			Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hAR transient transfection into human HepG2 cells, aromatase evaluation within the HepG2 cells, and MDA-MB453-kb2 cells	Glyphosate and formulations Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line. Method for aromatase activity evaluation is also part of OECD TG 456 for steroidogenesis.	MDA-MB453-kb2 cell line has a high content of glucocorticoid receptors in addition to androgen receptors The characterization of the cell line and discussion of such confounding factors is not considered in the paper. While glyphosate and formulations reduced AR transcription in this cell line, there appears to have been no control with androgen-specific responses to exclude glucocorticoid-specific responses	Low	Positive	Gasnier et al. (2009)
Steroidogenesis In vitro: Transformed and human aromatase–transfected cDNA in human embryonic kidney 293 cells and placental-	Glyphosate and formulations 0.01% (with 210 μmol/L glyphosate) to 2% glyphosate/glyphosate formulation	Relevant cell models, but limited characterization provided in the paper	Inhibition of aromatase noted in two different species by both parent compound and formulations The aromatase assay may be subject to variability, e.g. due to degradation of the enzyme, and therefore performance criteria are specified in guideline OPPTS 890.1200 to	Low–Med	Positive	Benachour et al. (2007)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
derived JEG3 cells Ex vivo: normal human placenta and equine testis			demonstrate that the assay is functioning correctly. This is addressed in the EDSP data, but is not evident in the Séralini lab. papers (Benachour et al., 2007; Gasnier et al., 2009), although OECD GD 150 is cited. An adequate response with the proficiency chemicals econazole, fenarimol, nitrofen (inhibitors) and atrazine (non-inhibitor) should be demonstrated and the inhibitor 4-hydroxyandrostenedione (formestane) used as a positive control chemical in each experiment. While the correct positive control was used, proficiency testing is not reported Compliance with the performance criteria should be checked before evaluating results from this assay. A positive result in GD OPPTS 890.1200 requires demonstration of inhibition of aromatase activity that fits a 4-parameter nonlinear regression model such that the concentration response curve crosses 50% inhibition. The concentration response curve allows the determination of potency, i.e. IC_{50}. In some cases, variability may be due to limited solubility of a chemical			
Steroidogenesis In vitro: Placenta-derived JEG3 cells	Glyphosate and formulation ingredients Top dose: 100 ppm		The coformulants were each tested independently and were reported to inhibit aromatase activity at concentrations 20–67% below the no-observed-effect concentration, at which levels glyphosate alone did not significantly inhibit aromatase. (See also comment above regarding proficiency testing of the assay)	Low–Med	Positive	Defarge et al. (2016)
Steroidogenesis In vitro: BLTK1 murine Leydig cells	Glyphosate 300 µmol/L	Relevant and well-characterized Leydig cell model	No effect on basal or rhCG	Med–High	Negative	Forgacs et al. (2012)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Steroidogenesis In vitro: StAR in a mouse MA-10 Leydig tumour cell	Glyphosate formulation (containing 180 g/L glyphosate)	Relevant and well-characterized cell model	Statistically significant reduction ($P < 0.01$) of $(Bu)_2$cAMP with the glyphosate formulation was observed after 2 hours of treatment. Statistical significance ($P < 0.01$) was also reported for P450scc, the enzyme responsible for the conversion of cholesterol to pregnenolone and for initiating the synthesis of all steroid hormones	Med–High	Positive	Walsh et al. (2000)
Overall conclusion: There is no convincing evidence of a potential interaction between glyphosate and the androgen-receptor pathway. Decreases in sperm count in the subchronic rat study (1 678 mg/kg bw per day; USEPA 2015) and a delay in preputial separation at 1234 mg/kg bw per day in the two-generation reproduction study in rats (the EDSP Tier 2 study) were observed at a dose that was above the limit dose (1000 mg/kg bw per day) and therefore of low physiological relevance. However, there is plausible evidence that glyphosate and glyphosate coformulants affect the steroidogenesis pathway, via P450scc and StAR. Further investigation is needed.						
Thyroid						
EDSP Tier 1 data 2014/2015: In vitro: No assays conducted. In vivo test battery: There were no treatment-related effects on T4 and TSH, thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. No thyroid-related effects were observed in the female pubertal assay. There were no developmental effects or alterations in thyroid histopathology in the amphibian metamorphosis assay. No thyroid-related effects were noted in any of the Part 158 studies.	Glyphosate	Relevant and validated test methods	No convincing evidence of potential interaction of glyphosate	High	Negative	USEPA 2015

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Overall conclusion: There is no convincing evidence of a potential interaction with the thyroid pathway for glyphosate						
Other endocrine mechanisms						
Retinoid system In vivo *Xenopus laevis* embryo model and chicken embryos	360 pg and 5 000 pg of glyphosate (Sigma)	Whole vertebrate models, two species	Experimental design and hypothesis based on medical observations of craniofacial defects with malformations observed in humans residing in areas chronically exposed to glyphosate formulations. Suspected to be resulting from a dysfunctional retinoic-acid or Sonic hedgehog pathway. Further investigation is needed	Med–High	Positive: increase in endogenous retinoic-acid activity	Paganelli et al. (2010)
Cortisol In vivo fish study *Rhamdia quelen* fingerlings	Glyphosate formulation 360 g/L	Stress response of *Rhamdia quelen* fingerlings acute exposure at 45, 90, 135 and 180 days	Stress responses important but difficult variable to control for, as stress is induced from handling, etc. This study included appropriate controls for stress confounders	Med	Negative for impaired cortisol release, but impaired growth and survival	Koakoski et al. (2014)
Hypolipidaemia and peroxisome proliferation In vivo rat	Glyphosate formulation 300 mg/kg single daily dose for 2 weeks, 5 animals/dose per group		No increase in number or size or peroxisomes	Med	Negative	Vainio et al. (1983)
AhR induction In vitro: Mouse hepatoma Hepa1c1c7 cells AhR Luciferase reporter gene transcriptional assay	Glyphosate (95–100% purity) Assay performed at concentrations of $\leq 10^{-5}$ mol/L	Relevant and recognized assay		Med	Negative	Takeuchi et al. (2008)
In vitro mPPARα, mAhR, hPXR	Glyphosate		Review, insufficient detail given. Concentration tested not given for negative test chemicals	Low	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
Overall conclusion: Suggestion of adverse effect upon retinoic-acid pathways. Further investigation required.						

AhR: aryl hydrocarbon receptor; AR: androgen receptor; CAS: Chemical Abstracts Service; CHO: Chinese hamster ovary; EDSP: Endocrine Disruptor Screening Program; ER: estrogen receptor; ERTA: estrogen receptor transcriptional activation; FSTRA: fish short-term reproduction assay; GD: guideline; hAR: human androgen receptor; HepG2: hepatocellular carcinoma; IC_{50}: median inhibitory concentration; no.: number; OECD: Organisation for Economic Co-operation and Development; PPAR: peroxisome proliferator-activated receptor; PXR: pregnane X receptor; rhCG: recombinant human chorionic gonadotrophin; StAR: steroidogenic acute regulatory protein; T4: thyroxine; TG: test guideline; TSH: thyroid-stimulating hormone; VTG: vitellogenin

[a] High: line of evidence could be sufficient on its own to be almost sure of entry (approaching 100% likelihood); Med: contributes importantly towards increasing likelihood; Low: minor contribution towards increasing likelihood.

(g) Microbiological effects

Bacteria

The herbicidal action of glyphosate is generated by chelating manganese required in the reduction of the flavin mononucleotide cofactor 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) (Cerdeira & Duke, 2006). Since bacteria have EPSPS and produce amino acids via the shikimate pathway, there is potential for glyphosate residues to disrupt microbes in the human gastrointestinal tract. However, no studies have specifically addressed whether glyphosate affects the microbiota in the human gastrointestinal tract or in mouse and rat animal models. What is known is that selected bacterial pathogens and probiotic bacteria from dairy cows and poultry can be affected differently by residual levels of glyphosate.

The minimum inhibitory concentration (MIC) of glyphosate on the growth and viability of poultry microbiota and pathogens was determined in triplicate in 24-well microtitre plates. Just 100 µL of the tested bacteria (105 colony-forming units [cfu] per mL) was added to 900 µL broth media containing different concentrations of glyphosate (0.075, 0.15, 0.30, 0.60, 1.20, 2.40 or 5.0 mg/mL). Plates containing glyphosate and bacteria were incubated at 37 ºC. MIC values were determined by quantitative analysis of bacteria on agar plates.

Clostridium perfringens, Salmonella gallinarum, S. typhimurium and S. enteritidis were highly resistant to glyphosate (MIC of 5 mg/mL*). Lactobacillus casei, L. buchneri, L. harbinensis, Staphylococcus aureus, S. lentus* and *S. haemolyticu*s were moderately resistant to glyphosate (MIC 0.60–0.30 mg/mL). All other tested bacteria including *Enterococcus faecalis, E. faecium, Bacillus badius, B. cereus* and *Bifidobacterium adolescentis* were highly sensitive to glyphosate, with MIC values ranging from 0.15 to 0.075 mg/mL (Table 48). Pathogenic *E. coli* and *E. coli* 1917 strain Nissle were also found to be resistant to glyphosate (MIC of 1.2 mg/mL).

In summary, most of the tested pathogenic bacteria were highly resistant to glyphosate; however, most other tested bacteria were moderate to highly susceptible (Shehata et al., 2013b).

Table 48. Inhibitory effects of glyphosate on different bacteria

		Bacterial count [a]	
Genus/species	**MIC (mg/mL)**	**Treated with glyphosate at MIC**	**Not treated with glyphosate**
Bacillus badius	0.15	2.24 ± 0.49	8.90 ± 0.44
B. cereus	0.3	2.75 ± 0.68	8.08 ± 0.12
Bacteriodes vulgatus	0.6	3.54 ± 0.31	7.37 ± 0.10
Bifidobacterium adolescentis	0.075	3.87 ± 0.50	8.67 ± 0.48
Campylobacter coli	0.15	3.07 ± 0.50	9.00 ± 0.70
C. jejuni	0.15	3.90 ± 0.50	9.54 ± 0.97
Clostridium perfringens	5.0	3.37 ± 0.89	8.30 ± 0.28
C. botulinum type A	1.2	4.00 ± 0.50	8.16 ± 0.32
C. botulinum type B	1.2	3.56 ± 0.45	7.60 ± 057
E. coli	1.2	3.15 ± 0.24	8.00 ± 0.34
E. coli 1917 strain Nissle	1.2	2.35 ± 0.24	7.26 ± 0.21
Enterococcus faecalis	0.15	2.00 ± 0.45	8.49 ± 0.58
E. faecium	0.15	2.01± 0.34	7.06 ± 0.95
Lactobacillus buchneri	0.6	4.00 ± 0.88	8.00 ± 0.34
L. casei	0.6	4.74 ± 0.56	8.28 ± 0.35
L. harbinensis	0.6	5.30 ± 0.44	8.40 ± 0.32

Genus/species	MIC (mg/mL)	Bacterial count [a]	
		Treated with glyphosate at MIC	Not treated with glyphosate
Riemerella anatipestifer	0.15	4.00 ± 0.50	7.88 ± 0.50
Salmonella enteritidis	5.0	2.35 ± 0.26	8.28 ± 0.16
S. gallinarum	5.0	2.15 ± 0.33	8.68 ± 0.20
S. typhimurium	5.0	2.75 ± 0.68	8.03 ± 0.16
Staphylococcus aureus	0.3	5.74 ± 0.58	9.00 ± 0.10
S. haemolyticus	0.3	5.74 ± 0.32	8.08 ± 0.16
S. lentus	0.3	3.90 ± 0.44	8.08 ± 0.14

MIC: minimum inhibitory concentration; SD: standard deviation

[a] Mean of $n = 3$ quantitative bacterial counts expressed as reciprocal $\log_{10} \pm$ SD.

Source: Shehata et al. (2013b)

An evaluation of the effects of Roundup and its glyphosate ingredients on the growth and viability of three food-associated microorganisms widely used as starters in traditional and industrial dairy technologies found that glyphosate inhibited the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* at a concentration of 1 mg/mL and *Lactococcus lactis* subsp. *cremoris*, which was more sensitive to glyphosate, with an MIC of 0.312 mg/mL (Table 49). The fungus *Geotrichum candidum* was more sensitive, with an MIC of 0.100 mg/mL (Clair et al., 2012).

Table 49. Effect of Roundup on three food-associated microorganisms

Microorganism strain	Concentration of glyphosate in Roundup (g/L)	MIC (ppm)	MMC (ppm)
G. candidum ATCC 204307	400	100	1000
	450	625	1000
L. lactis subsp. *cremoris* ATCC 19257	450	312	625
L. delbrueckii subsp. *bulgaricus* CFL1	450	1000	1250

MIC: minimum inhibitory concentration; MMC: minimum microbicidal concentration; ppm: parts per million

MIC and MMC measured after 24-hour incubation in growth media supplemented with Roundup or equivalent amount of glyphosate.

Source: Clair et al. (2012)

The minimal agricultural use of the herbicide is 10 000 ppm.

In a study of the impact of glyphosate on poultry microbiota and the production of botulinum neurotoxin during ruminal fermentation, ruminal microbiota were characterized by fluorescence in situ hybridization technique using 16S rRNA/23S rRNA-targeted oligonucleotide probes. After incubation with 0, 1, 10 or 100 µg/mL glyphosate in rumen fluids from donor cows, the cell counts of *Ruminococcus albus* and *R. flavefaciens* were significantly lower in the presence of 1 µg/mL glyphosate; *Streptococcus* spp. cell counts were significantly lower with 100 µg/mL glyphosate, and cell counts of the phylum Euryarchaeota were significantly lower on exposure to 10 and 100 µg/mL. In contrast, cell counts of *Clostridium histolyticum* and *Lactobacilli* and *Enterococci* were significantly higher with 100 µg/mL glyphosate. The study authors noted that more bacterial species were inhibited when cows were fed a crude fibre-rich diet than a lower-fibre diet, indicating a possible inhibitory effect on the microbiota responsible for fibre degradation (Ackermann et al., 2015).

In a study of the toxicity of glyphosate to the most prevalent *Enterococcus* spp. in the gastrointestinal tract, the lowest concentration of glyphosate and Roundup to show bactericidal or bacteriostatic effects was determined in 96-well microtitre plates. Serial dilutions of glyphosate from 10–0.001 mg/mL were made in nutrient broth. *Enterococcus* isolates were added at a final concentration of 10^4 cfu/mL, and the test plates with diluted glyphosate and *Enterococcus* incubated overnight at 37 °C before plating aliquots on citrate azide tween carbonate agar. Bacterial growth on each agar plate was evaluated.

Glyphosate and Roundup at 0.1–10 mg/mL inhibited the growth of *E. faecalis* but not of *C. botulinum* or the production of botulinum neurotoxin (Table 50). The study authors proposed that glyphosate may be a significant factor in the observed increased risk of *C. botulinum* infection in cattle in Germany over the past 10 to 15 years (Krüger et al., 2013). Glyphosate toxicity to *Enterococcus* spp. leads to an imbalance in the gut favouring overgrowth of *Clostridium* spp. because the common, beneficial bacteria, *Enterococcus* spp., suppress *Clostridium* growth in the gastrointestinal tract (Krüger et al., 2013; Shehata et al., 2013a,b).

***Table 50. Effect of glyphosate and Roundup on the growth of* C. botulinum *type B and* E. faecalis**

	Glyphosate			Roundup formulation		
Herbicide concentration (mg/mL)	***C. botulinum* type B (cfu/mL) [a]**	**BoNT (ng/mL) [b]**	***E. faecalis* (cfu/mL) [c]**	***C. botulinum* type B (cfu/mL) [a]**	**BoNT (ng/mL)**	***E. faecalis* (cfu/mL)**
0	6.9 ± 0.34	300 ± 47	8.2 ± 0.87	6.9 ± 0.34	270 ± 120	8.2 ± 0.87
0.1	5.3 ± 0.78	312 ± 20	0	5.1 ± 0.78	337 ± 50	0
1	5.4 ± 0.45	319 ± 60	0	3.3 ± 0.80	0	0
10	3.2 ± 0.43	0	0	3.0 ± 0.65	0	0

BoNT: botulinum neurotoxin; cfu: colony-forming unit; ELISA: enzyme-linked immunosorbent assay; SD: standard deviation

[a] *C. botulinum* type B (10^4/mL) cultured anaerobically in reinforced clostridial medium containing different concentrations of glyphosate or herbicide formulation for 5 days. *C. botulinum* quantified using the most probable number estimation method. Data express as reciprocal $\log_{10}$.

[b] *C. botulinum* type B quantified by ELISA.

[c] *E. faecalis* cultured aerobically in reinforced clostridial medium containing different concentrations of glyphosate or herbicide formulation for 8 hours and quantified on citrate-acid-tween-carbonate agar. Data expressed as reciprocal $\log_{10}$ ± SD.

Source: Krüger et al. (2013)

The neutralization ability of the antimicrobial effect of glyphosate by different humic acids was investigated by determining the MIC of glyphosate for different bacteria in different concentrations (0.25, 0.5 and 1.0 mg/mL) of humic acid. The MIC values of glyphosate for *E. faecalis, B. badius and B. adolescentis* were 0.3, 0.3 and 0.15 mg/mL, respectively. Humic acids neutralized the antimicrobial effect of glyphosate in different patterns. The WH67/2, WH67/4/3 and WH67/4 humic acids at 1 mg/mL showed the highest degree of neutralization of the antimicrobial effect of glyphosate. The MIC values of glyphosate for *E. faecalis*, *B. badius* and *B. adolescentis* in the presence of 1 mg/mL WH67/2, WH67/3, and WH67/4 humic acids were more than 2.4 mg/mL, while the MIC values in the presence of other humic acids ranged from 0.3 to 0.6 mg/mL (Shehata et al., 2014). Sorption of the glyphosate to humic acids varied, depending upon their macromolecular structure, but overall, these compounds neutralized the antimicrobial effect of glyphosate (Piccolo et al., 1995, 1996).

Rats

Toxicokinetics of glyphosate after single 100 mg/kg intravenous and 400 mg/kg oral doses were studied in rats. The oral bioavailability of glyphosate was 23.21% (Anadón et al., 2009). This was lower than the oral bioavailability in studies in which [^{14}C]glyphosate was administered orally at 10 mg/kg, when approximately 30–36% of the dose was absorbed (Howe, Chott & McClanahan, 1988; Ridley & Mirley, 1988; Brewster, Warren & Hopkins, 1991). A National Toxicology Program study showed that approximately 19–23% of the 1000 mg/kg dose was absorbed, as determined from urinary excretion data (Chan & Mahler, 1992). Conversely, when a single oral dose of glyphosate (6–9 mg/kg) was administered to New Zealand White rabbits, 80% of the test material appeared in the faeces (Colvin & Miller, 1973c). Glyphosate is poorly metabolized in rats, and the metabolite AMPA represented 6.49% of the parent drug plasma concentration. A similar metabolic characterization was indicated by Brewster et al. (1991). The production of this metabolite could have been the result of intestinal microbial action (Rueppel et al., 1977; Mueller et al., 1981). Taken together, the fraction of the oral dose of glyphosate bioavailable to intestinal microorganisms could range from 70–80% and be microbiologically active. The microbiological activity of the minor metabolite AMPA has not been determined.

Humans

A review of the published scientific literature found no specific information on whether glyphosate bioaccumulates or affects the microbiota in the human gastrointestinal tract. There are no data that show measurements of the amount of glyphosate residues in human gastrointestinal tract. However, several pharmacokinetic, toxicokinetic and bioavailability studies indicate that glyphosate is poorly absorbed after oral administration.

A review of the literature does not indicate that intestinal bacteria generally found in the human gastrointestinal tract have been tested for the ability to degrade glyphosate. However, the microbial capacity for glyphosate degradation has been shown in terrestrial and aquatic environments (Balthazor & Hallas, 1986; Rueppel et al., 1977; Sprankle, Meggitt & Penner, 1975; Mueller et al., 1981; Franz et al., 1997; Zaranyika & Nyandoro, 1993; Kryuchkova et al., 2014). Glyphosate is metabolized by several bacteria in soil to give sarcosine, which is then converted to glycine and ammonia by sarcosine oxidase. An alternative metabolic pathway involves the formation of AMPA by glyphosate oxidoreductase, which is found in colon tissue in rats (Brewster et al., 1991). Therefore, based on the enzymatic repertoire of the intestinal microbiota, there is potential for these microorganisms to metabolize glyphosate.

There are no specific studies on the effects of glyphosate on the mammalian gut microbiota in mouse, rat, rabbit or humans, that is, there is a lack of in vivo studies: all reports are on in vitro tests. In addition, there are no data on the microbiological activity of the glyphosate metabolites, for example, AMPA.

Many of the chronic and long-term in vivo studies reviewed in this monograph reported that high doses of glyphosate have an impact upon the gastrointestinal tract. While not uncommon with administration of high-dose chemical substances, this merits further investigation as glyphosate is known to be poorly absorbed in mammalian models and alterations in gut microbiota profiles, specifically reductions in the beneficial microbiota and increases in pathogenic bacteria, are known to affect the early initiation and progression of the multistep processes in carcinogenesis (Viljoen et al., 2015).

Evidence from livestock species indicates that pathogenic bacteria are more resistant to glyphosate, while beneficial microbiota are more sensitive, and thus more vulnerable (Shehata et al., 2013b). There is also evidence of intestinal metabolism of glyphosate to AMPA in the colon tissue of rats (Brewster, Warren & Hopkins, 1991).

While plausible mechanistic links could be postulated between chromosome breakage, Bcl-2 and p53, adverse gut microbiome profiles in relation to glyphosate formulations (including the

solvent/contaminant 1,4-dioxane), the (nonsignificant) association seen between glyphosate exposure and non-Hodgkin lymphoma (McDuffie et al., 2001) and mechanisms of action of several proteins closely associated with non-Hodgkin lymphoma (NHL) pathogenesis (Song et al., 2016), there are major knowledge gaps in addressing this question. This is because the available information does not specifically address measurement of glyphosate residues in the (gastro)intestinal tract or whether glyphosate adversely affects the normal functioning of the microbiota in the human gastrointestinal tract or the gastrointestinal tract of experimental mammalian models.

2.7 Studies on metabolites: AMPA

AMPA is the only identified metabolite found in the urine and faeces of orally treated rats. It was reviewed by the JMPR in 1997. The Meeting established an acceptable daily intake (ADI) of 0–0.3mg/kg bw (sum of glyphosate and AMPA) based on a NOAEL of 31 mg/kg bw per day, the highest dose tested in a 26-month study of toxicity in rats with glyphosate.

(a) Acute toxicity of AMPA

Mice

In an acute oral toxicity study, five male and five female ICR(Crj:CD-1) mice were orally dosed with AMPA (purity 99.33%) at 5000 mg/kg bw. The test material was administered as a 25% suspension in 1% CMC sodium solution at 20 mL/kg bw. There were no deaths and no signs of toxicity. All mice gained weight on days 0–7; one male and two females had slight weight losses on days 7–14. There were no observed abnormalities at necropsy.

The oral LD_{50} of AMPA in male and female mice was greater than 5000 mg/kg bw (Komura, 1996).

Rats

In an acute oral toxicity study, five male and five female Wistar-derived Alpk:AP_fSD(SPF) albino rats were orally dosed with 5000 mg/kg bw AMPA (assumed purity 100%). The test material was administered as a 50% suspension in 0.5% aqueous polysorbate 80 at a constant dose volume of 10 mL/kg bw.

There were no deaths. Signs of toxicity included diarrhoea, chromodacryorrhea, piloerection, stains around the nose and ungroomed appearance, with recovery by day 5. All the rats but one male gained weight on days 1–8; two males and three females had weight losses on days 8–15. No abnormalities were observed at necropsy.

The oral LD_{50} of AMPA in male and female rats was greater than 5000 mg/kg bw (Leah, 1988).

In a study of acute oral toxicity, five male and five female Sprague Dawley rats were administered AMPA (purity 99.2%) in 0.5% CMC as a single dose at 5000 mg/kg bw by gavage.

Clinical signs, observed 4 hours after dosing, included piloerection, diarrhoea, subdued behaviour, hunched appearance and soiled anal and perigenital areas. All the animals had normal body-weight gain throughout the experiment. No abnormalities were detected at necropsy after 14 days of observation.

The acute oral LD_{50} of AMPA in rats was greater than 5000 mg/kg bw (Cuthbert & Jackson, 1993a).

In a study of acute dermal toxicity, five male and five female Sprague Dawley rats were treated with a single 2000 mg/kg bw dose of AMPA (purity 99.2%). The test material was evenly spread on a 5 × 5 cm dressing moistened with distilled water that was then placed on the shaved back of each rat. The patch was covered with an occlusive dressing and kept in contact with the skin for 24 hours. At the end of the exposure period the patch was removed and the exposed skin wiped with distilled water to remove any excess test material.

There were no mortalities after a single dermal application of AMPA at 2000 mg/kg bw and no clinical signs or abnormalities were noted at necropsy. Thus, the acute dermal LD_{50} of AMPA to rats must be greater than 2000 mg/kg bw (Cuthbert & Jackson, 1993b).

In an acute dermal toxicity study, 2000 mg AMPA (purity 98.0%) suspended in 0.5% aqueous hydroxypropylmethylcellulose gel was applied at a volume of 10 mL/kg to five male and five female CD/Crl:CD rats as an occluded exposure for 24 hours. There were no deaths, no signs of toxicity, no dermal irritation and no observed abnormalities at necropsy.

The dermal LD_{50} of AMPA was greater than 2000 mg/kg (Leuschner, 2002a).

Guinea pigs

The sensitization potential of AMPA (purity 99.2%) was investigated by means of the Magnusson–Kligman Maximization Test in guinea pigs. A group of 20 female Dunkin Hartley guinea pigs were intradermally injected with AMPA at 10% w/v in CMC; 6 days later, 25% w/v in 0.5% CMC was topically applied. Challenge was at a concentration of 25% w/v in CMC.

At challenge, none of the test or control group animals showed a positive response. There was no evidence from the test results that AMPA is a sensitizer in guinea pigs (Cuthbert & Jackson, 1993c).

In a Magnusson–Kligman (maximization test) dermal sensitization study, 10 male Dunkin Hartley guinea pigs were injected with 5% AMPA (purity 98.0%) on day 0, had their application site skin treated with sodium lauryl sulfate on day 6, and then were topically treated with 2 mL of a 50% suspension of AMPA in *aqua ad iniectabilia* on day 7. They were challenged (along with five negative control animals) with 2 mL of a 50% suspension of AMPA in *aqua ad iniectabilia* on day 21. There was no resultant skin irritation in any guinea pig.

The evidence from the test results was that AMPA was a non-sensitizer in this assay (Leuschner, 2002b).

(b) Short-term toxicity studies of AMPA

In a short-term toxicity study, groups of five male and five female Sprague Dawley rats were administered AMPA (purity 99.2%) in CMC at concentrations of 0, 10, 100, 350 or 1000 mg/kg bw per day by oral gavage for 28 days.

There were no treatment-related effects on mortality, clinical signs, body weight, body-weight gains, feed or water consumption or macroscopic findings. There were slight but statistically significant increases in kidney weights in males at 350 and 1000 mg/kg bw per day compared with control group (by 7% and 8%, respectively). Histological examinations revealed a very slight reduction in serous secretion in the mandibular salivary gland of one high-dose male. Whether the minor salivary gland findings is related to treatment is equivocal.

The NOAEL is 100 mg/kg bw per day based on an increase in kidney weights seen at 350 mg/kg bw per day and greater (Heath, Strutt & Iswariah, 1993).

In a 90-day toxicity study, groups of 10 male and 10 female Sprague Dawley rats were administered AMPA (purity 99.2%; in CMC) at a concentrations of 0, 10, 100 or 1000 mg/kg bw per day by gavage for 13 weeks. Blood samples were taken from all animals during week 13 for investigation of haematology and clinical chemistry parameters. An ophthalmoscopic examination was undertaken on all animals during pre-trial and on all control and high-dose animals during week 12. All surviving animals were necropsied at termination as were all pre-terminal decedents. Histological examination was carried out on selected tissues from all control and high-dose animals and all pre-terminal decedents and on the kidneys, liver, lungs, submaxillary salivary gland, sublingual salivary gland and parotid salivary gland of all other animals.

There was no treatment-related effect on mortality, clinical signs, body weight, body-weight gain, feed consumption, water consumption, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights, macroscopic findings and histological examination. The NOAEL in this 90-day gavage toxicity in rats with AMPA was 1000 mg/kg bw per day (Strutt et al., 1993).

Table 51. Summary of acute toxicity studies of AMPA

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	(Crj:CD-1)	M + F	Oral	99.33	> 5 000	Komura (1996)
Rat	Alpk:AP_fSD, Wistar	M + F	Oral	100 (assumed)	> 5 000	Leah (1988)
Rat	Sprague Dawley	M + F	Oral	99.2	> 5 000	Cuthbert & Jackson (1993a)
Rat	Sprague Dawley	M + F	Dermal	99.2	> 2 000	Cuthbert & Jackson (1993b)
Rat	CD/Crl:CD	M + F	Dermal	98.0	> 2 000	Leuschner (2002a)
Guinea pig	Dunkin Hartley	F	Sensitization (Magnusson–Kligman Maximization Test)	99.2	Negative	Cuthbert & Jackson (1993c)
Guinea pig	Dunkin Hartley	M	Sensitization (Magnusson–Kligman Maximization Test)	98.0	Negative	Leuschner (2002b)

LD_{50}: median lethal dose

(c) Genotoxicity of AMPA

A much smaller number of studies have been conducted on the glyphosate metabolite, AMPA, as well as the plant metabolites, *N*-acetyl-glyphosate and *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo studies (Jensen, 1993c; Kier & Stegeman, 1993; Manas et al., 2009b; see Table 35) investigated the ability of AMPA to induce micronuclei in the bone marrow erythrocytes of mice and have largely been negative although a modest positive response was reported by Manas (2009b) when AMPA was administered by intraperitoneal injection to male mice.

Studies by other investigators using the more relevant oral route of administration did not show an increase in micronuclei in either male or female mice.

In the in vitro studies, increases in mutation in bacteria were not seen for AMPA or the acetylated metabolites. Both positive (Manas et al., 2009b) and negative (Jensen, 1993b,c; Roustan et al., 2014) results were reported in studies of chromosome aberrations and DNA damage for AMPA. AMPA was negative in two studies of unscheduled DNA synthesis in isolated rat hepatocytes (Bakke,

1991; Nesslany, 2002). Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

(d) Developmental toxicity of AMPA

In a developmental toxicity study, AMPA (purity 99.2%) suspended in CMC was administered to 10 copulated Sprague Dawley female rats per dose by oral gavage at concentrations of 0, 100, 350 or 1000 mg/kg bw per day from days 6 through 16 of gestation. On day 20 of gestation, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no mortalities or treatment-related clinical throughout the study. Body-weight gain and feed consumption of the test animals were similar to those of the controls. There were no notable intergroup differences in the incidence of intrauterine deaths or in mean fetal weights. Examination of fetuses for developmental abnormalities and variations of the viscera and skeleton (including state of ossification) showed no intergroup differences.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Hazelden, 1992).

2.8 Studies on metabolites: N*-acetyl-glyphosate and* N*-acetyl-AMPA*

Metabolism studies in genetically modified soya beans and maize containing the glyphosate-*N*-acetyltransferase gene demonstrated the formation of new metabolites not observed in conventional crops. The major metabolite in the new maize and soya bean varieties was *N*-acetyl-glyphosate (which may be degraded to glyphosate in the rat), whereas glyphosate, *N*-acetyl-AMPA and AMPA were found in low concentrations in the edible parts of the crops. *N*-Acetyl glyphosate and *N*-acetyl-AMPA were reviewed by the JMPR in 2011. The Meeting (2011) concluded that the group ADI of 0–1 mg/kg bw established by the 2004 JMPR for glyphosate and AMPA may also be applied to *N*-acetyl-glyphosate and *N*-acetyl-AMPA as the available toxicological data showed that these plant metabolites have no greater toxicity than the parent glyphosate. The 2004 JMPR decided that an acute reference dose (ARfD) for glyphosate was unnecessary. The 2011 JMPR confirmed that it is not necessary to establish an ARfD for *N*-acetyl-glyphosate or *N*-acetyl-AMPA in view of their low acute toxicity and the absence of any toxicological effects that would be likely to be elicited by a single dose.

(a) Biotransformation of N*-acetyl-glyphosate (company code IN-MCX20)*

A total of 45 male Crl:CD(SD)IGS BR rats were each administered a single oral dose of free acid at 15 mg eq/kg bw of [^{14}C]*N*-acetyl glyphosate (sodium salt; purity 84.3%, radiochemical purity 99.2%) in water. Blood was collected from four animals pre dose and at 0.5, 1, 2, 4, 8, 12, 48 and 72 hours post dose. Excreta were collected from five animals at specified intervals through 168 hours post dose. Plasma, excreta and carcasses were analysed for radioactive content. Selected samples of plasma, urine and faeces were analysed for unchanged parent compound and metabolites.

The mean total recovery was 95.5%, with 66.1% (61.3% within 12 hours of dosage) in urine, 26.4% (25.8% within 48 hours of dosage) in faeces, 2.79% in cage wash and wipe, and 0.23% in residual carcass. More than 90% of the total radioactivity was eliminated 48 hours post dose. C_{max} in blood and plasma were 2.93 and 5.31 µg eq/g at 1 and 2 hours post dose, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma AUC values indicates that ^{14}C-labelled *N*-acetyl-glyphosate distributed preferentially into plasma.

Unchanged ^{14}C-labelled *N*-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and

represented less than 1% of the total radioactivity. Plasma radioactivity consisted entirely of unchanged ^{14}C-labelled *N*-acetyl-glyphosate (Cheng & Howard, 2004).

(b) Acute toxicity of N-*acetyl-glyphosate and of* N-*acetyl-AMPA*

N-Acetyl glyphosate (purity 84.3% sodium salt, equivalent to 67.4% free acid) was suspended in water and administered to five male and five female Crl:CD(SD) IGS BR fasted (17–20 hours) rats at a dose of 5000 mg/kg bw of free acid, administered as a constant dose volume of 10 mL/kg bw.

One female was found dead at 6 hours after administration, and one female and one male were found dead the following day. Signs of toxicity (seen in all rats) included slight hypoactivity, irregular respiration, liquid faeces, soft faeces, light-brown perineal staining, squinted eyes and brown nasal crust. All survivors were normal 3 days after dosing. Necropsy findings of decedents included mottled or discoloured lungs, discoloured (black) liver, soft stomach, yellow fluid or gel-like clear liquid in stomach, fluid in abdominal cavity, fluid in duodenum, jejunum and ileum.

The LD_{50} of *N*-acetyl glyphosate in rats was greater than 5000 mg/kg bw of free acid (Vegarra, 2004).

N-Acetyl AMPA (purity 97%) suspended in deionized water was administered by oral gavage at a constant dose volume of 20 mL/kg bw at 5000 mg/kg to three Crl (CD)SD female rats.

There were no deaths. Signs of toxicity included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyperreactivity. All the rats had fully recovered 3 days after dosage. All the rats gained weight on days 0–7 and 7–14. There were no dose-related abnormalities at necropsy.

The LD_{50} of *N*-acetyl-AMPA in rats was greater than 5000 mg/kg bw based on the signs of toxicity (Carpenter, 2007).

(c) Subacute toxicity of N-acetyl-glyphosate

Five groups of young adult male and female Crl:CD(SD) rats (10/sex per group) were fed diets containing 0, 180, 900, 4500 or 18 000 ppm *N*-acetyl-glyphosate sodium salt (purity 81.8%) (equal to 0, 11.3, 55.7, 283 and 1157 mg/kg bw per day, respectively, for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw per day, respectively, for females) for 95 days (males) or 96 days (females).

No adverse effects on body weights or nutritional parameters were observed. The slight decrease in body weight (92% of the control) in the high-dose animals was not considered adverse since statistical significance was not achieved. Statistically significant lower overall mean body-weight gain (86% of control) was observed in males at 18 000 ppm but it was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean feed consumption of feed efficiency.

There were neither any treatment-related deaths nor any clinical, ophthalmological or neurobehavioural observations. There were no adverse effects on clinical pathology parameters, organ weights, gross pathology or microscopic pathology in male or female rats. The NOAEL for male and female rats was 18 000 ppm, equivalent to 1157 mg/kg bw per day in males and females, respectively (MacKenzie, 2007).

A supplemental report to the 90-day MacKenzie (2007) study tested dietary disodium *N*-acetyl-*N*-(phosphonomethyl)glycine (purity 63%, expressed as the weight per cent on a free acid basis).

Pooled urine samples for male rat groups I (control), III (180 ppm), V (900 ppm), VII (4500 ppm) and IX (18 000 ppm) were collected on day 82 and for female rats groups II (control), IV (180

ppm), VI (900 ppm), VIII (4500 ppm) and X (18 000 ppm) on day 83 for analysis of IN-MCX20 (*N*-acetyl-glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and IN-EY252 (*N*-acetyl AMPA). On the same days, plasma samples from individual rats were collected for the same analyses.

Concentrations of IN-MCX20 (*N*-acetyl-glyphosate) in the urine increased with the increasing dietary levels of *N*-acetyl-*N*-(phosphonomethyl)glycine. Concentrations of IN-B2856 and IN-EY252 were above the limit of detection at higher dietary levels (900–18 000 ppm) but at or below the limit of detection at 180 ppm. In addition, the concentrations of these metabolites were much higher in urine samples from male rats than from female rats at 4500 and 18 000 ppm. Neither IN-MCX20 nor its metabolites were detected in urine of control rats.

Concentrations of IN-MCX20 (*N*-acetyl-glyphosate) also increased in the plasma samples with increasing dietary levels of *N*-acetyl-*N*-(phosphonomethyl)glycine. Concentrations of IN-MCX20 were less than 1.0 µg/mL for males and females in the 180 ppm dietary group, but increased from a mean of about 2 µg/mL up to about 14.0 µg/mL for the other dietary groups. Little to no IN-B2856 (glyphosate) or IN-EY252 (*N*-acetyl AMPA) was detected in plasma at all dietary levels.

These results confirm that IN-MCX20 (*N*-acetyl-glyphosate) is metabolized in rats to small quantities of IN-B2856 (glyphosate) and IN-EY252 (*N*-acetyl AMPA) (Shen, 2007).

(d) Genotoxicity of N-*acetyl-glyphosate and of* N-*acetyl-AMPA*

A few studies have been conducted on the genotoxicity of the glyphosate metabolite, *N*-acetyl-glyphosate. The results are shown in Tables 36, 37 and 38.

The in vivo studies shown in Table 35 (Murli, 2004; Donner, 2006; Glatt, 2006) that investigated the ability of *N*-acetyl-glyphosate to induce micronuclei in the bone marrow erythrocytes of mice and gene mutations and chromosomal aberrations in CHO cells were negative.

Increases in mutation were also not seen in the in vitro studies.

A smaller number of studies have been conducted on the plant metabolites, *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo study (Donner, 2007; Table 35) investigating the ability of *N*-acetyl-AMPA to induce micronuclei in the bone marrow erythrocytes of mice was negative.

In the in vitro studies, increases in mutation in bacteria were not seen; nor were gene mutations in Chinese hamster cells (Glatt, 2007) or chromosomal aberrations in human peripheral blood lymphocytes (Gudi & Rao, 2007).

2.9 Studies on other formulation ingredients

Several publications have reported that glyphosate formulation ingredients and possible contaminants have a greater toxicity than the active ingredient, glyphosate.

Although it was pertinent to consider the toxicity of the known formulants, a detailed review and exhaustive analysis could not be undertaken due to lack of time and confidentiality constraints; producers often consider formulation ingredients proprietary and hence confidential and obtaining this information can be problematic. Nevertheless, based on the reports listed below, it is apparent that some of the formulants may have a greater toxicity than the active ingredient, glyphosate.

Polyethoxylated tallow amine (polyoxyethyleneamine [POEA]; MON 0818; CAS No. 61791-26-2 (tallow); POE n =15)

In a 30-day oral toxicity study, MON 0818 (purity and lot number not reported) was administered to groups of 10 male and 10 female Sprague Dawley rats in the diet at concentrations of

0, 800, 2000 or 5000 ppm (equal to 0, 51.7, 122.8 and 268.7 mg/kg bw per day for males and 0, 63.2, 159.9 and 324.8 mg/kg bw per day for females).

All the treated rats survived until scheduled termination. Soft stools were observed from three high-dose males on four occasions and from eight high-dose females on 23 occasions. Body weight, body-weight gain and feed consumption of high-dose male and female rats were significantly reduced during the study; this was consistent with poor diet palatability. Feed consumption of mid-dose male rats was statistically decreased during the first week of treatment, as was total body weight at the end of the study; however, the final body weight was decreased by only 7% relative to controls. No treatment-related effects were found in mid-dose female rats or in low-dose male and female rats. The absolute and relative organ weights of high-dose male and female rats were decreased consistent with the markedly decreased body weight. Prominent or enlarged lymphoid aggregates in the colon of five high-dose female rats were observed at necropsy.

Because a description of the test material, its lot number, its purity and its concentration, homogeneity and stability in the diet were not provided or determined, an estimate of the dose inducing treatment-related effects on male and female rats cannot be made. In addition, very limited in-life observations and, with the exception of selected organ weights and gross pathology, no post-termination studies or observations were made (Ogrowsky, 1989). As a result, this study was deemed unacceptable and it could not be used to establish a NOAEL or LOAEL.

In a subchronic oral toxicity study of MON 0818 in Sprague Dawley rats, the test material was administered in the diet ad libitum to three groups of 10 male and 10 female rats for 90 days. Target test diet concentrations were 0, 500, 1500 or 4500 ppm (equal to 0, 33.0, 99.3 and 291.6 mg/kg bw per day in males and 0, 39.9, 123.1 and 356.6 mg/kg bw per day in females). A similar, concurrent control group of rats were fed the basal diet only.

Exposure at 1500 and 4500 ppm resulted in statistically and toxicologically significant effects. Toxicity observed at 4500 ppm consists of clinical signs (soft stools, three incidences in two males and 86 incidences in all females) observed from day 16 through day 92 of the study, decreased mean body weights throughout the study (from 12–20% in males and 8–18% in females), and decreased mean total body-weight gains in males (31%) and females (35%). Feed consumption was also significantly reduced throughout most of the study (13 weeks for males and 10 weeks for females), particularly during the first week of the study (32% decrease in males and 27% decrease in females). Since a feed efficiency assessment was not conducted, it is not possible to determine if the decreases in body weights, body-weight gains, and feed consumption were due, in part, to the unpalatability of the diet. Statistically significant changes in haematological parameters observed in females may be a result of the inflammation observed in the intestines. Statistically significant changes in clinical chemistry parameters and organ weights observed in high-dose males and females are likely a result of decreased feed consumption/nutrient absorption and body weight.

At both 1500 and 4500 ppm, microscopic examination conducted at necropsy revealed lesions, including hypertrophy and/or vacuolation of histiocytes in the lamina propria of the ileum in all high-dose males and females, and four mid-dose males and four mid-dose females; hypertrophy and/or vacuolation of histiocytes in the lamina propria of the jejunum in four high-dose males, seven high-dose females and one mid-dose female; sinus histiocytosis in nine high-dose males, six high-dose females and two mid-dose males and females; and accumulation of macrophage aggregates in the cortex and medullary cords of the mesenteric lymph node in eight high-dose males, seven high-dose females and two mid-dose females. These inflammatory changes are likely the cause of the soft stools observed during the study and are considered treatment-related.

No statistically significant treatment-related effects on body weight, body-weight gain, feed consumption, haematological/clinical chemistry parameters and organ weights were observed at the low-dose level of 500 ppm. In addition, no gross abnormalities or histopathological findings related to treatment were observed at this dose level.

Based on treatment-related inflammatory changes at 1500 ppm (equal to 99.3 mg/kg bw per day), the NOAEL for MON 0818 was 500 ppm (equal to 33.0 mg/kg bw per day). The LOAEL was 1500 ppm (equal to 99.3 mg/kg bw per day) based on irritation in the intestines and colon (hypertrophy and vacuolation of histiocytes in the lamina propria of the jejunum and ileum, and histiocytosis and accumulation of macrophage aggregates in the mesenteric lymph node (Stout, 1990).

In a screening study, the potential reproductive toxicity and developmental (prenatal and postnatal) toxicity of MON 0818 (purity 69–73%) was evaluated in CD (Sprague Dawley) rats through two successive generations. The study was designed to evaluate the effects of MON 0818 on male and female reproduction within the scope of a screening study. The study was extended to a two-generation study when a decrease in live litter size was observed at the high-dose level. MON 0818 was administered orally via the diet to three groups of 20 male and 20 female CD rats. Target test diet concentrations were 0, 100, 300 or 1000 ppm (corrected for purity to doses equal to 0, 4.4, 13.4 and 44.5 mg/kg bw per day for males and 0, 9.6, 16.1 and 54.0 mg/kg bw per day for females). A similar concurrent control group of rats were fed the basal diet only. At approximately 10 weeks of age, the F_0 animals were dosed via diet for at least 70 days prior to mating and then to termination (males) or lactation day 21 (females). All F_0 adults were terminated following selection of the F_1 generation on postnatal day 21.

Parents for the F_1 generation were selected from the weaned F_1 litters. Between postnatal day 21 or 22 and 70, the weanling F_1 animals (3 per sex/litter, if possible) were administered the test diet on a mg/kg bw basis (so not to overexpose the rapidly growing F_1 animals) at target concentrations of 0, 6, 18 or 61 mg/kg bw per day for the F_1 males and 0, 7, 22 or 74 mg/kg bw per day for the F_1 females. Beginning on postnatal day 70, the F_1 animals selected for breeding from the control and high-dose groups only (2 per sex/litter) were administered the test diet at a constant concentration (0 or 1000 ppm) for at least 80–88 days prior to mating. The selected F_1 males continued to receive the test diet throughout mating and until termination (after lactation day 4). The selected F_1 females continued to receive the test diet throughout mating, gestation and lactation, until termination (after lactation day 4).

Mortality and clinical signs, body weights, body-weight gains, feed consumption, reproductive function, fertility and mating performance, absolute and relative organ weights, macroscopic abnormalities at necropsy and histopathological findings were recorded for all parental/adult animals. In addition, blood samples for testosterone and/or thyroid hormone concentration determinations were collected from one F_1 male and one F_1 female per litter at the scheduled necropsy. Sperm evaluation (motility and morphology) was also performed on all F_1 male animals at termination. Litter size, viability, clinical signs, body weights, body-weight gains, developmental (sexual and physical) parameters, and macroscopic abnormalities at necropsy were recorded for the F_1 and F_2 pups.

Survival and clinical conditions, mean body weights and feed consumption (pre-mating, gestation, and lactation), reproductive performance, mean organ weights, and macroscopic and microscopic morphology of the F_0 and F_1 parental generations were unaffected at all dose levels. Treatment-related effects were also not seen in estrous cyclicity, spermatogenic end-points and testosterone and thyroid hormone levels of the F_1 generation or in the clinical signs, mean body weights and developmental landmarks of the F_1 and F_2 pups, as well as the litter viability and postnatal survival of the F_2 litters.

Potential treatment-related effects were observed in litter loss, increased mean number of unaccounted-for implantation sites and decreased mean number of pups born, live litter size and postnatal survival from birth to lactation day 4 in the high-dose F_0 females and F_1 litters. These effects were limited to a small number of litters, were not always statistically significant and were not reproduced in the F_2 litters. However, the increased (statistically significant) mean number of unaccounted-for implantation sites exceeded the maximum mean value in the laboratory historical control data. While not statistically significant, the corresponding reduced number of pups born and

live litter size, as well as the reduced postnatal survival, were at or below the limits observed in the laboratory historical control data.

The LOAEL of MON 0818 for reproductive toxicity and offspring toxicity in rats was 1000 ppm (equal to 44.5 mg/kg bw per day) based on litter loss, increase mean number of unaccounted-for implantation sites and decreased mean number of pups born, live litter size and postnatal survival from birth to lactation day 4. The NOAEL for reproductive and offspring toxicity was 300 ppm (equal to 13.4 mg/kg bw per day). The NOAEL for parental systemic toxicity was 1000 ppm (equal to 44.5 mg/kg bw per day). A LOAEL for parental systemic toxicity was not determined (Knapp, 2007).

In a combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test, MON 8109 (coco amine ethoxylates, CAS No. 61791-31-9, (coco); Ave POE n = 2); purity 100%) or MON 0818 (purity 100%) was administered to 12 Crl:CD(SD) rats/sex per dose in the diet at dose levels of 0, 30, 100, 300 or 2000 ppm MON 8109 or 1000 ppm MON 0818. The mean compound intake for MON 8109 was 0, 2, 8, 23 and 134 mg/kg bw per day for males and 0, 3, 9, 26 and 148 mg/kg bw per day for females. The mean compound intake for MON 8108 was 0, 2, 8, 23 and 76 mg/kg bw per day for males and 0, 3, 9, 26 and 86 mg/kg bw per day for females. Males were fed the test or basal diets for a total of 71–72 days, and the females were fed the test or basal diets for a total of 69–72 days. Functional observational battery and locomotor activity data were recorded for six males per group near the end of diet administration and for six females per group on lactation day 4. Parental animals were terminated approximately 2.5 weeks after lactation day 4, and offspring were terminated on lactation day 4.

There was no treatment-related mortality. One female in the 1000 ppm MON 0818 group was found dead with dystocia on lactation day 1 and another was euthanized in extremis on gestation day 30 and found to have a ruptured uterus. Increased incidences of red material around the nose, reddened nose and reddened mouth at 2000 ppm MON 8109 in males and females were treatment-related. Mean body-weight losses were noted at 2000 ppm MON 8109 in male and females during the first week of test diet administration. Lower mean body weight and/or body-weight gain with corresponding reduction in feed consumption were usually observed in the animals from this group throughout the study. Absolute and relative organ-weight values that were statistically different from the corresponding control were not treatment related as this difference was due to the significantly lower body weight of the 2000 ppm MON 8109–treated animals. The females from this group had a lower number of implantation sites and lower live litter size. The offspring of these females had lower postnatal survival on postnatal day 0, postnatal day 0–1, postnatal days 1–4 and birth to postnatal day 4 compared to the control group. No effect of treatment was observed in male and female mating and fertility, male copulation and female conception indices, gestation length, functional observational battery, locomotor activity, haematology or serum chemistry. No test-substance–related findings were noted in the 30, 100 or 300 ppm MON 8109 or 1000 ppm MON 0818 group males, females or offspring.

The parental systemic LOAEL was 2000 ppm for MON 8109 (equal to 134 mg/kg bw per day), based on clinical findings and decreased mean body weight, body-weight gain and feed consumption. The parental systemic NOAEL was 300 ppm for MON 8109 (equal to 23 mg/kg bw per day).

The reproductive/developmental LOAEL was 2000 ppm MON 8109 (equal to 134 mg/kg bw per day) based on decreased postnatal survival, lower live litter size on postnatal day 0, lower number of pups born and lower number of implantation sites. The reproductive NOAEL is 300 ppm MON 8109 (equal to 23 mg/kg bw per day).

A parental LOAEL for MON 0818 was not demonstrated in this study. The parental NOAEL was 1000 ppm for MON 0818 (equal to 76 mg/kg bw per day).

The reproductive/developmental LOAEL for MON 0818 was not demonstrated in this study. The reproductive NOAEL was 1000 ppm MON 0818 (equal to 76 mg/kg bw per day) (Knapp, 2008; Nord, 2008).

In a developmental toxicity study, MON 0818 (purity 100%) was administered in Mazola Corn Oil to 25 Charles River Crl:CDBr female rats per dose by gavage at dose levels of 0 (corn oil only), 15, 100 or 300 mg/kg bw per day from gestation day 6 through 15. On gestation day 20, all surviving females were terminated for developmental examination. The developmental parameters noted included the number of viable fetuses, early and late resorptions, total implantations and total corpora lutea and the sex and weight of fetuses and external, visceral and skeletal examinations of all fetuses.

Six of the 25 high-dose females died during gestation days 6–15. Clinical signs observed in the high-dose females included rales (12/25), laboured respiration (3/25), yellow uro- (15/25) or anogenital (14/25) matting and mucoid faeces (22/25) compared to none for the control animals. Few to no clinical signs were observed in the mid-dose and low-dose females. High-dose females weighed significantly ($P < 0.01$) less than the controls from study day 9 until termination at study day 20. High-dose females also gained 59% less weight compared to controls during treatment (days 6–16). Body weight was similar to controls in the low- and mid-dose groups. Gravid uterine weight was not affected by treatment in any of the groups. High-dose females ate statistically ($P < 0.01$) less feed compared to the control rats, with the most significant decrease (55% less than controls) on days 6–9 before gradually improvement to comparability with controls by day 16. Overall, the high-dose group ate 29% less than the controls during days 6–16. Feed consumption for the low-dose and mid-dose females was comparable to that of controls throughout the study, except for days 6–9 when the mid-dose group had a statistically significant ($P < 0.05$) decrease. No treatment-related effects were observed on liver weight or gross pathology at necropsy in any of the treated dams.

No treatment-related differences were observed in the mean number of corpora lutea, implantations, live fetuses or resorptions or mean fetal weight. On external examination, the mean number of fetal malformations from the high-dose dams appeared to be high but most were observed in a single fetus and a dose–response relationship was not observed. On visceral examination of the fetuses from the high-dose group, one fetus was missing a urinary bladder, one had stenosis of the right carotid artery and two had situs inversus, but these were not considered treatment related as there was no dose–response relationship for the situs inversus and the others were within the historical control data range. Vertebral anomalies with or without rib anomalies were observed in one fetus in the high-dose group but this was within the range of historical control data. No malformations were observed in the low- or mid-dose groups. Several skeletal variations in the sternebrae and ribs were identified but they were observed in both the control and treated groups at similar incidences and are not considered treatment related.

The maternal toxicity LOAEL for MON 0818 in rats was 300 mg/kg bw per day, based on increased mortality, clinical signs and decreased body weight, body-weight gain and feed consumption. The maternal NOAEL for MON 0818 was 100 mg/kg bw per day.

The developmental toxicity LOAEL for MON 0818 in rats could not be determined as no effects were associated with treatment. The developmental toxicity NOAEL for MON 0818 is 300 mg/kg bw per day (Holson, 2006).

In independent trials of the reverse gene mutation assay in bacteria, strains TA1535, TA1537, TA98 and TA100 of *S. typhimurium* were exposed to MON 0818 (purity not stated). In the first trial, all tester strains were exposed to 0.001, 0.003, 0.01, 0.03 or 0.1 mg/plate with S9 activation and 0.0003, 0.001, 0.003, 0.01 or 0.03 mg/plate without S9 activation. (The S9-fraction was obtained from Aroclor 1254–induced male Sprague Dawley rat liver.) A repeat assay was performed on TA1535 and TA1537 (±S9) using the same concentrations as in in trial one. Because cytotoxicity was not observed with all tester strains, test material concentrations were adjusted for the subsequent mutagenicity trials (trials 3 and 4). Concentrations of MON 0818 from 0.01–1.0 mg/plate with S9 activation and 0.003–0.3 mg/plate without S9 activation were tested in strain TA98; 0.001–0.10 mg/plate with and without

S9 activation in TA100; 0.001–0.1 mg/plate without S9 in TA1535; 0.003–0.3 mg/plate with S9 activation and 0.001–0.1 mg/plate without S9 activation in TA1537.

No evidence of mutagenicity was observed in trial 1. A statistically significant ($P < 0.01$) increase in the number of revertant colonies was observed at 0.03 mg/plate (−S9) in TA98 and 0.0003 mg/plate in TA1535 (−S9); however, the increases were less than twofold and not concentration dependent. When the strains were retested in trials 3 and 4, cytotoxicity was seen at 0.3 mg/plate and above with S9 activation and 0.1 mg/plate and above without S9 activation in TA98; 0.03 mg/plate and above with and without S9 activation in TA100; at 0.1 mg/plate without S9 activation in TA1535; and at 0.1 mg/plate and above with and without S9 activation in TA1537. Although slight increases in the number of revertants were seen at non-cytotoxic concentrations of 0.01 and 0.1 mg/plate with S9 activation in TA98, the increases were less than twofold greater than the solvent controls and did not satisfy the criteria for a positive response. No concentration-dependent increase in the number of revertant colonies was observed in any of tester strains with or without S9 activation.

Overall, no evidence of mutagenicity was observed at non-cytotoxic concentrations with or without S9 activation.

MON 0818 was tested up to cytotoxic concentrations in all strains, but failed to induce a mutagenic response in this test system. The positive controls induced the expected mutagenic responses in the appropriate strain (Stegeman & Li, 1990).

In a bone marrow micronucleus assay, adult male and female ICR(Crl:CD-1) mice (5/sex per dose) were treated once via intraperitoneal injection with 0 or 100 mg/kg MON 0818, which was estimated to be about 61% of the LD_{50} (batch/lot no. PIT-8907-757-I; purity 100%, prepared in corn oil). Bone marrow cells were harvested at 24 and 48 hours following dosing and scored for micronucleated polychromatic erythrocytes. Cyclophosphamide (60 mg/kg) served as the positive control.

No deaths or overt signs of clinical toxicity or cytotoxicity of bone marrow were observed at this dose. Although no toxicity was seen at 100 mg/kg, the selected level was considered acceptable in accordance with the high dose recommended by the USEPA Gene-Tox Program (i.e. when a dose that is not less than 50% of the LD_{50} is used to define the maximum tolerated dose) for the micronucleus assay (Mavournin et al., 1990). Administration of 60 mg/kg cyclophosphamide caused a significant ($P < 0.01$) induction of micronucleated polychromatic erythrocytes in both sexes. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any harvest time up to the maximum tolerated dose (Stegeman & Kier, 1998).

N,N-bis-(2-hydroxyethyl) alkylamine; synthetic ethoxylated amine, ATMER 163 (CAS No. 70955-14-5; C13-C15; ave POE n =2)

In a 90-day oral gavage toxicity study, ATMER 163 (100% a.i. assumed and batch/lot no. not reported) was administered to 20 Sprague Dawley (Crl:CD[SD]BR) rats per sex per dose at concentrations of 0, 15, 30 or 150 mg/kg bw per day. Deionized water was administered to controls.

Numerous clinical signs were observed in animals at 150 mg/kg bw per day. The most notable signs were wheezing and salivation in all high-dose animals and in some at 30 mg/kg bw per day. Other clinical signs observed in both sexes at 150 mg/kg bw per day included blood crust and/or red discharge (nose), dyspnoea, rhinorrhea, opaque eyes, redness, hunched posture, thinness, urine stains, rough hair, desquamation and an increased incidence of alopecia. Two males at 30 mg/kg bw per day as well as four males and one female at 150 mg/kg bw per day died during the study. Statistically significant body weight and body-weight gain deficits were observed in both sexes at 150 mg/kg bw per day; overall body-weight gains were 30.5% and 15.3% lower than control values in males and females, respectively. Statistically significant decreased feed consumption was seen at 150 mg/kg bw per day in males only. An ophthalmoscopic assessment revealed posterior subcapsular cataracts in males at 30 and 150 mg/kg bw per day and in females at 150 mg/kg bw per day while

complete cataracts were found only at 150 mg/kg bw per day in both sexes. Increased mean values for platelet count, white blood cell count, segmented neutrophil count and lymphocyte count were seen at the 150 mg/kg bw per day dose in both males and females; all of the increases were statistically significant except the increased lymphocyte count in males. These findings are often associated with tissue inflammation which, together with other relevant findings, was observed in the lungs and stomach of both sexes at this dosage. The only noteworthy treatment-related gross pathology findings were in the nonglandular stomach and eyes. The findings in the nonglandular stomach, desquamation and alteration of mucosa, were primarily found in both sexes at 150 mg/kg bw per day, although some alterations of mucosa were also seen in animals at 30 mg/kg bw per day. Opaque eyes, seen in both sexes at 150 mg/kg bw per day, were consistent with the ophthalmoscopic findings of complete cataracts. Treatment-related histopathological findings included inflammation in the lungs of both sexes at 150 mg/kg bw per day and the nonglandular stomach of both sexes at 30 and 150 mg/kg bw per day. The inflammation in lungs might have been due to inadvertent aspiration since previous studies have established that ATMER 163 is a primary irritant. Dose-related incidences of acanthosis in the nonglandular stomach were seen in males and females at 30 and 150 mg/kg bw per day. The only noteworthy finding in the glandular stomach was suppurative inflammation in two females at 150 mg/kg bw per day. In addition, microscopic assessment showed cataracts, mostly bilateral, in the eyes of both sexes at 150 mg/kg bw per day.

There were no toxicologically significant treatment-related effects based on assessment of clinical chemistry and limited assessment of organ weights. Urine analysis was not conducted.

The LOAEL for ATMER 163 in Sprague Dawley rats was 30 mg/kg bw per day based on increased mortality, salivation and posterior subcapsular cataracts in males as well as wheezing and macro- and microscopic changes in the nonglandular stomach of both sexes. The NOAEL is 15 mg/kg bw per day (Zoetis, 1991).

In a subchronic 90-day oral toxicity study, ATMER 163 (purity 100%) was administered via capsule to three groups of four male and four female beagle dogs for 13 weeks at concentrations of 15, 30, or 100 mg/kg bw per day. A similar concurrent control group was given empty capsules.

There were no unscheduled deaths during the study. All the dogs survived until scheduled termination. Exposure at 100 mg/kg bw per day resulted in statistically and toxicologically significant effects. Clinical signs of toxicity included increased incidence of salivation, emesis and soft faeces (noted with mucus alone or mucus and bile-like material). Salivation was observed in all males and females beginning in week 3 of the study (six animals) and continuing over 5–11 weeks. Emesis was also observed in all of the males and females and was first observed during the first 2 weeks of the study in seven animals and continued over 1–11 weeks. Soft mucoid faeces were observed in three males and all the females over 2 to 7 weeks; soft mucoid or bile-particle–containing faeces were observed in the high-dose animals (three males and two females) over 1–3 weeks. All of these clinical signs are considered treatment related based on the high frequency of occurrence and clear dose–response relationship. In addition, mean alanine transaminase levels in females were significantly increased (154%) relative to controls. Microscopic examination at necropsy revealed increased pigment accumulation in the Kupffer cells and bile canaliculi in the livers of all high-dose females. The increased pigment accumulation was not observed in any of the treated males or in the low- and mid-dose females. Other microscopic findings were observed, but were not dose related or were found also found in control animals.

The statistically significant increase (22%) in mean erythrocyte counts observed in high-dose females was within the historical control range. The significant increases (6%) in mean calcium levels observed in the mid- and high-dose females were small, and the observed significant decrease (23%) in mean blood urea nitrogen levels in the mid-dose males did not follow a dose–response pattern. All of the changes are considered incidental to treatment.

No statistically significant effects on body weight, body-weight gain, feed consumption or organ weights were observed at any dose level. In addition no gross abnormalities or ophthalmological changes related to treatment were observed.

The NOAEL for ATMER 163 in rats was 30 mg/kg bw per day based on the clinical signs seen at 100 mg/kg bw per day. The LOAEL was 100 mg/kg bw per day based on clinical signs (increased incidence of salivation, emesis, and soft faeces (with mucus alone or mucus and bile-like material) in males and females, increased alanine transaminase levels in females, and an increased incidence of pigment accumulation in the Kupffer cells and bile canaliculi in the livers of females (Osheroff, 1991).

Armoblen 557 (CAS No. 68213-26-3 (Tallow, POE n = 5/12)

In a four-week oral toxicity study, Armoblen 557 (purity unknown) was administered daily by gavage to groups of five male and five female CD rats at concentrations of 0, 15, 75 or 200 mg/kg bw per day.

All the rats survived until scheduled termination. Salivation in males and females at 75 and 200 mg/kg bw per day was probably due to the taste of the test material and was not considered toxicologically significant. Rales reported in one to three high-dose females were not associated with other effects seen at necropsy and was therefore not considered toxicologically significant. The brown staining around the muzzle occasionally seen in females at 75 mg/kg bw per day and males and females at 200 mg/kg bw per day was also not considered toxicologically significant. Mean body weight was decreased in males (11–17% lower than controls) and females (4–7% lower than controls) at 200 mg/kg bw per day. Overall body-weight gain was decreased in males at 75 mg/kg bw per day (13% lower than controls) and in males and females at 200 mg/kg bw per day (27% and 14% lower than controls, respectively). Overall feed consumption for high-dose females was decreased (10% lower than controls), while it was decreased in high-dose males during week 1 only. Overall feed conversion efficiency was decreased in males at 75 and 200 mg/kg bw per day (13 and 23% lower than controls, respectively).

Changes in haematology and clinical chemistry parameters were either not treatment related or not toxicologically significant. Increases in the absolute and relative adrenal weights in males and females at 200 mg/kg bw per day were not accompanied by microscopic findings and were not considered toxicologically significant.

Based on decreased body weight, body-weight gain and food-conversion efficiency, a LOAEL of 200 mg/kg bw per day and a NOAEL of 75 mg/kg bw per day was established for Armoblen 557 in male CD rats. A LOAEL for Armoblen 557 in female CD rats was not established. The NOAEL in female CD rats was 200 mg/kg bw per day (Higgs, 1994).

MON 59112

In three independent reverse gene mutation assays, *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* WP2 uvrA were exposed to MON 59112 (assumed 100% purity) in deionized water at concentrations of 0, 1, 3.33, 10, 33.3, 100 or 333 μg/plate with and without S9 activation for the *S. typhimurium* strains and 0, 10, 33.3, 100, 333, 1000 or 3330 μg/plate with and without S9 activation for WP2 uvrA. The S9-fraction was derived from male Sprague Dawley rats induced with Aroclor 1254.

MON 59112 was tested up to cytotoxic concentrations in all strains (≥ 100 μg/plate +S9 and ≥ 33.3 μg/plate −S9 for *S. typhimurium* TA1535, TA1537, TA100 and TA98; ≥ 3330 μg/plate +S9 and ≥1000 μg/plate –S9 for WP2 uvrA) but failed to induce a mutagenic response in this test system. The positive controls induced the expected mutagenic responses in the appropriate strain. There was no evidence of induced mutant colonies over background (Lawlor, 2000).

In a bone marrow micronucleus assay, adult male and female ICR(Crl:CD-1) mice were treated once via oral gavage with MON 59112 (lot no. GLP-9708-8157-I) emulsified in corn oil. Doses of 0, 375, 750 or 1500 mg/kg bw were administered to groups of six male mice and doses of 0, 500, 1000 or 2000 mg/kg bw were administered to groups of six female mice. Bone marrow cells were harvested from the first five survivors at 24 hours (all dose groups) and 48 hours (1500 or 2000 mg/kg bw) following dosing. The harvested bone marrow cells were scored for micronucleated polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes. Cyclophosphamide (80 mg/kg bw) served as the positive control.

Based on the findings of no substantial differences in the toxicological response of the male or female mice, only the females were administered the limit dose of 2000 mg/kg bw. Two males in the 1500 mg/kg bw and one female in the 2000 mg/kg bw treatment groups died before the scheduled termination. Other toxic signs included hypoactivity, hunched posture, squinted eyes, rough hair coats and faecal stains (1500 mg/kg bw males) and hunched posture and urine stains (2000 mg/kg bw females). There were also significant reductions in the polychromatic to normochromatic erythrocyte ratio for the high-dose males but not the high-dose females. Administration of 80 mg/kg bw cyclophosphamide caused a significant ($P < 0.01$) induction of micronucleated polychromatic erythrocytes in both sexes. There was, however, no significant increase in the frequency of micronucleated polychromatic erythrocytes in any treatment group at either harvest time (Myhr, 2000).

Five glyphosate coformulants were tested for activation of the steroidogenic enzyme aromatase in an in vitro assay (Defarge et al., 2016).

The coformulants tested may have different CAS numbers as the formulations differ. Those tested were (1) pure polyethoxylated tallow amine (POEA; POE-15, CAS No.: 61791-26-2, trade name Emulson AG GPE 3SS) and formulated polyethoxylated tallow amine (POEA/F; CAS No.: 61791-26-2, trade name Emulson AG GPE 3/SSM) form containing 70% of POE-15; (2) alkyl polyglucoside (APG; CAS No.: 383178-66-3/110615-47-9, trade name Plantapon LGC); (3) a mixture of alkyl (C8–10) polyoxyethylene ether phosphates and polyoxyethylene alkyl ether phosphate (POE-APE; CAS Nos.: 68130-47-2 and 50769-39-6, trade name Rolfen Bio); and (4) quaternary ammonium compound (QAC, CAS No.: 66455-29-6, trade name Emulson AG CB 30; and (5) alkyl polyglycoside (CAS No. 110615-49-9, trade name Plantapon LGC).

Aromatase activity was measured by tritiated water release in human JEG3 cells (for discussion on this assay, see Section 2.6f, Table 50). Mitochondrial succinate dehydrogenase activity and membrane integrity were assayed after a 24-hour exposure to assess cytotoxic effects.

The concentrations tested for succinate dehydrogenase activity were derived based on those concentrations reported to be used in glyphosate formulations which can differ according to different formulations: for example, POEA (9 ppm); POEA (18 ppm); APG (800 ppm); POE-APE (100 ppm); and QAC (100 ppm). Statistically significant differences from the controls were determined by a Kruskal–Wallis nonparametric test followed by a post hoc test using significant levels. Aromatase assays were performed at 2.5 ppm of POEA, 120 ppm of APG. The authors report that aromatase activity was decreased by the coformulant alone (POEA, −43%; $P < 0.01$) and slightly by the formulation of the active ingredient plus the coformulant (−25%; $P < 0.05$). Formestane (4-hydroxyandrost-4-ene-3,17-dione), a known aromatase inhibitor, was used as a positive control to demonstrate the specificity of the effect.

1,4-Dioxane

1,4-Dioxane is used primarily as a solvent in the manufacture of chemicals and as a laboratory reagent; it has been noted as being a trace contaminant of glyphosate formulations.

1,4-Dioxane has been classified by the IARC as "possibly carcinogenic to humans (Group 2B)" (IARC, 1987) and, in the National Toxicology Program's fourteenth edition report on carcinogens, as "reasonably anticipated to be a human carcinogen" (NTP, 2016).

Studies in rodents show liver tumours to be consistently reported after chronic oral exposure to 1,4-dioxane. A weight-of-evidence evaluation re-examined mouse liver slides from the 1978 National Cancer Institute bioassay of 1,4-dioxane in drinking water. This re-examination clearly identified dose-related non-neoplastic changes in the liver; specifically, a dose-related increase in the hypertrophic response of hepatocytes, followed by necrosis, inflammation and hyperplastic hepatocellular foci. While 1,4-dioxane does not cause point mutations, DNA repair or initiation, it appears to promote tumours and stimulate DNA synthesis. The weight of the evidence suggests that 1,4-dioxane causes liver tumours in rats and mice through cytotoxicity followed by regenerative hyperplasia. A reference dose (RfD) of 0.05 mg/kg day was proposed to protect against regenerative liver hyperplasia based on a benchmark dose approach (Dourson et al., 2014).

FD&C Blue No. 1

FD&C Blue No. 1 is a blue colourant used in glyphosate formulations. As literature on this compound is sparse, it was run through predictive expert system software (Derek Nexus 5.0.1, Nexus 2.1.0, Lhasa Ltd., Leeds, United Kingdom) in February 2016. The parent compound indicated plausible toxicity with respect to chromosome damage in vitro in mammals due to an alert match with triarylmethane salt and irritation of the eye in mammals due to an alert matched with 4,4'-methylenedianiline.

Availability of supplementary Toxcast/Tox 21 data

In addition to supplementary literature review, Toxcast and Tox21 data searches were conducted on 29 April 2016 for glyphosate coformulants. The Tox21 toolbox (http://ntp.niehs.nih.gov/results/tox21/tbox/index.html) was utilized to access the databases and acquire the data. Data were obtained for two of the glyphosate coformulants: 1,4-dioxane and FD&C Blue No 1. Other typical coformulants were not tested.

In a broad sweep of testing (including AhR, FXR, PPARs, VDR, MMP, p53, NFkB, GR), for FD&C Blue No. 1, the AC50 (µmol/L) results were positive for estrogenic agonist (1 assay only: 1.00E-4) and antagonist activity (1 assay only: 4.79), AR antagonist activity (4.76), aromatase inhibition (1.00E-4), TR antagonism (1.00E-4) and retinoic acid–receptor-related orphan receptor antagonism (1.00E-4). For 1,4-dioxane, the AC50 (µmol/L) results were negative across all assays.

3. Observations in humans

3.1 Occupational exposure: Biomonitoring studies

Occupational exposure to glyphosate can occur via dermal and inhalation routes. However, in vitro and in vivo percutaneous absorption studies suggest that dermal penetration of glyphosate formulation is very limited and that exposure through inhalation is minimal due to the low vapour pressure of glyphosate.

Both passive dosimetry and biomonitoring have been used as techniques to assess exposure. Biomonitoring results represent systemic (internal) exposure, whereas passive dosimetry results quantify external deposition. There is general agreement that biological measurements obtained through biomonitoring provide the most relevant information for safety assessments (Franklin, Muir & Moody, 1986; Chester & Hart, 1986).

The Farm Family Exposure Study was a biomonitoring study supported by seven agricultural companies. In this study, eligible farm families from Minnesota and South Carolina were randomly

selected from a roster of licensed private pesticide applicators. Participant families consisted of a farmer, their spouse and at least one child between the ages of 4 and 17 years; lived on the farm; and planned to apply one of the target pesticides (glyphosate, chlorpyrifos, 2,4-D) to at least 10 acres (4.1 hectares) of land within 1 mile (1.6 kilometres) of their house. For each family member, geometric means were calculated for 24-hour composite urinary samples, with a 1 ppb limit of detection, the day before, the day of and for 3 days after the pesticide application. For the farmers, the peak geometric mean concentrations were 3 ppb for glyphosate, 64 ppb for 2,4-D and 19 ppb for the primary chlorpyrifos metabolite. For the spouses and children, the percentage with detectable values varied by chemical, although the average values for each chemical did not vary during the study period. The applicators had the highest urine pesticide concentrations, children had much lower values and spouses had the lowest values. Exposure to family members was largely, though not exclusively, determined by the degree of direct contact with the application process. The exposure profile varied for the three chemicals for each family member (Mandel et al., 2005).

As part of the Farm Family Exposure Study, urinary glyphosate concentrations were evaluated for 48 farmers, their spouses and their 79 children (4–18 years of age). The study authors stated that they evaluated 24-hour composite urine samples for each family member the day before, the day of and for 3 days after a glyphosate application. On the day of application, 60% of the farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. Those farmers who did not use rubber gloves had higher geometric mean urinary concentrations than the other farmers (10 ppb vs 2.0 ppb). For spouses, 4% had detectable levels in their urine on the day of application; their maximum value was 3 ppb. For children, 12% had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading or application. None of the systemic doses estimated in this study approached the USEPA reference dose for glyphosate of 2 mg/kg bw per day (Acquavella et al., 2004).

Some earlier biomonitoring studies were performed on silvicultural workers who sprayed a glyphosate formulation in a variety of forestry and tree farming activities. In one study, the United States Department of Agriculture's Forest Service, in collaboration with Monsanto and the University of Arkansas, sponsored a study to investigate exposure to glyphosate of workers at two forestry nurseries (Phipps Nursery in Oregon and Ashe Nursery in Massachusetts) where glyphosate was used for weed control. Urine samples were collected from the weeders and scouts prior to working with glyphosate and for an eight-month period thereafter. Continuous total urine sampling was conducted for the first 12 consecutive weeks of the study, after which a 24-hour sample was collected each Wednesday for the next five months.

Of the 355 daily urine samples analysed, none were found to contain quantifiable levels of glyphosate. The limit of quantification was 10 ppb (Lavy et al., 1992).

A separate collaborative study conducted by the United States Department of Agriculture (USDA) Forestry Service, Georgia Tech Research Institute and Monsanto examined the effects of exposure to glyphosate on applicators using a hand-held directed spray foliar application at three sites maintained by the USDA Forestry Service. Urinary samples were collected for 5 days after exposure. Of the 96 urine samples analysed, five were found to contain quantifiable levels of glyphosate. The highest glyphosate measure was 14 ppb and the highest estimated internal dose was 0.0006 mg/kg body weight (Cowell & Steinmetz 1990).

Two other studies have been conducted to measure exposure of forestry workers to glyphosate during normal silvicultural applications. In the Finnish study (Jauhiainen et al., 1991), urine samples were collected at the end of each day from workers spraying glyphosate for 5 consecutive days in August 1988. In addition, each worker had an ECG; underwent haematology, clinical chemistry and pulmonary function tests and a general clinical examination (including blood pressure, pulse rate and pressure craft of hands); and was interviewed for a health questionnaire on the first day and last day. All urine samples had less than detectable concentrations of glyphosate. There were no statistically significant differences in the findings of the medical examinations conducted before and after exposure (Jauhiainen et al., 1991).

The Canadian study of forestry workers following normal silviculture uses of glyphosate was conducted over two growing seasons (in 1986) and involved 45 workers conducting various operations. Glyphosate was not detected in the majority of urine samples. For the two flagmen and the operator, glyphosate concentrations in all urine samples were less than 0.03 ppm (the limit of quantitation). In contrast, 14 of 33 urine samples from the mixer and two urine samples for the foreman contained glyphosate concentrations greater than 0.03 ppm. Maximum glyphosate concentrations in the foreman's and mixer's urine were 0.043 and 0.055 ppm, respectively. In the follow-up study in 1987, glyphosate concentrations in urine of exposed workers were very low. In the majority of samples, glyphosate was not detectable. In those samples with detectable levels of glyphosate, concentrations were less than 0.1 ppm in all cases and typically less than 0.035 ppm (Centre de Toxicologie du Quebec, 1988).

3.2 Occupational exposure: Epidemiological studies with specific reference to cancer outcomes

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[9].

Identification of compound/cancer sites and screening of papers

This assessment was limited to studies of cancer outcomes; numerous studies have assessed risks for neurodevelopmental, neurodegenerative and reproductive outcomes, among other health outcomes. Restricting the assessment to cancer outcomes was partly driven by reasons of feasibility: 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 (Clewell & Crump, 2005; Nachman et al., 2011).

The pre-agreed evaluation process and Tier 1 screening criteria used to evaluate epidemiological studies on glyphosate (and malathion and diazinon) are described in "Section 2.2: Methods for the evaluation of epidemiological evidence for risk assessment" of the Meeting report[10].

The IARC monographs on glyphosate, malathion and diazinon refer to a total of 45 epidemiological studies. Two studies published since the IARC monographs, which evaluated at least one of malathion, diazinon or glyphosate in relation to cancer outcomes, were also identified (Lerro et al., 2015; Koutros et al., 2015).

[9] 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/).

[10] 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/).

The 45 publications referred to in the IARC monographs and the two publications since (Lerro et al., 2015; Koutros et al., 2015) covered a total of 48 compound/cancer site combinations. The current evaluation focuses on the six 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 the 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 the 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 compound/cancer site combination for glyphosate was NHL. The evaluation of the relevant publications is summarized in Table 52.

During the identification of relevant publications, stand-alone analyses for specific subtypes of NHL (of which there are many subtypes) were noted. The risk was not evaluated separately for subtypes of NHL as there was insufficient evidence (too few studies or small numbers of cases), or for other haematopoietic and lymphoid tumours as the positive associations identified by the IARC were for total NHL.

Overview of studies included in evaluation

The IARC monograph on malathion (IARC, 2015) already provides a good 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 to give context.

The Agricultural Health Study (AHS) 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 AHS has examined a range of cancer outcomes, and published updated analyses with longer periods of follow-up (e.g. Beane Freeman et al., 2005; De Roos 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 collected 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), Kansas (Hoar et al., 1986), which have subsequently been pooled (748 cases/2236 controls) for analysis of 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 collected retrospectively via 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 which may differ for cases and controls. De Roos et al. (2003) (same study population as Waddell et al., 2001) performed an extensive evaluation and adjustment for other pesticides.

The Cross-Canada Case–control Study of Pesticides and Health is a population-based case–control study of haematopoietic cancers in men diagnosed during 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 who 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.

These three sets of studies 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 & 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 reported in publication which hindered interpretation, lack of specificity due to high correlations between use of different pesticides, and limited power.

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 long follow-up time 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 AHS), 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 which can bias risk estimates either towards or away from the null. As a cohort study, the AHS 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. As a result, confounding, possible effect modification and additive/multiplicative effects due to co-exposures are all concerns. However, many studies were able to adjust risk estimates for other pesticide co-exposures, 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 NHL) or screening/diagnosis rates (prostate cancer) over time may limit comparability between studies.

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 funnel plot tests for asymmetry should be used only where there are at least 10 studies because otherwise statistical power is insufficient to distinguish true asymmetry from chance (Higgins & Green, 2011; Sterne et al., 2011). Other formal objective statistical tests require an even 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.

Summary of evidence for an association between glyphosate and NHL

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

The risk estimates findings for each study are summarized in Table 52, and findings for non-quantitative exposure assessment (predominantly ever- vs never-use) are shown in the forest plot below.

Table 52. Results of Tier 1 evaluation and summary of publications by glyphosate/cancer site

Study/ Location	Glyphosate / NHL	Reference
Meta-analysis	Qualitative exposure only – ever-/never-use of glyphosate Meta risk ratio: 1.5 (95% CI: 1.1–2.0) Meta-analysis includes McDuffie et al. (2001); Hardell et al. (2002); De Roos et al. (2003, 2005a); Eriksson et al. (2008); and Orsi et al. (2009). *N*s for each meta-analysis not presented	Schinasi & Leon (2014)
Agricultural Health Study	Quantitative exposure (cumulative exposure days; intensity-weighted cumulative exposure days [years of use × days/year × estimated intensity level]: in tertiles) Risk estimates – aRR (95% CI) Ever-use 1.1 (0.7–1.9) LED 1–20.0 1.0 (ref.) LED 21–56 0.7 (0.4–1.4) LED 57–2678 0.9 (0.5–1.6) *P* for trend 0.73 IW-LED 0.1–79.5 1.0 (ref.) IW-LED 79.6–337.1 0.6 (0.3–1.1) IW-LED 337.2–18241 0.8 (0.5–1.4) *P* for trend = 0.99 Total *N* = 54 315 (49 211/36 823, depending on the analysis), with 92 incident NHL cases (for ever-use; and 61 for analysis based on tertiles of exposure)	De Roos et al. (2005)
United States Midwest case–control studies	The study population overlaps with that of De Roos et al. (2003). See comment below Qualitative – ever/never (analysis stratified by asthmatics vs non asthmatics) Risk estimates – aRR (95% CI) Non-asthmatics: 1.4 (0.98–2.1) Asthmatics: 1.2 (0.4–3.3) Total *N* = 3208 (872 NHL cases, 2336 controls). *N* = 53/91 glyphosate-exposed NHL cases/controls for non-asthmatics and 6/12 glyphosate-exposed NHL cases/controls for asthmatics	Lee et al. (2004)

Study/ Location	Glyphosate / NHL	Reference
	The study population overlaps with Lee et al. (2004) and total *N* is smaller, but as an exception this study was not excluded in the assessment of consistency of risk estimates as it provides overall risk estimates which are comparable with other studies, while Lee et al. (2004) only provides risk estimates stratified by asthma diagnosis Qualitative (ever/never) Risk estimates – aOR (95% CI) From a logistic regression model: Exposed 2.1 (1.1–4.0) From the hierarchical regression model: Exposed 1.6 (0.9–2.8) Both adjusted for other pesticides Total *N* =2 583 (650 NHL cases, 1 933 controls). *N* = 36 exposed cases; *N* = 61 controls	De Roos et al. (2003)
	Excluded – as this study is pooled in De Roos et al. (2003) and Lee et al. (2004) Qualitative exposure only – ever-/never-use of glyphosate Risk estimates – OR (95% CI) Ever-use = 1.1 (0.7–1.9) Total *N* = 1867 (622 cases, 1245 controls) *N* = 26 exposed cases	Cantor et al. (1992)
Cross-Canada Study of Pesticides and Health	Quantitative exposure – days of use per year (3 categories – cutpoints are given). Risk estimates – OR (95% CI) Ever-use: 1.2 (0.83–1.74) Unexposed 1.0 (ref.) >0–<=2 days/year 1.0 (0.63–1.57) > 2 days/year 2.12 (1.20–3.73) *P* trend = NR Total *N* = 2 023 517 cases, 1 506 controls (overall) *N* = 51 exposed cases, 133 exposed controls	McDuffie et al. (2001)
Sweden – note that there is some overlap between Eriksson et al. (2008), Hardell et al. (2002) and Hardell & Eriksson (1999)	Quantitative exposure – days of use per year (2 categories – cutpoints are given). Risk estimates – aOR (95% CI) Ever-use: 2.02 (1.10–3.71) Risk estimates – aOR (95% CI) Non-farmers: 1.0 (ref.) ≤ 10 days/year: 1.69 (0.7–4.07) > 10 days/year: 2.36 (1.04–5.37) *P* trend = NR Total N = 1926 (910 cases, 1016 controls) *N* = 29 exposed cases; *N* = 18 exposed controls	Eriksson et al. (2008)
	Qualitative exposure only – ever-/never-use of glyphosate. A pooled analysis of Nordström et al. (1998) (NHL subtype only, not evaluated separately here) and Hardell & Eriksson (1999) Risk estimates – aOR (95% CI) Ever-use: 1.85 (0.55–6.20) Total *N* = 1 656 (515 cases, 1 141 controls) *N* = 8 exposed cases; *N* = 8 exposed controls.	Hardell et al. (2002)
	Exclude as this study is pooled in Hardell et al. (2002). Qualitative exposure only – ever-/never-use of glyphosate	Hardell & Eriksson (1999)

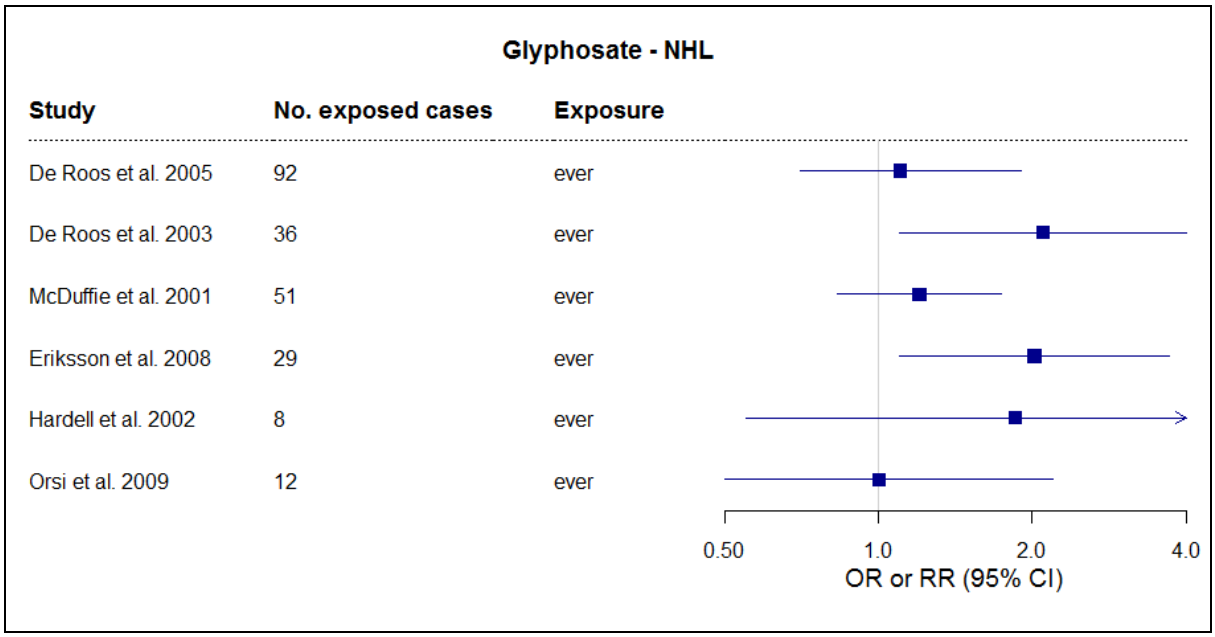
Study/ Location	Glyphosate / NHL	Reference
France	Qualitative – ever-/never-use of glyphosate Risk estimates – aOR (95% CI) Ever-use: 1.0 (0.5–2.2) *N* = 12 exposed cases; *N* = 24 exposed controls (The researchers report evaluating quantitative duration with respect to median duration of exposure among exposed controls as never exposed; duration < median; duration > median, but neither the median cutpoint nor ORs/test for trend results are presented in the paper, so this study cannot contribute any information for quantitative risk assessment.)	Orsi et al. (2009)

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; NHL: non-Hodgkin lymphoma; *N*: sample size; NR: not reported; OR: odds ratio; ref.: reference

The maximally adjusted risk estimates were extracted.

The Glyphosate / NHL evaluation included seven studies (McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Lee et al., 2004; De Roos et al., 2005; Eriksson et al., 2008; Orsi et al., 2009) and one meta-analysis (Schinasi & Leon, 2014). Three studies used quantitative exposure metrics, although, the units differed: lifetime exposure days and intensity-weighted lifetime exposure days (De Roos et al., 2005) and days of use per year (McDuffie et al., 2001; Eriksson et al., 2008). The AHS found no evidence of elevated risk of NHL or exposure–response associated with glyphosate exposure (De Roos et al., 2005). Elevated risks were reported in various case–control studies. De Roos et al. (2003) reported significant elevated risk of NHL associated with ever- versus never-use of glyphosate (OR: 2.1 [1.1–4.0] and a borderline nonsignificant OR (1.6 [0.9–2.8]) with an alternative Bayesian hierarchical model) from the United States Midwest pooled case–control studies. There was no evidence of effect modification by asthma diagnosis in the United States Midwest pooled case–control studies (Lee et al., 2004). Ever-use of glyphosate was not associated with risk of NHL in the Cross-Canada Case–control Study of Pesticides and Health, but using glyphosate for longer than 2 days per year was associated with a significant elevated risk (OR: 2.12; 95% CI: 1.20–3.73), although there was no indication of an exposure–response relationship across exposure categories (McDuffie et al., 2001). Eriksson et al. (2008) reported significant elevated risk of NHL associated with ever-use (OR: 2.02 [1.10–3.71]) and use of glyphosate for longer than 10 days per year (OR: 2.36 [1.04–5.37]) and indicate an exposure–response relationship. A pooled study of two Swedish case–control studies reported a nonsignificant elevated risk of NHL for ever-use of (OR: 1.85 [0.55–6.2]); however, with only eight exposed cases, this study had limited power to detect associations (Hardell et al., 2002). Orsi et al. (2009) found no evidence of association. Schinasi & Leon (2014) reported a meta risk ratio of 1.5 (95% CI: 1.1–2.0) for ever- versus never-use of glyphosate. The meta-analysis included the AHS (De Roos et al., 2005) and five out of the six case–control studies included in this evaluation (McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Eriksson et al., 2008; Orsi et al., 2009).

Fig. 3. Forest plot for risk estimates of NHL associated with glyphosate, from studies with qualitative exposure categories



Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS, which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level.

Comments

Biochemical aspects

In studies with radiolabelled glyphosate in rats, glyphosate was rapidly absorbed from the gastrointestinal tract following oral intake, but only to a limited extent (about 20–30%) (McEwen, 1995). Elimination was fast and virtually complete within 72–168 hours, with the majority being excreted during the first 48 hours (McEwen, 1995). Most of the excretion occurred in faeces, largely as unabsorbed dose, and in the urine. Biliary excretion of glyphosate was negligible. Less than 1% of the administered dose was retained in tissues 168 hours post-administration. Highest residues were detected in bone, followed by kidney and liver (Powles, 1992b; Ridley & Mirly, 1988). This pattern of absorption, distribution and elimination was independent of dose, treatment regimen and sex of the test animals. Peak plasma concentrations of radiolabel were observed at 6 and 2 hours after administration in male and female rats, respectively (McEwen, 1995). The estimated half-life for whole-body elimination of the radiolabel was about 5.9–8.3 hours (McEwen, 1995).

There was very little biotransformation of glyphosate; the only metabolite, AMPA, accounted for 0.2–0.7% of the administered dose in excreta; the rest was unchanged glyphosate (Macpherson, 1996).

Toxicological data

Glyphosate has low acute oral toxicity in mice (LD_{50} > 2000 to > 10 000 mg/kg bw; no lethality at 2000 mg/kg bw) (Shirasu & Takahashi, 1975)and rats (LD_{50} 5600 mg/kg bw) (Heenehan, 1979a), low acute dermal toxicity in rats (LD_{50} > 2000 mg/kg bw) (Cuthbert & Jackson, 1989b; Komura, 1995c; Doyle, 1996b; Talvioja, 2007b; Do Amaral Guimaraes, 2008b; Haferkorn, 2009b, 2010c,d; Simon, 2009b) and rabbits (LD_{50} > 5000 mg/kg bw) (Heenehan, 1979b; Blaszcak, 1988b;

Reagan, 1988a), and low acute inhalation toxicity in rats (LC_{50} > 5.48 mg/L). Glyphosate was not irritating to the skin of rabbits (Heenehan, 1979c; Reagan & Laveglia, 1988b; Hideo, 1995a; Doyle, 1996c; Arcelin, 2007c; Talvioja, 2007c; Canabrava Frossard de Faria, 2008a; You, 2009c; Leuschner, 2009a,c, 2010a). Glyphosate produced moderate to severe eye irritation in rabbits, with irreversible corneal opacity in one study as a consequence of the low pH of the test material in solution (Arcelin, 2007d; Blaszcak, 1988d; Hideo, 1995b; Johnson, 1997; Merkel, 2005e; Talvioja, 2007d; You, 2009d). Glyphosate was not sensitizing in guinea pigs (Doyle, 1996d; Haferkorn, 2009d, 2010f,g; Hideo, 1995c; Lima Dallago, 2008; Merkel, 2005f; Richeux, 2006; Talvioja, 2007e; Simon, 2009d; You, 2009e) or mice (Betts, 2007; Török-Bathó, 2011) as determined by the Magnusson–Kligman maximization test, the Buehler test and the local lymph node assay.

In short-term studies of toxicity in different species, the most notable effects were clinical signs related to gastrointestinal irritation, decreased body weight, salivary gland changes (hypertrophy and increase in basophilia of cytoplasm of acinar cells), histological findings in the caecum and hepatotoxicity.

In short-term studies in mice, reduced body weight was seen at a dietary concentration of 50 000 ppm (equal to 9710 mg/kg bw per day) (Tierney & Rinehart, 1979). The NOAEL for decreased body weight was 10 000 ppm (equal to 1221 mg/kg bw per day) (Kuwahara, 1995). Effects on the salivary glands were observed in mice in only one study out of four, at 6250 ppm (equal to 1065 mg/kg bw per day) (Chan & Mahler, 1992). The NOAEL for the salivary gland effects in mice was 3125 ppm (equal to 507 mg/kg bw per day) (Chan & Mahler, 1992). The overall NOAEL in short-term studies in mice was 3125 ppm (equal to 507 mg/kg bw per day), and the overall LOAEL was 6250 ppm (equal to 1065 mg/kg bw per day).

In 90-day toxicity studies in rats, common findings included soft faeces, diarrhoea, reduced body-weight gain and decreased food utilization at dietary concentrations of 20 000 ppm (equal to 1262.1 mg/kg bw per day) and above. The lowest NOAEL was 371.9 mg/kg bw per day. A decrease in urine pH was frequently noted owing to the acidic nature of the compound and excretion as glyphosate in the urine. In two 90-day dietary toxicity studies, an increase in caecum weight (at 10 000 ppm, equal to 569 mg/kg bw per day) and histological findings in the caecum (at 50 000 ppm, equal to 3706 mg/kg bw per day) (Kinoshita, 1995; Coles et al., 1996) were observed. In rats, effects on the salivary gland were seen in two out of seven 90-day studies starting at 12 500 ppm (equal to 811 mg/kg bw per day). The NOAELs for effects on the salivary gland were 300 and 410 mg/kg bw per day. The overall NOAEL in short-term studies in rats was 300 mg/kg bw per day, and the overall LOAEL was 10 000 ppm (equal to 569 mg/kg bw per day).

In four 90-day toxicity studies in dogs, the most notable effects were loose stools, decreased body weight and reduced feed consumption **(**Hodge, 1996; Yoshida, 1996; Prakash, 1999; Gaou, 2007**)**. In one study, there were no treatment-related effects at doses up to 40 000 ppm (equal to 1015 mg/kg bw per day) **(**Yoshida, 1996**)**. The lowest NOAEL and LOAEL were 300 mg/kg bw per day and 1000 mg/kg bw per day, respectively.

Seven 1-year toxicity studies in dogs are available. In one study, changes in faeces were observed at 100 mg/kg bw per day and above. The NOAEL was 30 mg/kg bw per day (Teramoto, 1998). However, these results were not reproduced in four other studies with administration via capsules at 300 or 500 mg/kg bw per day (Reyna & Ruecker, 1985; Goburdhun, 1991; Haag, 2008). In the remaining six studies, the NOAELs ranged from 8000 ppm (equal to 182 mg/kg bw per day; Nakashima, 1997) to 500 mg/kg bw per day (Reyna, 1985; Haag, 2008), and the LOAELs ranged from 30 000 ppm (equal to 926 mg/kg bw per day; Brammer, 1996) to 1000 mg/kg bw per day (Goburdhun, 1991).

The overall NOAEL in the 90-day and 1-year toxicity studies in dogs was 15 000 ppm (equal to 448 mg/kg bw per day), and the overall LOAEL was 30 000 ppm (equal to 926 mg/kg bw per day).

The Meeting compiled the tumour incidence data for all relevant mouse and rat studies in order to undertake statistical analysis and investigate any potential pattern of occurrence across studies. In addition, incidences of tumours of lymphatic tissues were summarized, as these were

identified as possible targets of relevance from the review of epidemiological cancer studies. However, the Meeting recognized that the relationship between tumours of lymphatic tissues in rodents and humans has not been clearly established.

Nine carcinogenicity studies in mice were available. Two studies were considered to be of insufficient quality to be included in the assessment (Bhide, 1988; Vereczkey & Csanyi, 1982, revised 1992). Effects such as loose stools, reduced body weights and decreased feed consumption were noted in most of the studies (Pavkov & Turnier, 1987; Atkinson et al., 1993a; Sugimoto, 1997; Takahashi, 1999a). The overall NOAEL for systemic toxicity in mice was 1600 ppm (equal to 153 mg/kg bw per day), and the overall LOAEL was 8000 ppm (equal to 787 mg/kg bw per day).

The Meeting concluded that there is equivocal evidence of induction of lymphomas in male mice in three out of seven studies (Sugimoto, 1997; Kumar, 2001; Wood et al., 2009a) and in female mice in one out of seven studies (Takahashi, 1999a) at high doses (5000–40 000 ppm, equal to 814–4348 mg/kg bw per day). The Meeting also noted that in the other three studies in which even higher doses (up to 50 000 ppm, equal to 7470 mg/kg bw per day) had been used, no effect was observed.

The Meeting concluded that there is some indication, by a trend test and not by pairwise comparison, of induction of kidney adenomas in male mice in four out of seven studies (Knezevich & Hogan, 1983; Sugimoto, 1997; Takahashi, 1999a; Kumar, 2001). The Meeting noted that the increases were marginal and occurred at the highest dose only and that other studies that used appreciably higher doses did not find any excess. However, the Meeting noted that kidney adenomas are uncommon in male mice.

Eleven combined chronic toxicity and carcinogenicity studies in rats were available (Lankas, 1981; Pavkov & Wyand, 1987; Strout & Ruecker, 1990; Atkinson et al., 1993b; Milburn, 1996; Suresh, 1996; Bhide, 1997; Enomoto, 1997; Takahasi, 1999a,b; Brammer, 2001; Wood et al., 2009b). One study was considered to be inadequate for carcinogenicity assessment due to its exposure duration (12 months). Toxicities variously reported in some of these studies included increased incidences of clinical signs, reduced body weights, degenerative lens changes (cataracts) in males, microscopic findings in the salivary gland, increased incidence of basophilia of parotid acinar cells, and microscopic findings in liver, prostate and kidneys. The overall NOAEL for systemic toxicity in rats was 100 mg/kg bw per day, and the overall LOAEL was 300 mg/kg bw per day.

The Meeting discussed the increased incidence of a variety of tumours observed in one or, in one case, two of the 10 studies in rats. The Meeting concluded that these findings were incidental, based on the following considerations:

- interstitial cell tumours of the testes: occurred in only one study (Lankas, 1981); and other studies that used appreciably higher doses did not find any excess;
- pancreatic islet cell adenoma: occurred in only one study in males only (Strout & Ruecker, 1990); other studies that used appreciably higher doses did not find any excess; there was no dose–response relationship; and the incidence in controls was unusually low (less than the lower bound of the historical control data); the Meeting also noted that there was a negative dose–response relationship in females;
- thyroid C-cell tumours: occurred in only one study (Strout & Ruecker, 1990); other studies that used appreciably higher doses did not find any excess; and these tumours are considered not to be relevant for humans;
- skin keratoma: occurred in two studies in males only; other studies that used appreciably higher doses did not find any excess; in one study (Strout & Ruecker, 1990), there was no dose–response relationship; and in the other study, only the test for trend was statistically significant, not the pairwise test at any dose (Enomoto, 1997); and
- lymphoma (in spleen and kidney): no evidence of induction in any of the studies.

The Meeting concluded that there is no reliable evidence for treatment-related tumours in rats at doses up to 32 000 ppm (equal to 1750 mg/kg bw per day).

The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses.

Glyphosate and its formulation products have been extensively tested for genotoxic effects using a variety of tests in a wide range of organisms. While no mutational effects have been detected in bacterial test systems, DNA damage and chromosomal effects have commonly been seen in cell culture models and in organisms that are phylogenetically distant from humans. However, these effects have not been seen in vivo in orally treated mammalian models. The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg bw by the oral route, the route most relevant to human dietary exposure, was not associated with genotoxic effects in an overwhelming majority of studies conducted in mammals, a model considered to be appropriate for assessing genotoxic risks to humans.

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

Seven reproductive toxicity studies in rats were available. No evidence of reproductive toxicity was observed at doses up to 30 000 ppm (equal to 1983 mg/kg bw per day). In one study, an increased incidence of histopathological findings in the parotid (both sexes) and submaxillary salivary glands in females was observed in both generations at 10 000 ppm (equal to 668 mg/kg bw per day). The NOAEL was 3000 ppm (equal to 197 mg/kg bw per day) (Brooker et al., 1992). In a separate study, an increased incidence of loose stools and caecum distension was observed in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day), and the NOAEL was 6000 ppm (equal to 417 mg/kg bw per day) (Takahashi, 1997). Slight reductions in pup weight or weight gain were observed in most studies, but were confined to very high, parentally toxic dose levels (Moxon, 2000; Takahashi, 1997). In addition, a significant delay in sexual maturation in male pups (F_1) was seen at 15 000 ppm (equal to 1063 mg/kg bw per day) (Dhinsa, 2007). The overall NOAEL for parental toxicity was 6000 ppm (equal to 417 mg/kg bw per day), and the overall LOAEL was 10 000 ppm (equal to 668 mg/kg bw per day). The overall NOAEL for offspring toxicity was 6000 ppm (equal to 417 mg/kg bw per day), and the overall LOAEL was 10 000 ppm (equal to 985 mg/kg bw per day).

No evidence of teratogenicity was observed in four developmental toxicity studies in rats at doses up to 3500 mg/kg bw per day. There was some variation in the extent of toxicity observed in the four studies. The lowest NOAEL for maternal toxicity was 300 mg/kg bw per day, based on loose stools and reduced body weights seen at 1000 mg/kg bw per day (Hatakenaka, 1995). The lowest NOAEL for embryo and fetal toxicity was 300 mg/kg bw per day, based on delayed ossification and an increased incidence of fetuses with skeletal anomalies observed at 1000 mg/kg bw per day.

Seven developmental toxicity studies in the rabbit were available. Maternal toxicity was primarily manifested as an increased incidence of soft stool and diarrhoea at doses of 175 mg/kg bw per day and above. The overall NOAEL for maternal toxicity was 100 mg/kg bw per day. In three studies, the occurrences of a variety of low-incidence fetal effects (e.g. cardiac malformation, absent kidney) were slightly increased at higher dose levels (Bhide & Patil, 1989; Brooker et al., 1991b; Suresh, 1993c). These increases are considered secondary to maternal toxicity. The overall NOAEL for embryo and fetal toxicity was 250 mg/kg bw per day (Bhide & Patil, 1989), based on effects at 450 mg/kg bw per day. The Meeting considered that these effects were secondary to local irritation from unabsorbed glyphosate in the colon administered by gavage dosing and concluded that they were not relevant for establishing health-based guidance values.

The Meeting concluded that glyphosate is not teratogenic.

Glyphosate was tested in a range of validated in vivo and in vitro assays for its potential to interact with the endocrine system. The studies that the Meeting considered adequate for the evaluation clearly demonstrate that there is no interaction with estrogen or androgen-receptor pathways or thyroid pathways.

There was no evidence of neurotoxicity in an acute neurotoxicity study in rats at doses up to 2000 mg/kg bw. The NOAEL for systemic toxicity was 1000 mg/kg bw, based on a single death and general signs of toxicity at 2000 mg/kg bw (Horner, 1996a). In a 90-day neurotoxicity study in rats,

no evidence of neurotoxicity or systemic toxicity was seen at doses up to 20 000 ppm (equal to 1546.5 mg/kg bw per day) (Horner, 1996b).

No evidence of immunotoxicity was seen in a 28-day dietary study in female mice at doses up to 5000 ppm (equal to 1448 mg/kg bw per day) (Haas, 2012).

Effects on the salivary glands were observed in several repeated-dose toxicity studies in rats. The pH of glyphosate in solution is low, and it has been shown that exposure to organic acids can cause such changes in salivary glands. Therefore, the changes are likely secondary to the effects caused by the pH of the test compound in solution.

In many of the long-term repeated-dose studies reviewed, glyphosate was reported to have an impact on the gastrointestinal tract at high doses. Although this is not uncommon with high-dose chemical substance administration, this was investigated further, as glyphosate is known to be poorly absorbed in mammalian models, and alterations in gut microbiota profiles, specifically reductions in the beneficial microbiota and increases in pathogenic bacteria, are known to have impacts on carcinogenesis. There is evidence from livestock species that pathogenic bacteria are more resistant to glyphosate, whereas beneficial microbiota are more sensitive, and thus more vulnerable.

This is an emerging area of scientific investigation. The extent to which glyphosate adversely affects the normal functioning of the microbiota in the human gastrointestinal tract or the gastrointestinal tract of mammalian models is unclear. However, it is unlikely, given the available information on MIC values, that this would occur from glyphosate residues in the diet.

Toxicological data on metabolites and/or degradates

AMPA is the only identified metabolite found in the urine and faeces of orally treated rats. AMPA was of low acute oral and dermal toxicity in rats (LD_{50} > 5000 [Leah, 1988; Cuthbert & Jackson, 1993a] and > 2000 mg/kg bw [Leuschner, 2002a], respectively) and was not sensitizing in guinea pigs, as determined by the Magnusson–Kligman maximization test. In a 90-day study of toxicity in rats, the NOAEL was 1000 mg/kg bw per day, the highest dose tested (Strutt et al., 1993). AMPA administered orally in mammalian test systems showed no evidence of genotoxicity (Leah, 1988; Cuthbert & Jackson, 1993a; Komura, 1996). Only negative results were seen in studies in vitro (Callander, 1988b; Jensen, 1993a; Akanuma, 1996). The Meeting concluded that AMPA is unlikely to be genotoxic in vivo by the oral route.

In a study of developmental toxicity in rats, no evidence for embryo or fetal toxicity was observed; the NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

Following single gavage administration of radiolabelled *N*-acetyl-glyphosate, a plant-specific metabolite, at 15 mg/kg bw in rats, about 66.1% of the administered dose was excreted in urine (61.3% within 12 hours post dosing), 26.4% in faeces (25.8% within 48 hours post dosing), 2.79% in cage wash and wipe, and 0.23% in residual carcass. Radioactivity was eliminated rapidly from blood and plasma, with half-life values of 20.1 and 15.6 hours, respectively. Unchanged [^{14}C]*N*-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. Glyphosate, a metabolite of *N*-acetyl-glyphosate, was detected in faeces and represented less than 1% of the total radioactivity (Cheng & Howard, 2004).

The acute oral toxicity LD_{50} of *N*-acetyl-glyphosate in rats is greater than 5000 mg/kg bw, expressed as the free acid (Vegarra, 2004). In a 90-day toxicity study in rats, the NOAEL was 18 000 ppm (equal to 1157 mg/kg bw per day) (MacKenzie, 2007).

N-Acetyl-glyphosate was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that *N*-acetyl-glyphosate is unlikely to be genotoxic.

N-Acetyl-AMPA, another plant-specific metabolite, was of low acute oral toxicity; the LD_{50} was greater than 5000 mg/kg bw in rats (Carpenter, 2007).

N-Acetyl-AMPA was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that *N*-acetyl-AMPA is unlikely to be genotoxic.

Human data

Routine medical surveillance of workers in production and formulation plants revealed no adverse health effects attributable to glyphosate. In operators applying glyphosate products, cases of eye, skin and/or respiratory tract irritation have been reported. Acute intoxication was reported in humans after accidental or intentional ingestion of concentrated glyphosate formulations, resulting in gastrointestinal, cardiovascular, pulmonary and renal effects and, occasionally, death. The acute toxicity of glyphosate formulations was likely caused by the surfactant in these products (JMPR, 2004).

Several epidemiological studies on cancer outcomes following occupational exposure to glyphosate were available. The evaluation of these studies focused on the occurrence of NHL, as outlined in Section 2.2 of the Meeting report. One meta-analysis and one prospective cohort study, the AHS, with a large sample size and detailed exposure assessment, were available. 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 cohort study found no evidence of a positive association of NHL with glyphosate exposure or an exposure–response relationship (De Roos et al., 2005). Elevated risks were reported in various case–control studies. A significant elevated risk of NHL associated with ever- versus never-use of glyphosate (OR = 2.1; 95% CI = 1.1–4.0) was reported (De Roos et al., 2003). Ever-use of glyphosate was not associated with risk of NHL in the Cross-Canada Case–control Study of Pesticides and Health (McDuffie et al., 2001), but when analysing days of use per year, there was a significant elevated risk in the highest usage category (OR = 2.12; 95% CI = 1.20–3.73; for > 2 days/year glyphosate use). There was, however, no indication of an exposure–response relationship across exposure usage categories (McDuffie et al., 2001). In another case–control study, a significant increased risk of NHL associated with ever-use (OR = 2.02; 95% CI = 1.10–3.71) as well as the highest usage category (OR = 2.36; 95% CI = 1.04–5.37; for greater than 10 days/year glyphosate use) was observed, with some suggestion of an exposure–response gradient (Eriksson et al., 2008). Two smaller case–control studies with few exposed cases and limited statistical power reported a nonsignificant elevated risk (Hardell et al., 2002) and no association (Orsi et al., 2009), respectively, for risk of NHL and ever-use of glyphosate. The meta-analysis, including the AHS, found a significant 50% excess risk ratio for ever- versus never-use of glyphosate (Schinasi & Leon, 2014).

Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS (De Roos et al., 2005), which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level.

In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans via exposure from the diet.

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

Toxicological evaluation

The Meeting reaffirmed the group ADI for the sum of glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA of 0–1 mg/kg bw on the basis of the NOAEL of 100 mg/kg bw per day for effects on the salivary gland in a long-term study of toxicity and carcinogenicity in rats and

application of a safety factor of 100. The Meeting noted that these effects may be secondary to local irritation due to the low pH of glyphosate in solution, but was unable to establish this unequivocally.

The Meeting concluded that it was not necessary to establish an ARfD for glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA in view of their low acute toxicity, the absence of relevant developmental toxicity in rats and rabbits that could have occurred as a consequence of acute exposure, and the absence of any other toxicological effect that would be elicited by a single dose.

Levels relevant to risk assessment of glyphosate

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen- to 24-month studies of toxicity and carcinogenicity[a,b]	Toxicity	1 600 ppm, equal to 153 mg/kg bw per day[c]	8 000 ppm, equal to 787 mg/kg bw per day
		Carcinogenicity	The Meeting could not exclude the possibility that glyphosate is carcinogenic in mice at very high doses.	
Rat	Acute neurotoxicity study[a]	Neurotoxicity	2 000 mg/kg bw[c]	–
	Two-year studies of toxicity and carcinogenicity[b]	Toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Carcinogenicity	32 000 ppm, equal to 1 750 mg/kg bw per day[c]	–
	Two-generation studies of reproductive toxicity[a,b]	Reproductive toxicity	30 000 ppm, equal to 1 983 mg/kg bw per day[c]	–
		Parental toxicity	6 000 ppm, equal to 417 mg/kg bw per day	10 000 ppm, equal to 668 mg/kg bw per day
		Offspring toxicity	6 000 ppm, equal to 417 mg/kg bw per day	10 000 ppm, equal to 985 mg/kg bw per day
	Developmental toxicity studies[b,d]	Maternal toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
Rabbit	Developmental toxicity studies[b,d]	Maternal toxicity[e]	100 mg/kg bw per day	175 mg/kg bw per day
		Embryo and fetal toxicity[e]	250 mg/kg bw per day	450 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[b,f]	Toxicity	15 000 ppm, equal to 448 mg/kg bw per day	30 000 ppm, equal to 926 mg/kg bw per day
AMPA				
Rat	Thirteen-week study of toxicity[d]	Toxicity	1 000 mg/kg bw per day[c]	–
	Developmental toxicity study[d]	Maternal toxicity	1 000 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[c]	–

[a] Dietary administration.

[b] Two or more studies combined.

[c] Highest dose tested.

[d] Gavage administration.

[e] Secondary to local irritation of the colon.

[f] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–1 mg/kg bw (for sum of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA)

Estimate of acute reference dose (ARfD)

Unnecessary

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly, but only to a limited extent (about 20–30%)
Dermal absorption	About 1–3%
Distribution	Widely distributed (low levels occurring in all tissues)
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid and nearly complete in 48 h (about 20–30% in urine and about 60–70% in faeces)
Metabolism in animals	Very limited (< 0.7%), by hydrolysis leading to AMPA
Toxicologically significant compounds in animals and plants	Parent compound, AMPA, *N*-acetyl-glyphosate, *N*-acetyl-AMPA
Acute toxicity	
Rat, LD_{50}, oral	5 600 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.48 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Moderately to severely irritating
Guinea-pig, dermal sensitization	Not sensitizing (Magnusson and Kligman test, Buehler test)
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Clinical signs (loose stools, diarrhoea), liver, salivary glands and reduced body weights
Lowest relevant oral NOAEL	300 mg/kg bw per day (90 days; rat)
Lowest relevant dermal NOAEL	> 5 000 mg/kg bw per day (21 days; rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weights, loose stools, liver (toxicity), salivary glands (organ weight, histology), eye (cataracts, lens fibre degeneration)
Lowest relevant NOAEL	100 mg/kg bw per day (2 years; rat)
Carcinogenicity	Not carcinogenic in rats; could not exclude possibility of carcinogenicity in mice at very high doses[a]
Genotoxicity	

Absorption, distribution, excretion and metabolism in mammals	
	No genotoxic potential via oral route in mammals[a]
Reproductive toxicity	
Target/critical effect	Reduced body weights and delayed development (absence of maternal toxicity)
Lowest relevant parental NOAEL	417 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	417 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	1 983 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Slight increase in malformations at maternally toxic doses
Lowest relevant maternal NOAEL	100 mg/kg bw per day (rabbit)[b]
Lowest relevant embryo/fetal NOAEL	250 mg/kg bw per day (rabbit)[b]
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw, highest dose tested
Subchronic neurotoxicity NOAEL	1 547 mg/kg bw per day, highest dose tested
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	No immunotoxicity; NOAEL 1 448 mg/kg bw per day, highest dose tested (28 days; mouse)
Studies on toxicologically relevant metabolites	Toxicological studies on AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA reveal the metabolites to be less toxic than the parent compound
Human data	
	Medical surveillance of workers in plants producing and formulating glyphosate did not reveal any adverse health effects. In operators applying glyphosate products, cases of eye, skin and/or respiratory irritation have been reported. Cases of acute intoxication have been observed after accidental or intentional ingestion of glyphosate formulation.

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

[b] Secondary to local irritation of the colon.

Summary

	Value	Study	Safety factor
ADI	0–1 mg/kg bw	Two-year studies of toxicity (rat)	100
ARfD	Unnecessary	–	–

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Appendix 1(a). Results of in vitro genotoxicity with glyphosate in nonmammalian species

End-point	Test object	Concentration	Purity %	GLP (Yes/No)	Results	Reference
Chromosome damage	*Allium* root cells	w/o S9; 720–2880 µg/L	Glyphosate isopropylamine (96%)	No	Negative	Rank et al. (1993)
Chromosome alterations	*Allium* root cells	w/o S9; 720–2 880 µg/L calculated as glyphosate isopropylamine	Roundup	No	Positive	Rank et al. (1993)
Chromosome alterations	*Allium cepa* onion root tips	0.036– 0.146%	Springbok, glyphosate isopropylamine formulation (48%)	No	Positive	Asita & Makhalemele (2008)
Chromosome alterations	*Trigonella foenum-graecum*n root tips	0.1–0.5%	Glyphosate	No	Positive	Siddiqui et al. (2012)
Chromosome alterations	*Allium cepa* onion root tips	3%	Glyphosate	No	Positive	Frescura et al. (2013)
Micronucleus	*Allium cepa* onion root tips	35. 70, 105, 140, 350, 700. 1050 and 1400 µg/g	Glyphosate formulation (21%)	No	Equivocal	De Marco et al. (1992)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L	Glyphosate	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L active ingredient	Roundup	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	Frog (*Eleutherodactyl us johnstonei*) blood cells	4.6–37 mg a.e./cm^2	Roundup SL–Cosmoflux 411F (360 g/L glyphosate)	No	Positive	Meza-Joya, Ramirez-Pinilla & Fuentes-Lorenzo (2013)
DNA strand breaks (Comet assay)	Tilapia (*Oreochromis niloticus*) erythrocytes	w/o S9; 0.000 7– 0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Inconclusi ve	Alvarez-Moya et al. (2014)

S9: 9000 × *g* supernatant fraction

Appendix 1(b). Results of in vivo genotoxicity with glyphosate, Roundup and other formulations in nonmammalian species

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Glyphosate						
Mutation	*Drosophila larvae*	0.1 ppm	Pondmaster	N/S	Positive	Kale et al. (1995)
Mutation	*Drosophila larvae*	1 ppm	Roundup	N/S	Positive	Kale et al. (1995)
Mutation	*Drosophila* standard cross	0.1–10 mmol/L	Glyphosate (96%)	No	Weak positive	Kaya et al. (2000)
Mutation	*Drosophila* high bioactivation cross	0.1–10 mmol/L	Glyphosate (96%)	No	Negative	Kaya et al. (2000)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L	Glyphosate	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	17.9 35.7 µg/L	Glyphosate	No	Positive	Guilherme et al. (2012a)
DNA strand breaks (Comet assay)	Nile tilapia *Oreochromis niloticus* erythrocytes	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.0007–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Weak positive /inclusive	Alvarez-Moya et al. (2014)
DNA damage	Zebrafish (*Danio rerio*) sperm	5 & 10 mg/L	Glyphosate	No	Positive	Lopes et al. (2014)
DNA strand breaks (Comet assay)	Sabalo fish (*Prochilodus lineatus*) erythrocytes and gill cells	0.48 & 2.4 mg/L	Glyphosate	No	Positive	Moreno, Sofia & Martinez (2014)
Mutation (sex-linked recessive lethal)	*Drosophila* standard cross	1 ppm	Roundup	No	Positive	Kale et al. (1995)
Mutation (sex-linked recessive lethal)	*Drosophila* standard cross	0.1 ppm	Pondmaster	No	Positive	Kale et al. (1995)
Chromosomal aberrations	Plant meristems of *Crepis capillaris*	0.05–1%	Roundup (> 90% purity)	No	Negative	Dimitrov et al. (2006)
Chromosome abnormalities	Mitotic plant meristems of *Hordeum vulgare*	0.1–2%	Roundup	No	Positive	Truta et al. (2011)
Micronucleus	Nile tilapia fish *Oreochromis niloticus* erythrocytes	42–170 mg/kg bw	Glyphosate (Roundup 69)	N/S	Negative	Nascimento & Grisolia (2000)

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Micronucleus	*Tilapia rendalli* peripheral erythrocytes	42–170 mg/kg bw	Roundup (480 g/L)	No	Positive	Grisolia (2002)
Micronucleus	Plant meristems of *Crepis capillaris*	0.05–1%	Roundup (> 90% purity)	No	Negative	Dimitrov et al. (2006)
Micronucleus	The freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	No	Positive	Cavas & Konen (2007)
Micronucleus	Neotropical fish (*Prochilodus lineatus)* erythrocytes and gill cells	10 mg/L	Roundup (41%)	No	Negative	Cavalcante, Martinez & Sofia (2008)
Micronucleus	*Caiman latirostris* erythrocytes	50–1 750 µg/egg	Roundup (66.2%)	No	Positive	Poletta et al. (2009)
Micronucleus	European eel (*Anguilla anguilla*) blood cells	58 & 116 µg/L	Roundup (30.8%)	No	Negative	Guilherme et al. (2010)
Micronucleus	*Caiman latirostris* erythrocytes	3%	Roundup (66.2%)	No	Positive	Poletta et al. (2011)
Micronucleus and Nuclear abnormalities	Brazilian freshwater fish *Astyanax* sp.	0.006 mL/L	Roundup	No	Positive	Rossi et al. (2011)
Micronucleus	The fish *Corydoras paleatus* erythrocytes	6.67 µg/L	Roundup (48%)	No	Negative	De Castilhos, Ghisi & Cestari (2013)
Micronucleus	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	No	Positive	De Souza Filho et al. (2013)
Micronucleus	*Caiman latirostris* erythrocytes	2.5–21 mg/L	Roundup	No	Positive	López Gonzáles et al. (2013)
Micronucleus	Ten spotted live-bearer fish *Cnesterodon decemmaculatus* erythrocytes	22.9–68.8 mg/L	Glyphosate formulation Credit (48%)	No	Positive	Vera-Candioti, Soloneski & Larramendy (2013)
Micronucleus	Ten spotted fish *Cnesterodon decemmaculatus* erythrocytes	3.9–11.8 mg/L	Glyphosate formulation Panzer (48%)	No	Weak positive	Vera-Candioti, Soloneski & Larramendy (2013)
Micronucleus	Indian skittering frog (*Euflictis cyanophlyctis*) tadpole erythrocytes	1–8 mg a.e./L	Roundup (41%)	No	Positive	Yadav et al. (2013)
Micronucleus	Earthworm (*Pheretima peguana*) coelomocytes	47–432 µg cm^{-2}	Glyphosate formulation (36%)	No	Positive	Muangphra, Kwankua & Gooneratne (2012)
Micronucleus	*Channa punctatus* blood cells	8.1–24.4 mg/L	Roundup (41%)	No	Positive	Nwani et al. (2014)

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Micronuclei and meiotic anomalies	Black lentil beans *Vigna mungo*	Not specified	Glyphosate	No	Positive	Singh & Srivastava (2014)
DNA strand breaks (Comet assay)	Bullfrog *(Rana catesbeiana*) tadpoles	1.69–27 mg/L	Roundup (356 g/L)	No	Positive	Clements, Ralph & Petras (1997)
DNA strand breaks (Comet assay)	Freshwater mussels (*Utterbackia imbecillis*)	2.5 and 5 mg/L	Roundup (18%)	No	Negative	Conners & Black (2004)
DNA strand breaks (Comet assay)	Freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	No	Positive	Cavas & Konen (2007)
DNA strand breaks (Comet assay)	Neotropical fish (*Prochilodus lineatus)* erythrocytes and gill cells	10 mg/L	Roundup (41%)	No	Weak positive	Cavalcante, Martinez & Sofia (2008)
DNA strand breaks (Comet assay)	*Caiman latirostris* erythrocytes	50–1 750 µg/egg	Roundup (66.2%)	No	Positive	Poletta et al. (2009)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2010)
DNA strand breaks (Comet assay)	*Caiman latirostris* erythrocytes	3%	Roundup (66.2%)	No	Positive	Poletta et al. (2011)
DNA strand breaks (Comet assay)	Snail (*Biomphalaria alexandrina*) haemocytes	10 mg/L	Roundup (48%)	No	Positive	Mohamed (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L active ingredient	Roundup	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) gill and liver cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2012b)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2012a)
DNA strand breaks (Comet assay)	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	No	Positive	De Souza Filho et al. (2013)
DNA strand breaks (Comet assay)	Frog (*Eleutherodactylus johnstonei*) blood cells	0.5–1.7 mg a.e./cm^2	Roundup SL–Cosmoflux 411F (360 g/L glyphosate)	No	Positive	Meza-Joya, Ramirez-Pinilla & Fuentes-Lorenzo (2013)
DNA strand breaks (Comet assay)	Fish *Corydoras paleatus* erythrocytes	6.67 µg/L	Roundup (48%)	No	Positive	De Castilhos, Ghisi & Cestari (2013)

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
DNA strand breaks (Comet assay)	Freshwater clam (*Corbicula fluminea*) haemocytes	2 and 10 ppm	Roundup	No	Negative	Dos Santos & Martinez (2014)
DNA strand breaks (Comet assay)	Common carp (*Cyprinus carpio*) erythrocytes	2 mg/L	Roundup (480 g/L)	No	Positive	Gholami-Seyedkolaei et al. (2013)
DNA strand breaks (Comet assay)	*Channa punctatus* blood and gill cells	3.25–6.51 mg/L	Roundup (41%)	No	Positive	Nwani et al. 2013
DNA strand breaks (Comet assay)	Earthworm (*Eisenia andrei*) coelomocytes	15 and 30 $\mu g/cm^{-1}$	Roundup FG (71%)	No	Positive	Piola et al. (2013)
DNA strand breaks (Comet assay)	Earthworm (*Eisenia andrei*) coelomocytes	15–240 $\mu g/cm^{-1}$	Glyphosate formulation (85.4%)	No	Negative	Piola et al. (2013)
DNA strand breaks (Comet assay)	Ten spotted live-bearer fish *Cnesterodon decemmaculatus* erythrocytes	3.9 mg/L	Glyphosate formulation Panzer (48%)	No	Positive	Vera-Candioti Soloneski & Larramendy (2013b)
DNA strand breaks (Comet assay)	Ten spotted live-bearer fish *Cnesterodon decemmaculatus* erythrocytes	22.9 mg/L	Glyphosate formulation Credit (48%)	No	Positive	Vera-Candioti, Soloneski & Larramendy (2013b)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2014a)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) liver cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Marques et al. (2014)
DNA strand breaks (Comet assay)	Sabalo fish (*Prochilodus lineatus*) erythrocytes and gill cells	1 and 5 mg/L	Roundup Transorb (480 g/L)	No	Positive	Moreno, Sofia & Martinez (2014)
DNA strand breaks (Comet assay)	Earthworm (*Pheretima peguana*) coelomocytes	47–432 $\mu g\ cm^{-2}$	Glyphosate formulation (36%)	No	Negative	Muangphra Kwankua & Gooneratne (2012)
DNA strand breaks (Comet assay)	Tambaqui (Colossoma macropomum) fish	10–15 mg/L	Roundup (360 g/L)	No	Positive	Braz-Mota et al. (2015)
DNA breakage (Comet assay)	Neotropical fish *Prochilodus lineatus* blood cells	0.15–1.5 mg/L	Polyoxyethylene amine	N/S	Positive	Navarro & Martinez (2014)
AMPA						
Micronucleus	European eel (*Anguilla anguilla*) blood cells	11.8, 23.6 µg/L	N/A	No	Negative	Guilherme et al. (2014b)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	11.8, 23.6 µg/L	N/A	No	Positive	Guilherme et al. (2014b)

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Nuclear abnormalities	European eel (*Anguilla anguilla*) blood cells	11.8, 23.6 µg/L	N/A	N/S	Positive	Guilherme et al. (2014b)

AMPA: aminomethylphosphonic acid; bw: body weight; GLP: good laboratory practice; N/A: not applicable; N/S: not stated; ppm: parts per million; S9: 9000 × g supernatant fraction

References to Appendix 1

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MALATHION

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Explanation

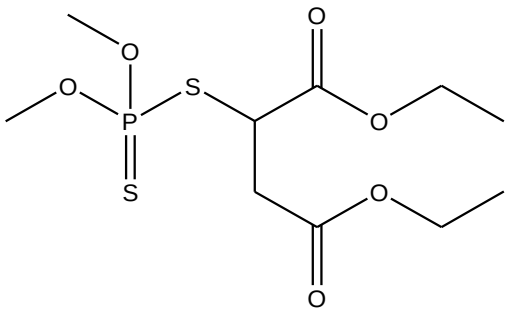
Malathion is the International Organization for Standardization–approved common name for *S*-1,2-*bis*(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorothioate (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service (CAS) number 121-75-5. The chemical structure of malathion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

	Mean % of administered radioactivity					
	40 mg/kg bw (single dose)		800 mg/kg bw (single dose)		40 mg/kg bw (repeated dose)	
Tissue	**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

	% of administered radioactivity		
Parameter	**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

	% of urinary metabolite					
Treatment	**MMCA**	**MMDA**	**DMP**	**DMTP**	**DMDTP**	**Total**
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).

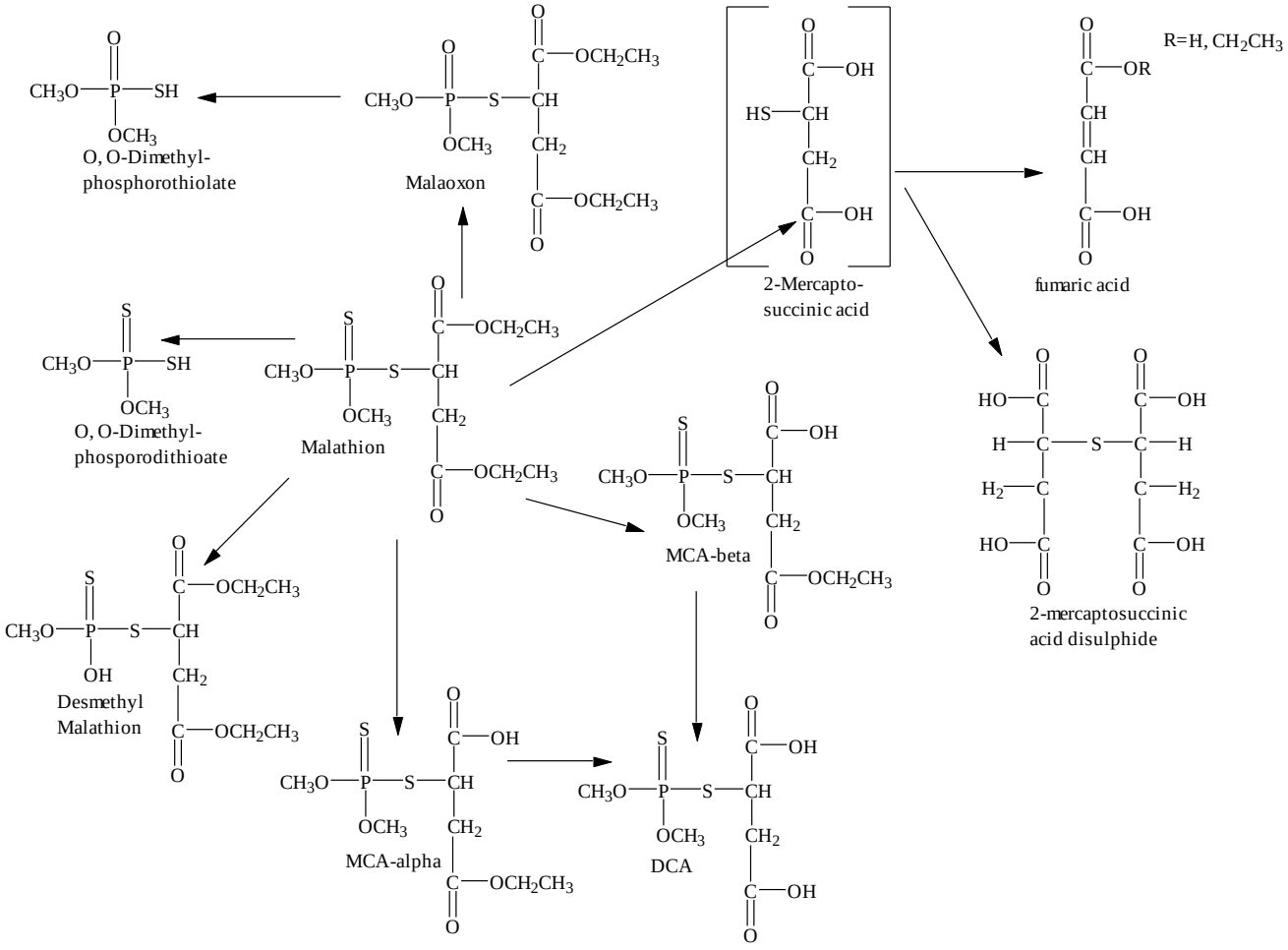
The disposition kinetics of malathion and its metabolites in humans (MMCA, MDCA, dimethyl dithiophosphate, dimethyl thiophosphate and dimethyl phosphate) following oral, dermal or intravenous exposure was estimated using a heuristic toxicokinetic model, based on data from

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

	Measure per dietary concentration					
Parameter	**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

	No. of findings per aerosol concentration			
Parameter	**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

	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**
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				

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**
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

	Measure of cholinesterase activity per dietary concentration				
Parameter	**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

	Measure per dietary concentration				
Parameter	**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

	No. and incidence per dietary concentration			
Neoplasm	**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

	Measure per dietary concentration				
Parameter	**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%)

	Measure per dietary concentration				
Parameter	**0 ppm**	**100 or 50 ppm**	**500 ppm**	**6 000 ppm**	**12 000 ppm**
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					

	Measure per dietary concentration				
Parameter	**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

	Measure per dietary concentration				
Parameter	**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

	Measure per dietary concentration				
Parameter	**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

	No. per dietary concentration				
Parameter	**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.

[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		Comments	Reference
				−S9	+S9		
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	Results +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
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 −S9	Results +S9	Comments	Reference
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 −S9	Results +S9	Comments	Reference
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		Comments	Reference
				−S9	+S9		
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	Results +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		Comments	Reference
				−S9	+S9		
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 × *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		Comments	Reference
				−S9	+S9		
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 −S9	Results +S9	Comments	Reference
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 −S9	Results +S9	Comments	Reference
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	Results +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 $1/10^{th}$ 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 2 ppm injection, in water	94.4%	Positive (feed) Negative (injection)		Foureman et al. (1994)

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)	$1/15^{th}$ 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)	Swiss albino mice, sex unspecified (bone marrow)	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 Quimica 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 = $1/9^{th}$ 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

	Achieved dose in mg/kg bw per day							
	Males				Females			
Phase	**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 $\log IC_{50}$ of −4.3 mol/L while the $\log IC_{50}$ 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 $\log IC_{50}$ of −4.4 mol/L, while the $\log IC_{50}$ 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 $\text{Log}IC_{50}$ of −4.6 mol/L while the $\log IC_{50}$ 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.

Source: Barnett Jr (2012d)

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

	No. or % measure per dose			
Parameter	**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

	Cholinesterase activity per dietary concentration			
Parameter	**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)				

	Cholinesterase activity per dietary concentration			
Parameter	**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

Parameter	Cholinesterase activity per dietary concentration			
	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

	Erythrocyte AChE activity		
Time	**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

			Erythrocyte AChE	
Compound	**Sex**	**Brain AChE BMD_{10}**	**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

			Erythrocyte AChE	
Compound	**Sex**	**Brain AChE BMD_{10}**	**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

			Erythrocyte AChE	
Compound	**Sex**	**Brain AChE BMD_{10}**	**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 preweanling 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

	AChE activity per treatment per dose			
	Males		Females	
Treatment	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

	AChE activity per treatment per dose			
	Males		Females	
Treatment	**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)

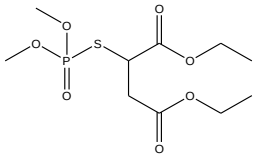
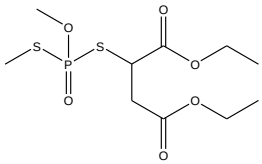
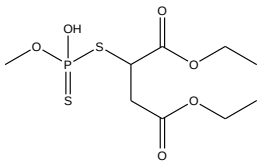
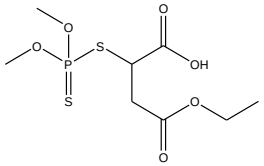
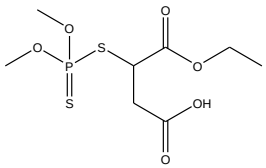
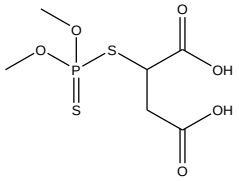
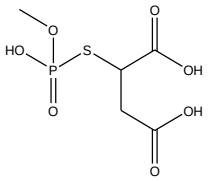
(e) Intestinal microbiota effects

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

	No. and per cent change compared to control per dietary concentration			
Parameter	**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

	No. per dietary concentration			
Parameter	**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.

[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) 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	Schinasi & Leon (2014)
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)

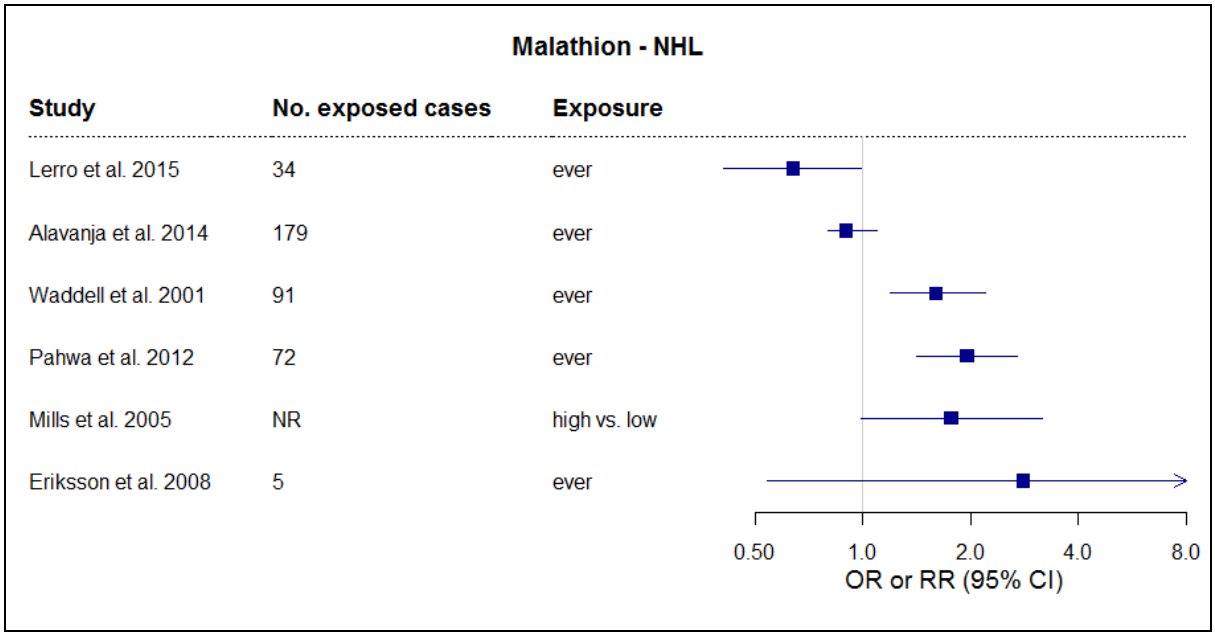
AHS: Agricultural Health Study; aOR: adjusted odds ratio; aRR: adjusted risk ratio; CI: confidence interval; IW-LED: intensity-weighted lifetime-exposure days, defined as number of years of use × number of days used per year × personal protective equipment use reduction factor × intensity level score (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)).

[14] We thank the Agricultural Health Study, and Dr Laura Beane Freeman in particular, for providing the additional information we requested relating to this publication.

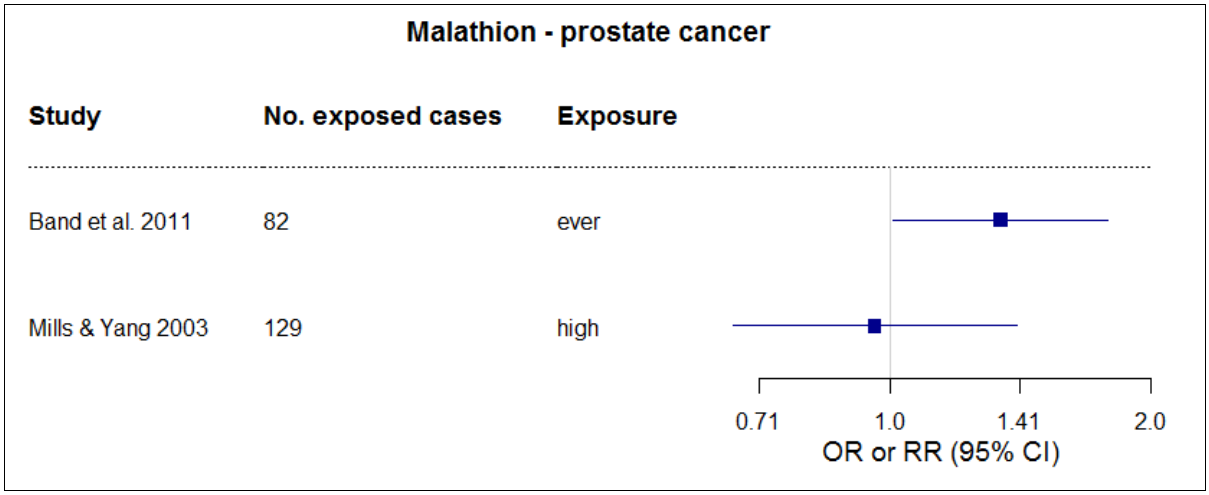
Fig. 3. Forest plot for risk estimates of studies with qualitative exposure categories



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



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;

[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[b,e]	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[b,e]	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

	No. and incidence per dietary concentration (ppm)				
Parameter	**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 epithlelium 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

	Males		Females	
Finding	**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

	Males					Females				
Finding	**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

	Aerosol concentration (mg/L)			
Parameter	**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

	Dietary concentration (ppm)				
Parameter	**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

	Dietary concentration (ppm)				
Parameter	**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 epithlelium (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

	Dietary concentration (ppm)			
Parameter	**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

	Dietary concentration (ppm)			
Parameter	**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.

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ANNEX 1

Reports and other documents resulting from previous Joint Meetings of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO Core Assessment Group on Pesticide Residues

1. Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240, 1962.
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This volume contains toxicological monographs that were prepared by the 2016 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Geneva on 9–13 May 2016.

The monographs in this volume summarize the safety data on three pesticides that could leave residues in food commodities. These pesticides are diazinon, glyphosate and malathion. The data summarized in the toxicological monographs served as the basis for the acceptable daily intakes and acute reference doses that were established by the Meeting.

This volume and previous volumes of JMPR toxicological evaluations, many of which were published in the FAO Plant Production and Protection Paper series, contain information that is useful to companies that produce pesticides, government regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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