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International Agency for Research on Cancer

World Health Organization

MALATHION

Malathion was previously considered by the Working Group and evaluated as *not classifiable as to its carcinogenicity to humans* (Group 3) (IARC, 1983, 1987). The Working Group concluded that there was *inadequate evidence* for the carcinogenicity of malathion or its metabolite malaoxon in experimental animals, and no data for humans were available at that time. New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 121-75-5

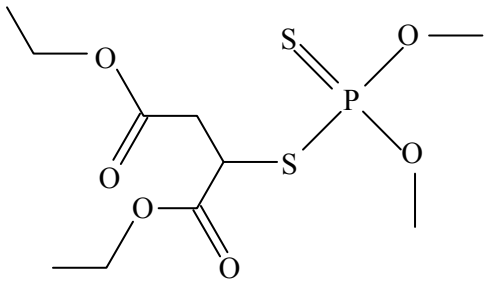
Chem. Abstr. Serv. Name: diethyl 2-[(dimethoxyphosphinothioyl)thio]butanedioate

Preferred IUPAC Name: diethyl 2-dimethoxyphosphinothioylsulfanylbutanedioate

Selected Synonyms: American Cyanamid 4049, Carbafos, Carbofos, Carbophos, Cythion, Fyfanon, Karbofos, Maldison, Mercaptothion, Mercaptotion, Prioderm, Sadophos

Trade Names: Malathion is marketed under at least 17 different trade names (including Agrothion, Heckthion, Hilmala, Hilthion, Malatox, and Tragumal) in several countries (Farm Chemicals International, 2015).

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_{10}H_{19}O_6PS_2$

Relative molecular mass: 330.36

Additional chemical structure information is available in the PubChem Compound database (NCBI, 2015).

1.1.3 Chemical and physical properties of the pure substance

Description: Clear to amber liquid with an odour variously reported as garlic-like, skunk-like, or similar to mercaptan (Tomlin, 2000; NCBI, 2015).

Solubility: Slightly soluble in water (145 mg/L at 25 °C) (NCBI, 2015); soluble in ethanol, benzene and ethyl ether (NCBI, 2015), and miscible with most organic solvents, e.g. alcohols, esters, ketones, ethers, and aromatic hydrocarbons (NCBI, 2015).

Volatility: Vapour pressure, 5.3 mPa at 30 °C (negligible) (Tomlin, 2000; NCBI, 2015); relative vapour density (air = 1.0), 11.4 (IPCS, 2005)

Stability: Relatively stable in neutral, aqueous media (Tomlin, 2000) but rapidly hydrolysed at pH > 7.0 or < 5.0 (HSDB, 2015); hydrolysis produces thiomalic acid and dimethyl thiophosphate (Mulla et al., 1981). Generally stable to photolysis (Katagi, 2004). Decomposes on heating and on burning, producing toxic fumes including phosphorus oxides and sulfur oxides; reacts violently with strong oxidants (IPCS, 2005).

Reactivity: Attacks iron, some other metals, some forms of plastic and rubber (IPCS, 2005)

Octanol/water partition coefficient: log K_{ow}, 2.89 (IPCS, 2005).

Henry's law: 4.9×10^{-9} atm m^3 $mole^{-1}$ at 25 °C (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), $mg/m^3 = 13.5 \times ppm$.

Additional chemical and physical properties are described in the PubChem Compound database (NCBI, 2015).

1.1.4 Technical products and impurities

The technical product contains 90–95% malathion (Tomlin, 2000; ATSDR, 2003). Fourteen impurities have been identified in technical-grade malathion, including isomalathion and malaoxon (ATSDR, 2003). Isomalathion may be formed during both manufacture and storage (EPA, 2009; WHO, 2013). Some formulations also contain gamma-cyhalothrin (NCBI, 2015).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Malathion, an aliphatic organophosphate introduced in 1950, is one of the oldest and most heavily used insecticides in the family of organophosphate chemicals (Ware & Whitacre, 2004).

Malathion is typically manufactured using a condensation reaction (at 70–80 °C) of *O,O*-dimethyl phosphorodithioic acid and diethyl maleate or diethyl fumarate in the presence of hydroquinone (Sittig, 1980). Other processes are available for producing malathion for pharmaceutical purposes and for the two enantiomers of malathion (e.g. Berkman et al., 1993; Arava et al., 2010).

Malathion is formulated as a dust, wettable powder, emulsifiable concentrate (active ingredient, up to 82%), ready-to-use liquid (active ingredient, up to 97%), or pressurized liquid. The liquids containing 97% active ingredient are typically intended for ultra-low-volume applications, such as in mosquito abatement programmes. Several end-use products containing malathion also contain other active ingredients such as captan and methoxychlor (EPA, 2009).

(b) Production volume

Malathion is manufactured in 10 countries by 49 producers; the majority are located in China (22 producers) and India (12 producers), with others in Singapore, the USA, the United Kingdom, Denmark, Egypt, Japan, Mexico, and Switzerland (Farm Chemicals International, 2015). In the USA market, 31 unique malathion products are available from 20 companies (NPIRS, 2015).

In 1978, about 14 000 tonnes of malathion were reportedly produced (IARC, 1983). Although information on current production volume was not available to the Working Group, production of malathion probably peaked in 1999 due to high

demand in the USA for eradication of the boll weevil (EPA, 2004). It is reasonable to assume that production of malathion has decreased, and will continue to decrease as worldwide demand for organophosphate pesticides declines (FAO, 2014). Nevertheless, malathion has been among the best-selling generic organophosphate insecticides worldwide since the 1980s (EPA, 2004; PAN, 2006).

1.2.2 Uses

Malathion is a non-systemic broad-spectrum insecticide used widely in agriculture for various food and feed crops, grain storage facilities, lawns, gardens and outdoor residential areas, ornamental nursery stock, building perimeters, roadways, pastures and rangeland, and regional pest eradication programmes (ATSDR, 2003). It is applied to control a large variety of insect pests, including ants, aphids, caterpillars, flies, fruit flies, grasshoppers, hornets, moths, mites, mosquitoes, scorpions, spiders, wasps, and weevils, as well as ectoparasites of cattle, horses, swine, poultry and pets (including fleas on dogs and cats). Additionally, malathion is used to treat head and body lice on humans (EPA, 2009).

Malathion is applied mainly as ground and aerial sprays, aerosols and baits (ATSDR, 2003). Application techniques include spraying by aircraft or ground-based equipment, fogger, ground boom, airblast sprayer, and various hand-held equipment such as backpack sprayers, low-pressure handwands, hose-end sprayers, power dusters, and shaker cans (ATSDR, 2003; EPA, 2009).

(a) Agriculture

Malathion is applied to a wide variety of food and feed crops, including alfalfa, berries, broccoli, cabbage, celery, citrus, cotton, fruit, garlic, hay, greens, mushrooms, nuts, rice, root crops, squash, and wheat (EPA, 2009). In the USA, the greatest use of malathion has been associated with a campaign to eradicate the boll weevil from cotton-growing areas (EPA, 2004). Annual use of malathion in the USA reached a peak at 12 700–14 500 tonnes in 1999, but fell to 2000–4000 tonnes by 2007, near the completion of the boll-weevil eradication campaign (EPA, 2011). Malathion has also been used in several fruit-fly eradication efforts in the USA (EPA, 2009).

Malathion was among the most commonly observed pesticides in four African countries (selected to cover a range of policy scenarios, market contexts, and production zones) (Williamson et al., 2008).

(b) Public health

Malathion is used for mosquito abatement in public-health programmes in industrialized and less industrialized countries. In the USA and Canada, treatments are typically performed using ultra-low volume aerial and truck-fogger applications (ATSDR, 2003; Health Canada, 2003). In tropical areas such as India and Brazil, it is used in malaria-control efforts as a residual insecticide that is applied to interior walls and roofs (Lal et al., 2004; Singh et al., 2011a).

(c) Pharmaceuticals

Malathion (formulated as a 0.5% lotion) is used pharmaceutically as a pediculicide for the treatment of head and body lice, and their ova (EPA, 2009).

(d) Regulation

Although approval of malathion for the European Union market was revoked in 2008, Member States of the European Union voted in 2010 to allow malathion end-use products to be registered for the control of insect pests in agricultural crops; malathion has been re-authorized at the national level in Austria, the Czech Republic, France, Poland, Romania, and Slovakia, and authorization is in progress in Bulgaria and Italy (European Commission, 2015).

Table 1.1 Representative methods for the analysis for malathion

Sample matrix	Assay procedure	Limit of detection	Reference
Air	GC-MS	0.3 ng/m³	Elflein et al. (2003)
Water	GC-FPD (phosphorus mode)	NR	EPA (2007)
	GC-MS (selected ion monitoring mode)	0.01 µg/L	Zaugg et al. (1995)
Urine	GC-MS/MS	< 0.001 µg/L	Cruz-Márquez et al. (2001)
	GC-MS-ECNI-SIM	0.2 µg/L (as MDA) 0.2 µg/L (as MMA)	Bouchard et al. (2006)
Fruits and vegetables	GC-MS	0.04 ng/g	Fillion et al. (2000)
Dust	GC-MS	10 ng/g	Harnly et al. (2009)

GC-FID, gas chromatography/flame ionization detection; GC-FPD, gas chromatography/flame photometric detection; GC-MS, gas chromatography-mass spectrometry; GC-MS-ECNI-SIM, gas chromatography-mass spectrometry with electron capture negative ionization in single-ion monitoring mode; MDA, malathion dicarboxylic acid; MMA, malathion monocarboxylic acid; NR, not reported

Occupational exposure limits for malathion ranging from 1 mg/m³ to 15 mg/m³ have been established in several countries (IFA, 2015).

1.3 Measurement and analysis

Historically, the analysis of organophosphate pesticides has presented challenges, since many are photosensitive or easily degraded during standard preparation, storage, and analysis. Additionally, the large number of organophosphate pesticides that could potentially be present in a sample may hinder identification of the individual analytes. Before the relatively recent increase in the sensitivity of gas chromatography-mass spectrometry (GC-MS), ion-specific detectors (e.g. flame photometric detector in the phosphorus mode) were used routinely to detect organophosphate pesticides at low ppb levels (RESTEK, 2002).

Due to its uses for agricultural, public health, and residential pest-control purposes, malathion may be present in soil, air, surface water and groundwater, and food, in addition to occupational exposure. Exposure to malathion may be assessed using urinary biomarkers, including three non-specific metabolites of dimethyl phosphate – namely, dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP) – and two specific metabolites – namely malathion dicarboxylic acid (MDA) and malathion monocarboxylic acid (MMA). Representative methods of chemical analysis are listed in Table 1.1.

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Occupational exposure to malathion has been measured in greenhouse workers, strawberry farm workers, date farmers, and pest- and vector-eradication workers. Exposure has been found to vary significantly according to factors such as task (e.g. application or re-entry activities), application method, extent of leaks and spills, use of personal protective equipment, and personal hygiene (Machera et al., 2003; Edwards et al., 2007; Salvatore et al., 2008).

(i) Air

Monitoring of air is not a useful way of determining exposure in workers since most exposure occurs via the dermal route (Tuomainen et al., 2002a; ATSDR, 2003; Machera et al., 2003). In one study in malathion-spraying workers, personal air samples were negative for malathion (Edwards et al., 2007), while other studies estimated potential exposures from inhalation to be

Table 1.2 Concentrations of malathion metabolites in the urine of occupationally exposed workers

Country, year	No. of workers	Occupation	Tasks	Results	Reference
USA, 2003	72	Farm workers	Picking strawberries	Urinary MDA, 93% detects; geometric mean, 44.4 µg/g; maximum, 971.3 µg/g (adjusted for creatinine)	Salvatore et al. (2008)
Thailand, year NR	25	Farmers	Producing a variety of crops	Urinary MDA, 18.4% detects, maximum, 3.194 µg/L (939 µg/g creatinine); geometric mean, NR	Panuwet et al. (2008)
Canada, 2003	18	Greenhouse workers	Spraying (2), working on treated plants (5), unexposed (1)	Urinary MDA median, 0.085 µg/L; 95th percentile, 4.1 µg/L Urinary MMA median, 1.3 µg/L; 95th percentile, 10 µg/L	Bouchard et al. (2006)
Finland, year NR	3	Greenhouse workers	Spraying	Urinary MMA, 2–24 h after spraying, range, 0–600 µg/L (max. observed when leaks occurred); mean, NR	Tuomainen et al. (2002b)
Haiti, year NR	5	Sprayers	Spraying for mosquito control	Urinary MMA mean, 3600 µg/L before weekend and 90 µg/L after weekend (creatinine adjusted)	Warren et al. (1985)

GM, geometric mean; MDA, malathion dicarboxylic acid; MMA, malathion monocarboxylic acid; NR, not reported

several orders of magnitude lower than dermal exposures (Tuomainen et al., 2002a; Machera et al., 2003).

(ii) Skin

Dermal contact is the most important route of exposure to malathion. Studies have used a variety of interception methods, including shirts, patches and whole-body coveralls from which malathion is extracted in attempts to determine the extent of exposure for the worker (Krieger & Dinoff, 2000; Machera et al., 2003; Edwards et al., 2007). Factors such as the time spent spraying and the pressure of the spray influence the dose received (Machera et al., 2003). Accidental exposure due to spills, leaks, or dripping of malathion can contribute significantly to exposure (Machera et al., 2003; Edwards et al., 2007). Most studies found that higher levels of exposure occur on the hands than on other parts of the body (Tuomainen et al., 2002a; Machera et al., 2003).

Exposure can be reduced by wearing gloves, hats, long-sleeved shirts, trousers, and closed shoes, changing clothes daily, and washing hands with soap (Salvatore et al., 2008).

(iii) Biological markers

The carboxylic acids MMA and MDA are metabolites that are specific to malathion and can be used to assess malathion exposure. After exposure to malathion, excretion of MMA in the urine increases and reaches a maximum about 6–7 hours after completion of the application (Tuomainen et al., 2002b). After about 2 days of non-exposure, MMA and MDA decline to undetectable levels in the urine (Warren et al., 1985; Krieger & Dinoff, 2000).

Urinary concentrations of MDA and MMA have been measured in farm workers, greenhouse workers, and sprayers in mosquito-control programmes. Concentrations ranged widely, but there were too few studies to identify patterns of exposure according to task or crop (Table 1.2). [The Working Group noted that exposures were far lower in a study in Canada carried out by Bouchard et al. (2006) than in other studies, but only two workers included in this study were

engaged in spraying and both used personal protective equipment.]

Urinary concentrations of MMA and MDA in workers occupationally exposed to malathion have been observed to decrease significantly after several days of absence from work (Warren et al., 1985; Krieger & Dinoff, 2000). MMA and MDA were not detected in the urine of family members of an occupationally exposed date-palm worker. Urinary concentrations of MMA and MDA for the wife and two children were less than the limit of detection at the end of the working week, while detectable concentrations were found in the worker and in two other date-palm workers who lived with the family (Krieger & Dinoff, 2000).

Malathion also exhibits cholinesterase-inhibitory activity; however, this effect is not specific to malathion and is common to other organophosphate and carbamate pesticides (ATSDR, 2003).

Several studies in the USA, Australia, and Haiti have shown no inhibition of cholinesterase activity among workers employed in spraying with malathion (Warren et al., 1985; Krieger & Dinoff, 2000; Edwards et al., 2007), although two studies found reductions in cholinesterase activity in mosquito-control sprayers in India (Lal et al., 2004; Singh et al., 2011b). In one study in six workers spraying malathion formulation for the control of the vectors of kala-azar (visceral leishmaniasis), the mean cholinesterase activity of the workers after spraying decreased to about 83% of the value before spraying ($P < 0.01$), but was still within the normal range (Lal et al., 2004). The workers wore masks and gloves, and washed their hands with soap after spraying. Another study found significantly reduced acetylcholinesterase activity in erythrocytes of 70 workers who sprayed organophosphate pesticides for community-health programmes when compared with healthy volunteers (Singh et al., 2011b). However, this decrease cannot be linked definitively with exposure to malathion, since the workers sprayed several different organophosphate pesticides.

(b) Community exposure

The general population can be exposed to malathion from residues on food, from living near areas where malathion is sprayed, or through personal use of products containing malathion (ATSDR, 2003). Measured concentrations of malathion in environmental media are generally very low and malathion is not persistent, since it degrades relatively quickly. Nevertheless, the use of sensitive analytical methods has found that malathion can be detected at low concentrations in the urine of a notable proportion of subjects, including among those who live near sprayed areas (ATSDR, 2003).

(i) Drinking-water

Malathion has been detected in < 1% of groundwater samples from the USA (ATSDR, 2003). Because of rapid degradation, and the fact that malathion is usually applied to foliage, groundwater contamination is not widespread (Newhart, 2006).

In Kanpur, India, three groundwater samples from six agricultural locations were found to be positive for malathion, with the highest value being 2.61 µg/L. Seven out of 12 samples from industrial areas contained malathion in the range of 0.85 to 16.24 µg/L (Sankararamakrishnan et al., 2005).

Surface-water contamination is also relatively low. The California Department of Pesticide Regulation collects pesticide monitoring data in the Surface Water Database (CDPR, 2014). Of the 12 941 measurements of malathion, 602 (4.7%) were "non-zero" and only 37 were > 1 µg/L. Of the 1064 measurements of malaoxon, only one was non-zero.

The United States Geological Survey National Water Quality Assessment Data Warehouse has systematically collected data on water quality from 51 basins since 1991 (USGS, 2014). Of 13 890 non-zero measurements for malathion, 99.97% were < 0.1 µg/L. Of 5522 non-zero measurements

for malaoxon, 99.93% were < 0.1 µg/L [analysis by the Working Group].

Contamination of surface water appears to be higher in less industrialized countries. In India, one out of six samples taken from different locations on the River Ganges contained malathion at a detectable level (2.61 µg/L ± 0.05) (Sankararamakrishnan et al., 2005). In the Philippines, concentrations of malathion in unfiltered water samples ranged from below the detection limit (0.1 µg/L) to 3.3 µg/L, with a mean of 0.85 µg/L (Varca, 2012). The maximum concentration was measured at a time when insecticide was being applied in rice farms nearby.

(ii) Air

Concentrations of malathion in air are generally very low (ATSDR, 2003). However, exposures may be greater for residents living around sites where malathion is sprayed for mosquito control and other reasons. In the USA, the maximum concentrations detected in indoor, outdoor, and personal air at one spraying site were 20.8, 0.3, and 16.8 ng/m^3, respectively (ATSDR, 2003). In California, the highest concentrations (averaged over three sites) of malathion and malaoxon in air were 61.6 ng/m^3 and 47.9 ng/m^3 after spraying, and 28.0 and 48.1 ng/m^3 at 24–48 hours after spraying, respectively (Brown et al., 1993a).

(iii) Residues in food

Malathion residues have been measured in a variety of foods. The reported concentrations are below the limit of detection in most countries for which data were available, but the limits of detection varied widely and were not always reported (Dogheim et al., 2002; Rawn et al., 2004; FDA, 2006; Bhanti & Taneja, 2007; Darko & Akoto, 2008; EFSA, 2011; NRS, 2011; Health Canada 2014; Li et al., 2014).

(iv) Household exposure

In a survey of 246 households in California, USA, 2% were storing a product containing malathion (Guha et al., 2013).

(v) Biological markers

There are few available studies of specific malathion metabolites in representative samples, and most of these studies tested for MDA and were carried out in the USA (Table 1.3). MDA was detected in 1–7% of urine samples from adults in the 1970s to 1990s (Kutz et al., 1992; MacIntosh et al., 1999), but was found more frequently (52% of samples) in data for 1999–2000 from the largest study, the National Health and Nutrition Examination Survey (NHANES) in the USA, with a geometric 95th percentile of 1.6 µg/L (1.8 µg/g of creatinine) (Barr et al., 2005).

A study of community residents exposed to malathion formulations used for vector control in India reported that the mean level of cholinesterase activity for the population was 79% of the pre-spraying level after 1 week ($P < 0.01$), 82% after 1 month ($P < 0.01$), and was back to the pre-spraying level after 1 year (Lal et al., 2004).

1.4.2 Exposure assessment

This section summarizes the exposure assessment and assignment for epidemiological studies of cancer and exposure to the pesticides considered in the present volume (diazinon, malathion, glyphosate, tetrachlorvinphos, and parathion).

Almost all the epidemiological studies of occupational exposure reviewed in this volume considered pesticide exposure of licensed applicators, farmers, farmworkers, and their spouses. The challenges faced in the exposure assessment are substantial, given the nature of agricultural production and typical use of these chemicals. Exposure to pesticides can occur directly by mixing and applying pesticides, but also takes place when performing re-entry tasks among treated crops. For most pesticides, dermal exposure is much more important than exposure by inhalation. Agricultural work is often seasonal and exposures to pesticides will therefore vary in a temporal sense due to task variety, meteorological conditions, and the inherent intermittent

Table 1.3 Concentrations of malathion dicarboxylic acid in urine samples from the general population

Country, year, reference	No.	Age (years)	Percentage detectable, levels	Comments	Reference
USA, 1976–80 NHANES II	6990	12–74	0.5% detectable; maximum, 250 µg/L; mean and median, NR	Not standardized for creatinine	Kutz et al. (1992)
USA, 1995–96	80	Adults	6.6% detectable; median, < 0.4 µg/g creatinine; range, < 0.2–51 µg/g		MacIntosh et al. (1999)
USA, 1997	262	3–13	37% detectable; geometric mean, 0.7 µg/g creatinine		Adgate et al. (2001)
USA, 1999–2000 NHANES	1920	6–59	52% detectable; median, < LOD (0.31 µg/L); 75th percentile, 0.49 µg/g creatinine	Highest at age 6–11 years (median, 0.44 µg/g creatinine)	Barr et al. (2005)
USA, 1998	13	2–5	71% detectable; median, 1.5 µg/g	Not standardized for creatinine	Kissel et al. (2005)
USA, 2004	60	1–6	28% detectable; median, 0.33 µg/g creatinine	Not adjusted for creatinine	Arcury et al. (2007)
USA, 1999–2000	445	≥ 18	39% detectable; median, 0.82 µg/L (not adjusted for creatinine)	Adjusting metabolites by creatinine yielded similar results	Eskenazi et al. (2007)
Thailand, year NR	207	12–13	25% detectable; geometric mean, 0.32 µg/g creatinine		Panuwet et al. (2009)

LOD, limit of detection; MDA, malathion dicarboxylic acid; MMA, malathion monocarboxylic acid; NHANES, National Health and Nutrition Examination Survey; NR, not reported

nature of most agricultural exposures (Kromhout & Heederik, 2005). However, farmers often have stable careers and tend to stay in the same working and living environments. Such stability also makes them reliable sources of information on past production patterns, machinery, and chemical use (Blair et al., 2002, Hoppin et al., 2002). A study in the USA carried out annual surveys of pesticide use among farmers (Engel et al., 2001). Compared to what they had initially reported, participants interviewed 20 years after the start of the study reported using fewer insecticides (including organophosphates) and more herbicides and fungicides at the time of the initial study. Sensitivity and specificity for individual pesticides ranged from 0.22 to 0.72, and 0.48 to 0.84, respectively.

Exposure patterns are also often complex in terms of the specific chemicals involved, and frequently entail mixed exposure situations (either due to use of multiple active ingredients in one season, or use of different active ingredients for the same purpose consecutively over a lifetime). The number of active ingredients to which a farmer may have been exposed can vary between types of agriculture, from a handful over a lifetime in large farms predominantly growing one or a few crops (Hoppin et al., 2012), to more than 15 active ingredients in 1 year for intensive culture of a variety of flowers and vegetables in greenhouses in horticulture (Tielemans et al., 2007).

The intrinsic correlation structure of exposure patterns will be highly dependent on the number of crops being grown, the homogeneity of the population studied, the authorization policies in force, and other factors such as climatological conditions, agronomical guidelines, and recommendations from agricultural extension services. Exposure assignment based on information collected at the level of the individual study subject will in principle provide insight into this

matter, provided that reporting of the information is reliable and accurate. In the Agricultural Health Study for which pesticide-use information was collected at the individual level, it was shown that correlation between active ingredients was higher for pesticides within the same type, such as herbicides or insecticides, ranging from 0.30 to 0.70, but considerable lower or close to zero for pesticides of different types (Samanic et al. 2005). Pairwise correlation between individual organophosphate insecticides ever used was low: more than 90% were less than 0.2, with a maximum of 0.58 (Hoppin et al., 2012).

The method used to assess and assign exposure, and the type of information collected or available might increase the correlation between active ingredients and therefore limit the possibility of disentangling the effects of one active ingredient from another. For instance, in a case–control study, the correlation between active ingredients can increase dramatically if information is obtained on crops grown, and a crop-exposure matrix based on linkage of crops and authorization data of pesticides is then used to assign exposure to individual cases and controls. This can make it impossible to distinguish the effects of one insecticide from another in such a study.

To reduce measurement error, some studies have used known determinants of pesticide exposure in questionnaires for retrospective assessment of exposure, both in studies of the general population and within agricultural populations (Dosemeci et al., 2002). It is possible to use generic questions about exposure determinants in case–control studies since they will result in considerable contrast between persons exposed and unexposed to pesticides. On the other hand, studies within agriculture might lack sufficient contrast to discriminate different intensities of exposure. Use of quantitative measurement data does not necessarily result in more accurate exposure assessment, since in such mixed exposure situations there is enormous temporal variability in exposure intensity, and often only limited numbers of exposure measurements are available (due to logistic problems). Good exposure-modelling practices, combined with additional information collection, can remedy this problem to a large extent (Kromhout & Heederik, 2005).

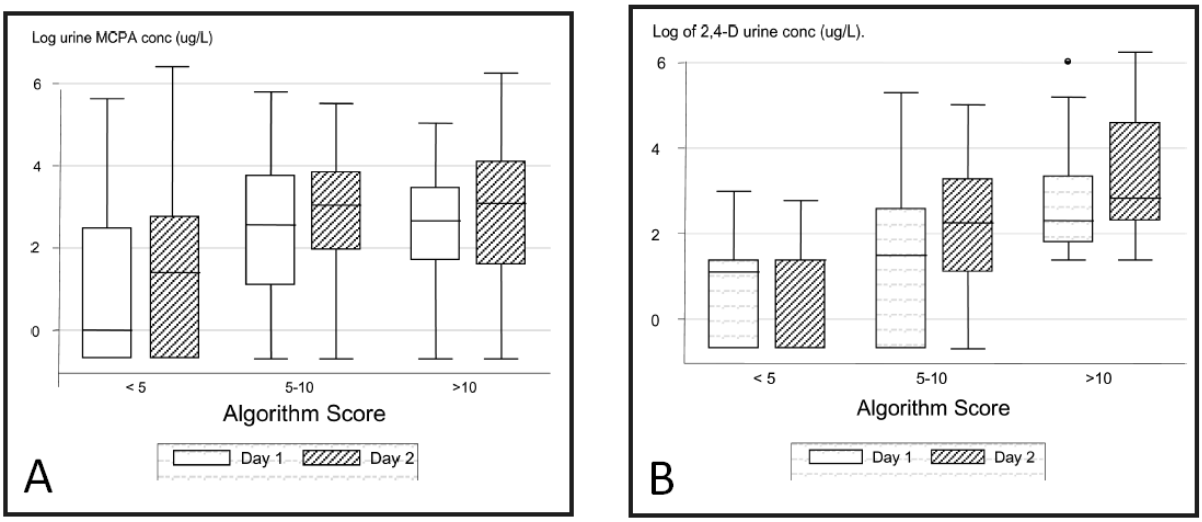
(a) Agricultural Health Study

Great efforts were made in the Agricultural Health Study (AHS) to assess exposure among agricultural pesticide applicators and their spouses. These questionnaires and algorithms have been extensively described and have undergone several tests for reliability and accuracy that have provided considerable insight into the quality of this exposure assessment.

A semiquantitative exposure assessment method was developed based on self-reported information from 58 000 applicators in Iowa and North Carolina, USA, on determinants of exposure intensity, such as mixing condition, duration and frequency of application, application methods, maintenance or repair of mixing and application equipment, work practices, use of personal protective equipment and personal hygiene. For each study subject, chemical-specific lifetime cumulative levels of pesticide exposure were derived by combining intensity of pesticide exposure (estimated using self-reported information on determinants of exposure intensity in formal algorithms) and self-reported years and annual frequency of pesticide application (Dosemeci et al., 2002). Using logic checks, the accuracy of self-reported use of the pesticides on the initial questionnaires in the AHS was studied by comparing self-reported decade of first use and total years of use to the year the pesticide active ingredient was first registered. The majority of respondents provided plausible responses for decade of first use and total duration of use (Hoppin et al., 2002).

More direct validation of the algorithm used to estimate exposure intensity scores was

Fig. 1.1 Urine concentrations of MCPA and 2,4-D in applicators, grouped by pesticide exposure



(A) Box plot of day 1 and day 2 urine concentration of MCPA for applicators grouped by pesticide exposure algorithm score (n = 84); (B) Box plot of day 1 and day 2 urine concentration of 2,4-D for applicators grouped by pesticide exposure algorithm score (n = 41).
2,4-D, 2,4-dichlorophenoxyacetic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid
From Coble et al. (2005), Taylor & Francis Ltd, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com)

performed through comparison of algorithm scores with biological monitoring data from 84 farmers who had applied the herbicide MCPA and 41 farmers who had applied 2,4-D. Urinary concentrations of MCPA ranged from < 1.0 to 610 µg/L, while urinary concentrations of 2,4-D ranged from < 1.0 to 514 µg/L. A direct comparison of algorithm scores and urine concentrations showed weak correlation for MCPA (Spearman correlation, 0.17–0.18), and moderate correlation for 2,4-D (Spearman correlation, 0.34–0.45). Categorizing the population based on algorithm scores into three groups showed that the geometric mean urinary concentration was 20 µg/L in the group with highest exposure, and 5 µg/L in the group with lowest exposure, for those applying MCPA. For those applying 2,4 D, the geometric means were 29 µg/L in the group with highest exposure, and 2 µg/L in the group with lowest exposure (Coble et al., 2005; see Fig. 1.1).

The second validation study in the AHS focused on appraising the intensity algorithm using actual measurements of fungicide exposure for applicators working in orchards. Personal air, hand rinses, 10 dermal patches, a pre-application first-morning urine and a subsequent 24-hour urine sample were collected from 74 applicators for 2 days after application. Environmental samples were analysed for captan, and urine samples for *cis*-1,2,3,6-tetrahydrophthalimide (THPI). Captan and THPI were more frequently detected in samples from applicators who used air-blast rather than manual application. The exposure intensity algorithm was marginally predictive of concentrations on the thigh and forearm, but did not predict exposures in air, hand rinse, or urine for THPI (Hines et al., 2008).

A third validation study compared algorithm intensity scores with measured exposures in the field. Pre- and post-application measurements of urinary biomarkers were made for applicators of 2,4-D (n = 69) and chlorpyrifos (n = 17). Personal dermal exposure was measured by patches and hand wipes, and inhalation exposure was measured by personal air samples. Intensity scores

were estimated using information collected from technicians and applicators. Scores from the two groups were highly correlated (Spearman's r = 0.92 and r = 0.84 for 2,4-D and chlorpyrifos, respectively). Correlations between the algorithm intensity scores and post-application urinary concentrations were moderate for both 2,4-D and chlorpyrifos (r = 0.42 and r = 0.53 respectively. Correlations between intensity scores and estimated hand loading, estimated body loading, and air concentrations were weak to moderate for 2,4-D applicators (r = 0.28–0.50) but lower for chlorpyrifos applicators using granular products (r = 0.02–0.58) (Thomas et al., 2010). Based on the results of this validation study, the algorithm used for the AHS was modified, but the new algorithm containing modified weighting factors for personal protection efficiency and application method was not validated in a new exposure study (Coble et al., 2011).

[The Working Group noted that these validity studies suggested that the AHS exposure intensity algorithm has some capacity to discriminate between extremes of the exposure intensity range; however, validity was evaluated only for exposure during application days, while the epidemiological analyses used estimates of long-term exposure intensity.]

(b) Other epidemiological studies

A summary of the methods of exposure assessment used in epidemiological studies discussed in this volume is presented in Table 1.4. Most these studies were carried out in North America.

All of the studies addressed historical exposure to pesticides, therefore the use of biomarkers or monitoring data was not feasible at the individual subject level. Almost all of the studies relied on self-reported data, which (as discussed above) is reasonably reliable and valid when applicators are reporting their own use, but may not be suitable for spouses or other farm workers, particularly those exposed by re-entry. Proxy respondents are unlikely to know the details of use of specific pesticides by their next-of-kin.

Apart from the AHS, few of the studies included expert review of the data or performed validity or reliability studies.

In most community-based studies, the numbers of subjects exposed to individual pesticides were low, and analyses were performed on a simple assessment of whether a subject had been ever exposed or not. Some studies were able to subdivide the exposed subjects by number of years exposed or number of days of use per year. No study was able to make a quantitative estimate of cumulative exposure.

[In conclusion, the Working Group noted that the exposure assessment methods used in in most studies were relatively crude.]

2. Studies of Cancer in Humans

Malathion was previously considered by the *IARC Monographs* in 1983 and 1987 (IARC, 1983, 1987). No data on exposure in humans were available at that time. New data have become available since the previous evaluation, including several epidemiological studies that are described below.

2.1 Scope of available epidemiological studies

The frequently cited epidemiological studies that contributed to the decision of the Working Group regarding the strength of the evidence for carcinogenicity in humans associated with the pesticides considered in the present volume of the *IARC Monographs* (malathion, parathion, diazinon, glyphosate, and tetrachlorvinphos) are summarized in Section 2.2. These pesticides have been used for many decades worldwide, sometimes in large quantities, in both agricultural and domestic situations. Despite this widespread use, there are surprisingly few studies

Table 1.4 Methods of assessment of pesticide exposure used in epidemiological studies

Study or reference, country	Information-collection method	Exposure assessed	Respondents	Exposure assessment method	Exposure metrics	Comments
Cohort studies						
Agricultural Health Study, USA	Prompted questionnaire with list of 50 pesticides	Retrospective, occupational and residential	Individual licensed applicators and spouses	Exposure intensity algorithm developed by experts (Dosemeci et al., 2002) frequency of application and duration of use	Cumulative exposure, intensity, frequency, duration	Quality of pesticide use reporting was checked against pesticide registration data. Validation studies of the exposure intensity score were performed, indicating limited power to assess intensity
Settimi et al. (1999), Italy	Human resources records	Employment in a cigarette factory using tobacco treated with TCVP	Workers employed at least 6 months in the factory		Employment for ≥ 6 months	Tobacco used in the factory was treated with TCVP and other pesticides
Nested case–control studies						
Mills et al. (2005), USA (California)	Data linkage (union records and cancer registry)	Ecological, occupational		State pesticide database provided pounds of each pesticide used by county, crop, and year; assigned as a JEM	Ever exposed, and tertiles of pounds used	Exposure categorization based on ecological information results in misclassification
Pesatori et al. (1994), USA (Florida)	Questionnaire	Retrospective, occupational	Next-of-kin of cases and controls	Next-of-kin report	Yes/no	Questionnaire details were unclear; very basic exposure categorization; next-of-kin are likely to be a poor source of information on exposure to specific active ingredients
Case–control studies						
NCI studies, USA (Nebraska, Iowa and Minnesota, Kansas)	Open-ended questionnaire in Kansas; list of 19 pesticides in other areas	Retrospective, occupational	General-population cases and controls or next-of-kin	Self-report	Yes/no, frequency, duration	Questions on specific pesticides may improve recall

Table 1.4 (continued)

Study or reference, country	Information-collection method	Exposure assessed	Respondents	Exposure assessment method	Exposure metrics	Comments
Cross-Canada Case–control Study of Pesticides and Health Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia)	Screening questionnaire for pesticide use; telephone interview with list of specific pesticides if > 10 hours use per year and 15% of others	Retrospective, occupational	General-population cases and controls or next-of-kin	Self-reporting	Yes/no	No validity checks were done. Very basic exposure categorization
Band et al. (2011) Canada (British Columbia)	Questionnaire	Retrospective, occupational	General population cases and controls	Job-exposure matrix for 6 jobs, 290 chemical agents, including 180 pesticides (Wood et al., 2002)	Cumulative exposure	Although the JEM included detailed information on regions, crops and tasks, it is unclear how this information was obtained from cases and controls
Upper Midwest Health Study USA (Iowa, Michigan, Minnesota, Wisconsin)	Questionnaire. Ever exposed and list of pesticides for farm workers or residents	Retrospective, occupational and residential	General population cases and controls or proxies (50% of subjects)	Self-report	Yes/no	No validity checks were done. Very basic exposure categorization
Mills et al. (2005) USA (California)	Data linkage (union records and cancer registry)	Ecological, occupational		State pesticide database provided pounds of each pesticide used by county, crop and year. Assigned as a JEM	Ever exposed, and tertiles of pounds used	Exposure categorization based on ecological information will result in misclassification
Davis et al. (1993) USA (Missouri)	Questionnaire on residential pesticide use with list of specific pesticides including diazinon	Retrospective, residential	Parents of cases and controls		Ever exposed during pregnancy	Self-reported data for ever use. Required to remember very specific period (potential telescoping). No validity checks, basic exposure categorization
Pogoda & Preston-Martin (1997) USA (California and Washington)	Questionnaire with list of specific pesticides including diazinon	Retrospective, residential and occupational	Parents of cases and controls		Ever exposed during pregnancy	Self-reported data for ever use. Required to remember very specific period (potential telescoping). No validity checks, basic exposure categorization

Table 1.4 (continued)

Study or reference, country	Information-collection method	Exposure assessed	Respondents	Exposure assessment method	Exposure metrics	Comments
EPILYMPH study 6 European countries (Italy, France, Germany, Czech Republic, Spain, Ireland)	Job-specific questionnaire regarding pesticides, pests and application methods for study subjects reported having worked in agriculture	Retrospective, occupational (agriculture)	General population cases and controls	Hygienist reviewed responses and categorized exposure with assistance from a crop-exposure matrix	Yes/no	Self-reported data with expert review using crop-exposure matrix. No validity checks, basic exposure categorization
Nordström et al. (1998) Sweden	Questionnaire	Retrospective, occupational	General population cases and controls		Yes/no	Details of what was asked are not clear. Self-reported data. No validity checks, basic exposure categorization
Hardell & Eriksson (1999) Sweden	Questionnaire regarding pesticides, pests and application methods	Retrospective, occupational	General population cases and controls		Yes/no	Self-reported data. No validity checks, basic exposure categorization
Eriksson et al. (2008) Sweden	Questionnaire regarding pesticides use, and duration of use	Retrospective, occupational	General population cases and controls		Yes/no	Self-reported data. No validity checks, basic exposure categorization
Orsi et al., 2009 France	Job-specific questionnaire on use of pesticides, duration of use, and application methods for farmers or gardeners, followed by a telephone interview for some	Retrospective, occupational and residential (garden and household)	General population cases and controls	Questionnaire reviewed by expert to ensure consistency of information with crop, pest and availability	Yes/no	Self-reported data with expert review regarding face validity. Two exposure definitions: possible or definite and definite only

JEM, job-exposure matrix; NCI, National Cancer Institute; TCVP, tetrachlorvinphos

on cancer outcomes. Most of the studies were performed in North America, with some studies in Europe. Very few studies have been performed in less industrialized countries, where exposure is likely to be much higher. Some of these studies were of good quality, but tended to focus more on acute effects such as poisoning and inhibition of acetylcholinesterase activity, rather than on cancer.

There were few studies of use of specific pesticides in women. This is particularly a problem for assessing the association of pesticides with cancers such as cancer of the breast.

Occupational exposure, which tends to be higher than residential exposure, is of relatively low prevalence in the general population. Thus the numbers of exposed cases in population-based studies are low, particularly when considering individual pesticides. To overcome this problem, there is a tendency to combine exposures to individual pesticides into larger groupings either by use (e.g. herbicides, insecticides, fungicides) or chemical group (e.g. carbamates, organophosphates) for end-points such as cancer. Consequently, the literature contains many more studies on the general class of organophosphate pesticides than on individual active ingredients, and thus few studies contributed to the evaluation of individual pesticides of the Working Group.

2.1.1 Chance, bias, and confounding

The studies considered in the evaluation of human carcinogenicity in this volume were primarily of case–control design. The advantage of such studies is the larger number of cases, particularly of rare cancers such as non-Hodgkin lymphoma (NHL); however, as discussed above, the number of exposed cases is often low in general population-based studies, so chance is often a factor in the results.

In addition, case–control studies can be subject to the problem of recall bias in the reporting of past use of pesticides. A particular type of recall bias that may occur in studies of exposure at a particular time (such as during pregnancy) is "telescoping" of exposure (in which respondents have difficulty in placing limits on the time period about which they are being asked). The AHS, being a cohort study, avoids recall bias since exposure was obtained before the onset of cancer. Misclassification of pesticide exposure in the AHS cannot however be excluded, because exposure was retrospective and self-reported (as is typical for most case–control studies), but the error would be non-differential and in most scenarios would not inflate risk estimates.

While there is high potential for confounding by use of multiple pesticides (see Section 2.1.2), there are few other co-exposures with pesticide use (e.g. diesel exposure in farm workers should be considered in analyses of cancer of the lung), and these can be measured and taken into account in case–control studies.

2.1.2 Exposure assessment

The quality of the exposure assessment is a major issue in studies of pesticide exposure. Exposure assessments are almost entirely dependent on self-reported data. The pesticides studied in this volume are not persistent and there are no valid long-lived biomarkers. Therefore, the type of pesticide used is likely to be reasonably accurate when reported by the pesticide applicators themselves, but less accurate for other potentially exposed subjects such as farm workers (who are exposed mainly through "re-entry"– going back into the field after it has been sprayed), or when next-of-kin have to answer questions on actual frequency and type of application for (deceased) relatives. Self-reporting is also more difficult in farms growing crops that use a very large number of active ingredients per season (e.g. apples, potatoes, vineyards, greenhouses) and thus during a lifetime. For the applicators, frequency and duration of use are likely to be

reasonably well reported. However, it is very difficult to measure the intensity of exposure to an individual pesticide over a long period. The combination of few exposed cases, and difficulty in assessing the amount of exposure, has meant that it is difficult to examine or detect an exposure–response association in some studies.

Exposures to multiple pesticides are very difficult to disentangle, in part due to their correlated nature. To examine these properly, sample sizes must be very large and there must be heterogeneity to control for multiple exposures. This is especially a problem when exposure information is not collected at the individual study subject level. In addition, even in large studies, missing data for some pesticides may make it difficult to adjust for potential confounding by multiple substance use. Dropping the subjects with missing data for multiple pesticide adjustment not only results in loss of precision, but also has the potential to result in selection bias.

Encouraging signs are seen in some studies (e.g. Alavanja et al., 1996; Monge et al., 2007) where researchers have identified determinants of exposure (e.g. type of equipment, characteristics of tasks) that can be used in epidemiological questionnaires. The construction of algorithms can be seen as a way to improve exposure assessment and to investigate exposure–effect relationships.

In summary, the assessment of carcinogenicity in humans for agents in the present volume was limited by the relatively small number of high-quality epidemiological studies available. There is a lack of studies with good exposure assessment, large numbers of exposed cases, the ability to control for multiple pesticides, and set in a wide range of geographical regions with variation in pesticide usage patterns.

2.2 Summary of frequently cited epidemiological studies

Several informative epidemiological studies conducted over the past few decades have assessed the risk of cancer in association with exposure to several of the pesticides evaluated in the present volume of the *IARC Monographs* (i.e. malathion, parathion, diazinon, glyphosate, and tetrachlorvinphos) (see Table 2.1). These studies are described here in detail, and the results for specific pesticides are presented in the individual *Monographs* in this volume.

2.2.1 Agricultural Health Study

The Agricultural Health Study (AHS) (Alavanja et al., 1996, 2003; NIH, 2015) is a prospective cohort of licensed pesticide applicators (n = 52 395) and their spouses (n = 32 347) from Iowa and North Carolina, USA. The cohort was established in 1993–1997 to answer questions about the health of the farming populations, and in particular the incidence of cancer. In Iowa, 4916 commercial pesticide applicators were also enrolled. Farmers and pesticide applicators were identified when seeking a license to apply restricted-use pesticides from state departments of agriculture; they were asked to complete an enrolment questionnaire (which included detailed questions on pesticide use, application methods, use of protective equipment, and demographic and lifestyle factors). Individuals willing to participate in the study were also given take-home questionnaires to be completed by themselves and their spouses that sought more extensive information on occupational activities (completion rate, 46% of applicators and 62% of spouses). Two follow-up telephone interviews have been completed since enrolment (phase 2: 1999–2003; and phase 3: 2005–2010) to update data on farming practices, lifestyle and health. A new follow-up effort began in 2013. Recent publications concerning the AHS have drawn

Table 2.1 Main characteristics of frequently cited epidemiological studies on agents reviewed in Volume 112 of the *IARC Monographs*

Study Location	Design and population description	Exposure assessment	Pesticides assessed	Cancers assessed	Comments	References
Agricultural Health Study Iowa and North Carolina, USA	Prospective cohort of licensed pesticide applicators (n = 52 395) and their spouses (n = 32 347); mostly farmers, but 4916 commercial applicators also enrolled in Iowa; reference group was farmers not exposed to the evaluated pesticides	Interview, individual assessment of pesticide exposure (ever vs never, cumulative, intensity), validation	Malathion, parathion, diazinon, and glyphosate	Bladder, brain, breast, lung, colon, head and neck, kidney, leukaemia, liver, melanoma, mesothelioma, multiple myeloma, NHL, ovary, pancreas, prostate, rectum, stomach, thyroid, uterus	Several reports, including cohort and nested case–control analyses; adjustment for multiple exposures; high-quality study	Alavanja et al. (1996, 2003); NIH (2015)
United States Midwest case–control studies of lymphatic and haematopoietic cancers and soft tissue sarcoma Iowa, Minnesota, Kansas, Nebraska, USA	Three case–control studies in Iowa and Minnesota, Kansas, and Nebraska, and pooled analyses of these data for NHL	Interview, individual assessment of pesticide exposure (ever vs never, cumulative)	Malathion, parathion, diazinon, glyphosate, tetrachlorovinphos	Leukaemia, NHL, multiple myeloma, Hodgkin lymphoma, and soft tissue sarcoma	Adjustment for multiple exposures; high quality study	Hoar et al. (1986); Brown et al. (1990); Hoar Zahm et al. (1990); Cantor et al. (1992); Waddell et al. (2001); De Roos et al. (2003)

Table 2.1 (continued)

Study Location	Design and population description	Exposure assessment	Pesticides assessed	Cancers assessed	Comments	References
Cross-Canada Case–control Study of Pesticides and Health Six provinces of Canada	Population-based, case–control study; white men (age ≥ 19 yr) diagnosed between 1991 and 1994; cases: 517 NHL, 316 Hodgkin lymphoma, 342 multiple myeloma, 357 soft tissue sarcoma; 1506 controls	Interview, individual assessment of pesticide exposure (ever vs never, frequency)	Malathion, parathion, diazinon, and glyphosate	NHL, Hodgkin lymphoma, multiple myeloma, soft tissue sarcoma	High quality study The strengths included the large sample size, detailed collection of pesticide exposures, and the attempt to disentangle the effect of other pesticides; however, as a population-based case–control study carried out across diverse geographical regions, there was broad diversity in exposures, and lower prevalence of pesticide use than in other studies that focused on specific occupational groups. Typical of case–control studies with retrospective exposure assessment, this study was limited by the need to rely on self-reported exposure data	McDuffie et al., (2001); Hohenadel et al., (2011); Pahwa et al. (2011, 2012b) ; Karunanayake et al. (2012)
Florida Pest Control Worker Study Florida, USA	Cohort of pesticide workers in Florida (n = 4411), licensed in 1965–66; nested case–control study of lung cancer with 65 deceased cases	Next-of-kin interviews	Diazinon, malathion, parathion	Lung	Substantial limitations to the pesticide exposure assessment based on proxy interviews, the potential for considerable variation in the degree of exposure misclassification given the wide range of dates of the follow-up (1965–1982), and the likelihood of differential exposure misclassification resulting from the use of next-of-kin interviews for living and deceased study subjects	Blair et al. (1983); Pesatori et al. (1994)

Table 2.1 (continued)

Study Location	Design and population description	Exposure assessment	Pesticides assessed	Cancers assessed	Comments	References
United Farm Workers of America California, USA	Cohort of Hispanic farm workers in California (n = 139 000); plus nested case–control studies	Linking county/ month and crop-specific job history information from union records with California Department of Pesticide Regulation pesticide-use reports	Diazinon, malathion	Leukaemia, NHL, multiple myeloma, breast	Ecological exposure assessment method: advantage is that it does not rely on self-reporting, thus eliminating the potential for recall bias; disadvantage is that it reflected ecological rather than individual exposure to pesticides, and was therefore likely to be associated with substantial exposure misclassification	Mills & Kwong (2001); Mills et al. (2005); Mills & Yang (2005)
Case–control study of cancer of the prostate in British Columbia British Columbia, Canada	Population-based case–control study; patients with prostate cancer (n = 1516) recruited between 1983 and 1990; controls (n = 4994) had other cancers (age-matched)	Exposures assigned by JEM	Malathion, parathion, diazinon, and glyphosate	Prostate	There was high correlation between the use of specific pesticides as assessed through JEM. This, together with the large number of pesticides showing dose–response associations, suggests that associations for specific pesticides may be due to intercorrelations with other pesticides. While strengthened by its large number of cases, the results must be interpreted with caution due to the many comparisons examined, the correlated nature of occupational exposures, and the potential misclassification that derives from using a JEM to estimate individual exposures to a particular chemical	Band et al. (2011)

Table 2.1 (continued)

Study Location	Design and population description	Exposure assessment	Pesticides assessed	Cancers assessed	Comments	References
Upper Midwest Health Study (UMHS) Iowa, Michigan, Minnesota, Wisconsin, USA	Case–control study; 798 histologically confirmed cases of intracranial glioma identified through participating medical facilities and neurosurgeon offices by a rapid ascertainment system; 1175 controls without glioma were randomly selected within 10-yr age-group strata	In-person interview asking about exposure to specific pesticides (based on research on crops grown and pesticides used in recent years in the participating states)	Malathion, diazinon, glyphosate	Intracranial glioma		Ruder et al. (2004); Carreón et al. (2005); Yiin et al. (2012)

JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; yr, year

information on pesticide use and other information from the enrolment questionnaire, as well as from the first follow-up questionnaire. Incidence of cancer (at each site) and mortality are determined by periodically linking the cohort with the two state cancer registries and with the national death index.

At enrolment, detailed questions were posed about exposure to 50 pesticides selected because of their importance in agriculture in Iowa and North Carolina, or because data from humans or animals had suggested potential health effects (Karami et al., 2013). For 22 of these pesticides, detailed questions on use duration (number of exposed years), and frequency (average number of days of mixing and/or application per year) were posed in the enrolment questionnaire. For the other 28 pesticides, detailed information on frequency and duration of use were solicited in a second take-home questionnaire. Because not all of the cohort members returned the take-home questionnaire, the number of individuals may differ by analysis of pesticide. Methodological studies were completed to assess the reliability and validity of the pesticide information provided by the applicators (Blair et al., 2002; Hoppin et al., 2002). Monitoring studies on pesticide application among AHS participants were completed to assess the accuracy of the exposure intensity algorithm and new algorithm weights were estimated (Hines et al., 2008; Thomas et al., 2010). The original exposure intensity algorithm (Dosemeci et al., 2002) was modified slightly based on these weights (Coble et al., 2005), and since then the modified algorithm has been used in hazard analyses in the AHS. For individuals in the AHS who did not complete a phase 2 re-interview 5 years after enrolment, an imputation method was used that permitted inclusion of all participants in phase 2 analyses. The imputation method was based on their baseline data, even if portions of subsequent data were missing, which led to the observation that neither missing data nor imputation had major impacts on the main results for many of the pesticides, including parathion, diazinon, and malathion (Heltshe et al., 2012).

Blair et al. (2011) assessed the possible impact of misclassification of occupational pesticide exposure on relative risks, demonstrating that nondifferential exposure misclassification biases relative risk estimates towards the null in the AHS and tends to decrease the study precision. [The Working Group considered the AHS to be a highly informative study.]

2.2.2 Case–control studies in the midwest USA

Three population-based case–control studies conducted in the 1980s by the National Cancer Institute in Nebraska (Hoar Zahm et al., 1990), Iowa and Minnesota (Brown et al., 1990; Cantor et al., 1992), and Kansas (Hoar et al., 1986) provided information on several pesticides. All three studies assessed the risk for NHL. NHL cases and controls were combined from these studies to create a pooled data set to increase study precision to enable analyses for specific pesticides (Waddell et al., 2001; De Roos et al., 2003).

These studies also assessed other cancer sites. The study in Iowa and Minnesota included leukaemia (Brown et al., 1990) and NHL (Cantor et al., 1992), the study in Iowa included multiple myeloma (Brown et al., 1993b), the study in Nebraska included NHL, Hodgkin lymphoma, multiple myeloma, and chronic lymphocytic leukaemia (Hoar Zahm et al., 1990), and the study in Kansas included NHL, soft tissue sarcoma, and Hodgkin lymphoma (Hoar et al., 1986). In Iowa and Minnesota, 622 cases of NHL (Cantor et al., 1992), and 669 cases of leukaemia (Brown et al., 1990) among white men aged ≥ 30 years were identified from the Iowa state cancer registry and from a surveillance system of hospital and pathology laboratory records in Minnesota. In Iowa, cases of multiple myeloma (n = 173) were

identified from the state cancer registry (Brown et al., 1993b). In Nebraska, cases of NHL among white men and women aged ≥ 21 years (cases of NHL in men, 227; [the numbers of cases for the other cancers were not cited]) were identified through the Nebraska Lymphoma Study Group and area hospitals (Hoar Zahm et al., 1990). In Kansas, cases were white men aged ≥ 21 years identified from the state cancer registry (NHL, 170; Hodgkin lymphoma, 121; and soft tissue sarcoma, 133) (Hoar et al., 1986). Controls were identified by random-digit telephone dialling (< 65 years), Medicare records (≥ 65 years), or state mortality records (if matched to deceased cases). Controls were frequency-matched to the cases by race, sex, age (± 2 years), and vital status at the time of interview. Tumour tissue was reviewed by expert pathologists to confirm diagnosis in each of the three studies.

The exposure assessment differed somewhat between the three studies. The questionnaires for these studies were administered in person in Iowa and Minnesota (Brown et al., 1990; Cantor et al., 1992), and by telephone in Kansas (Hoar et al., 1986), and Nebraska (Hoar Zahm et al., 1990). Proxy respondents were selected to provide information if the cases or controls were deceased or incapacitated. [Because information obtained from proxies may not be as accurate as direct interviews, the possibility of misclassification of exposure may be greater.] Questionnaires included detailed questions about the use of pesticides and other relevant lifestyle, medical, and occupational factors for these cancers. Some of the pesticides that were assessed were malathion, parathion, diazinon, glyphosate, and tetrachlorovinphos. In Nebraska, Iowa, and Minnesota, participants were asked about a list of specific pesticides, while questions about pesticide use were open-ended in Kansas (without prompting for information on specific pesticides). In Nebraska, the total number of years of use and average number of days per year were collected for each pesticide, and for a predetermined list of approximately 90 pesticides (including malathion) (Hoar Zahm et al., 1990). In Iowa and Minnesota, dates of first and last use were collected. In Kansas, information was collected on days of use per year for pesticides, and years of use for herbicides and insecticides overall, not by specific pesticide, and participants were asked to volunteer information on the pesticides they had used (Hoar et al., 1986).

Waddell and colleagues reported on the association between several pesticides and NHL as investigated in the pooled database of the three United States Midwestern case–control studies in Iowa and Minnesota, Kansas, and Nebraska (Waddell et al., 2001). The evaluation ncluded total of 748 white men (age, ≥ 21 years) newly diagnosed with NHL were included (Iowa and Minnesota, 462; Kansas, 150; Nebraska, 136), and 2236 population-based controls (Iowa and Minnesota, 927; Kansas, 823; Nebraska, 486).

De Roos and colleagues also reported on the association between specific pesticides and NHL in the three pooled United States Midwestern case–control studies. This study was based on the same study population as Waddell et al. (2001), but the focus of analysis was on exposure to multiple pesticides to evaluate risk associated with realistic exposure scenarios; thus, detailed adjustment of risk estimates for other pesticides was made (De Roos et al., 2003). The analyses focused on 47 pesticides to which 20 or more persons were exposed. Any subject with a missing or "don't know" response for any of the 47 pesticides was excluded from all analyses, leaving 650 cases and 1933 controls (of the 870 cases and 2569 controls that comprised the study population). [Considering the detailed adjustment for other pesticides in De Roos et al. (2003), it is likely that any elevation of odds ratio is not due to confounding. A limitation of this analysis was that the results excluding proxy respondents were not presented, although it can be assumed that excluding individuals with missing and "don't know" responses would eliminate many

of the proxy interviews. The strengths of this report included the large sample size, which enabled assessment of pesticides with infrequent exposure).]

[The Working Group considered this set of studies to be highly informative.]

2.2.3 The Cross-Canada Case–control Study of Pesticides and Health

A population-based, case–control study of cancers of the haematopoietic tissue was conducted in white men (age, ≥ 19 years) with occupational and non-occupational exposures to pesticides (including malathion, parathion, diazinon, and glyphosate) in six provinces of Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, and Saskatchewan) with diverse agricultural practices. Incident cases of NHL (McDuffie et al., 2001; Hohenadel et al., 2011, which explored specific pesticide combinations), Hodgkin lymphoma (Karunanayake et al., 2012), soft tissue sarcoma (Pahwa et al., 2011), and multiple myeloma (Pahwa et al., 2012b) diagnosed in 1991–1994 were ascertained from provincial (population-based) cancer registries in all except one province where recruitment was based on hospital and clinical records (Quebec), and diagnosis was confirmed by pathology reports and reviewed by a central reference pathologist. Population controls were selected from provincial health insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or "voters' lists," and frequency-matched by age (± 2 years) to the distribution in the case group within each province. The response rates were 67.1% and 48% among cases and controls, respectively. A questionnaire sent by post (self-administered) collected information on a wide range of known and potential risk factors and a brief screen to identify general use of pesticides, and was followed by a telephone interview for subjects with > 10 hours per year of pesticide exposure and a 15% random sample of the remainder. A list of chemical and brand names was sent by post to the participants before the telephone interview to explain which agents would be referred to in the interview. The postal questionnaire was based on a revised version of the questionnaire used in the case–control studies by the National Cancer Institute (Hoar et al., 1986; Hoar Zahm et al., 1990). Environmental or incidental exposures and more intensive exposures were identified on the basis of number of hours of pesticide use per year (≥ 10 or < 10 hours). Information on pesticides was collected at several levels, from broadest categories to major classes, chemical groups, and individual compounds. Adjusted odds ratios were computed using conditional logistic regression analysis, stratified by the matching variables of age and province of residence, and analyses for each particular cancer type took into account a wide range of potential confounders (e.g. positive history of cancer in a first-degree relative) and certain, pre-defined potential effect-modifiers. Analyses to assess effect gradients were examined by categorizing by the average number of days per year of exposure. As in other epidemiological studies in humans, it was not possible to fully distinguish the effects of individual agents in the context of complex and multiple exposures, although attempts were made in this study to assess the effects of specific pesticides by controlling for the effects of other pesticides; however, such modelling in the initial publication did not include the pesticides reviewed by the Working Group for the present volume of the *IARC Monographs* (McDuffie et al. 2001), and subsequent publications relevant to the present volume reported only a few combined exposures to specific pesticides (Hohenadel et al., 2011, Pahwa et al., 2011). [The strengths of this study included its large sample size, detailed collection of pesticide exposures, and the attempt to disentangle the effect of other pesticides; however, as a population-based case–control study carried out across diverse

geographical regions, there was broad diversity in exposures, and lower prevalence of pesticide use than in other studies that focused on specific occupational groups. Typical of case–control studies with retrospective exposure assessment, this study was limited by the need to rely on self-reported exposure data. The Working Group considered this study to be highly informative.]

2.2.4 Florida Pest Control Worker Study

A cohort of pest-control workers in Florida, USA, was assembled to evaluate the risk of cancer among commercial pesticide applicators (Blair et al., 1983; Pesatori et al., 1994). The cohort (n = 4411) was established from licence records of pest-control workers in the state between 1965 and 1966. Since 1947, the Florida Department of Health and Rehabilitative Services has required that all persons engaged in pest control in houses, commercial buildings, and lawns and gardens be licensed annually. Licence records contained sociodemographic information and some data on occupation (city where they were employed, job task, and duration licensed). The cohort was followed for mortality (until 1977 in Blair et al., 1983, and until 1982 in Pesatori et al., 1994) using files from the social security administration and motor vehicle departments of Florida and other states, telephone and street directories, post offices, personal contacts, and the National Death Index. Among the 541 deaths, there were 54 cancers of the lung among white men, corresponding to elevated mortality for this cancer compared with the general population (standardized mortality ratio, 1.4; 95% CI, 1.0–1.8) (Pesatori et al., 1994). To further evaluate this excess, a nested case–control study of cancer of the lung was conducted that included 65 deceased cases (some occurred after 1982) with 294 (deceased, 122; living, 172) controls matched to cases on year of birth and death. Controls were randomly matched to each case by age. Questionnaires on tobacco use, occupation, dietary habits, and specific chemicals including pesticides were administered by telephone, with next-of-kin of deceased cases and surrogates for living and deceased controls (Pesatori et al., 1994).

[The Working Group noted substantial limitations to the pesticide exposure assessment based on proxy interviews, the potential for considerable variation in the degree of exposure misclassification given the wide range of dates of the follow-up (1965–1982), and the likelihood of differential exposure misclassification resulting from the use of next-of-kin interviews for living and deceased study subjects.]

2.2.5 United Farm Workers of America cohort study

Within a cohort of 139 000 members of the United Farm Workers of America, a largely Hispanic farm-workers' union in California (Mills & Kwong, 2001), two nested case–control studies were conducted on cancer of the breast (Mills & Yang, 2005) and incident cases of lympho-haematopoietic cancers (including leukaemia, NHL, and multiple myeloma) (Mills et al., 2005) to assess the role of occupational exposure to certain crops and to 15 most commonly used chemicals. Cases of lympho-haematopoietic cancers (including leukaemia, NHL, and multiple myeloma; n = 131) and cancer of the breast (n = 128) that were newly diagnosed between 1987 and 2001 in California were included. Five controls were selected for each case from the cohort who had not been diagnosed with any cancer and matched on sex, Hispanic ethnicity and ± 1 year of birth. Risk of cancer associated with several pesticides, including malathion and diazinon, was reported. Cases were identified by linkage with the California cancer registry (state-wide population-based cancer registry that has monitored all newly diagnosed cancers and cancer-related mortality since 1988) for 1987–2001. Crop and

pesticide exposures were estimated by linking county/month and crop-specific job history information from union records with California Department of Pesticide Regulation pesticide-use reports during the 20 years before cancer diagnosis. [The Working Group noted that these methods enabled estimation of whether a cohort member worked in an area with high pesticide use. The Working Group also noted that this is an ecological exposure assessment method, not an individual exposure assessment method.] After matching job histories with the pesticide database, applications (in pounds of active ingredient applied) were summed and used as a surrogate for pesticide exposures. For the 15 most commonly used pesticides (including diazinon and malathion), odds ratios for high versus low use were estimated by categorizing pounds of the active ingredient applied in the counties where the farm workers were employed.

[The Working Group noted that although some elevated relative risks were observed, these were difficult to interpret because the number of exposed cases on which these estimates were based was not reported. The exposure assessment method used had the advantage that it did not rely on self-reporting, thus eliminating the potential for recall bias, with the disadvantage that it reflected ecological rather than individual exposure to pesticides, and was therefore likely to be associated with substantial exposure misclassification. International Classification of Disease (ICD) codes were not provided.]

2.2.6 Case–control study of cancer of the prostate in British Columbia, Canada

Band and colleagues conducted a case–control study including 1516 patients with cancer of the prostate who were ascertained from the population-based cancer registry for the province of British Columbia, Canada, for the years 1983–1990, and 4994 age-matched cancer controls (all other sites excluding the lung and cancers of unknown primary site) (Band et al., 2011). Lifetime occupational history was obtained through a self-administered questionnaire, also including questions on sociodemographic characteristics, and smoking and alcohol consumption. A job-exposure matrix (JEM) was developed that covered 1950–1998 (45 animal and crop commodities), and provided quantitative information on specific active ingredients regarding combinations of region, crop, task (application, re-entry), and job title. The quantitative information was derived from models used for pesticide registration in the USA. The JEM was used to estimate participants' lifetime cumulative exposures to approximately 180 active compounds in pesticides, and the paper provided results for 100 individual pesticides (including malathion, parathion, diazinon, and glyphosate). Lifetime cumulative exposures were estimated as days of use. For pesticide exposures for which there were at least 15 exposed cases, low and high exposure categories were defined based on the median for exposed controls to assess whether there was a gradient of effect with increasing exposure. Conditional logistic regression was used to assess risk of cancer of the prostate and, after considering potential confounding by many factors, reported estimates were adjusted for age, alcohol consumption, cigarette-years, pipe-years, education, and respondent type (self or proxy). Band et al. (2011) reported the correlation between exposure to specific pesticides as assessed by the JEM, showing high correlation of use between several pesticides. [The Working Group noted that there was high correlation between the use of specific pesticides as assessed through JEM. This, together with the large number of pesticides showing dose–response associations similar to diazinon, suggests that associations for specific pesticides may be due to intercorrelations with other pesticides. While strengthened by its large number of cases, the results must be interpreted with caution due to the many comparisons examined, the correlated nature of occupational

exposures, and the potential misclassification that derives from using a JEM to estimate individual exposures to pesticides.]

2.2.7 Upper Midwest Health Study

The association between exposure to pesticides used on farms and risk of intracranial glioma in adults was studied in the Upper Midwest Health Study conducted among rural residents (aged 18–80 years) in Iowa, Michigan, Minnesota, and Wisconsin (Ruder et al., 2004; Carreón et al., 2005; Yiin et al., 2012). Cases with a histologically confirmed primary intracranial glioma [International Classification of Diseases for Oncology (ICD-O) codes 938–948] (Percy et al., 2001), diagnosed between 1 January 1995 and 31 January 1997, were identified via participating medical facilities and neurosurgeon offices by a rapid ascertainment system to try to complete case eligibility determination and physician consent within 2–3 weeks. Cases with a previous malignancy other than a glioma were not excluded. Case ascertainment completeness was determined by comparison with the corresponding cases of glioma in state cancer registries in all four states. Ascertainment percentages were 78.2% for Iowa, 82.7% for Michigan, 86.5% for Minnesota, and 65.5% for Wisconsin. Controls had no diagnosis of glioma, but those with a previous diagnosis of cancer or any other disease were not excluded. They were randomly selected from within 10-year age-group strata, with the proportion/stratum determined by the age distribution of glioma.

Cases or proxies and controls received two lists of pesticides by post before the face-to-face interview, which included a farm section asking about exposure to these specific pesticides (based on research on crops grown and pesticides used in recent years in the participating study states), distinguishing between direct and indirect exposure. Participants who had ever lived or worked on farms were asked to report their lifetime exposure to agricultural pesticides until 1 January 1993.

Data were collected on years of pesticide use, application days, or acreage covered, only for those applying pesticides directly. Questions covering a wide range of farm activities, including washing pesticide-contaminated clothes and whether specific crops were grown or animals were raised were asked only of those who had lived or worked on a farm after age 18 years. Odds ratios were adjusted for 10-year age group, education, farm residence, and exposure to any other pesticide.

2.2.8 Meta-analysis

Schinasi & Leon (2014) conducted a meta-analysis of NHL and exposure to several pesticides (including glyphosate, malathion, and diazinon) in agricultural settings. Case–control and cohort studies were included if they had been published in English, had used interviews, questionnaires, or exposure matrices to assess occupational exposure to agricultural pesticides, and reported quantitative associations for NHL overall or by subtype with specific active ingredients or chemical groups.

2.3 Cohort studies on malathion

See Table 2.2

Since the 1990s, one cohort study (the Agricultural Health Study) and two case–control studies nested in occupational cohorts (the Florida Pest Control Worker cohort and the United Farm Workers of America cohort) have assessed the association between exposure to malathion and cancer.

Table 2.2 Cohort studies of cancer and exposure to malathion

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Engel et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2000	30 454 wives of licensed pesticide applicators with no history of breast cancer at enrolment; because of the small number of cases in North Carolina, they were excluded from the analyses. Exposure assessment method: questionnaire	Breast	Wife's use (direct exposure)	63	0.9 (0.7–1.2)	Age, race, state	AHS [Strengths: large cohort; studied women's exposures; collection of detailed exposure information at enrolment, before disease outcome. Limitations: based on self-reported exposure; potential exposure to multiple pesticides]
			Husband's use (indirect exposure)	101	1.4 (1.0–2.0)		
			Premenopausal women:				
			Wife's use (direct exposure)	16	0.9 (0.5–1.5)		
			Husband's use (indirect exposure)	25	1.5 (0.7–3.0)		
			Postmenopausal women:				
			Wife's use (direct exposure)	41	0.9 (0.6–1.2)		
			Husband's use (indirect exposure)	69	1.5 (1.0–2.3)		
Flower et al. (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, 1975–1998	50 incident cases of childhood cancer; 17 357 children of Iowa pesticide applicators were matched against the Iowa Cancer Registry to identify cases of childhood cancer (aged < 19 yr) arising between 1975 and 1998 Exposure assessment method: questionnaire	Childhood cancer	Maternal use (ever)	11	1.12 (0.57–2.20)	Child's age at enrolment	AHS There were few cases from North Carolina, so analyses focused on children from Iowa [Strengths: large cohort. Limitations: based on self-reported exposure; potential exposure to multiple pesticides]
			Paternal use (prenatal)	8	0.78 (0.34–1.79)		

Table 2.2 (continued)

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bonner et al. (2007) Iowa, North Carolina, USA 1993–2002	19 717 licensed pesticide applicators with complete information on malathion (enrolment and take-home questionnaire), excluding prevalent cancer cases, those with no information on malathion or key potential confounders Exposure assessment method: questionnaire; state tumour registries, national death index for deaths	Lympho-haematopoietic	LED			Age, sex, smoking, alcohol consumption, education, family history of cancer, year of enrolment, state of residence (for all except melanoma)	AHS Having applied malathion was not associated with all cancers combined, and the quantitative metrics did not show associations, whichever referent group was considered. None of the individual cancer sites had statistically significant rate ratios, although for the highest category of LED (> 39) when comparing with a non-exposed referent group, an increase was observed for leukaemia and inverse associations were observed for melanoma, colorectal cancer, bladder cancer, and NHL. Lung, prostate, and kidney cancers were also assessed [Strengths: large numbers of exposed individuals. Limitations: limited numbers for some cancers]
			> 0–9	21	0.87 (0.50–1.50)		
			10–39	17	0.90 (0.50–1.61)		
			> 39	24	1.27 (0.75–2.16)		
			Trend-test *P* value: 0.23				
		Leukaemia	LED				
			> 0–9	7	0.8 (0.31–2.08)		
			10–39	5	0.74 (0.26–2.15)		
			> 39	11	1.65 (0.71–3.86)		
			Trend-test *P* value: 0.11				
		NHL	LED				
			> 0–9	7	0.62 (0.24–1.56)		
			10–39	7	0.69 (0.27–1.78)		
			> 39	9	0.81 (0.33–2.01)		
			Trend-test *P* value: 0.96				
		Colorectum	LED				
			> 0–9	29	1.06 (0.65–1.71)		
			10–39	20	0.92 (0.54–1.59)		
			> 39	18	0.84 (0.48–1.48)		
		Bladder	LED				
			> 0–9	9	0.81 (0.35–1.87)		
			10–39	10	1.14 (0.51–2.55)		
			> 39	7	0.71 (0.29–1.77)		
			Trend-test *P* value: 0.51				
		Melanoma	LED			Age, sex, smoking, alcohol consumption, education, family history of cancer, year of enrolment, state, carbaryl use, parathion use	
			> 0–9	15	1.16 (0.54–2.49)		
			10–39	9	0.79 (0.32–1.91)		
			> 39	7	0.48 (0.17–1.3)		
			Trend-test *P* value: 0.09				

Table 2.2 (continued)

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bonner et al. (2007) (cont.)		Lung	LED				
			> 0–9	16	0.75 (0.41–1.38)		
			10–39	18	1.08 (0.6–1.84)		
			> 39	22	0.94 (0.53–1.65)		
			Trend-test *P* value: 0.98				
Lee et al. (2007) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2002	56 813 licensed pesticide applicators with no prior history of colorectal cancer Exposure assessment method: questionnaire	Colorectum	Ever use	169	0.8 (0.6–1.1)	Age, smoking, state, total days of pesticide use	AHS [Strengths: large cohort. Limitations: based on self-reported exposure, limited to licensed applicators, potential exposure to multiple pesticides]
		Colon	Ever use	112	0.8 (0.5–1.1)		
		Rectum	Ever use	57	1.0 (0.6–1.7)		
Koutros et al. (2013a) Iowa and North Carolina, USA Enrolment, 1993–2007; follow-up to 31 December 2007	54 412 licensed private pesticide applicators (Iowa and North Carolina) and 4916 licensed commercial applicators (Iowa); 1962 incident cases including 919 aggressive cancers Exposure assessment method: questionnaire	Prostate, aggressive cancer	Unlagged IW-LED			Age, state, race, smoking, fruit servings, family history of prostate cancer, and physical activity	AHS Extension of the analysis by Alavanja et al. (2003) [Strengths: prospective design; large number of prostate cancers; subanalysis of aggressive tumours, defined on histological and clinical parameters; adjustments for other pesticides. Limitations: missing data on specific pesticides were imputed (validation on a subsample)]
			Quartile 1	95	1.19 (0.89–1.59)		
			Quartile 2	93	1.27 (0.97–1.67)		
			Quartile 3	93	1.28 (0.98–1.68)		
			Quartile 4	93	1.43 (1.08–1.88)		
			Trend-test *P* value: 0.04				
		Prostate, total cancers	Unlagged IW-LED				
			Quartile 1	189	1.03 (0.84–1.26)		
			Quartile 2	187	1.13 (0.94–1.36)		
			Quartile 3	184	1.11 (0.93–1.34)		
			Quartile 4	186	1.08 (0.9–1.29)		
			Trend-test *P* value: 0.62				

Table 2.2 (continued)

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Andreotti et al. (2009) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2004 Nested case–control study	Cases: 93 (response rate, NR); identified from population-based state cancer registries; incident cases diagnosed between enrolment and 31 December 2004 (> 9 yr follow-up) included in the analysis; participants with any type of prevalent cancer at enrolment were excluded; vital status was obtained from the state death registries and the National Death Index; participants who left North Carolina or Iowa were not subsequently followed for cancer occurrence Controls: 82 503 (response rate, NR); cancer-free participants enrolled in the cohort Exposure assessment method: questionnaire providing detailed pesticide use, demographic and lifestyle information. Ever-use of 24 pesticides and IW-LED [(LED) × (exposure intensity score)] of 13 pesticides was assessed	Pancreas	Ever use	15	0.4 (0.2–0.9)	Age, cigarette smoking, diabetes, applicator type	AHS [Strengths: large cohort. Limitations: based on self-reported exposure; potential exposure to multiple pesticides]

Table 2.2 (continued)

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Alavanja et al. (2014) Iowa and North Carolina, USA Enrolment and follow-up, 1993–2011	54 306 licensed pesticide applicators (523 incident cases of NHL) with no prevalent cancer at baseline, not living outside the catchment area of cancer registries in Iowa and North Carolina, and with complete data on potential confounders Exposure assessment method: questionnaire	NHL	Ever vs never	332	0.9 (0.8–1.1)	Age, state, race, AHS herbicides (tertile of total herbicide use-days)	AHS [Strengths: prospective design; adjustment for other pesticides. Limitations: missing data on specific pesticides were imputed (validation on a subsample)]
		SLL/CLL/MCL		99	1.0 (0.7–1.4)		
		DLBCL		72	0.9 (0.6–1.4)		
		FBCL		46	1.3 (0.7–2.4)		
		Other B-cell lymphoma		30	0.6 (0.3–1)		
		Multiple myeloma		61	0.9 (0.6–1.5)		
		NHL	Low (LED ≤ 8.75)	75	0.97 (0.7–1.3)		
			Medium (LED > 8.75–38.75)	47	0.7 (0.5–1.1)		
			High (LED > 38.75–737.5)	57	0.9 (0.6–1.3)		
			Trend-test *P* value: 0.63				
		FBCL	LED				
			Low	12	[1.0 (0.4–2.9)]		
			High	11	[1.6 (0.6–4.4)]		
			Trend-test *P* value: 0.25				

Table 2.2 (continued)

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mills et al. (2005) California, USA 1988–2001 Nested case–control study	Cases: 131 (response rate, NR) identified by the California cancer registry Controls: 651 (response rate, NR) from the United Farm Workers of America cohort Exposure assessment method: union records to identify farms where the worker had worked (work histories collected); link to obtain potential exposure to pesticides from the California Department of Pesticide Regulation (pesticide databank)	Total leukaemia	High	NR	1.83 (0.91–3.67)	Age, sex, duration of union affiliation, date of first union affiliation	United Farm Workers of America cohort study [Strengths: availability of a historical databank of pesticide use in the region; indirect assessment of exposures that avoid recall bias. Limitations: possible misclassification of pesticides exposures since no information on treatment tasks was collected from the participants]
		Lymphocytic leukaemia	High	NR	2.88 (0.94–8.80)		
		Granulocytic leukaemia	High	NR	1.79 (0.63–5.08)		
		Total NHL	High	NR	1.77 (0.99–3.17)		
		NHL, nodal	High	NR	1.25 (0.60–2.64)		
		NHL, extranodal	High	NR	3.52 (1.24–10)		
		Leukaemia (women)	High	NR	4.91 (1.21–19.89)		
		Leukaemia (men)	High	NR	1.19 (0.51–2.76)		
Mills & Yang (2005) California, USA 1988–2001 Nested case–control study	Cases: 128 (response rate, NR); identified by the California cancer registry Controls: 640 (response rate, NR); from the United Farm Workers of America cohort Exposure assessment method: union records to identify the farms where the worker had worked (work histories collected). Link to obtain potential exposure to pesticides from the California Department of Pesticide Regulation (pesticide databank)	Breast (diagnosed 1988–1994)	Low	14	1.89 (0.72–4.94)	Fertility, age, socioeconomic level, date and duration of first union affiliation	United Farm Workers of America cohort [Strengths: availability of a historical databank of pesticide use in the region for indirect assessment of exposures to avoid recall bias. Limitations: possible misclassification of pesticide exposures since no information on treatment tasks collected from the participants; surrogate variables for reproductive histories: county level measures of fertility and socioeconomic status]
			Medium	16	2.95 (1.07–8.11)		
			High	9	1.68 (0.50–5.62)		
		Breast (diagnosed 1995–2001)	Low	17	0.79 (0.40–1.56)		
			Medium	18	0.68 (0.33–1.43)		
			High	14	0.5 (0.21–1.23)		

Table 2.2 (continued)

Reference, study location, enrolment/ follow-up period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pesatori et al. (1994) Florida, USA Enrolment, 1965–66; follow-up until 1982 Nested case–control study	Cases: 65 (response rate, 83%); identified from the Florida pest control workers cohort Controls: 294 (122 deceased, 172 living) (response rates: deceased controls, 80%, living controls, 75%) Exposure assessment method: questionnaire	Lung	Ever vs never Deceased controls Living controls	 11 29	 1.6 (0.5–4.6) 1.0 (0.4–2.6)	Age, smoking	Florida Pest Control Worker Study [Strengths: occupational cohort with high exposure to pesticides. Two referent groups (deceased, living controls). Limitations: mortality cohort: information obtained from interview with proxies (possible information bias); small number of cases in the cohort; possible healthy worker effect]

AHS, Agricultural Health Study; CLL, chronic lymphocytic leukaemia; DLBCL, diffuse large B-cell lymphoma; FBCL, follicular B-cell lymphoma; IW-LED, intensity-weighted lifetime exposure days; JEM, job-exposure matrix; LED, lifetime exposure days; MCL; mantle cell lymphoma; NHL, non-Hodgkin lymphoma; NR, not reported; SEER, Surveillance, Epidemiology, and End Results Program; SLL, small lymphocytic lymphoma; vs, versus

2.3.1 Agricultural Health Study

(a) Nine cancer sites

Within the Agricultural Health Study (AHS; see Section 2.2 for a detailed description of this study), the association of malathion with cancer (n = 1000) at different organ sites was analysed for a subset of 19 717 applicators with complete information on the substance (collected on the take-home questionnaire), with no prevalent cancer, and with data on key potential confounders, for 1993–2002 (Bonner et al., 2007). The analysis was separately run for nine cancer sites with a sufficient number of cases (> 5 cases per category of exposure): lympho-haematopoietic cancers combined (including multiple myeloma, leukaemia, Hodgkin lymphoma, and NHL), leukaemia, NHL, lung, prostate, colon and rectum, kidney, bladder, and melanoma. Analyses were adjusted for age, sex, education, cigarette smoking, alcohol consumption, history of cancer in first-degree relatives, year of enrolment, state of residence, and use of the five pesticides most highly correlated with exposure to malathion (carbaryl, parathion, diazinon, chlordane, and lindane). Two reference groups were used in the analysis: applicators who had never used malathion; and applicators whose use of malathion was in the lowest tertile of exposure. Exposure metrics considered were lifetime exposure days (LED), intensity-weighted lifetime exposure days (IW-LED), frequency (days of use per year), and duration (years of use).

There was no association between having applied malathion and risk of all cancers combined, nor was there any association with the quantitative metrics, for any referent group considered. Rate ratios were not statistically significant for any individual cancer site; however, an inverse association was observed with the highest category of LED (> 39), when compared with a non-exposed referent group, for melanoma (relative risk, RR, 0.48; 95% CI, 0.17–1.30) and to a lesser extent for cancers of the colorectum (RR, 0.84; 95% CI, 0.48–1.48) and bladder (RR, 0.71; 95% CI, 0.29–1.77). The relative risk of NHL associated with ever use of malathion was 0.82 (95% CI, 0.43–1.55). The relative risk of leukaemia in the highest category of LED (> 39) was 1.65 (95% CI, 0.71–3.86).

(b) Cancer of the prostate

The follow-up of the cohort by Alavanja et al. (2003) was extended through 2007 and a new analysis included 1962 incident cases of cancer of the prostate among 54 412 white male pesticide applicators (Koutros et al., 2013a). Cases were characterized by stage, histological grade, and Gleason score, which were used to identify 919 aggressive cancers. Updated information on pesticide use was obtained from the phase-2 questionnaire (5 years after enrolment) and a data-driven multiple imputation procedure was used to estimate use of specific pesticides for participants who did not complete the phase-2 questionnaire. No increase in risk was observed with quartile of exposure to malathion (unlagged IW-LED) when considering all cancers of the prostate, nor was risk elevated among applicators with a family history of cancer of the prostate. However, a significant trend (P = 0.04) was observed for aggressive cancers of the prostate: the relative risk was 1.43 (95% CI, 1.08–1.88) in the highest quartile of exposure, and persisted after simultaneous adjustment for use of fonofos, terbufos, and aldrin (for all of which a positive association was also found). [The Working Group observed that this study included well-characterized exposures and outcomes, and a large sample size that enabled relative-risk estimation while controlling for multiple potential confounders and stratifying for features such as tumour traits, resulting in the detection of an association between exposure to malathion and aggressive, but not all, cancers of the prostate.]

Additional information on genetic susceptibility, pesticide exposure, and risk of cancer of the prostate was provided in a complementary

case–control study nested in the same cohort of white male pesticide applicators (841 cases; 1659 controls frequency-matched to cases by date of birth ± 1 year) (Koutros et al., 2013b). DNA was obtained from 72% of all applicators during a follow-up (1999–2003). Thirty-two single nucleotide polymorphisms (SNPs) identified by genome-wide association studies for cancer of the prostate were evaluated. Among men carrying two alleles TT at rs2710647 in EH domain binding protein 1 (EHBP1), the risk of cancer of the prostate in those with low exposure to malathion (based on LED) compared with those with no use was 2.17 (95% CI, 0.91–5.14), and in those with high exposure was 3.43 (95% CI, 1.44–8.15) (*P*-value for multiplicative interaction = 0.003). [EHBP1 encodes a protein that is involved in clathrin-mediated endocytosis; alterations (fusions, somatic mutations, over -and underexpression) of clathrin-mediated endocytosis proteins have been reported in numerous cancers, including prostate.]

(c) Non-Hodgkin lymphoma

Malathion and other insecticides were evaluated for their association with the risk of NHL among 54 306 pesticide applicators, with no prevalent cancer at baseline, living within the catchment area of the cancer registries of Iowa and North Carolina, and with complete data on potential confounders (Alavanja et al., 2014). During the follow-up period (until 2010 in North Carolina, and 2011 in Iowa), 523 incident cases of NHL were identified. The analysis was conducted for NHL and its subtypes, including chronic lymphocytic leukaemia and multiple myeloma, as classified by the Surveillance, Epidemiology, and End Results Program (SEER) coding scheme, and also for the original definition of NHL as per the International Classification of Diseases for Oncology, 3rd Edition (ICD-O-3), so that results could be compared with those of earlier reports (Percy et al., 2001; NCI, 2012). The exposure metrics used for the analysis were: (i) ever versus never use; (ii) LED; and (iii) IW-LED. The effect of lagging exposure data for 5 years was explored, but the unlagged data were generally presented. LEDs or IW-LED for malathion were not associated with risk of NHL (for ever versus never use: RR, 0.9; 95% CI, 0.8–1.1) or any of its subtypes, including follicular B-cell lymphoma (ever versus never use, RR, 1.3; 95% CI, 0.7–2.4; and high use: RR 1.6, 95% CI, 0.6–4.4), after adjustment for age, state, race, and total days of herbicide use. [The Working Group noted that the analyses accounted for total herbicide use days. Total pesticide use days was also examined, but was not included in the final model because it did not change the effect estimates by more than 10%.]

(d) Cancer of the breast

Pesticide use and cancer of the breast (excluding prevalent and in situ cancers) was investigated among 30 454 wives of farmers enrolled in the AHS (Engel et al., 2005). At enrolment, famers' wives were given a questionnaire to investigate personal ever versus never use of specific pesticides, while information on potential indirect exposure to pesticides was obtained from their husbands' responses concerning use of specific pesticides. During the follow-up period (from enrolment until 2000), 309 cases of cancer of the breast were identified. No elevation in risk was observed when considering wives' use of malathion in the entire cohort (RR, 0.9; 95% CI, 0.7–1.2), while an increase was observed when restricting the analysis to wives who had never used pesticides themselves, but whose husband had used malathion (RR, 1.4; 95% CI, 1.0–2.0), after adjusting for age, race, and state of residence. There was no apparent trend in relation to husband's use of malathion [data not shown]. [The Working Group noted inconsistency in the results in that there was no elevation in risk for personal use of malathion, but an increase was noted only for husband's use. The strengths of this study included its large sample

size, comprehensive exposure assessment, extent of potential confounder control, and exploration of potential effect modulation, such as by family history. Because of the small number of cases in North Carolina, these were excluded from the analyses.]

(e) Cancer of the colorectum

The association between cancer of the colorectum (305 incident cases that occurred between 1993–2002) and exposure to specific pesticides, including malathion, was assessed among 56 813 pesticide applicators with no prior history of cancer of the colorectum who were enrolled in the AHS (Lee et al., 2007). No association was seen between exposure to malathion and risk of all cancers of the colorectum (RR, 0.8; 95% CI, 0.6–1.1) or separately for cancer of the colon (RR, 0.8; 95% CI, 0.5–1.1) and rectum (RR, 1.0; 95% CI, 0.6–1.7), after adjusting for age, smoking, state, and total number of days of pesticide application. [The Working Group noted the large sample size, and that among the many potential confounders considered, the final models included an indicator of exposures to other pesticides.]

(f) Cancer of the pancreas

In a case–control analysis nested within the AHS of farmers and pesticide applicators and their spouses, which included 93 incident cases (applicators, 64 cases; spouses, 29 cases) of primary cancer of the pancreas, (all of which were exocrine, except for one), and 82 503 cancer-free controls, an inverse association was observed with ever use of malathion (OR, 0.4; 95% CI, 0.2–0.9) (Andreotti et al., 2009). [The Working Group noted that this analysis was based on only 15 exposed cases. Negative associations were found for several pesticides, which was statistically significant in the case of DDT.]

(g) Childhood cancer

The AHS also provided the opportunity to examine risk of cancer among children of farmers and pesticide applicators whose exposure to pesticides had been characterized. The study did not detect an association between risk of cancer and either paternal (prenatal) or maternal (ever) exposure to malathion. Among 17 280 children of participants in Iowa, the odds ratio for cancer in children related to paternal prenatal use of malathion was 0.78 (95% CI, 0.34–1.79; 8 exposed cases) and 1.12 (95% CI, 0.57–2.20; 11 exposed cases) for maternal exposure to malathion (Flower et al., 2004).

2.3.2 United Farm Workers of America

In a case–control study nested within a cohort of members of the United Farm Workers of America union (Mills et al., 2005; see Section 2.2 for a detailed description of this study), an increased risk was associated with high (compared with low) exposure to malathion for all types of lympho-haematopoietic cancers (131 cases), including all types of leukaemia (OR, 1.83; 95% CI, 0.91–3.67), lymphocytic leukaemia (OR, 2.88; 95% CI, 0.94–8.80), granulocytic leukaemia (OR, 1.79; 95% CI, 0.63–5.08), total NHL (OR, 1.77; 95% CI, 0.99–3.17), NHL nodal (OR, 1.25; 95% CI, 0.60–2.64), and extranodal NHL (OR, 3.52; 95% CI, 1.24–10.0). For leukaemia, odds ratios were higher in women (OR, 4.91; 95% CI, 1.21–19.89) than in men (OR, 1.19; 95% CI, 0.51–2.76). No elevated risk was observed for multiple myeloma, but only 20 cases were analysed. Associations did not change after simultaneous adjustment was made for exposure to all 15 chemicals. [The Working Group noted that this was an ecological exposure assessment method, not an individual exposure assessment method.]

Cancer of the breast was also analysed within this cohort (Mills & Yang, 2005). An increase in risk of cancer of the breast was observed in

malathion users versus non-users, only among those diagnosed in 1988–1994, after adjustment for age, date of first union affiliation, duration of union affiliation, fertility, and socioeconomic level (low use: OR, 1.89; 95% CI, 0.72–4.94; medium use: OR, 2.95, 95% CI, 1.07–8.11; high use: OR, 1.68, 95% CI, 0.50–5.62). [The Working Group noted that the exposure assessment was obtained through record linkage; this method of indirect assessment of exposure avoids recall bias. Since this method of assessment is independent of disease status, there is no differential exposure misclassification. Level of exposure was based on the county, crop, and period when the person worked, and there was no information on job tasks collected from the participants, resulting in possible exposure misclassification.]

2.3.3 Florida Pest Control Worker Study

Pesatori et al. (1994) conducted a case–control study of cancer of the lung nested within the cohort of the Florida Pest Control Worker Study and included 65 deceased cases and 194 controls (deceased, 122; living, 172) (see Section 2.2 for a detailed description of this study). Ever versus never use of malathion was associated with an elevated odds ratio of cancer of the lung after adjusting for age and smoking, when comparing cases with deceased controls (OR, 1.6; 95% CI, 0.5–4.6), but not with living controls (OR, 1.0; 95% CI, 0.4–2.6). [The Working Group noted that there were substantial limitations to exposure assessment based on proxy interviews, the degree of exposure misclassification may vary considerably given the wide range of dates of the follow-up (1965–1982), and that there was probably differential exposure misclassification because of the use of next-of-kin interviews for living and deceased study subjects.]

2.4 Case–control studies on lympho-haematopoietic cancers and malathion

See Table 2.3

2.4.1 Studies in the midwest USA

Three population-based case–control studies conducted in the 1980s by the National Cancer Institute in the USA in Nebraska (Hoar Zahm et al., 1990), Iowa and Minnesota (Brown et al., 1990; Cantor et al., 1992), and Kansas (Hoar et al., 1986) provided information on the risk of haematopoietic cancer associated with exposure to several pesticides, including malathion (see Section 2.2 for a detailed description of these studies).

A case–control study in Iowa and Minnesota found modest increases in the risk of NHL in white men who had handled malathion for treatment of animals (ever use: OR, 1.3, 95% CI, 0.9–2.1; before 1965: OR, 1.8; 95% CI, 1.0–3.3; without protective equipment: OR, 1.4; 95% CI, 0.8–2.2), or for treatment of crops (ever use: OR, 1.5; 95% CI, 0.8–2.7; before 1965: OR, 2.9; 95% CI, 1.1–7.4; without protective equipment: OR, 1.9; 95% CI, 0.9–4.1) (Cantor et al., 1992). Risks appeared to be higher in Minnesota than in Iowa. [These data were included in the study by Waddell et al. (2001) and are therefore not presented in Table 2.3.]

The risk of leukaemia was 0.9 (95% CI, 0.4–1.9) for ever use of malathion for treatment of crops, 1.2 (95% CI, 0.8–2.2) for ever use of malathion for treatment of animals, and 3.2 (95% CI, 1.0–10.0) for treatment of animals with malathion on ≥ 10 days per year [the *P*-value for trend was not presented] (Brown et al. 1990).

The association between exposure to malathion and multiple myeloma was also assessed in Iowa and Minnesota; no increase in risk was seen for use of malathion as an insecticide on animals (OR, 0.8; 95% CI, 0.3–1.9; 6 exposed cases),

Table 2.3 Case–control studies on lympho-haematopoietic cancers and exposure to malathion

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown et al. (1990) Iowa and Minnesota, USA 1981–1984	Cases: 578 (340 living, 238 deceased) (response rate, 86%); cancer registry or hospital records Controls: 1245 (820 living, 425 deceased) (response rate, 77–79%); random-digit dialling for those aged < 65 yr and Medicare for those aged ≥ 65 yr Exposure assessment method: questionnaire	Leukaemia (including myelodysplasias)	Use on animals			Vital status, age, state, tobacco use, family history of lympho-haematopoietic cancer, high-risk occupations, high-risk exposures	Studies in midwest USA [Strengths: large population based study in a farming area; in-person interviews; detailed questionnaires including quantification of pesticide exposure; collection of other potential risk factors; reviewed diagnosis. Limitations: multiple comparisons; self-report of pesticide use; not controlled for exposure to other pesticides]
			Ever	30	1.2 (0.8–2)		
			Handled > 20 yr ago	15	1.5 (0.8–2.9)		
			1–4 days/yr	5	0.5 (0.1–1.3)		
			5–9 days/yr	0	0		
			≥ 10 days/yr	7	3.2 (1–10)		
			Use on crops				
			Ever use	10	0.9 (0.4–1.9)		
			1–4 days/yr	4	1.2 (0.3–3.9)		
			5–9 days/yr	2	0.8 (0.2–4.4)		
			≥ 10 days/yr	0	–		
Waddell et al. (2001) Nebraska, Iowa & Minnesota, Kansas, USA 1979–1986	Cases: 748 (response rate, NR); three previous studies Controls: 2236 (response rate, NR); three previous studies Exposure assessment method: detailed questionnaire on agricultural practices (Nebraska and Kansas): years of use, days per year of use, protective practices, livestock reared and crops grown	NHL	Ever use	91	1.6 (1.2–2.2)	Age, state, respondent type (proxy/direct)	Studies in midwest USA (pooled) Reference group: non-farmers [Strengths: large numbers; possibility to analyse subtypes; detailed information on pesticide use; cases reviewed by pathologists. Limitations: differences in the collection of pesticide information within the three studies (days per year for each active ingredient only available in Iowa & Minnesota and Kansas); no list of pesticides in Kansas]
			< 20 yr ago	22	0.9 (0.5–1.6)		
			> 20 yr ago	35	1.7 (1.1–2.9)		
			< 10 yr	22	1.1 (0.6–1.9)		
			10–19 yr	23	1.9 (1.0–3.5)		
			> 20 yr	10	1.1 (0.5–2.4)		
			< 5 days/yr (Nebraska only)	7	2.1 (0.7–6.1)		
			≥ 5 days/yr (Nebraska only)	5	1.5 (0.5–5.2)		
		Follicular NHL	Ever	29	1.3 (0.8–2.2)	Age, state	
		Diffuse NHL	Ever	19	1.1 (0.6–1.9)	Age, state	
		Small lymphocytic NHL	Ever	10	1.9 (0.8–4.7)	Age, state	
		Other type NHL	Ever	10	0.9 (0.4–2.0)	Age, state	

Table 2.3 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos et al. (2003) Nebraska, Iowa, Minnesota, Kansas, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files Exposure assessment method: questionnaire; interview (direct or next-of-kin)	NHL	Ever use Hierarchical regression Logistic regression	 53 53	 1.1 (0.7–1.7) 1.1 (0.6–1.8)	Other pesticides	Studies in midwest USA (pooled) [Strengths: large number of exposed subjects; analysis of combined pesticide exposure (same person but not necessarily at the same time; analysis with the number of pesticides used; use of hierarchical models; adjustments for other pesticides; evaluation of potential more than additive effects of pesticides. Limitations: no quantification of exposure; no information on the timing of pesticide use; exclusions due to missing data] Both logistic and hierarchical regression analyses were used, the latter providing more conservative estimates Included participants from Cantor et al. (1992), Zahm et al. (1990), Hoar et al. (1986), and Brown et al. (1990)

Table 2.3 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McDuffie et al. (2001) Six provinces in Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia) 1991–1994	Cases: 517 (response rate, 67.1%), from cancer registries and hospitals Controls: 1506 (response rate, 48%); random sample from health insurance and voting records Exposure assessment method: questionnaire by post and telephone	NHL (ICD-9 200 and 202)	Ever use Indoors as a fumigant > 0 and ≤ 2 days/yr ≥ 2 days/yr	72 12 50 22	1.83 (1.31–2.55) 1.54 (0.74–3.22) 1.82 (1.25–2.68) 1.75 (1.02–3.03)	Age, province of residence	Cross-Canada Case–control Study Risk increased with the total number of pesticides used [Strengths: large number of cases; population-based; diversity in the occupational exposures; pathological material reviewed; collected information on the number of pesticides used; analysis of use of multiple pesticides; non-occupational use of pesticides considered. Limitations: potential recall bias; low response rate; multiple comparisons; no quantitative exposure data]
Hohenadel et al. (2011) Six provinces in Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, Saskatchewan) 1991–1994	Cases: 513 (response rate, 66.6%); from registries in five provinces and hospital records in Quebec Controls: 1506 (response rate, 48.0%); population-based study; health insurance records, computerized telephone listing or voters' lists. Frequency matched on age ± 2 yr and province of residence Exposure assessment method: questionnaire by post and telephone	NHL (ICD-9 200 and 202)	Malathion use with the following pesticides: + 2,4-D + Carbaryl + DDT + Glyphosate + Mecoprop	 61 20 20 31 28	 2.06 (1.45–2.93) 3.34 (1.77–6.31) 2.11 (1.17–3.80) 2.10 (1.31–3.37) 3.04 (1.80–5.15)	Age, province, respondent (direct/proxy)	Cross-Canada Case–control Study [Strengths: pathology review; large numbers; information on potential confounders. Limitations: self-reported lifetime use of pesticides; no time scale concerning combinations of pesticides]

Table 2.3 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pahwa et al. (2012a) Six provinces in Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, Saskatchewan) 1991–1994	Cases: 513 (response rate, NR); from cancer registries in five provinces, hospital records in Quebec Controls: 1506 (response rate, NR); randomly selected from provincial health insurance, telephone listings and voters' lists. frequency matched by age (± 2 yr) and province of residence Exposure assessment method: questionnaire by post and telephone	NHL (ICD-9 200 and 202)	Ever vs never Ever + No asthma/ allergy + Asthma/ allergy	 72 55 17	 1.96 (1.42–2.70) 2.42 (1.65–3.56) 1.22 (0.65–2.29)	Age, province of residence, respondent type (proxy/ direct), diesel-oil exposure	Cross-Canada Case–control Study Analysis of the role of asthma or allergy [Strengths: large numbers; consideration of effect modification of asthma/ allergy in the association between malathion and NHL; control for risk factors of NHL. Limitations: self-reported use of pesticides; self-reported data for immunological condition; no information on duration, intensity or frequency of use]
Eriksson et al. (2008) Four health service areas in Sweden (Lund, Linkoping, Orebro and Umea) 1999–2002	Cases: 910 (response rate, 91%); incident NHL cases were enrolled from university hospitals Controls: 1016 (response rate, 92%); national population registry Exposure assessment method: questionnaire	NHL	Ever vs never	5	2.81 (0.54–14.7)	Age, sex, year of diagnosis or enrolment	[Strengths: population-based; males and females included; controls general population; no next-of-kin interviewed; subtypes of NHL were considered; exposure to other pesticides (e.g. MPCA) controlled for in the analysis. Limitations: self-reported information on pesticides; few individuals exposed to malathion]

Table 2.3 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Monge et al. (2007) Costa Rica 1995–2000	Cases: 300 (response rate, 90%) from cancer registry and National Children's Hospital Controls: 579 (response rate, 90.5%) from national birth registry, matched by birth year Exposure assessment method: questionnaire; face-to-face interviews with parents, more detailed for parents involved in agriculture (16.9% in cases, 15.6% in controls)	Childhood leukaemia	Father's exposure in the year before conception			Residence (urban/rural)	[Strengths: population-based study; detailed information on tasks, and ranking of their potential exposures; consideration of five periods: year before conception, first trimester, second trimester, third trimester, first year of life. Limitations: possible recall bias; correlations of exposure between time-periods]
			Boys	5	8.5 (1.1–74.1)		
			Girls	2	0.9 (0.2–4.9)		
		Childhood ALL	Father's exposure in the year before conception			Residence (urban/rural)	
			Boys	5	10.4 (1.2–91.1)		
			Girls	1	0.5 (0.1–4.8)		
Brown et al. (1993b) Iowa, USA 1981–1984	Cases: 173 (response rate, 84%); Iowa health registry Controls: 650 (response rate, 78%); random-digit dialling (aged < 65 yr) and Medicare (age > 65 yr) Exposure assessment method: questionnaire	Multiple myeloma	Ever vs never			Vital status, age	[Strengths: population-based; conducted in areas with high prevalence of farming; distinction of malathion use on animals or crops. Limitations: self-reported exposure; deceased cases and controls (interviews with proxies)]
			On animals	6	0.8 (0.3–1.9)		
			On crops	8	1.9 (0.8–4.6)		

Table 2.3 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pahwa et al. (2012b) Six provinces of Canada (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, British Columbia) 1991–1994	Cases: 342 (response rate, 58%); men newly diagnosed (age ≥ 19 yr) Controls: 1506 (response rate, 48%); men (age ≥ 19 yr), frequency-matched to province and ± 2 yr to the age distribution of entire case group (which also included soft tissue sarcoma, Hodgkin lymphoma, NHL) Exposure assessment method: questionnaire	Multiple myeloma (ICD-O M 9732/3)	Ever use Use as a fumigant	32 6	0.97 (0.62–1.53) 1.16 (0.44–3.11)	Medical history, age, province of residence	Cross-Canada Case–control Study [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders. Limitations: men only; most men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides]
Kachuri et al. (2013) Six provinces in Canada (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and Quebec) 1991–1994	Cases: 342 (response rate, 58%); men aged ≥ 19 yr diagnosed between 1991 and 1994 were ascertained from provincial cancer registries except in Quebec, where ascertained from hospitals Controls: 1357 (response rate, 48%); men aged ≥ 19 yr selected randomly using provincial health insurance records, random-digit dialling, or voters' lists, frequency-matched to cases by age (± 2 yr) and province of residence Exposure assessment method: questionnaire	Multiple myeloma	Ever use Ever use (proxy respondents excluded) > 0 and ≤ 2 days/yr > 2 days/yr	32 29 17 12	1.12 (0.71–1.74) 1.28 (0.79–2.07) 1.04 (0.58–1.88) 1.37 (0.68–2.77)	Age, province of residence, smoking, family history of cancer, select medical conditions, respondent (direct/proxy)	Cross-Canada Case–control Study [Strengths: large population-based case–control study; detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders. Limitations: relatively low response rates; men only; most men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides]

ALL, acute lymphocytic leukaemia; NHL, non-Hodgkin lymphoma; yr, year; NR, not reported

but an increase in risk was observed for use of malathion on crops (OR, 1.9; 95% CI, 0.8–4.6; 8 exposed cases), after adjusting for vital status and age (Brown et al., 1993b).

Together with two other case–control studies of NHL – one in Nebraska (Hoar Zahm et al., 1990) and the other in Kansas (Hoar et al., 1986) – the study in Iowa and Minnesota provided information on malathion from a pooled analysis that included 748 cases and 2236 controls (Waddell et al., 2001). The analysis included white men only, since there were too few women for analysis. The risk of NHL associated with exposure to malathion (ever versus never) was 1.6 (95% CI, 1.2–2.2; 91 exposed cases), with variations according to histological type: the relative risk observed for small lymphocytic NHL was 1.9 (95% CI, 0.8–4.7). When proxies were excluded, the relative risks were attenuated (RR, 1.2; 95% CI, 0.9–1.8) and proxies were excluded from subsequent analyses. [Because information obtained from proxies may not be as accurate as direct interviews, the possibility of misclassification of exposure may be greater.] When the first use of malathion was 20 years ago or more, the risk was 1.7 (95% CI, 1.1–2.9), but no clear trend was observed concerning the number of days of use per year.

On the same pooled data from the three studies, an analysis was conducted to adjust for use of multiple pesticides, to take into account the frequent combinations of active ingredients, and to test for potential more-than-additive effects of the active ingredients (De Roos et al., 2003). [The Working Group noted that the difference between the two pooled analyses were that De Roos et al. (2003) adjusted for use of many other pesticides and used fewer subjects for the analysis than Waddell et al., 2001. This pooled analysis included subjects with data on each pesticide from all three studies and to which at least 20 people were exposed.] For malathion, combinations with DDT, aldrin, lindane, alachlor, atrazine, and 2,4-D were analysed. While fully adjusted for other pesticides, the results from the hierarchical regression did not indicate that malathion used in combination increased the risk of NHL (OR, 1.1; 95% CI, 0.7–1.7; 53 exposed cases). [The Working Group noted that the strengths of this study were the large number of subjects, that it was population-based and conducted in farming areas with high exposure, there was detailed exposure information, and adjustment for multiple exposures that accounted for potential confounding from use of multiple pesticides. A limitation was that the fully adjusted risk estimates were based on fewer numbers of cases than in the study by Waddell et al. (2001) because only subjects with no missing data on pesticide use were included.]

2.4.2 The Cross-Canada Case–control Study of Pestides and Health

In an analysis of 517 cases of NHL and 1506 controls from the Cross-Canada Case–control Study (see Section 2.2 for a detailed description of this study), the odds ratios associated with use of malathion were 1.83 (95% CI, 1.31–2.55) for ever versus never use and 1.54 (95% CI, 0.74–3.22) for use of malathion as a fumigant indoors, adjusted for statistically significant medical variables, age, and province (McDuffie et al., 2001). No clear trend was observed with the number of days of use per year (for < 2 days of use per year: OR, 1.82; 95% CI, 1.25–2.68; and for > 2 days of use per year: OR, 1.75, 95% CI, 1.02–3.03; adjusted for age and province).

In further analysis of 513 cases and 1506 controls, Hohenadel et al. (2011) evaluated exposure to malathion in combination with several insecticides (carbaryl, DDT, dimethoate) and herbicides (2,4-D, glyphosate, mecoprop, methyoxychlor) [an odds ratio for ever versus never malathion use was not reported]. Statistically significant increased risks of NHL were observed for use of malathion in combination with 2,4-D, mecoprop, carbaryl, glyphosate, or DDT

(adjusted for age, province, and use of a proxy respondent), with odds ratios that were much higher than those for the use of any pesticide alone.

Data from the same study were used to explore whether the effect of pesticide exposure was modified by asthma and/or allergies (Pahwa et al., 2012a). Use of malathion was associated with an increased risk of NHL (OR, 1.96; 95% CI, 1.42–2.70; 72 exposed cases; adjusted for age, province, respondent type, diesel-oil exposure). For use of malathion, results indicated that the risk of NHL was higher for people without asthma, allergies, or hay fever, than for people with any of these conditions of the immune system. The *P* value for interaction of malathion with asthma, allergies, and hay fever was 0.07. [The Working Group noted that there was some evidence of effect modification among people with asthma and allergies, which was contrary to reports from earlier studies.]

No significant increase in risk associated with use of malathion was observed in an analysis of 342 cases of multiple myeloma (32 exposed) and 1506 controls (matched by age and province) from the Cross-Canada study for ever use (OR, 0.97; 95% CI, 0.62–1.53) (Pahwa et al., 2012b), and when excluding proxy respondents (OR, 1.28; 95% CI, 0.79–2.07), or when considering the number of days of use per year (OR, 1.37; 95% CI, 0.68–2.77; for > 2 days per year) (Kachuri et al., 2013).

No increase in risk of Hodgkin lymphoma was observed in an analysis of the 316 cases and 1506 controls (OR, 0.97; 95% CI, 0.58–1.63; 27 exposed cases; adjusted for medical history variables, age, and province) (Karunanayake et al., 2012). [Response rates in this study were relatively low.]

2.4.3 NHL in Sweden

Eriksson et al. (2008) reported the results of a population-based case–control study of exposure to pesticides as a risk factor for NHL. The study included men and women aged 18–74 years living in Sweden and enrolled between 1 December 1999 and 30 April 2002. Incident cases of NHL were enrolled from university hospitals in Lund, Linköping, Örebro, and Umeå. Controls (matched by age and sex) were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total, 910 cases (response rate, 91%) of NHL (819 cases of B-cell lymphoma, 53 cases of T-cell lymphoma, and 38 cases of unspecified lymphoma) and 1016 controls (response rate, 92%) participated. Multivariable models included agents associated with a statistically significant increased odds ratios (MCPA, 2-methyl-4-chlorophenoxyacetic acid), or with an odds ratio of > 1.50 and at least 10 exposed subjects (2,4,5-T and/or 2,4-D; mercurial seed dressing, arsenic, creosote, tar), age, sex, and year of diagnosis or enrolment. There was an increase in risk in individuals ever exposed to malathion (OR, 2.81; 95% CI, 0.54–14.7; 5 exposed cases), after adjustment for age, sex, and year of diagnosis or enrolment. [This was a large study; there was possible confounding from use of other pesticides including MCPA, but this was considered in the analysis.]

2.4.4 Childhood leukaemia in Costa Rica

In Costa Rica, the risk of childhood leukaemia in relation to parental occupational exposure to pesticides was investigated in a population-based case–control study covering 1995–2000 (Monge et al., 2007). Cases of childhood leukaemia (n = 300) were identified at the cancer registry and the National Children's Hospital. Population controls (n = 579) were drawn from the national birth registry. Detailed information on environmental and occupational exposure to pesticides

was collected during a face-to-face interview using both conventional and icon-based calendar forms. Exposure was assessed for 25 pesticides in five time periods in relation to pregnancy. Father's exposure to malathion in the year before conception was associated with an elevated risk of childhood leukaemia in boys (OR, 8.5; 95% CI, 1.1–74.1; 5 exposed cases), but not in girls (OR, 0.9; 95% CI, 0.2–4.9; 2 exposed cases), after adjustment for place of residence (urban or rural).

2.5 Case–control studies on other cancers

See Table 2.4

2.5.1 Cross Canada Case–control Study of Pesticides and Health

In an analysis of 357 men with soft tissue sarcoma and 1506 controls within the Cross Canada Case–control Study of Pesticides and Health, the odds ratio for risk of soft tissue sarcoma associated with exposure to malathion was 1.23 (95% CI, 0.81–1.85), after adjusting for medical history, age, and province (Pahwa et al., 2011; see Section 2.2 for a detailed description of this study).

2.5.2 Cancer of the prostate

A case–control study among patients from the cancer registry of British Columbia assessed the risk of cancer of the prostate in relation to exposure to several specific pesticides, including malathion. Exposure was assessed through a JEM that covered 1950–1998 (45 animal and crop commodities), and provided quantitative information for specific active ingredients regarding combinations of region, crop, task (re-entry, application), and job title (Band et al., 2011; see Section 2.2 for a detailed description of this study). A significant excess risk was shown for ever use of malathion (OR, 1.34; 95% CI, 1.01–1.78), with a dose–response relationship: in men with low exposure, the risk was 1.18 (95% CI, 0.78–1.78), while in men with high exposure the risk was 1.49 (95% CI, 1.02–2.18; *P* for trend, 0.03), after adjusting for alcohol and tobacco use, education level, and respondent type (self-reported versus proxy). An association was observed for several other pesticides. [The Working Group noted that there was no adjustment for exposure to other pesticides, despite a large number of other pesticides showing associations with cancer of the prostate.]

2.5.3 Cancer of the brain

Several publications from the Upper Midwest Health Study in the USA reported on the association between exposure to pesticides, including malathion, and the risk of glioma (see Section 2.2 for a detailed description of this study).

Carreón et al. (2005) evaluated the effects of exposure to pesticides on the risk of intracranial glioma among women aged 18–80 years who were rural residents of Iowa, Michigan, Minnesota, or Wisconsin, in the Upper Midwest Health Study. A total of 341 cases of glioma and 527 controls were enrolled. Exposure assessment was carried out via an in-person interview. The response rates were 90% and 72%, respectively. After adjusting for age, age group, and education, generally no association with glioma was observed for exposure to several pesticide classes or individual pesticides. There was a non-significant increase in risk for malathion when considering direct interviews (excluding proxy respondents) (OR, 1.5; 95% CI, 0.7–3.0) (Carreón et al., 2005).

Yiin et al. (2012) reported on the Upper Midwest Health Study, including men and women (798 cases and 1175 controls), with the aim of investigating quantitative estimated lifetime cumulative exposure (gram-years) in farmers, and also investigating non-farm use of pesticides. In non-farming jobs, the risk of glioma associated with use of malathion was not

Table 2.4 Case–control studies of other cancers and exposure to malathion

Reference, location, enrolment period/follow-up, study design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Carreón et al. (2005) Iowa, Michigan, Minnesota, Wisconsin, USA 1995–1997	Cases: 341 (response rate, 90%); female patients with a histologically confirmed primary intracranial glioma Controls: 527 (response rate, 72%); women with no diagnosis of glioma randomly selected within 10-yr age group strata frequency matching within the state; selection from the state driver's licence/non-drivers identification records (for those aged 18–64 yr) and from Medicare (aged 65–80 yr) Exposure assessment method: questionnaire; postal questionnaire with a list of pesticides – including malathion – and collecting lifetime pesticide use in farming and not-farming jobs, in the house and the garden. Followed by an interview collecting additional information (first year of use, number of years of use, days per year of use, use on animals and crops, use on buildings or lots)	Brain, intracranial glioma (ICD-O 938–948)	Ever use Including proxy respondents Excluding proxy respondents	 18 13	 1.0 (0.5–1.8) 1.5 (0.7–3.0)	Age, 10-yr age group, education, other pesticides	Upper Midwest Health Study [Strengths: large number of cases; extensive questionnaire on farm and rural risk factors and pesticide use; cases histologically confirmed and limited to glioma. Limitations: controls older than cases; large proportion of proxy respondents (43% of cases, 2% of controls)]

Table 2.4 (continued)

Reference, location, enrolment period/follow-up, study design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Band et al. (2011) British Columbia, Canada 1983–1990 Nested case–control study	Cases: 1153 (response rate, NR); British Columbia cancer registry; with histological confirmation Controls: 3999 (response rate, NR); cancer patients from the same registry: other sites, excluding lung cancer and cancer of unknown primary site Exposure assessment method: JEM for 1950–1998, including 45 animal and crop commodities; information on exposure (quantitative or never vs ever) to 180 pesticide active ingredients were determined for "type-of-work" (combination of region, crop, task – application or re-entry, and job-title) and time; quantification was derived from models used for pesticide registration in the USA	Prostate	Ever Low High Trend-test *P* value: 0.03	210 105 105	1.34 (1.01–1.78) 1.18 (0.78–1.78) 1.49 (1.02–2.18)	Alcohol consumption, pipe years, cigarette years, education level, respondent type (proxy/ direct)	Men only [Strengths: incident cancers, histologically confirmed; before the period of early detection of prostate cancer; large number of cases and controls; lifetime cumulative exposure; no recall bias on pesticide exposure assessment. Limitations: lack of information on family history; quantification from models; potential exposure misclassification; multiple comparisons; high intercorrelations between active ingredients]
Yiin et al. (2012) Iowa, Michigan, Minnesota, Wisconsin, USA 1995–1997	Cases: 798 (response rate, 93%); patients with a histologically confirmed primary intracranial glioma identified through participating medical facilities and offices of neurosurgeon Controls: 1175 (response rate, 70%); selected from the state driver's license/nondriver identification records and Centers for Medicare & Medicaid Services Exposure assessment method: questionnaire; based on self-report	Brain, intracranial glioma (ICD-O 938–948)	Ever use Non-farm job Non-farm job (excluding proxies) In house/ garden In house/ garden (excluding proxies)	 9 9 45 24	 0.69 (0.30–1.56) 1.04 (0.45–2.40) 0.82 (0.56–1.20) 0.72 (0.44–1.18)	Age, 10-yr age group, education, sex, farm pesticide exposure (yes/ no)	Upper Midwest Health Study [Strengths: large number of cases; extensive questionnaire on farm and rural risk factors and pesticide use; population-based design; cases histologically confirmed and limited to glioma. Limitations: controls older than cases; large proportion of proxy respondents (45% of cases)]

Table 2.4 (continued)

Reference, location, enrolment period/follow-up, study design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pahwa et al. (2011) Six provinces in Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia) 1991–1994	Cases: 357 (response rate, 60.8%); men newly diagnosed, age ≥ 19 yr Controls: 1506 (response rate, 48.0%); men aged ≥ 19 yr, frequency matched to province and ± 2 years to the age distribution of entire case group (which also included NHL, Hodgkin lymphoma, multiple myeloma) Exposure assessment method: self-administered postal questionnaire and telephone interview for subjects with ≥ 10 hours/yr of pesticide exposure and 15% random sample of the remainder; a list of chemical and brand names was mailed to these participants before the telephone interview; exposure defined as use at work, in home garden, or as hobby	Soft tissue sarcoma	Ever vs never	38	1.23 (0.81–1.85)	Age, province, medical history	Cross-Canada Case–control Study [Strengths: population based study; large number of cases; tumour slides reviewed by pathologists; detailed questionnaires on pesticides. Limitations: diversity in exposure situations (crops and animals) but no distinction in analysis; self-reported information]

Table 2.4 (continued)

Reference, location, enrolment period/follow-up, study design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lee et al. (2004) Nebraska 1988–1993	Cases: 170 (stomach) + 137 (oesophageal) incident cases of adenocarcinoma identified from the Nebraska cancer registry or discharge diagnosis and pathology records at 14 hospitals; white men and women (aged ≥ 21 yr) Controls: 502 (response rate, 83%); controls from a previous case–control study in Nebraska (see Hoar Zahm et al., 1990), from random-digit dialling, and from Medicare files Exposure assessment method: detailed questions on pesticide use and agricultural activities; separate analysis for nitrosatable pesticides (4 insecticides, 10 herbicides)	Stomach (adeno-carcinoma) Oesophagus (adeno-carcinoma)	Ever use Ever use	14 12	0.8 (0.4–1.6) 0.7 (0.4–1.5)	Age, sex Age, sex	[Limitations: many proxy-questionnaires; self-report of pesticide use; possible misclassification of exposures]

NHL, non-Hodgkin lymphoma; NR, not reported; yr, year

elevated (OR, 0.69; 95% CI, 0.30–1.56; including proxy respondents; OR, 1.04; 95% CI, 0.45–2.40; excluding proxy respondents). Similar results were observed in subjects reporting house and garden use of malathion (OR, 0.82; 95% CI, 0.56–1.20; including proxy respondents; and OR, 0.72, 95% CI, 0.44–1.18; excluding proxy respondents). [The Working Group noted the large proportion of proxy respondents in this study, approximately 45% of cases, and the potential for differential exposure misclassification; however the results, with and without proxy respondents, were reported for many chemicals, and did not differ significantly.]

2.5.4. Cancer of the stomach and oesophagus

Lee et al. (2004) evaluated the risk of adenocarcinomas of the oesophagus or stomach associated with farming and agricultural use of pesticides (including malathion) in a population-based case–control study in eastern Nebraska, USA. Men and women diagnosed with adenocarcinoma of the stomach (n = 170) or oesophagus (n = 137) between 1988 and 1993 were enrolled. Controls (n = 502) were randomly selected from the population registry of the same geographical area (Hoar Zahm et al., 1990). The response rates were 79% for cancer of the stomach, 88% for cancer of the oesophagus, and 83% for controls. Adjusted odds ratios were estimated for use of individual and chemical classes of insecticides and herbicides, with non-farmers as the reference category. No association was found with farming or ever-use of insecticides or herbicides, or with individual pesticides. No increase in risk associated with use of malathion was observed. [The study was conducted in a farming area, but the power to detect an effect of glyphosate use was limited.]

2.6 Meta-analysis

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including malathion. The meta-analysis for malathion included three studies (Waddell et al., 2001; Mills et al., 2005; Pahwa et al., 2012a), and yielded a meta-risk ratio of 1.8 (95% CI, 1.4–2.2) (see Section 2.2 for a detailed description of this study). [The Working Group noted that the relative risk estimate from Bonner et al. (2007), which was 0.82 (95% CI, 0.43–1.54), was not included in this analysis.]

3. Cancer in Experimental Animals

Studies of carcinogenicity with malathion and malaoxon (a metabolite of malathion) in experimental animals were available to the Working Group. In all except one study, tumour incidences were determined in rats or mice given diets containing either malathion or malaoxon for 18–26 months. A single study in rats examined the effect of subcutaneous injection of malathion for 5 days on the development and incidence of cancer of the mammary gland for up to 28 months. The results of these studies are summarized in Table 3.1, Table 3.2, Table 3.3 and Table 3.4. The present monograph also includes studies of carcinogenicity by the National Toxicology Program (NTP, 1978, 1979a, b) that were reviewed at a previous meeting of the Working Group (IARC, 1983) and lead to the previous evaluation of *inadequate evidence* for the carcinogenicity of malathion in experimental animals (IARC, 1987).

3.1 Mouse

3.1.1 Oral administration

See Table 3.1

Table 3.1 Studies of carcinogenicity with malathion in mice

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, $B6C3F_1$ (M, F) 94–95 wk NTP (1978)	Diet containing malathion (technical grade; purity, ≥ 95%; dissolved in acetone) given at concentrations of 0 (matched control), 0 (pooled control), 8000, or 16 000 ppm, ad libitum, 7 days/wk, for 80 wk, then held untreated for an additional 14–15 wk 50 M and 50 F/treated group; 10 M and 10 F/matched-control group (age, 35 days) Since the numbers of mice in the matched-control groups were small, statistical comparisons also made use of pooled-control groups, which consisted of matched controls from the malathion bioassay combined with matched controls from contemporary bioassays of tetrachlorvinphos, toxaphene, endrin, and lindane, resulting in groups of 50 M and 50 F	*Males* Hepatocellular neoplastic nodule [adenoma]: 0/10, 3/49 (6%), 0/48, 6/49 (12%) Hepatocellular carcinoma: 2/10 (20%), 5/49 (10%), 7/48 (15%), 11/49 (22%) Hepatocellular adenoma or carcinoma (combined): 2/10 (20%), 8/49 (16%), 7/48 (15%), 17/49 (35%)* Hepatocellular adenoma or carcinoma (combined) (time-adjusted): 2/9 (22%), 8/48 (17%), 7/47 (15%), 17/49 (35%) *Females* No exposure-related tumours	*Males* Neoplastic nodule [adenoma]: $P = 0.016$ (trend) (vs matched) Adenoma or carcinoma (combined): $P = 0.041$ (trend) (vs matched); time-adjusted analysis eliminating mice not at risk, NS for trend and pairwise comparison to matched control group *$P = 0.031$ (vs pooled) *Females* NS	There was a dose-related decrease in mean body weights compared with controls. Low number of matched controls. Mice fed malathion were housed in the same room as mice fed dieldrin or tetrachlovinphos *Males* No significant dose-related trend in mortality: survival was 94% at higher dose, 80% in matched-control group. Historical control rate for hepatocellular carcinoma in males in the laboratory was 35–40% (incidence, NR) *Females* No significant dose-related trend in mortality: survival was 88% at higher dose; 80% in matched-control group Cystic endometrial hyperplasia: 1/9 (11%), 12/47 (25%), 10/42 (24%) Cystic endometrial hyperplasia is a potential estrogenic effect of the exposure; values for pooled controls, NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, B6C3F$_1$ BR (M, F) 18 mo EPA (1994, 2000b)	Diets containing malathion (purity, 96.4%) given at concentrations of 0 (control), 100, 800, 8000, or 16 000 ppm, ad libitum, 7 days/wk, for 18 mo 55 M and 55 F/group [age NR]	*Males* Hepatocellular adenoma: 1/54 (2%), 6/54 (11%), 2/55 (4%), 13/55 (24%)*, 49/51 (96%)* Hepatocellular carcinoma: 0/54, 6/54 (11%)*, 3/55 (5%), 6/55 (11%)*, 1/51 (2%) Hepatocellular adenoma or carcinoma (combined): 1/54 (2%), 10/54 (19%)*, 5/55 (9%), 18/55 (33%)**, 49/51 (96%)** Pathology Working Group re-read (EPA, 1998, 2000b): Hepatocellular adenoma: 4/54 (7%)*, 8/54 (15%), 7/55 (13%), 14/55 (25%)**, 49/51 (96%)** Hepatocellular carcinoma: 0/54, 4/54 (7%), 2/55 (5%), 2/55 (4%), 0/51 Hepatocellular adenoma or carcinoma (combined): 4/54 (7%)*, 10/54 (19%), 9/55 (16%), 15/55 (27%)**, 49/51 (96%)**	Adenoma: *$P \leq 0.001$, Fisher exact test; $P < 0.001$, trend test Carcinoma: *$P \leq 0.014$, Fisher exact test Adenoma or carcinoma (combined): *$P = 0.004$, Fisher exact test; **$P < 0.001$, Fisher exact test; $P < 0.001$, trend test *$P < 0.001$, trend test **$P \leq 0.01$, Fisher exact test	Significant reduction in body weight at 78 wk; 48–54 mice per group at terminal kill; two higher doses chosen to duplicate NTP (1978) study; liver hypertrophy at 12 mo in two highest-dose groups Hepatocellular hypertrophy: 0/54, 0/55, 0/55, 55/55*, 51/51*; [hepatocellular hypertrophy, *$P < 0.001$, $P < 0.001$ (trend)] Historical controls: hepatocellular adenoma, 14.3–21.7%; hepatocellular carcinoma, 0.0–6.4%

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Mouse, B6C3F$_1$ BR (M, F) 18 mo EPA (1994, 2000b) (cont.)		*Females* Hepatocellular adenoma: 0/55, 1/53 (2%), 0/53, 9/52 (17%)*, 42/51 (82%)* Hepatocellular carcinoma: 1/55 (2%), 0/53, 2/53 (4%), 1/52 (2%), 2/51 (4%) Hepatocellular adenoma or carcinoma (combined): 1/55 (2%), 1/53 (2%), 2/53 (4%), 10/52 (19%)*, 43/51 (84%)**	Adenoma: *$P \leq 0.001$, Fisher exact test; $P < 0.001$, trend test Adenoma or carcinoma (combined): *$P = 0.003$, **$P < 0.001$, Fisher exact test; $P < 0.001$, trend test	Significant reduction in body weight at 78 wk 51–55 mice/group at terminal kill Two highest doses chosen to duplicate NTP (1978) study; liver hypertrophy at 12 mo in two highest dose groups Hepatocellular hypertrophy: 0/55, 0/55, 0/54, 53/53*, 52/52*; [hepatocellular hypertrophy, *$P < 0.001$, $P < 0.001$ (trend)] Historical controls: hepatocellular adenoma, 0.0–10.6%; hepatocellular carcinoma, 0.0–2.3%

F, female; M, male; mo, month; NR, not reported; NS, not significant; vs, versus; wk, week

In a study by the National Cancer Institute (NCI), groups of 50 male and 50 female B6C3F$_1$ mice were given diets containing malathion (purity, ≥ 95%) at a concentration of 8000 or 16 000 ppm, respectively, for 80 weeks, and then held untreated for an additional 14–15 weeks (NTP, 1978). The matched-control group consisted of 10 male and 10 female mice. Because the number of matched-control mice was small, pooled controls were also used for statistical comparisons. The pooled-control groups consisted of the matched controls from the bioassay of malathion combined with matched controls from the contemporary bioassays of tetrachlorvinphos, toxaphene, endrin, and lindane, giving groups of 50 male and 50 female mice. There was a high percentage survival at the highest dose (males, 94%; females, 88%) compared with the matched-control groups (males, 80%; and females, 80%). Throughout the study, there was a dose-related decrease in mean body weights of males and females compared with controls.

In males, significant positive trends were noted in the incidence of hepatocellular neoplastic nodules [adenoma] (matched controls, 0/10; pooled controls, 3/49; 8000 ppm, 0/48; 16 000 ppm, 6/49; P = 0.016, versus matched controls) and of hepatocellular adenoma or carcinoma (combined) (matched controls, 2/10; pooled controls, 8/49; 8000 ppm, 7/48; 16 000 ppm, 17/49; P = 0.041, versus matched controls). At the highest dose in male mice, there was a non-significant increase in incidence (pooled controls, 8/49 (16%); 16 000 ppm, 17/49 (35%); P = 0.031, which is above P = 0.025 level required to meet Bonferroni criterion) of these hepatocellular tumours (combined). The incidence of hepatocellular tumours (combined) was within the range for historical controls (35–40% [incidence not reported]) for that laboratory. When a time-adjusted analysis eliminated those male mice not at risk, trend values, and tumour incidence for hepatocellular tumours (combined) were non-significant when matched controls were used.

There was no significant increase in the reported incidence of tumours in female mice, but an increase in the incidence of cystic endometrial hyperplasia was reported in the females in the groups receiving malathion at either dose [no statistics reported]. [The Working Group noted the low number of matched controls, that survival in the group of matched controls was lower than in the treated groups, and that the mice in this experiment were housed in the same room concurrently with mice exposed to dieldrin or tetrachlorvinphos. There were no available data on uterine weights, but the increased incidence of cystic endometrial hyperplasia pointed to a possible estrogen-like effect.]

In a second study, groups of 55 male and 55 female B6C3F$_1$ mice were given diets containing technical-grade malathion (purity, 96.4%) at a concentration of 0, 100, 800, 8000, or 16 000 ppm for 18 months (EPA, 1994, 2000b). The incidence of hepatocellular hypertrophy was significantly increased in males and females at 8000 and 16 000 ppm. The incidence of hepatocellular adenoma was significantly increased in males and females at 8000 and 16 000 ppm; statistical analysis showed a significant positive trend (P < 0.001) and pairwise significance (P ≤ 0.001). In males, the incidence of hepatocellular adenoma or carcinoma (combined) had a significant positive trend (P < 0.001) with pairwise significance at 100 ppm (P = 0.004), 8000 ppm (P < 0.001), and 16 000 ppm (P < 0.001); significant increases in the incidence of hepatocellular carcinoma (P ≤ 0.014) were reported at 100 and 8000 ppm. [The Working Group estimated that the significant increases in the incidence of hepatocellular adenoma or carcinoma (combined) reported at 8000 and 16 000 ppm in females were driven only by the incidences of hepatocellular adenoma.]

Subsequent to this study, the United States Environmental Protection Agency (EPA) requested a re-read of the liver pathology slides

for males by a pathology working group (PWG) due to the increase in the incidence of hepatocellular tumours at the lowest (100 ppm), and two higher doses (8000 ppm and 16 000 ppm), but not at the lower intermediate dose (800 ppm). Additionally, there was an apparently low incidence of tumours in the concurrent controls in this strain of mice (EPA, 1998, 2000b). Re-evaluation of the hepatocellular tumours by the PWG suggested that there was no increase in the incidence of hepatocellular tumours at 100 ppm, and no increase in the incidence of hepatocellular carcinoma in any group. In the group at 100 ppm, the PWG considered that two of the six carcinomas were in fact adenomas. In the group at 800 ppm, the study pathologist had identified two adenomas and three carcinomas, while the consensus opinion of the PWG was to upgrade all observed basophilic foci to adenomas, and to downgrade one carcinoma to adenoma, yielding seven adenomas and two carcinomas. In the group at 8000 ppm, the PWG downgraded some adenomas to eosinophilic foci, and some carcinomas to adenomas. In the group at 16 000 ppm, there was little difference between the study pathologist's interpretation and that of the PWG; adenomas (often multiple) were found in most of the animals; the study pathologist had identified one carcinoma that the PWG called adenoma (EPA, 1998). The PWG carried out a blind review of the slides (without knowledge of the treatment received). The review resulted in a shift in the identification of adenomas versus carcinomas in favour of adenomas. [The Working Group noted that the morphological appearance of most of the adenomas in animals at 16 000 ppm and the majority of those observed at 8000 ppm was quite different from that of the adenomas in the control group and in groups receiving the lower doses (100 or 800 ppm). The biological significance of this finding was not investigated in further detail. In addition, most of the hepatocellular carcinomas had been considered as single solitary masses at gross necropsy, and were diagnosed by light microscopy by the study pathologist, and multiple carcinomas were diagnosed in two mice at 100 ppm by the PWG. The Working Group highlighted the finding of hepatocellular hypertrophy and the different histological patterns identified in the groups at 8000 ppm and 16 000 ppm, the occurrence of intra-hepatic metastasizing hepatocellular carcinomas, and the polyphenotypical presentation of the histology of the hepatocellular carcinomas.]

3.1.2 Carcinogenicity of metabolites

See Table 3.2

In a 2-year study of carcinogenicity, groups of 50 male and 50 female B6C3F_1 mice were given diets containing malaoxon (purity > 95%), a metabolite of malathion, at a concentration of 0 (control), 500, or 1000 ppm for 103 weeks (NTP, 1979a). The mice were held untreated for up to 2 additional weeks. Mean body weight of females at the highest dose was lower than that of controls. There were no significant treatment-related changes in body weight in males. Survival at 103 weeks was 90%, 84%, and 74%, respectively, for male mice, and 78%, 76%, and 90%, respectively, for female mice. There was no significant increase in tumour incidence in groups of treated males or females. [The Working Group had minimal concerns regarding the quality of this study.]

3.2 Rat

3.2.1 Oral administration

See Table 3.3

In a first study by the NCI, groups of 50 male and 50 female Osborne-Mendel rats (age, 35 days) were given diets containing malathion (purity, 95%) at a concentration of 4700 or 8150 ppm for 80 weeks (time-weighted exposure). For matched controls (15 males and 15 females per group), the study duration was 108–113 weeks, and the study duration was 113 and 109 weeks for the

Table 3.2 Studies of carcinogenicity with malaoxon in mice

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start (control)	Incidence of tumour	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103–105 wk NTP (1979b)	Diets containing malaoxon (purity, > 95%; dissolved in acetone) at 0 (control), 500, or 1000 ppm, ad libitum, 7 days/wk, for 103 wk, then mice held untreated for up to 2 additional weeks 50 M and 50 F/group (age, 7 wk)	*Males* Hepatocellular adenoma: 0/50, 3/50 (6%), 4/50 (8%) Hepatocellular carcinoma: 12/50 (24%), 2/49 (4%), 13/50 (26%) Hepatocellular adenoma or carcinoma (combined): 12/50 (24%), 5/49 (10%), 17/50 (34%) *Females* No exposure-related tumours reported	*Males* NS NS (for increase) NS (for increase) *Females* NS	*Males* No significant changes in body weight. Survival: 90%, 84%, and 74% at 103 wk *Females* Mean body weight of mice at higher dose was lower than that of controls. Survival: 78%, 76%, and 90% at 103 wk

F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week

lower-dose and higher-dose groups, respectively (NTP, 1978). Time-weighted doses were used to assess the results as the concentration of malathion was reduced after study start due to toxicity with initial exposures. Since the numbers of rats in the matched-control groups were small, pooled controls were also used for statistical comparisons. The pooled-control groups consisted of the matched controls from the bioassay of malathion combined with matched controls from the contemporary bioassays of tetrachlorvinphos, toxaphene, endrin, and lindane to give groups of 55 male and 55 female rats. Body weight and survival were not significantly affected by treatment. A significant positive trend in tumour incidence was noted for follicular cell adenoma or carcinoma (combined) of the thyroid gland in females compared with pooled controls. The National Toxicology Program (NTP) in consultation with NCI re-evaluated the histopathology of the NTP (1978) study by convening a PWG, and the revised data on tumour incidence were reported by Huff et al. (1985). The positive trend in incidence of follicular cell adenoma or carcinoma (combined) was no longer significant in treated females after the PWG review. There were no other substantive changes in interpretation of the original data on tumour incidence. [The Working Group noted the low number of matched controls. The Working Group also noted that the highest dose was reduced from 12 000 ppm to 8000 ppm at 14 weeks due to excessive toxicity.]

In a second NCI study, groups of 50 male and 49–50 female Fischer 344 rats were fed diets containing malathion (purity, 95%) at a concentration of 0 (control), 2000, or 4000 ppm for 103 weeks, and killed at 105–106 weeks (NTP, 1979a). Males, but not females, showed a dose-related decrease in body weight and survival. In males, there was a significant positive trend ($P = 0.013$) and a significant increase in the incidence of pheochromocytoma at the lower dose (controls, 2/49 (4%); lower dose, 11/48 (23%)*; higher dose, 6/49 (12%); *$P = 0.006$), and also evidence for a dose-related increase in the incidence of gastric inflammation and gastric ulcers. There was no significant treatment-related increase in the

Table 3.3 Studies of carcinogenicity with malathion in rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Osborne Mendel (M, F) 108–113 wk NTP (1978); Huff et al. (1985)	Diets containing malathion (purity, ≥ 95%; dissolved in acetone) at concentrations of 0 (matched control), 0 (pooled control), 4700 ppm (time-weighted average: 14 wk at 8000 ppm then 66 wk at 4000 ppm), and 8150 ppm (time weighted average: 3 wk at 12 000 ppm and then 77 wk at 8000 ppm) Fed ad libitum, 7 days/wk for 80 wk, and rats then held untreated for an additional 28–33 wk 50 M and 50 F/treated group (age, 35 days); 15 M and 15 F/matched-control group Since the numbers of rats in the matched-control groups were small, statistical comparisons also made use of pooled-control groups, which consisted of matched controls from the malathion bioassay combined with matched controls from contemporary bioassays of tetrachlorvinphos, toxaphene, endrin, and lindane, resulting in 55 M and 55 F/group	*Males:* Thyroid gland: C-cell adenoma: 1/14 (7%), 3/41 (7%), 1/35 (3%), 7/40 (18%) Follicular cell adenoma: 1/14 (7%), 6/41 (15%), 7/35 (20%), 8/40 (20%) Follicular cell carcinoma: 1/14 (7%), 2/41 (5%), 2/35 (6%), 4/40 (10%) *Females* Thyroid gland: C-cell adenoma: 2/14 (14%), 9/41 (22%), 2/44 (5%), 4/42 (10%) Follicular cell adenoma: 0/14, 1/41 (2%), 1/44 (2%), 1/42(2%) Follicular cell carcinoma: 0/14, 0/41, 0/44, 3/42 (7%)	*Males* NS *Females* NS	*Males* Body weight and survival not significantly affected; survival at highest dose, 58% Low number of matched controls housed together with dosed rats; pooled controls included rats on test as controls for four other chemicals *Females* Body weight and survival not significantly affected; survival at highest dose, 67% Low number of matched controls housed together with dosed rats; pooled controls included rats on test as controls for four other chemicals NTP in consultation with NCI re-evaluated the histopathology of the study by convening a PWG and the tumour incidence data were reported by Huff et al. (1985).

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 105–106 wk NTP (1979a)	Diets containing malathion (purity, 95%; dissolved in acetone) at 0 (control), 2000, or 4000 ppm, fed ad libitum, 7 days/wk for 103 wk and rats then held untreated for an additional 2–3 wk 49–50 M and 50 F/group (age, 35 days)	*Males* Pheochromocytoma of the adrenal gland: 2/49 (4%), 11/48 (23%)*, 6/49 (12%)	*Males* *P = 0.006, Fisher exact test (see Comments) P = 0.013 (trend), Cochran-Armitage test (see Comments)	*Males* Dose-related decrease in body weight Survival at 78 wk: controls, 88%; lower dose, 86%; higher dose, 80% Stomach: chronic inflammation: 2/49, 6/46, 11/47; gastric ulcers: 1/49, 9/46, 15/47
		Females No exposure-related tumours	*Females* NS	*Females* Body weight not significantly affected. Survival at 78 wk: controls, 94%; lower dose, 98%; higher dose, 90%. Individual clinical signs of toxicity were not reported, but it is unlikely that the MTD was achieved Stomach: chronic inflammation: 0/50, 2/44, 4/47; gastric ulcers: 1/50, 2/44, 2/47 NTP in consultation with NCI re-evaluated the histopathology of the study by convening a PWG and the tumour incidence data were reported by Huff et al. (1985). The positive trend and the increase in the incidence of pheochromocytoma of the adrenal gland (5/49, 10/48, 6/46) were no longer significant for males. There were no other substantive changes in the original data on tumour incidence

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) Up to 24 mo EPA (1997, 2000b)	Diets containing malathion (purity, 97.1%) at 0 ppm for 24 mo (control), 100 ppm for 3 mo then 50 ppm for 21 mo, 500 ppm for 24 mo, 6000 ppm for 24 mo, or 12 000 ppm for 24 mo Fed ad libitum, 7 days/wk for up to 24 mo 55 M and 55 F/group [age NR]	*Males* Nasal pharyngeal cavity: one very rare adenoma (acanthoma) at 6000 ppm, and one very rare carcinoma (malignant acanthoma) at 12 000 ppm in nasoturbinate tissues, olfactory region. These tumours originated in the stratum spinosum layer of the epithelium	*Males* (see Comments)	*Males* Survival at 24 mo: 67%, 75%, 53%, 26%, 0%; most deaths due to nephrotoxicity and leukaemia Nasal tumours are exceedingly rare, with a historical control rate (NTP) of 6/4000 (0.15%) Group at highest dose was terminated at 94 wk because of excessive mortality PWG re-read (EPA, 2000b): Males: one nasal olfactory epithelium adenoma at 6000 ppm, one nasal respiratory epithelium adenoma at 12 000 ppm, and one squamous cell papilloma of the palate at 100/50 ppm
		Females Oral cavity: rare squamous cell carcinoma of the squamous epithelium lining of the alveolus of a tooth was identified in two females; one at 100/50 ppm and one at 12 000 ppm Liver: Hepatocellular adenoma: 0/40, 1/48 (2%), 1/43 (2%), 3/39 (8%)*, 3/29 (10%)** Hepatocellular carcinoma: 0/41, 1/50 (2%), 1/44 (2%), 0/41, 3/38 (8%) Hepatocellular adenoma or carcinoma (combined): 0/41, 2/50 (4%), 2/44 (5%), 3/41 (7%)*, 6/38 (16%)**	*Females* Peto's prevalence test Hepatocellular adenoma: $P = 0.007$ (trend), $^{*}P = 0.032$ (6000 ppm), $^{**}P = 0.008$ (12 000 ppm) Hepatocellular adenoma or carcinoma (combined): $P = 0.002$ (trend), $^{*}P = 0.032$ (6000 ppm), $^{**}P = 0.003$ (12 000 ppm)	*Females* Survival at 24 mo: 69%, 74%, 75%, 62%, 36% Historical controls (NTP, 1999): squamous-cell carcinoma of the oral cavity, 5/1001 (0.5%) Historical controls (NTP): hepatocellular adenoma, 8/1351 (0.59%); hepatocellular carcinoma, 1/1351 (0.07%) PWG re-read (EPA, 2000b): Females: one nasal respiratory epithelium adenoma at 6000 and one at 12 000 ppm, one squamous cell papilloma of the palate at 6000 ppm, one squamous cell carcinoma of the palate at 12 000 ppm, and one squamous cell carcinoma of the alveolus of the tooth at 100/50 ppm

Table 3.3 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 24 mo EPA (1984)	Diets containing malathion (purity, 92.1%) at 0 (control), 100, 1000, or 5000 ppm, given ad libitum, 7 days/wk for 24 mo 50 M and 50 F/group [age NR]	*Males* Mammary gland: Fibroadenomas: 0/49, 0/48, 1/49 (2%), 3/47 (6%) Adenocarcinoma: 0/49, 0/48, 0/49, 1/47 (2%) *Females* Mammary gland: Fibroadenomas: 9/50 (18%), 13/50 (26%), 15/50 (30%)*, 6/50 (12%) Adenocarcinoma: 1/50 (2%), 0/50, 3/50 (6%), 1/50 (2%) Uterus: Polyps: 3/50 (6%), 10/50 (20%)*, 9/50 (18%), 10/50 (20%)*	*Males* NS (see comments) *Females* *$P < 0.05$, Fisher exact test	*Males* Body weights at highest dose were 6–11% lower than controls Survival: 58%, 58%, 50%, 48% Prejean et al. (1973) reported historical-control incidence of fibroadenoma of 2/60 (3.3%) in male Sprague-Dawley rats *Females* Body weights at the highest dose were 4–9% lower than those of controls Survival: 74%, 59%, 62%, 88% Fibroadenomas in males and females included combined adenomas, fibromas, fibroadenomas, and papillary cystadenomas
Rat, Sprague-Dawley (F) 28 mo Cabello et al. (2001)	Subcutaneous injections of saline (control), malathion (17 mg/100 g bw), or malathion (17 mg/100 g bw) plus intraperitoneal injection of atropine (250 µg/100 g bw) Injected 2 × per day for 5 days and held for 28 mo 70 F/group (age, 39 days)	Mammary gland adenocarcinoma: 0/70, 17/70*, (24%) 0/70	*[$P < 0.0001$, Fisher exact test]	Body weights and survival, NR Tumour latency, 54–653 days Rats killed 1 mo after first mammary tumour detected by palpation. No tumours observed in heart, lungs, intestines, ovaries, and uterus [the authors did not report how these tissues were examined] In a separate experiment, of the same design, density of terminal end buds increased at age 45 days, 16 h after injections

bw, body weight; F, female; M, male; mo, month; MTD, maximum tolerated dose; NCI, National Cancer Institute; NR, not reported; NS, not significant; NTP, National Toxicology Program; PWG, pathology working group; wk, week

incidence of tumours in females. NTP in consultation with NCI re-evaluated the histopathology of the study by convening a PWG, and the revised data on tumour incidence were reported by Huff et al. (1985). The positive trend and the increase in the incidence of pheochromocytoma of the adrenal gland were no longer significant in treated males after the PWG review (revised incidences: controls, 5/49; lower dose, 10/48; higher dose, 6/46). There were no other substantive changes in the original data on tumour incidence. [The Working Group noted that body weights and survival of females were not significantly affected by malathion at the doses tested, and it was unlikely that the maximum tolerated dose was achieved. The Working Group had minimal other concerns with regard to the quality of this study.]

In addition to the two studies described above and previously reviewed by IARC (1983), two additional studies were identified in which male and female rats were given diets containing malathion for 24 months.

Groups of 55 male and 55 female Fischer 344 rats were fed diets containing malathion (purity, 97.1%) at a concentration of 0 ppm for 24 months (control), 100 ppm for 3 months and then 50 ppm for 21 months, 500 ppm for 24 months, 6000 ppm for 24 months, or 12 000 ppm for 24 months. Survival of male rats at 24 months was 67%, 75%, 53%, 26%, and 0%, respectively, with the majority of deaths attributed to nephrotoxicity and leukaemia. Because of excessive mortality, male rats in the group at the highest dose were killed after 94 weeks. A rare nasoturbinate adenoma (acanthoma) in a male at 6000 ppm, and another rare nasoturbinate carcinoma (malignant acanthoma) in a male at 12 000 ppm were reported (EPA, 1997, 2000b). [These nasal tumours are exceedingly rare, with a historical control rate reported by the NTP of 0.15% (6/4000) in males, and this outcome was considered to be treatment-related by the Working Group.] No other exposure-related tumours were reported in males. In the same study, survival of female rats was 69%, 74%, 75%, 62%, and 36%. Rare squamous cell carcinomas of the squamous epithelium lining the alveolus of a tooth [historical control rate: 5/1001 (0.5%), as reported by NTP (1999)] were identified in two female rats; one each was identified in the groups at 100/50 ppm and at 12 000 ppm. There were significant positive trends in the incidence of hepatocellular adenoma ($P = 0.007$), and of hepatocellular adenoma or carcinoma (combined) ($P = 0.002$), and pair-wise statistical significance at 6000 ppm ($P = 0.032$) and 12 000 ppm ($P = 0.008$) for hepatocellular adenoma, and 12 000 ppm ($P = 0.003$) for hepatocellular adenoma or carcinoma (combined). A subsequent PWG convened by the EPA (2000b) confirmed the observation of one nasal olfactory epithelium adenoma in each of the groups at 6000 ppm and 12 000 ppm, and identified one squamous cell papilloma of the palate at 100/50 ppm in males. In females, one squamous cell carcinoma of the alveolus of the tooth at 100/50 ppm was confirmed, and one nasal respiratory epithelium adenoma at 6000 ppm and one at 12 000 ppm, one squamous cell papilloma of the palate at 6000 ppm, and one squamous cell carcinoma of the palate at 12 000 ppm were identified (EPA, 1997, 2000b). [The Working Group considered that the increase in the incidence of hepatocellular tumours and the observation of squamous cell carcinomas of the oral cavity in females were treatment-related.]

In another study (EPA, 1984), groups of 50 male and 50 female Sprague-Dawley rats were given diets containing malathion (purity, 92.1%) at a concentration of 0 (control), 100, 1000, or 5000 ppm for 24 months. There was no significant effect on survival, but there was a slight decrease in body weight in treated males and females. A significant increase ($P < 0.05$) in the incidence of fibroadenoma (combined adenomas, fibromas, fibroadenomas, and papillary cystadenomas) of the mammary gland [the Working Group noted that the listed tumours are histogenetically

and morphologically different] was reported in females at 1000 ppm, but not at the higher dose of 5000 ppm. [It was uncertain whether this outcome was treatment-related since there was no positive trend in tumour incidence, and the range of historical controls for this tumour was not reported for males or females.] There was no significant positive trend or increase in tumour incidence in males. [The Working Group noted that the reported incidence of fibroadenoma of the mammary gland in males at 5000 ppm – 3/47 (6.4%) – was greater than that for historical controls for Sprague-Dawley rats – 2/60 [3.3%] – as reported by Prejean et al. (1973).] An apparent dose-related increase in the incidence of uterine polyps was also reported in female rats [there were no available data on uterine weights, but this result suggested that malathion may have an estrogen-like effect.] [The Working Group had moderate concerns with respect to the quality of this study, including interpretation of histopathological findings.]

3.2.2 Subcutaneous administration

See Table 3.3

Cabello et al. (2001) examined the effect of injection into the inguinal region of saline (control), or malathion, or malathion plus atropine, on development of the mammary gland (ductal morphogenesis) and formation of tumours of the mammary gland in groups of 70 female Sprague-Dawley rats (age, 39 days). Rats were injected with saline (subcutaneous), malathion (subcutaneous; 17 mg per 100 g body weight, bw), or malathion (subcutaneous; 17 mg per 100 g bw) plus atropine (intraperitoneal; 250 µg per 100 g bw) twice per day for 5 days and held for 28 months. Changes in body weight and survival were not reported. Rats with mammary tumours were killed 1 month after detection of the tumour by palpation. Tumours were examined by light microscopy. Tumour latency was 54–653 days. [No further information was provided on the protocol for tumour assessment, nor were data provided for individual animals.] A significant increase in the incidence of adenocarcinoma of the mammary gland (17/70, 24% [$P < 0.0001$]) was reported in the group receiving malathion only; no tumours of the mammary gland were reported in the groups receiving saline only, or malathion plus atropine. In another experiment with a similar protocol, 16 hours after the malathion injections (i.e. at age 45 days) there was an increase in terminal end bud (TEB) density and a decrease in branching to alveolar buds (ABs) compared with control animals. [TEBs and ABs represent two of the most important histogenetic milestones during the development of the normal mammary gland in rats. TEBs are club-shaped endings of secondary ducts and composed of 3–6 layers of medium-sized epithelial cells, while ABs represent further sprouting of lateral buds and cleaving of numerous TEBs. Mammary-gland differentiation is characterized by a progressive decrease in the number of TEBs and a concomitant increase in the number of ABs. The results suggested that subcutaneous injection of malathion affects ductal morphogenesis of the mammary gland in rats.]

3.2.3 Carcinogenicity of metabolites

See Table 3.4

In a 2-year study, groups of 50 male and 50 female Fischer 344 rats were given diets containing malaoxon (purity, > 95%) at a concentration of 0 (control), 500, or 1000 ppm for 103 weeks, and then held untreated for up to 2 weeks (NTP, 1979b). At 78 weeks, the rats were placed on fresh control diet for 4 days due to food rejection, before resuming the original diets. Mean body weights of males or females were not significantly affected by treatment with malaoxon. Survival at 90 weeks for male rats was 80%, 82%, and 64%, respectively. Survival at 90 weeks for female rats was 82%, 90%, and 80%, respectively. In males, there was a significant increase in the incidence

Table 3.4 Studies of carcinogenicity with malaoxon in rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 103–105 wk NTP (1979b)	Diets containing malaoxon (purity, > 95%; dissolved in acetone) at 0 (control), 500, or 1000 ppm, fed ad libitum, 7 days/wk for 103 wk and rats then held untreated for an additional up to 2 wk 50 M and 50 F/group (age, 6 wk)	*Males* Thyroid gland: C-cell adenoma or carcinoma (combined): 2/49, 0/45, 4/49	*Males* NS (see Comments)	*Males* Mean body weights were not significantly affected by malaoxon. At 78 wk, rats were given fresh control diet for 4 days due to food rejection before resuming test diet Survival at wk 90: 80%, 82%, 64% Gastric ulcers: 2/48, 6/50, 7/48 Thyroid gland C-cell hyperplasia: 0/49, 6/45 (13%)*, 10/49 (20%)** [*P = 0.010, **P < 0.001; P < 0.001 (trend)] NTP in consultation with NCI re-evaluated the histopathology of the study by convening a PWG and the tumour incidence data were reported by Huff et al. (1985). The increase in the incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland reached significance in males at 1000 ppm (3/49, 3/45, 10/49*; *P < 0.05) with a significant positive trend (P < 0.05)
		Females Thyroid gland: C-cell adenoma or carcinoma (combined): 0/50, 1/49 (2%), 5/47* (11%)	*Females* *P = 0.024 (Fisher exact test), P = 0.009 (trend, Cochran-Armitage test)	*Females* Mean body weights were not significantly affected by test chemical. Some hyperexcitability at 72 and 73 wk. At 78 wk, on control diet for 4 days due to food rejection and then test resumed. Survival at wk 90: 82, 90, 80% Historical control incidence for thyroid gland C-cell adenoma or carcinoma (combined) for the laboratory: 16/223 (7.2%) Gastric ulcers: 0/49, 1/49, 3/49
		C-cell carcinoma: 0/50, 0/49, 1/47 (2%)	NS	
Rat, F344 (M, F) 24 mo EPA (2000b)	Diets containing malaoxon (purity, 96.4%) at 0 (control), 20, 1000, or 2000 ppm, fed ad libitum, 7 days/wk for 24 mo 55 M and 55 F/group (age NR)	*Males* Mononuclear cell leukaemia: 13/55 (24%), 12/55 (22%), 19/55 (34%)*, 16/55 (29%)	Peto's test *Males* *P < 0.05 P = 0.03 (trend),	*Males* Body weight not reported Mortality: 29% (controls) and 53% (2000 ppm). Severe inhibition of cholinesterase at high dose. Historical control incidence for the laboratory, mononuclear cell leukaemia, 15–36%
		Females No exposure-related tumours	*Females* NS	*Females* Body weight not reported Mortality: 13% (controls) and 49% (2000 ppm). Severe inhibition of cholinesterase activity at highest dose

bw, body weight; F, female; M, male; mo, month; NS, not significant; PWG, pathology working group; wk, week

of C-cell hyperplasia of the thyroid gland – 0/49, 6/45 (13%)*, 10/49 (20%)**; *[P = 0.010], **[P < 0.001] – with a significant positive trend [P < 0.001], but no treatment-related tumours were reported. In females, there was a significant pairwise increase in the incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland at the higher dose – 0/50, 1/49 (2%), 5/47* (11%); *P = 0.024 – with a significant positive dose-related trend (P = 0.009). NTP in consultation with NCI re-evaluated the histopathology of the study by convening a PWG and the revised data on tumour incidence were reported by Huff et al. (1985). There was an increase in the incidence of C-cell adenoma or carcinoma (combined) of the thyroid gland (3/49, 3/45, 10/49*; *P < 0.05) in males, with a significant positive trend (P < 0.05). There were no other substantive changes in the original data on tumour incidence.

In a 2-year study, groups of 55 male and 55 female Fischer 344 rats were given diets containing malaoxon (purity, 96.4%) at a concentration of 0 (control), 20, 1000, or 2000 ppm for 24 months (EPA, 2000b). There was a dose-related decrease in survival in males and females. In males, the increase in the incidence of mononuclear cell leukaemia was significant for the group at 1000 ppm – 13/55 (24%), 12/55 (22%), 19/55 (34%)*, 16/55 (29%); *P < 0.05 – with a significant positive trend (P = 0.03). [The Working Group noted that this type of leukaemia, commonly found in male Fischer 344 rats, may not be a suitable model for development of certain human haematopoietic neoplasms, and also that the incidences were within the range (15–36%) for historical controls for that laboratory.] There was no significant increase in tumour incidence in females.

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

An extensive literature is available on the toxicokinetics of malathion in humans and in experimental animals.

4.1.1 Absorption

(a) Humans

Malathion is rapidly absorbed by mammals, including humans. It is likely that absorption of malathion, a lipophilic, non-ionized molecule, occurs via passive diffusion. On the basis of studies in humans, dermal exposure occurring occupationally and oral exposure via the diet are important routes of exposure to malathion. Although malathion has a low vapour pressure (Knaak et al., 2004), it can be detected in house dust and is applied in aerosol sprays (Lioy et al., 2000). However, data on the extent of inhalation and absorption in humans are few. Several case reports of accidental or intentional poisoning through oral ingestion of malathion indicate ready absorption from the gastrointestinal tract; malathion is found in the bloodstream post mortem, although it is difficult to obtain quantitative data on the absorption fraction because the actual doses ingested are often unknown (Zivot et al., 1993).

Absorption of malathion after oral exposure was evident from the urinary output of malathion metabolites in male volunteers who received a single oral dose of between 0.5 and 15 mg/kg bw (Bouchard et al., 2003). In another study in male volunteers who ingested malathion at 8, 16, or 24 mg per day for up to 56 days, malathion was efficiently absorbed, based on significant decrements in plasma and erythrocyte cholinesterase activities compared with baseline levels (Moeller & Rider, 1962).

Malathion was less efficiently absorbed after dermal exposure than after oral exposure in controlled experimental settings in vivo (Maibach et al., 1971). Two studies in volunteers showed that when [^{14}C]-malathion was applied in an aqueous ethanol solution to naked skin beneath the forearm, the absorption was ~7% of the applied dose (Maibach et al., 1971; Wester et al., 1996). This in-vivo absorption was assessed by measuring the levels of [^{14}C]-malathion-derived residues in the urine, and comparing with the amount of [^{14}C]-malathion applied to the skin. The dermal absorption rate decreased to 4% of the applied dose if malathion was added to cotton sheets that were placed immediately on the skin. If the cotton sheets treated with malathion solution were dried for 1 or 2 days before being applied to the skin, the rate of absorption was reduced to 0.6% of the applied dose (Wester et al., 1996). This suggested that a fraction of the malathion found in fabric (e.g. clothing, rug, upholstery, etc.) is transferred from the fabric into and through human skin. [The Working Group noted that on the basis of the studies reviewed above, it is expected that only a small fraction of the malathion applied would be internalized after dermal exposure.]

The extent of dermal absorption in greenhouse workers applying malathion with hand-held lance sprayers was monitored by measuring urinary biomarkers of malathion exposure (malathion metabolites). The applicators' lower limbs accounted for 48% of the dermal exposure, while hand and upper limb exposures accounted for 30% and 19%, respectively (Tuomainen et al., 2002b).

Using an in-vitro static diffusion cell, the maximal flux of malathion through human skin was measured directly (0.89 ± 0.11 μg/cm^2·h) (Guy et al., 1985). Approximately 20% of the applied dermal dose was recovered in the receptor cell beneath the skin flap after 48 hours, while 9% of the dose remained in the skin (Guy et al., 1985). In another in-vitro skin-flap study on human skin and malathion, the percentage of the applied dose that was directly absorbed and retained within the stratum corneum and underlying skin was evaluated after 24 hours (Capt et al., 2007). Of the applied dose, 7% directly penetrated the skin flap (when using an aqueous solution of bovine serum albumin to mimic plasma in the receptor cell), while 2% and 32% of the dose remained in the skin and stratum corneum, respectively (Capt et al., 2007). [The Working Group noted that on the basis of this study in vitro, ~40% of the applied dose would potentially be absorbed via the dermal route; this value is significantly higher than that found in studies in volunteers in vivo.]

(b) Non-human mammalian experimental systems

In fasted female ICR mice, a single dose of [^{14}C]-labelled malathion (1 mg/kg bw) administered by injection into the stomach was rapidly absorbed, with ~90% of the administered dose being absorbed, mostly in the intestine, within 60 minutes (Ahdaya et al., 1981).

Several studies of dermal exposure to malathion in rodents and pigs in vivo, and in rat and porcine skin-flap models in vitro have been reported. A study in female Duplin ICR mice in vivo showed that dermal application of [^{14}C]-labelled malathion (1 mg/kg bw; in acetone vehicle) to the shaved upper shoulder resulted in rapid and extensive penetration through the skin; 25% of the applied dose was absorbed within 1 hour, and 98% was absorbed within 48 hours (Shah et al., 1981). The extent of absorption of [^{14}C]-labelled malathion was determined by radiocarbon assay of blood, major tissues, collected urine, and the remaining carcass at each time-point. In contrast, instant electronic autoradiography in a study of dermal exposure in shaved male Sprague-Dawley rats indicated that a mean total of 6% of the applied dose of malathion was absorbed within 1 hour (Dary et al., 2001).

In a study in a rat skin-flap model, 56% of the applied dose directly penetrated the skin flap while 14% and 9% remained in the skin and stratum corneum, respectively (Capt et al., 2007). Thus nearly 80% of the applied dose was potentially absorbed by rat skin. Similar findings were obtained in rats in vivo, with ~53% of the dermal dose being potentially absorbed. These amounts are significantly higher than those found either in vitro in human skin, or in volunteers (see above); when human skin was grafted onto nude mouse (HuSki model), dermal absorption for malathion was similar to that in in-vitro models of human skin and in volunteers (Capt et al., 2007).

4.1.2 Distribution

(a) Humans

After fatal poisoning with malathion, malathion residues were detected in the lungs, liver, kidneys, spleen, brain, heart, blood, muscles, urine, and gastric contents (Jadhav et al., 1992).

(b) Experimental systems

Malathion is uniformly distributed systemically after absorption in mice, with no evidence of accumulation in any particular tissue, including fat (Ahdaya et al., 1981).

Malathion distribution was analysed 4, 8, 12, 16, 20, and 30 days after a single dose given by gavage (malathion, 467 mg/kg bw; in olive oil) in male albino rats. Malathion was detected in the blood only on day 4 (3.58 µg/g). The adipose tissue concentration was highest on day 4 (2.63 µg/g) and then declined until day 12. The concentration in muscle was 4.24 µg/g on day 4 and decreased until day 16. In the liver, malathion concentrations increased until day 16 (1.13 µg/g) and declined by day 20. Brain concentration peaked on day 16 (0.88 µg/g) and was not detected on day 30 (Garcia-Repetto et al., 1995).

Within 1–3 minutes after injection of [^{14}C]-labelled malathion (0.9 mg/kg bw) into the tail vein of male Wistar rats, radiolabel was found throughout the body, with highest levels in the kidney, liver, lung, heart, skin, muscle, and blood (Muan & Nafstad, 1989). After 10 minutes, the amount of radiolabel in the liver had decreased, and the largest amounts were found in the renal cortex, the medulla of the kidney, and the intestine. After 12 and 24 hours, radiolabel was barely detectable.

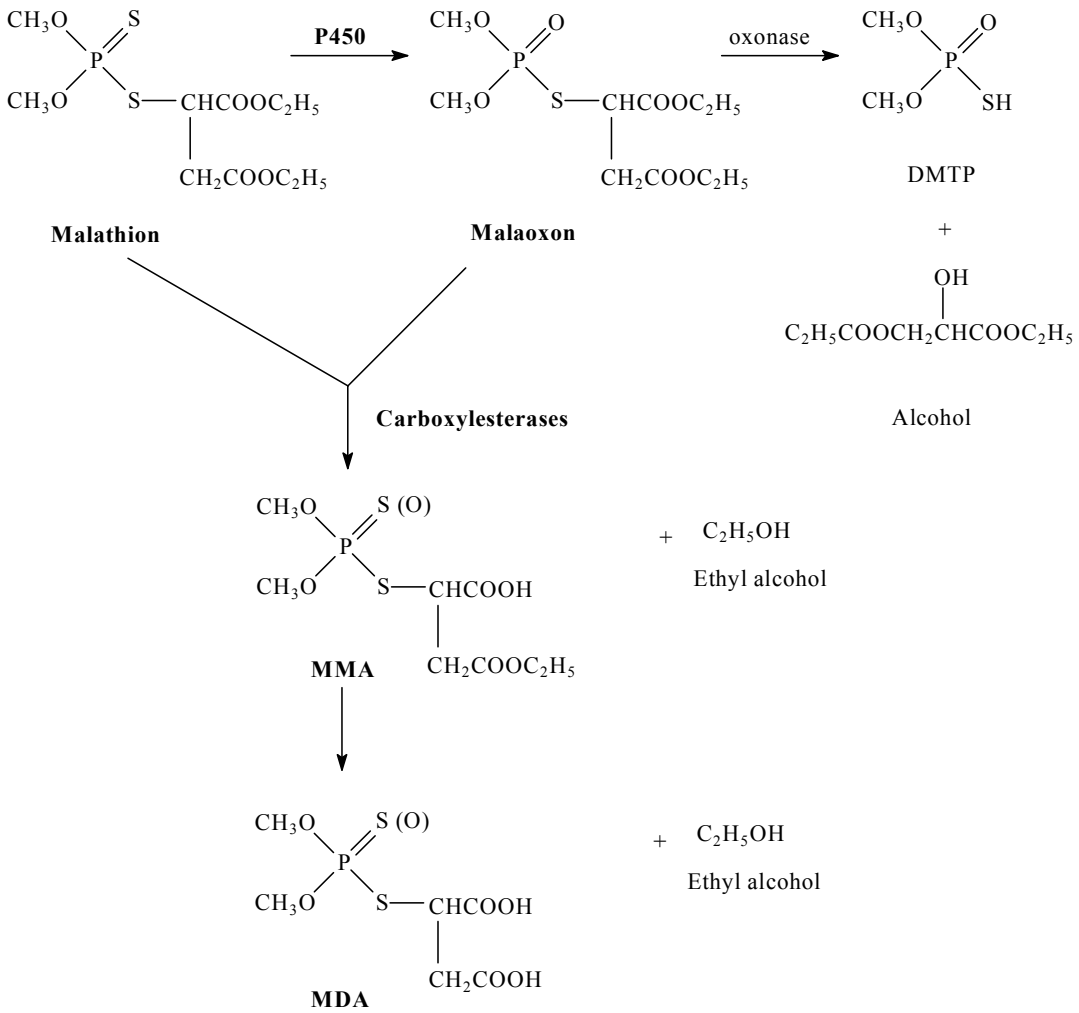
4.1.3 Metabolism

(a) Overview of metabolic pathways

In general, organophosphate pesticides (including malathion) follow metabolic pathways that are conserved across species (Casida & Quistad, 2004). Oxidation and hydrolytic biotransformation of malathion are key enzymatic pathways of metabolism. Biotransformation of malathion occurs primarily in the liver and, to a lesser extent, in the small intestine, after oral exposures. Malathion metabolites and their glucuronide or sulfate conjugates are mainly excreted in the urine (Barr & Angerer, 2006). After dermal or oral exposure, malathion is rapidly biotransformed by several enzymes – including cytochrome P450 (CYP), paraoxonases, and carboxylesterases – to water-soluble metabolites that are rapidly eliminated (see Fig. 4.1). One important reason for the rapid metabolism of malathion in mammals is that it is a diethyl succinate derivative containing two carboxylic acid ethyl ester moieties that are hydrolytically labile (Talcott et al., 1979). Most of the metabolites excreted in the urine are malathion monocarboxylic acids, which are hydrolytic products of the reaction catalysed by carboxylesterases (Fig. 4.1; Buratti & Testai, 2005).

The bioactive metabolite malaoxon is generated by CYP-catalysed desulfuration (Buratti et al., 2005; Barr & Angerer, 2006). If malaoxon is not degraded by hepatic paraoxonase or carboxylesterases, it can escape the liver and instead covalently modify (and inhibit) various serine hydrolase enzymes, including the

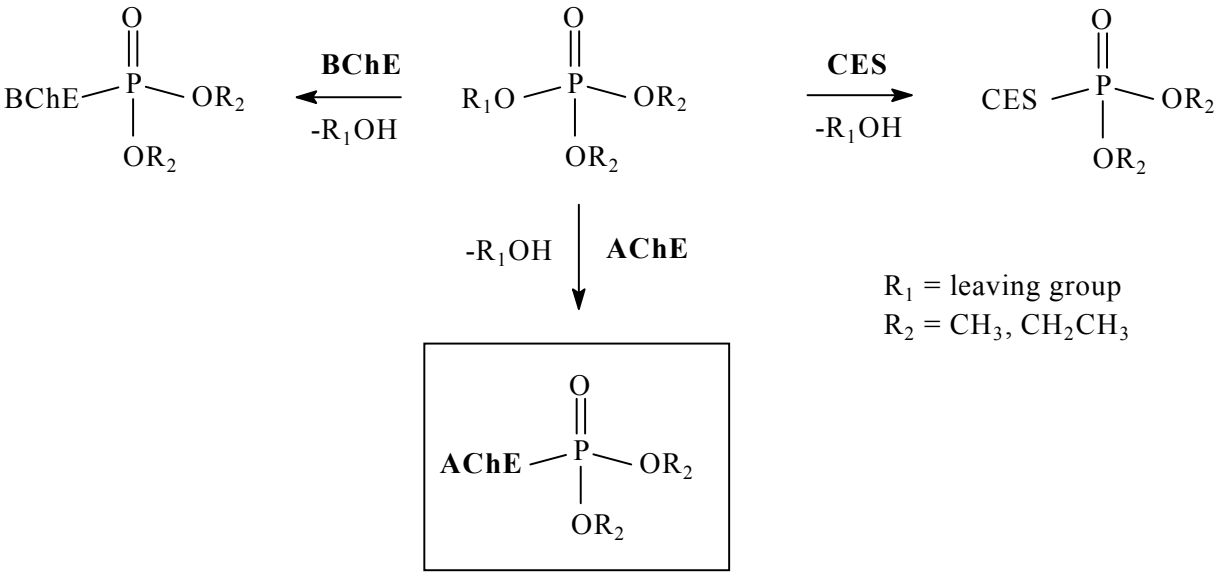
Fig. 4.1 Major pathways of biotransformation of malathion



From Buratti & Testai (2005)

B-esterase targets butyrylcholinesterase, acetylcholinesterase, and carboxylesterases (Casida & Quistad, 2004; see Fig. 4.2). Generation of the oxon metabolite is a bioactivation reaction, because the oxon is a much more potent inhibitor of B-esterases than the parent compound (Casida & Quistad, 2004). In general, analytical measurement of the oxons in blood is difficult due to the small quantities of metabolite that are formed and its relative instability (Timchalk et al., 2002). Nevertheless, the oxons are potent inhibitors of serine hydrolases, exhibiting bimolecular rate constants of inhibition varying from 10^3 to 10^7 $M^{-1}s^{-1}$, depending on the hydrolase and the specific oxon (Casida & Quistad, 2004; Crow et al., 2012). Most important with respect to the insecticidal and toxicological activity of malaoxon is acetylcholinesterase, the esterase responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems (Casida & Quistad, 2004; Crow et al., 2012).

Fig. 4.2 Reactions of a generic oxon metabolite with esterases



The reaction of the oxon metabolite common to several organophosphate pesticides (in this case, malathion, malaoxon) with the canonical target leads to inhibition of CES, AChE, and BChE activity. The neurotoxicity displayed by organophosphate pesticides is attributed to the product (shown in the box) of reaction between the oxon metabolite and AChE
AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CES, carboxylesterase
Adapted with permission from Casida & Quistad (2004); copyright (2004) American Chemical Society

(b) Humans or human-derived tissues

During in-vitro reactions with individual recombinant human CYP isoforms and malathion at low concentrations, malaoxon formation was shown to be catalysed by human CYP1A2 and, to a lesser extent, by CYP2B6; the role of CYP3A4 was relevant only at high concentrations of malathion (Buratti et al., 2005). The activity of human hepatic carboxylesterases on malathion was also assessed in a panel of liver microsomes from 18 individuals (Buratti & Testai, 2005). Carboxylesterase activity showed a low level (fourfold) of variation among individuals, suggesting minimal inter-individual variability in malathion hydrolysis. When Michaelis-Menten kinetic constants (K_m and V_{max}) for four samples of human liver microsomes were assessed, the intrinsic clearance values ($Cl_{int} = V_{max}/K_m$) for malathion were about tenfold greater with human hepatic carboxylesterases than with rat hepatic carboxylesterases; the hydrolysis of malathion by liver esterases is thus more efficient in humans than in rats (Buratti & Testai, 2005).

(c) Non-human mammalian experimental systems

In general, the profile of malathion metabolites formed is similar in human and rodent tissues (Barr & Angerer, 2006). A desmethyl malathion metabolite resulting from a glutathione transferase-catalysed reaction was observed when malathion was incubated with glutathione in the presence of a soluble fraction from mouse liver (Nomeir & Dauterman, 1978). Glutathione transferase-mediated demethylation of organophosphate pesticides is another metabolic pathway (Abel et al., 2004).

4.1.4 Excretion

(a) Humans

The elimination half-life of malathion in blood of volunteers was estimated to be only 12 minutes after absorption of an oral dose (0.5–15 mg/kg bw), highlighting its rapid turnover in vivo (Bouchard et al., 2003). By 48 hours, it was estimated that the systemic body burden of malathion and its metabolites was < 1% of the orally administered dose (0.5–15 mg/kg bw). The systemic body burden of malathion and its metabolites by 48 hours was estimated to be ~0.1% of the dermally administered dose in volunteers (4 μg/cm^2) (Feldmann & Maibach, 1974; Bouchard et al., 2003).

In volunteers exposed to malathion, about 35% of the orally administered dose was excreted as malathion monocarboxylic acids in the urine, while 8% was excreted as malathion dicarboxylic acid (Bouchard et al., 2003). The time taken to recover half of the absorbed dose of malathion in the urine as metabolites after dermal, oral, or intravenous administration was 11.8, 3.2, or 4 hours, respectively. The rate of dermal absorption is much slower than the rate of biotransformation or renal clearance for malathion (Bouchard et al., 2003), accounting for the longer half-lives of metabolites in the urine. However, direct ingestion of malathion degradates, i.e. malathion dicarboxylic acid, malathion monocarboxylic acids, dimethylphosphate, and dimethyltiophosphate, from the environment could potentially confound biomonitoring of urinary metabolites of pesticides such as malathion. Indeed, exposure to the environmental degradates of malathion may potentially increase urinary metabolite levels, thus leading to overestimation of malathion exposure and a false measure of the extent of excretion (Chen et al., 2013).

Approximately 90% of the administered dose was excreted in the urine after 24 hours as metabolites, with no unchanged parent compound detected, after male volunteers were intravenous administrated [^{14}C]-labelled malathion (1 μCi radioactivity; neither the dose of malathion nor the specific radioactivity of [^{14}C]-malathion was reported) (Feldmann & Maibach, 1974). After dermal administration of [^{14}C]-labelled malathion to the ventral forearm of male volunteers, approximately 5.5% of the dose had been excreted in the urine after 24 hours and ~6.8% by 120 hours (Maibach et al., 1971). Again, the excreted radiolabel in the urine entirely comprised metabolites of malathion. In another study, the excreted radiolabel in the urine ranged from 6% to 29% of the dermally applied dose, depending on the site of application (Maibach et al., 1971). The cumulative urinary excretion of [^{14}C] residues (as a percentage of the administered dose) in male volunteers, after dermal application of [^{14}C]-labelled malathion (4 μg/cm^2) to various anatomical regions, showed the following trend after 120 hours: axilla (~29%) > forehead (~23%) > hand dorsum (~12.5%) > abdomen (9.4%) > ventral forearm (~6.8%) > palm of hand (~6%) (Maibach et al., 1971).

Bouchard et al. (2003) showed that malathion is rapidly absorbed and eliminated from the body after a single oral exposure (dose range, 0.5–15 mg/kg bw). By 48 hours, the systemic body burden of malathion and its metabolites was < 1% of the administered dose. Nearly 70% of the oral dose was found as metabolites in the urine after 48 hours, in the following rank order: malathion monocarboxylic acids (~36% of oral dose) > phosphoric metabolites or derivatives (~21% of the administered dose) > malathion dicarboxylic acid (~10% of oral dose). In contrast, after a single dermal exposure to malathion, the systemic body burden of malathion and its metabolites by 48 hours was only ~0.1% of the administered dose. The relative abundance of individual metabolites in the urine after dermal exposure to malathion followed the same rank order observed after oral exposure, but in aggregate accounted for only ~6.5% of the applied dose.

(b) Non-human mammalian experimental systems

After oral or dermal administration of [^{14}C]-labelled malathion in rats, more than 90% of the radiolabel was excreted in the urine as metabolites after 24 hours, supporting the rapid metabolism and excretion of malathion (Abou Zeid et al., 1993).

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Malathion has been studied for genotoxic potential in a variety of assays. Table 4.1, Table 4.2, Table 4.3, Table 4.4, Table 4.5, and Table 4.6 summarize the studies carried out in exposed humans, in human cells in vitro, in other mammals in vivo, in other mammals in vitro, and in non-mammalian systems in vivo and in vitro, respectively.

(a) Humans

(i) Studies in exposed humans

See Table 4.1

Workers exposed to a mixture of pesticides, including malathion, showed increased rates of DNA damage in blood lymphocytes by the comet assay (Garaj-Vrhovac & Zeljezic 2001; Singh et al., 2011b). Malathion did not increase mutation frequencies in exposed workers (Windham et al., 1998). Workers exposed for 8 months to several pesticides, including malathion, did show an increase in the frequency of micronucleated lymphocytes (Garaj-Vrhovac & Zeljezic 2001), although malathion did not induce micronucleus formation in peripheral lymphocytes of workers in the Mediterranean Fruit Fly Eradication Program (Titenko-Holland et al., 1997; Windham et al., 1998).

A malathion-based formulation caused chromosomal aberrations in peripheral lymphocytes of patients treated in hospital for acute intoxication (van Bao et al., 1974), and in workers regularly exposed to malathion (Singaravelu et al. 1998). In workers exposed to several pesticides, including malathion, studies found increased frequencies of chromosomal aberration (Rupa et al., 1989, 1988; Garaj-Vrhovac & Zeljezic 2001), and sister-chromatid exchange (Rupa et al., 1988, 1991; Garaj-Vrhovac & Zeljezic 2001; Zeljezic & Garaj-Vrhovac 2002) in peripheral blood lymphocytes.

(ii) Human cells in vitro

See Table 4.2

Malathion induced DNA damage in the absence of metabolic activation in HepG2 liver cells in vitro by the comet assay (Moore et al. 2010). This assay gave negative results in isolated human lymphocytes treated with malathion, but positive results after treatment with malaoxon or isomalathion (Błasiak et al. 1999). Malathion induced an increase in levels of 8-hydroxydeoxyguanosine (8-OH-dG) in peripheral blood cells (Ahmed et al., 2011) but did not cause unscheduled DNA synthesis in fetal lung fibroblasts (Walter et al., 1980).

Malathion caused mutations in the *HPRT* gene of human T lymphocytes (Pluth et al. 1996). Chromosomal aberrations were induced in human lymphocytes after treatment in vitro in the absence of metabolic activation (Walter et al., 1980; Garry et al., 1990; Balaji & Sasikala 1993). Micronucleus formation was induced in isolated human lymphocytes after treatment in the absence of metabolic activation, but not in Molt-4 lymphocytes (Szekely et al., 1992); anti-kinetochore antibody staining showed that malathion mostly induced chromosome breakage (Titenko-Holland et al., 1997). Malathion gave positive results in assays for sister-chromatid exchange in human lymphocytes and fetal fibroblasts (Nicholas et al., 1979; Sobti et al., 1982; Garry et al., 1990; Balaji & Sasikala 1993).

Table 4.1 Genetic and related effects of malathion in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ Significance	Comments	Reference
Blood	Lymphocytes	DNA damage	Comet assay	20 workers in pesticide production and simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion)	(+) $P < 0.001$	P values for exposed group after 8 mo of exposure vs control group ($n = 20$)	Garaj-Vrhovac & Zeljezic (2001)
Blood	Lymphocytes	DNA damage	Comet assay	70 workers spraying pesticides for community health programmes in Delhi, India, and exposed to pirimiphos methyl, chlorpyrifos, temephos, and malathion	(+) $P < 0.001$	P value in workers vs controls ($n = 70$) [pellets used]	Singh et al. (2011b)
Blood	Lymphocytes	Mutation	Glycophorin A assay	Workers in the Mediterranean Fruit Fly Eradication Programme, California	–		Windham et al. (1998)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	20 workers working in pesticide production and simultaneously exposed to a complex mix of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	(+) $P < 0.05$	P values for exposed group after 8 mo of exposure vs control group ($n = 20$)	Garaj-Vrhovac & Zeljezic (2001)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	38 malathion-exposed workers involved in the Mediterranean Fruit Fly Eradication Programme, California	–	P values for exposed group after 6 mo of exposure vs control group ($n = 16$)	Titenko-Holland et al. (1997)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	Workers in the Mediterranean Fruit Fly Eradication Programme, with malathion as ground treatment	–		Windham et al. (1998)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberrations	14 patients suffering acute intoxication with a malathion-based formulation: blood analyses immediately (3–6 days), 1 mo, and 6 mo after intoxication	+ $P < 0.001$	P values for intoxicated group vs control group ($n = 15$)	van Bao et al. (1974)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberration	20 workers working in pesticide production and simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	(+) $P < 0.001$	P values for exposed group after 8 mo exposure vs control group ($n = 20$)	Garaj-Vrhovac & Zeljezic (2001)

Table 4.1 (continued)

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ Significance	Comments	Reference
Blood	Blood cells	Chromosomal damage	Chromosomal aberrations	50 smoking workers for 1–25 yr to 11 pesticides including malathion	(+) $P < 0.05$	Significant increase in gaps, breaks, fragments, deletions, and dicentrics in smokers ($n = 27$) exposed to a mixture of pesticides compared with unexposed smokers ($n = 20$).	Rupa et al. (1989)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberrations	25 vegetable-garden male workers, smokers and alcohol consumers, exposed to seven pesticides including malathion	(+) $P < 0.05$	P value for exposed workers, irrespective of the duration of exposure, vs control I (20 healthy non-smokers and non-alcohol consumers) or control II (10 healthy smokers and alcohol consumers)	Rupa et al. 1988
Blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	20 workers working in pesticide production and simultaneously exposed to five pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	(+) $P < 0.001$	P value for exposed group vs control group ($n = 20$)	Zeljezic & Garaj-Vrhovac (2002)
Blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	20 workers in pesticide production and simultaneously exposed to a complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, malathion)	(+) $P < 0.001$	P value for exposed group after 8 mo exposure vs control group ($n = 20$)	Garaj-Vrhovac & Zeljezic (2001)
Blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	61 non-smoking, cotton-field workers regularly exposed to 11 pesticides including malathion for several years	(+) $P < 0.05$	P value for pesticide applicators vs controls ($n = 45$)	Rupa et al. (1991)
Blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	25 vegetable-garden male workers, smokers and alcohol consumers, exposed to seven pesticides including malathion	(+) $P < 0.05$	P value for exposed workers, irrespective of the duration of exposure, vs control I (20 healthy non-smokers and non-alcohol consumers) or control II (10 healthy smokers and alcohol consumers)	Rupa et al. 1988

[a] +, positive; –, negative; (+) or (–), positive or negative result in a study of limited quality

mo, month; vs, versus

Table 4.2 Genetic and related effects of malathion (and its metabolites) in human cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
HepG2 hepatocellular carcinoma cell line	DNA damage	Comet assay	+	NT	24 mM	Purity, 98.2%; cell viability decreased by > 70%	Moore et al. (2010)
Lymphocytes	DNA damage	Comet assay	–	NT	200 µM	Purity, > 99.8% Malaoxon and isomalathion induced damage at 25 µM	Błasiak et al. (1999)
Peripheral blood mononuclear cells	DNA damage	Adduct 8-OH-dG	+	NT	20 µM	Malondialdehyde concentrations were also increased	Ahmed et al. (2011)
Human fetal lung fibroblasts (WI-38)	DNA damage	Unscheduled DNA synthesis	–	–	NR		Waters et al. (1980)
T lymphocytes	Mutation	*HPRT* mutation	+	NT	450 µg/mL		Pluth et al. (1996)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	10 µg/mL	Purity, 99%	Walter et al. (1980)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	+	132 µg/mL		Garry et al. (1990)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	2 µg/mL		Balaji & Sasikala (1993)
Lymphocytes (isolated)	Chromosomal damage	Micronucleus formation	+	NT	75 µg/mL	Purity, 95% (5% impurities, including malaoxon). Kinetochore-negative micronuclei (malathion mostly induced chromosome breakage). No clear increase in micronucleus formation in whole blood culture	Titenko-Holland et al. (1997)
Molt-4 lymphocytes	Chromosomal damage	Micronucleus formation	–	NT	120 µg/mL	Purity, > 99%	Szekely et al. (1992)
Fetal fibroblasts	Chromosomal damage	Sister-chromatid exchange	+	NT	20 µg/mL	Purity, 99%	Nicholas et al. (1979)
Lymphoid cells	Chromosomal damage	Sister-chromatid exchange	+	+	0.2 µg/mL	Only 20 µg/mL was tested + S9	Sobti et al. (1982)
T lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	+	660 µg/mL		Garry et al. (1990)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	20 µg/mL		Balaji & Sasikala (1993)

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine

Table 4.3 Genetic and related effects of malathion (and its metabolites) in non-human mammals in vivo

Species, strain (sex)	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Rat, Sprague-Dawley (M)	Lymphocytes	DNA damage	Comet assay	+	2.5 mg/kg bw per day	i.p. × 5	Purity, 98.2%	Moore et al. (2011)
Rat, Wistar (M)	Liver, brain, kidney, spleen	DNA damage	Comet assay	+ +	23 mg/kg bw per day 687.5 mg/kg bw	In diet, × 60 In diet, × 1		Ojha et al. (2013)
Mouse, Q strain (M)	Ovary	Mutation	Dominant-lethal test	–	300 mg/kg bw	i.p. × 1	Purity, > 99% Single dose level tested No increase in pre- or postimplantation fetal lethality	Degraeve & Moutschen (1984)
Mouse, Q strain (M)	Ovary	Mutation	Dominant-lethal test	–	300 mg/kg bw	i.p. × 1	Purity, > 99% Increase in preimplantation fetal lethality	Degraeve et al. (1985)
Mouse, $B6C3F_1$ (M)	Ovary	Mutation	Dominant-lethal test	–	5000 mg/kg bw	Oral dose, × 1		Waters et al. (1980)
Rat, Sprague-Dawley (M)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	5 mg/kg bw per day	i.p. × 5	Purity, 98.2%	Moore et al. (2011)
Mouse, Swiss Albino (NR)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	2 mg/kg bw per day	Intubation × 7		Kumar et al. (1995)
Mouse, Q strain (M)	Bone marrow, spermatogonia	Chromosomal damage	Chromosomal aberrations	–	300 mg/kg bw	i.p. × 1	Purity, > 99% Single dose level tested	Degraeve & Moutschen (1984)
Mouse, Swiss Webster (M)	Bone marrow, spermatogonia	Chromosomal damage	Chromosomal aberrations	+	Bone marrow, 250 mg/kg bw Sperm, 500 mg/kg bw	Dermal × 5	Single dose at up to 2000 mg/kg bw gave negative results	Salvadori et al. (1988)
Mouse, BALB/c (NR)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	230 mg/kg bw	i.p. × 1	Purity, 95.5%	Dulout et al. (1983)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss Albino (M)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	1/15th of LD_{50}	i.p. × 35; four mice killed at weekly intervals	Statistically significant increase in micronuclei (no *P* value calculated); level declined back to control value 2 wk after end of treatment	Abraham et al. (1997)
Mouse, Swiss Albino (M, F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	2.5 mg/kg bw	i.p. × 1		Giri et al. (2002)
Mouse, Swiss Albino (M, F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	5 mg/kg bw	Gavage × 1		Giri et al. (2002)
Mouse, White Swiss (M)	Bone marrow, spermatocytes, spleen cells	Chromosomal damage	Chromosomal aberrations	+	41.80 mg/kg grain, stored for 12 wk	Mice fed for 6 or 12 wk with treated grain (8.36, 25.08 or 41.80 mg/kg grain, for 4, 12, or 24 wk)	Positive results also obtained with any grain stored for 24 wk Negative results with mice fed with grain stored for 4 wk	Amer et al. (2002)
Hamster, Syrian (F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+/–	2400 mg/kg bw	i.p. × 1	One statistically significant increase only at highest dose of 2400 mg/kg bw $P < 0.05$	Dzwonkowska & Hübner (1986)
Mouse, Swiss Albino (NR)	Bone marrow	Chromosomal damage	Micronucleus formation	+	2.5 mg/kg bw	i.p. × 1, sampled after 24 or 48 h	Purity, 95%	Giri et al. (2011)
Mouse, Swiss Albino (NR)	Bone marrow	Chromosomal damage	Micronucleus formation	+	5 mg/kg bw	i.p. × 1		Giri et al. (2011)
Mouse, Swiss (Rockland) (M)	Bone marrow	Chromosomal damage	Micronucleus formation	+	120 mg/kg bw	i.p. × 1 Dermal × 1	Same LED for both routes	Dulout et al. (1982)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss Albino (M)	Bone marrow	Chromosomal damage	Micronucleus formation	+	1/15th of LD_{50}	i.p. × 35; four mice killed at weekly intervals	Statistically significant increase in frequency of micronucleus formation [no *P* value calculated]; increase directly proportional to treatment duration; level returnedto control value within 1 wk after end of treatment Precise dose, NR	Abraham et al. (1997)
Mouse, strain and sex, NR	Bone marrow	Chromosomal damage	Micronucleus formation	–	$0.8 \times LD_{50}$ $0.4 \times LD_{50}$ $0.2 \times LD_{50}$ $0.1 \times LD_{50}$	i.p. × 4 (4 days, once per day)	Purity, 99%	Ni et al. (1993)
Mouse White Swiss (M)	Spleen cells	Chromosomal damage	Sister-chromatid exchange	+	41.80 mg/kg grain, stored for 12 wk	Mice fed for 6 or 12 wk with treated grain (8.36, 25.08 or 41.80 mg/kg grain, stored for 4, 12, or 24 wk)	Positive results also obtained with any grain stored for 24 wk Negative results with mice fed with grain stored for 4 wk	Amer et al. (2002)
Mouse, Swiss Albino (M, F)	Bone marrow	Chromosomal damage	Sister-chromatid exchange	+	2.5 mg/kg bw	i.p.		Giri et al. (2002)

[a] +, positive; –, negative; +/–

DMSO, dimethyl sulfoxide; F, female; h, hour; HID, highest ineffective dose; i.p., intraperitoneal; LD_{50}, median lethal dose; LED, lowest effective dose; M, male; NR, not reported; NT, not tested; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; wk, week

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

In rats, malathion caused DNA damage, as detected by the comet assay, in lymphocytes after repeated intraperitoneal doses (Moore et al., 2011), and in the liver, brain, kidney and spleen after single or repeated oral doses (Ojha et al., 2013). Malathion did not induce mutations in mouse spermatogonia (dominant-lethal test) after intraperitoneal (Degraeve & Moutschen, 1984; Degraeve et al., 1985), or oral exposure (Waters et al., 1980), although combined intraperitoneal treatment with trichlorfon did induce dominant-lethal mutation (Degraeve & Moutschen 1984).

Chromosomal aberrations were induced by malathion in most studies in rats, mice, and hamsters in vivo: chromosomal aberrations were observed in bone marrow or spermatogonia after intraperitoneal administration (Dulout et al., 1983; Abraham et al., 1997; Giri et al., 2002; Moore et al., 2011), intubation (Kumar et al., 1995), or dermal administration (Salvadori et al., 1988), and in bone marrow (Giri et al., 2002) or bone marrow, spermatocytes, and spleen cells after oral administration (Amer et al. 2002). Malathion did not cause chromosomal aberrations in one study on bone marrow and spermatogonia of mice after intraperitoneal dosing (Degraeve & Moutschen, 1984).

In mice, malathion caused micronucleus formation in bone marrow after intraperitoneal dosing in several studies (Dulout et al., 1982; Abraham et al., 1997; Giri et al., 2011), but not in one study (Ni et al., 1993). Sister-chromatid exchange was also induced in the mouse, in spleen cells after oral administration (Amer et al., 2002) and in bone marrow after intraperitoneal administration (Giri et al., 2002). An increase in the frequency of sperm with abnormal head morphology was also reported in mice exposed intraperitoneally (Giri et al., 2002).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Malathion induced DNA breaks (as detected by the comet assay) in rat lymphocytes in the absence of metabolic activation (Ojha & Gupta 2014) and in rat PC12 adrenal gland cells (Lu et al., 2012), and also caused DNA–protein crosslinks (Ojha & Gupta 2014). Malathion produced micronucleus formation in Chinese hamster lung cells (Ni et al., 1993), and sister-chromatid exchange in Chinese hamster ovary cells (Nishio & Uyeki 1981; Ivett et al., 1989), and V79 cells (Chen et al., 1981); however, a study in V79 cells gave negative results (Szekely et al., 1992). Malathion did not induce chromosomal aberrations in Chinese hamster ovary cells (Ivett et al., 1989).

(iii) Non-mammalian systems in vivo

See Table 4.5

In fish (*Channa punctatus* Bloch), malathion caused DNA damage (comet assay) in gills, kidney, and lymphocytes, and also micronucleus formation in erythrocytes (Kumar et al., 2010). No increase in the hepatic levels of 8-OH-dG in fish (sea bream) was reported after intraperitoneal administration (Rodríguez-Ariza et al. 1999). Conflicting results were obtained in assays for mutation in *Drosophila melanogaster* (Waters et al., 1980; Velázquez et al., 1987; Foureman et al., 1994; Kumar et al., 1995; Osaba et al., 1999).

(iv) Non-mammalian systems in vitro

See Table 4.6

Malathion induced DNA damage in isolated DNA from *Escherichia coli* K-12 (Griffin & Hill 1978), and in *E. coli* in the SOS test (Venkat et al., 1995). Malathion did not demonstrate mutagenicity in *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102, TA1535, TA1537, or TA1538 (Pednekar et al., 1987; Wong et al., 1989; EPA, 1990b), in *E. coli* WP2 (Dean 1972; EPA, 1990b), in *Bacillus subtilis* (Shirasu et al., 1976) or in yeast (Gilot-Delhalle et al., 1983).The mutation spot test gave negative results in *B.*

Table 4.4 Genetic and related effects of malathion in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Rat	Lymphocytes	DNA damage	Comet assay (alkaline and neutral version measuring DNA SSB and DSB)	+	NT	0.52 mg/L	Time of exposure, 2, 4, 8, or 12 h	Ojha & Gupta (2014)
Rat	Adrenal gland, PC12 cells	DNA damage	Comet assay	+	NT	40 µg/mL	Malaoxon was more genotoxic than malathion; both chemicals increased intracellular ROS levels	Lu et al. (2012)
Rat	Lymphocytes	DNA damage	DNA–protein crosslink (assay based on binding of SDS to proteins, and lack of binding to DNA)	+	NT	0.52 mg/L	Time of exposure, 2, 4, 8 or 12 h	Ojha & Gupta (2014)
Hamster, Cinese	CHL cells	Chromosomal damage	Micronucleus formation	+	NT	200 µg/mL		Ni et al., (1993)
Hamster, Chinese	CHO cells	Chromosomal damage	Chromosomal aberrations	–	–	3010 µg/mL		Ivett et al. (1989)
Hamster, Chinese	V79 cells	Chromosomal damage	Sister-chromatid exchange	+	NT	40 µg/mL		Chen et al. (1981)
Hamster, Chinese	V79 cells	Chromosomal damage	Sister-chromatid exchange	–	NT	30 µg/mL	Strong increase in polyploidy (at 20–40 mg/L)	Szekely et al. (1992)
Hamster, Chinese	CHO cells	Chromosomal damage	Sister-chromatid exchange	+	(+)	50 µg/mL		Ivett et al. (1989)
Hamster, Chinese	CHO cells	Chromosomal damage	Sister-chromatid exchange	+	NT	Malathion 0.3 mM		Nishio & Uyeki (1981)
Hamster, Chinese	CHO cells	Chromosomal damage	Sister-chromatid exchange	+	NT	Malaoxon 0.1 mM	Malaoxon produced higher level of sister-chromatid exchange than malathion	Nishio & Uyeki (1981)

[a] +, positive; –, negative; (+), weakly positive

CHL, Chinese hamster lung; CHO, Chinese hamster ovary; DSB, DNA double-strand breaks; h, hour; HID, highest ineffective dose; LED, lowest effective dose, NT, not tested; ROS, reactive oxygen species; SDS, sodium dodecyl sulfate; SSB, DNA single-strand breaks

Table 4.5 Genetic and related effects of malathion (and its metabolites) in non-mammals in vivo

Species, strain (sex)	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Fish, *Channa punctatus* (Bloch)	Gill, kidney, lymphocytes	DNA damage	Comet assay	+	0.59 ppm (one tenth of LC_{50})	Fish maintained in water containing malathion, 1 day	Semi-static system, water changed every second day LC_{50}, 5.93 ppm	Kumar et al. (2010)
Fish (seabream)	Liver	DNA damage	Adduct (8-OH-dG)	–	6.38 mg/kg bw	i.p. × 1		Rodríguez-Ariza et al. (1999)
Fish, *Channa punctatus* (Bloch)	Blood erythrocytes	Chromosomal damage	Micronucleus formation	+	0.59 ppm (one tenth of LC_{50})	Fish maintained in water containing malathion	Semi-static system, water changed every second day LC_{50}, 5.93 ppm	Kumar et al. (2010)
Drosophila		Mutation	Dominant lethal	+	2 µg/L	In feeding solution		Kumar et al. (1995)
Drosophila melanogaster		Mutation	Sex linked recessive lethal	+	3.5 µg/L	In feeding solution		Kumar et al. (1995)
Drosophila melanogaster		Mutation	Wing-spot test	–	NR	In feeding solution	Malathion (in 3% Tween 80 and 3% ethanol) was used to rehydrate *Drosophila* instant medium in the ratio of 0.3 g of dry medium to 1 mL of test solution	Osaba et al. (1999)
Drosophila melanogaster		Mutation	Sex-linked recessive lethal	–	0.5 ppm	In feeding solution		Waters et al. (1980)
Drosophila melanogaster		Mutation	Sex-linked recessive lethal	–	NR	In feeding solution	Malathion dissolved in DMSO then diluted in 5% sucrose to give a final DMSO concentration of 0.1%	Velázquez et al. (1987)
Drosophila melanogaster		Mutation	Sex chromosome losses	–	NR	In feeding solution	Malathion dissolved in DMSO then diluted in 5% sucrose to give a final DMSO concentration of 0.1%	Velázquez et al. (1987)
Drosophila melanogaster		Mutation	Sex-linked recessive lethal	+	NR	In feed	Test on malaoxon Purity, 94.4% Negative results by injection	Foureman et al. (1994)

[a] +, positive; –, negative

DMSO, dimethyl sulfoxide; F, female; h, hour; HID, highest ineffective dose; i.p., intraperitoneal; LD_{50}, median lethal dose; LED, lowest effective dose; M, male; NR, not reported; NT, not tested; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine

Table 4.6 Genetic and related effects of malathion in non-mammalian systems in vitro

Phylogenetic class	Test system (species, strain)	End-point	Test	Results[a]		Agent, concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Acellular systems	Isolated DNA from *Escherichia coli* K-12	DNA damage	NR	+	NT	0.1 mg/mL	Purity, NR Malathion, 1 mg/mL Breakage at a slow rate (0.12 breaks/week)	Griffin & Hill (1978)
Prokaryote (bacteria)	*Escherichia coli PQ37*	DNA damage	SOS test	+	NT	NR		Venkat et al. (1995)
	Salmonella typhimurium; TA97a, TA98, TA100	Mutation	Reverse mutation	–	–	1650 mg/L (0.2 mL per plate)	Metabolic activation by S9 or caecal microbial extract	Pednekar et al. (1987)
	Salmonella typhimurium; TA98, TA100, TA1535, TA1537, TA1538	Mutation	Reverse mutation	–	–	5000 µg/plate		EPA (1990b)
	Salmonella typhimurium; TA98, TA102, TA1535, TA1537	Mutation	Reverse mutation	–	–	400 ppm		Wong et al. (1989)
	Escherichia coli WP2	Mutation	Reverse mutation	–	NT	NR	Tested dose not specified; semiquantitative paper disk method	Dean (1972)
	Bacillus subtilis	Mutation	Rec assay	–	NT	NR	10 mm paper disk containing 0.02 mL of solution	Shirasu et al. (1976)
	Bacillus subtillis TKJ5211	Mutation	Spot test	–	–	300 µg		Shiau et al. (1980)
	Bacillus subtillis TKJ6321	Mutation	Spot test	+	+/–	300 µg		Shiau et al. (1980)
Lower eukaryote (yeast)	*Schizosaccharomyces pombe (ade6)*	Mutation	Forward mutation	–	–	182 mM		Gilot-Delhalle et al. (1983)

[a] +, positive; –, negative; +/–, addition of S9 eliminated the maleic hydrazide-induced mutagenicity

HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant

subtilis TKJ5211, but positive results in *B. subtilis* TKJ6321 (Shiau et al., 1980).

4.2.2 Receptor-mediated mechanisms

(a) Neurotoxicity-pathway receptors

Malathion is metabolized to malaoxon in insects and mammals (Section 4.1.3) (Casida & Quistad, 2004). Malaoxon can covalently modify the catalytic serine residue and inhibit the activity of several B-esterases, including the recognized target acetylcholinesterase, resulting in the acute neurotoxicity elicited by malathion in insect and mammalian species. Acetylcholinesterase is responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems. Blockage results in acetylcholine overload and the overstimulation of nicotinic and muscarinic acetylcholine receptors.

Additional receptor targets of malaoxon that may affect neurotoxicity include butyrylcholinesterase and muscarinic receptors (Ward & Mundy, 1996; Quistad et al., 2002; Ahmed et al., 2007). As reviewed in Sections 4.2.4 and 4.2.5, some mechanistic effects of relevance to the potential carcinogenicity of malathion are blocked or mitigated by co-administration of the anticholinergic drug atropine, and may be at least partly related to acetylcholinesterase inhibition.

(b) Thyroid-hormone disruption

(i) Humans

In a study of exposed humans, the association between thyroid disease and pesticide use among male pesticide applicators was evaluated. Malathion was one of the eight insecticides for which "ever use" was associated with increased odds of hypothyroidism (Goldner et al., 2013). Among spouses of pesticide workers who had ever used malathion, the risk of hypothyroidism was slightly but not statistically elevated (odds ratio, 1.1; 95% CI, 0.92–1.3) (Goldner et al., 2010).

In an in-vitro assay, malathion and 64 other xenobiotics were tested for competitive binding at thyroxine (T4) binding sites on human transthyretin, a plasma protein that can bind to thyroid hormone and and distribute it to target sites (Van den Berg et al., 1991). Using a reaction mixture of malathion, radiolabelled thyroxine, and transthyretin, malathion was found to have positive but low competitive affinity for human transthyretin.

(ii) Non-human mammalian experimental systems

In rats given malathion at an oral dose of 0.06 mg per day (approximately 0.2 mg/kg bw per day) for 21 days, levels of triiodothyronine (T3) and T4 were reduced, while levels of thyroid stimulating hormone were increased (Akhtar et al., 1996). In rats given malathion at considerably higher levels for 3.5 months (10 or 100 mg/kg bw per day), T3 and T4 levels were unaffected (Ozmen & Akay, 1993).

Thyroid function was diminished in male albino rats given malathion (44 mg/kg bw by gavage for 12 weeks [not reported if given daily]) (Balasubramanian et al., 1986). Thyroid uptake of radiolabeled iodine in malathion-treated rats was considerably less than in controls (10.7 ± 0.9 versus 31.7 ± 1.2; $P < 0.001$), as was the proportion of serum protein bound iodine ($P < 0.001$). In a second group of rats in which malathion exposure was discontinued for 2 weeks after 10 weeks of exposure, uptake of iodine in the thyroid and serum protein-bound iodine levels were comparable to control values.

(ii) Non-mammalian experimental systems

T3 and T4 levels were reduced in freshwater catfish (*Clarias batrachus*) exposed to malathion at a concentration of 0.1 or 1 ppm in aquaria water for 30 days in the preparatory and prespawning phases of their reproductive cycle; the ratio of T3 to T4 was depressed at the higher dose (Lal et al., 2013). In catfish in the quiescent phase, T3 and T4

levels were reduced in the group at higher dose. In an earlier study, T3, and the T3 to T4 ratio, but not T4 levels, were reduced in the same species of catfish exposed to malathion at a concentration of 7 ppm in aquaria for 4 days in the vitellogenic or post-vitellogenic phase (Sinha et al., 1991). In a different catfish species (*Heteropneustes fossilis*) exposed to malathion at 10 or 20 ppm in aquaria water, T4 levels decreased after 4 weeks (Yadav & Singh 1986).

A significant dose-dependent reduction in uptake of radioactive iodine by the thyroid, along with other structural changes in the thyroid (see Section 4.2.4), was observed in teleost fish (*Channa punctatus* Bloch) exposed to malathion at 2 or 4 ppm in aquaria water for 6 months (Ram et al., 1989).

In an in-vitro study, malathion inhibited the binding of 3,3′,5-L-[^{125}I]triiodothyronine to purified transthyretin from the plasma of Japanese quail (Ishihara et al., 2003). The ligand-binding domain of thyroid hormone receptor β was unaffected by exposure to malathion.

(c) Androgen-pathway disruption

(i) Humans

No studies of exposed humans were available to the Working Group.

In an in-vitro study, testosterone production was significantly elevated above values for solvent controls by malathion (12.5 μM and above for 48 hours) in exposed human adrenal corticocarcinoma (H295R) cells Taxvig et al. (2013). At the tested concenetrations (1.6–100 μM), malathion had no effect on cell viability.

Malathion in a mixture with four other pesticides, but not alone, induced aromatase activity in human choriocarcinoma JEG-3 cells. Malathion additively antagonized androgen-receptor transactivation in hamster ovary CHO-K1 cells co-transfected with a luciferase reporter vector and a human androgen-receptor expression plasmid (pSVAR0) Kjeldsen et al. (2013).

In a human androgen-receptor reporter-gene assay based on a Chinese hamster ovary cell line (CHO-K1), malathion was not an androgen-receptor antagonist or an agonist (Kojima et al., 2004, 2010).

(ii) Non-human mammalian experimental systems

In adult male rats, a single subcutaneous dose of malathion at 23 mg/kg bw (1/50th of the LD_{50}) caused reductions in the levels of testosterone and luteinizing hormone at 24, 36 and 48 hours after injection (Prakash & Venkatesh, 1996). Administration of human chorionic gonadotropin for 2 days before malathion exposure had a protective effect.

In Wistar rats, daily dosing with malathion at 27 mg/kg bw (1/50th of LD_{50} for an oral dose) for 4 weeks similarly reduced levels of plasma testosterone, follicle-stimulating hormone, and luteinizing hormone. An additional group of rats receiving malathion plus vitamins C and E had similarly reduced levels, but was somewhat protected against adverse effects on sperm and histopathological testicular changes (Uzun et al., 2009).

Dose-dependent reduction in testosterone levels was also observed in Wistar rats given malathion as an oral dose at 50, 150, or 250 mg/kg bw per day for 60 days; there were also biochemical changes in the testes and profound structural and functional effects on the male reproduction system (weights of the prostate, testes, and other organs, sperm density in epididymis and testes, sperm motility, and fertility) (Choudhary et al., 2008).

Ozmen & Akay (1993) reported no significant changes in testosterone levels in Swiss albino rats receiving malathion at oral doses of 10 or 100 mg/kg bw for 15 weeks, but did observe a few degenerated testicular tubuli.

Bustos-Obregón & González-Hormazabal (2003) studied the time course of testicular dysfunction in CF1 mice given a single

intraperitoneal injection of malathion at 240 mg/kg bw (1/12th of the LD_{50}) and evaluated 1, 8, 16, 35, and 40 days thereafter. Testosterone levels steadily decreased over time to approximately 25% of the control value at day 16 and then began to rebound, approaching control values by day 40. Various effects on sperm and testicular histopathology were also reported.

(iii) Non-mammalian experimental systems

Testosterone levels were reduced in freshwater catfish (*Clarias batrachus*) exposed to malathion at 0.1 or 1 ppm for 30 days; the level of reduction increased with increasing dose. This occurred for each of the three reproductive phases tested – quiescent, preparatory, and prespawning (Lal et al., 2013).

(d) Estrogen-pathway disruption

(i) Humans

No studies in exposed humans were available to the Working Group.

In the in-vitro experiment by Taxvig et al. (2013) described above, production of progesterone and estradiol was significantly elevated from control levels in H295R cells exposed to malathion (12.5 to 100 μM).

Malathion did not have estrogenic activity in breast adenocarcinoma MCF7 cells by the E-screen assay, estrogen-receptor competitive-binding assay, or pS2 expression assay at concentrations of 0.00 001 to 1 μM (Chen et al., 2002). Sonnenschein & Soto (1998) found malathion to be inactive in the E-screen assay. Malathion weakly induced estrogen-receptor activity in human breast carcinoma MLVN cells (Kjeldsen et al., 2013).

Malathion was neither an agonist nor antagonist for human estrogen receptors α or β in transactivation assays in CHO-K1 cells (Kojima et al., 2010).

(ii) Non-human mammalian experimental systems

Hormonal changes were seen in rats given malathion (37 mg, intraperitoneal, per rat once per 2–3 days for 16 days) (Uluitu et al., 1981). Based on daily vaginal smears, treated and control rats were evaluated as being either in inactive ("diestrus + metestrus") or active ("estrus + proestrus") estral phases. In the pituitary of malathion-exposed rats, luteinizing hormone was substantially lower in active or inactive estral phases, and prolactin was strongly elevated in the inactive group, whereas follicle-stimulating hormone appeared to be unaffected. In the blood, however, follicle-stimulating hormone was significantly elevated during the active phase. While blood levels of luteinizing hormone and prolactin were lower in both groups, this was only significant for luteinizing hormone in the inactive group. Serotonin was higher in each brain section (hypothalamus, rhinencephalon, mesencephalon, cerebral cortex) taken from inactive-phase rats treated with malathion, but only in the cerebral cortex of active-phase treated rats.

Prakash et al. (1992) exposed dairy cattle intraruminally to malathion at 1 mg/kg bw at the onset of estrus, which was induced by injection of cloprostenol. No significant differences were observed in plasma concentrations of FSH or estradiol between treated and control animals. However, progesterone, which was followed for a longer period, was significantly ($P < 0.05$, Student's t test) lower than control values on post-estrus days 6–18 (measured every second day). Conception occurred in fewer of the treated (16%, 1 out of 6) compared with controls (50%, 3 out of 6) cattle, but sample sizes were small.

(ii) Non-mammalian experimental systems

Singh & Singh (1980) exposed gravid catfish (*Heteropneustes fossilis*) to malathion at concentrations of 9 or 38 ppm in aquaria for 96 hours, and among other findings, reported that the

gonadotropic potency of serum was significantly reduced in all fish.

In an in-vitro study of oocytes of a freshwater catfish native to southern India, malathion substantially reduced germinal-vesicle breakdown (induced by bovine luteinizing hormone), the first step towards oocyte maturation. This occurred at all three concentrations used (0.01, 0.1, and 1 ppm) (Haider & Upadhyaya, 1986).

(e) Other receptor-mediated mechanisms

Malathion was not found to be an agonist to a human pregnane X receptor (PXR) in a reporter-gene assay in a CHO-K1 cell line (Kojima et al., 2010).

Malathion was not an agonist for the aryl hydrocarbon receptor (AhR) in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing copies of dioxin-responsive element (Takeuchi et al., 2008; Kojima et al., 2010). Malathion was also not an agonist for mouse peroxisome proliferator-activated receptors α or γ in-vitro reporter-gene assays in CV-1 monkey kidney cells (Takeuchi et al., 2006; Kojima et al., 2010).

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

Studies in exposed humans

Oxidative stress marker alterations were reported in blood, lymphocytes, and erythrocytes collected immediately after hospital admission of 30 individuals acutely poisoned by ingestion of malathion Banerjee et al., (1999). Exposure was confirmed by serum malathion measurement (range of 382 to 1000 mg/L, by an HPLC-UV method, for the admitted subjects). All subjects had confirmed inhibition of acetylcholinesterase in erythrocytes, but no other significant alterations in routine haematological or biochemical measures. All subjects recovered with symptomatic treatment for 7–21 days in hospital. Statistically significant ($P < 0.05$) effects were found in malathion-poisoned subjects in the following parameters: in blood, increased thiobarbituric acid-reactive substance levels, reduced glutathione levels, and increased activity of gamma glutamyl transpeptidase, glutathione *S*-transferase and glutathione reductase; in erythrocytes, increased activity of superoxide dismutase, catalase, and glutathione peroxidase; in lymphocytes, decreased glutathione levels and increased gamma glutamyl transpeptidase activity.

Human cells in vitro

Several studies examined the potential of malathion to increase levels of oxidative stress markers in various types of human cells in vitro. In cultured human erythrocytes, malathion (25, 75, 200 μM) led to a dose-dependent increase in levels of malondialdehyde (that was statistically significant at all concentrations tested), and a decrease in the activity of superoxide dismutase, catalase, and glutathione peroxidase (Durak et al., 2009). These effects of malathion on oxidative stress markers were reduced by co-treatment with vitamins C and E at supra-physiological concentrations. In human liver carcinoma HepG2 cells, significant increases in cellular levels of malondialdehyde were observed 48 hours after all tested malathion concentrations (0, 6, 12, 18, and 24 mM) Moore et al. (2010). Cytotoxicity exceeded 50% at malathion concentrations of 18 and 24 mM. The comet assay showed a significant increase in the frequency of DNA damage only with malathion at 24 mM, when cell viability was reduced by more than 70%.

Ahmed et al. (2009) investigated the effects of malathion (5–100 μM) in human peripheral blood mononuclear cells cultured for 6, 12, or 24 hours. Intracellular concentrations of glutathione were significantly reduced at concentrations exceeding 20 μM, concomitant with an

increase (25–50%) in the number of apoptotic and necrotic cells in culture. These effects were only partially lessened by co-incubation with *N*-acetylcysteine. These data are similar to the results reported by Rodgers & Ellefson (1990) and Xiong & Rodgers (1997), who showed that exposure of human peripheral blood mononuclear cells to malathion in vitro enhanced their ability to produce hydrogen peroxide.

(ii) Non-human mammalian experimental systems

In vivo

Most of the studies of oxidative stress and malathion in experimental animals were conducted in rats and examined a range of exposure durations, doses, administration routes, and tissues. In addition, various end-points were evaluated to assess induction of oxidative stress.

One of the first reports of induction of lipid peroxidation in vivo (as assessed by thiobarbituric acid-reactive) in rat liver was that of Pawar & Makhija (1975), who observed statistically significant increases in lipid peroxidation 24 hours after treatment in male and female CF rats given an intraperitoneal injection of *O*,*O*-dimethyl malathion at a dose of 150 mg/kg bw on two consecutive days. Comparable acute doses of malathion were confirmed to induce oxidative stress in subsequent studies. Specifically, oxidative stress, as demonstrated by lipid peroxidation, protein oxidation, DNA damage and/or changes in antioxidant enzymes, was also reported in the liver, kidney, lung, blood, and in cardiac and skeletal muscle, and various brain regions of rats treated with one to three daily doses of malathion at a dose of between 25 and 825 mg/kg bw administered either intraperitoneally or orally (John et al., 2001; Brocardo et al., 2005; Possamai et al., 2007; Franco et al., 2009; Shafiee et al., 2010; Ojha & Srivastava, 2012). Acker et al. (2009) stated that there was no increase in oxidative stress markers in a rats given intraperitoneal injections of malathion at a dose of 50 mg/kg bw once per day for three consecutive days. [The Working Group noted that, although this study appeared to report negative results with respect to oxidative stress end-points, the data to support this conclusion were not presented, thus making the study uninterpretable.]

Several reports examined the potential of malathion to cause oxidative stress in rats in vivo for periods of 28 to 60 days. It was shown that repeated doses of malathion (25 to 687.5 mg/kg bw per day), given intraperitoneally or orally, resulted in oxidative stress in the liver, brain, kidney, and other tissues surveyed (Akhgari et al., 2003; Fortunato et al., 2006; Rezg et al., 2008; Franco et al., 2009; Mostafalou et al., 2012a; Ojha et al., 2013; Coban et al., 2014; Lasram et al., 2014a).

Selmi et al. (2012; 2013) exposed lactating female rats to malathion (200 mg/kg bw) by gavage for 21 days and examined pups on postnatal days 21 and 51. In the pups, lactation exposure to malathion increased oxidative stress in the liver, kidneys, brain, plasma, and erythrocytes (as assessed by an increase in levels of malondialdehyde, a decrease in thiol group content, and a decrease superoxide dismutase and catalase activities).

Fewer studies examined malathion-induced oxidative stress in the mouse in vivo. In the first of three studies of similar design, da Silva et al. (2008) injected female Swiss Albino mice subcutaneously with a single dose of malathion (1 g/kg bw, dissolved in saline) and studied effects on oxidative stress at 3 or 24 hours after treatment. A marked increase in the amount of malondialdehyde was found in prefrontal cortex 24 hours (but not 3 hours) after treatment, but there were no effects at either time-point on glutathione levels, or activity of glutathione peroxidase and glutathione reductase in this tissue. In the second report (dos Santos et al., 2011), male Swiss Albino mice were given a single subcutaneous injection of malathion (1.25 g/kg bw) and killed after 24

hours; no change in the activity of glutathione reductase, glutathione peroxidase, or catalase was observed in either the prefrontal cortex or hippocampus of mice treated with malathion only. No other markers of oxidative stress were evaluated. The third report (da Silva et al., 2006) described the effects of exposure to malathion during lactation (subcutaneous injections to the dams; doses of 20, 60, or 200 mg/kg bw per day) on acetylcholinesterase activity and on oxidative stress in the brain of suckling mice. Exposure to malathion during lactation markedly inhibited brain acetylcholinesterase activity in the offspring (even at the lowest dose of 20 mg/kg bw) and in mothers (only at the highest dose of 200 mg/kg bw). No changes in either dams or pups were observed in brain oxidative stress markers (glutathione levels, lipid peroxidation, and glutathione reductase and glutathione peroxidase activity).

Two independent reports provided evidence for oxidative stress in mice exposed to large doses of malathion in vivo. Significant increases were reported in lipid peroxidation, total thiol groups, and activity of antioxidant enzymes (superoxide dismutases and catalase) in testes and epididymis of male Swiss mice after a single oral dose of malathion (500 mg/kg bw) (Slimen et al., 2014). In male ICR mice, both tested doses of malathion (25.2 and 126 mg/kg bw per day by oral gavage for 30 consecutive days) affected liver oxidative-stress markers such as malondialdehyde, protein carbonyls, and superoxide dismutase and catalase activity (Wang et al., 2014). Serum and liver metabolomics analysis were conducted using ^{1}H nuclear magnetic resonance spectroscopy. [The Working Group interpreted the changes in liver and serum as also supportive of the induction of oxidative stress by malathion].

In vitro

Three reports were identified that investigated the effects of malathion on oxidative stress end-points in rat cells in vitro. In primary hepatocytes isolated from male Sprague-Dawley rats and exposed to malathion (purity, 90%; 0.5–1.5 mM for up to 3 hours), significant increases in oxidant production (as measured by fluorescence of 2',7'-dichlorofluorescein diacetate) and reduced mitochondria membrane potential were only seen at malathion concentrations of > 1 mM that were also overtly cytotoxic (50–100% loss in viability) (Mostafalou et al., 2012b). Co-incubation with *N*-acetyl cysteine prevented increases in oxidant production and cytotoxicity, an observation indicative of oxidant-mediated cytotoxicity of malathion in this in-vitro model (Mostafalou et al., 2012b). [The Working Group noted the recognized limitations of using dichlorofluorescein as a marker of oxidative stress (Bonini et al. 2006; Kalyanaraman et al., 2012), and that the studies that reported this end-point as the sole evidence for oxidative stress should thus be interpreted with caution.]

Lu et al. (2012) treated PC12 adrenal gland cells with malathion (5–80 mg/L). The two higher concentrations (40 and 80 mg/L) were weakly cytotoxic (< 20% loss of cell viability); however, the oxidative stress end-points (2',7'-dichlorofluorescein diacetate fluorescence, amounts of malondialdehyde, and activity of catalase, glutathione peroxidase, and superoxide dismutase) were significantly elevated at concentrations of > 20 mg/L. Pre-treatment with vitamin E (600 μM) caused significant attenuation in cytotoxicity, and elevation in oxidative-stress markers, also indicating a probable relationship between the two. Finally, Ojha & Srivastava (2014) exposed peripheral blood lymphocytes from male Wistar rats to malathion (0.25–1.3 mg/L) for up to 4 hours, and measured production of superoxide anion and hydrogen peroxide. At the concentrations tested, cytotoxicity ranged from 20% to 30%, and production of superoxide and hydrogen peroxide was significantly elevated by 20–100% compared with untreated cells.

In primary thymocytes from male C57BL/6 mice, malathion (37.5–300 μM) increased production of superoxide anion and hydrogen peroxide within 5–15 minutes (Olgun & Misra, 2006). There was no effect on the activity of superoxide dismutase, catalase, glutathione peroxidase, or glutathione reductase 12 hours after treatment.

(iii) Non-mammalian experimental systems

Several studies investigated whether malathion causes oxidative stress in wildlife toxicity models. Positive associations between exposure to malathion and oxidative-stress parameters were reported in cyanobacteria (Ningthoujam et al., 2013), insects (Büyükgüzel, 2006; Velki et al., 2011; Wu et al., 2011), amphibians (Ferrari et al., 2008), and fish (Rodríguez-Ariza et al., 1999; Rosety et al., 2005; Huculeci et al., 2009; Patil & David 2013; Yonar et al., 2014).

(b) Inflammation

No data in humans were available to the Working Group.

In male Wistar rats, malathion (200 mg/kg bw per day by oral intubation for 28 days) caused significant elevation in levels of serum markers of liver injury, and an increase in the number of leukocytes, monocytes, lymphocytes, and neutrophils in circulating blood (Lasram et al., 2014b). [While the Working Group agreed with the authors' conclusion that this study demonstrated that malathion promotes liver inflammation under these conditions, no histopathological examination of the tissues was conducted to corroborate the haematological parameters assessed in this study]. In a separate histopathological analysis of male Wistar rats, histological signs indicative of inflammatory and necrotic degenerative changes in the liver and kidney were reported after malathion given as a single dose (687.5 mg/kg bw, by gavage; evaluated 24, 48, or 72 hours after dosing) or repeated doses (23 mg/kg bw per day, by gavage for 60 days) (Ojha et al., 2013).

(c) Immunosuppression

Immunotoxicity of pesticides, including malathion, has been reviewed by Pruett (1992) and Galloway & Handy (2003).

(i) Humans

Several studies on occupational exposure to malathion have observed effects on the immune system. Milby & Epstein (1964) reported allergic contact dermatitis after exposure to malathion. Hypersensitivity reactions of the skin were also reported by Schanker et al. (1992) in a survey of 1874 reports of illness in workers applying malathion to crops in southern California, USA. These included 47 reports of urticaria, 38 reports of angioedema, and 213 reports of a nonspecific skin rash, but it was not possible to confirm that these cases were attributable to malathion.

In an in-vitro study, Xiong & Rodgers (1997) found that malathion and its metabolites can cause rapid release of histamine by cultured human peripheral blood basophils (but not cutaneous mast cells).

(ii) Non-human mammalian experimental systems

In vivo

Studies of hypersensitivity have demonstrated that malathion can cause histamine release and mast-cell degranulation in mice or rats exposed orally or dermally. For example, Rodgers & Xiong (1997a) showed that oral administration of malathion (dose range, 10–700 mg/kg bw) to mice or rats increased the level of serum histamine by 4 and 8 hours after administration. After application of malathion to the skin of mice or rats, the level of histamine in the blood was also increased. In female C57BL/6 mice, oral administration of malathion (dose range, 0.1–10 mg/kg bw per day) for 90 days resulted in degranulation of mast cells from the skin and peritoneum at a

dose of 1.0 mg/kg bw per day or greater (Rodgers & Xiong, 1997b). In the uterus, the percentage of mast cells that were undegranulated was decreased and the number of severely degranulated cells was increased at a dose of 0.1 mg/kg bw per day or greater. Similar effects were reported by Rodgers & Xiong (1997c) in a 90-day study in female C57BL/6 mice treated with malathion at an identical dose range by gavage.

Pathological effects of malathion on the spleen have been reported. Baconi et al. (2013) found that repeated doses of malathion (85 mg/kg bw per day, by gavage for 35 days) increased the number of mononuclear cells by weight in the spleen of Wistar rats. In the study reported above, Ojha et al. (2013) found histological signs indicative of degenerative changes in the spleen of male Wistar rats treated by gavage with malathion either as single or repeated doses. Rodgers (1997) showed that a single dose of malathion (300 mg/kg bw) to MRL-lpr mice (age, 6 weeks) resulted in elevated basal and mitogen-induced proliferation of splenocytes. Increased spleen weight was observed in males at the two higher doses in the long-term study conducted by the EPA (1996) in rats. Atrophy and depletion in splenic lymphoid follicles was seen at the two higher doses in males and females. At the same time, long-term studies conducted by the National Cancer Institute (NCI) did not find increases in non-neoplastic pathology in the spleen of mice or rats treated with malathion (NTP, 1978, 1979a), or malaoxon (NTP, 1979b), for 2 years.

Suppression of the humoral immune response has been reported when malathion was administered at doses that caused inhibition of acetylcholinesterase activity. Casale et al. (1983) showed that immunoglobulin IgG and IgM responses were suppressed in male C57BL/6 mice given a single oral dose of malathion (720 mg/kg bw) at 2 days after immunization with sheep erythrocytes. However, at a lower dose of malathion (240 mg/kg bw per day) administered four times over 8 days, no such effect was observed. Banerjee et al. (1998) reported that in rats and mice treated with repeated doses of malathion, there was suppression of the humoral immune response (serum IgM and IgG concentrations, and antibody titre against antigens and splenic plaque-forming cells). In BALB/c mice, no significant effect on the humoral immune response was found using an enzyme-linked immunosorbent assay (ELISA) to quantify production of antibodies to sheep erythrocytes after a single oral dose of a 2% or 8% water solution of malathion (Relford et al., 1989). In mast cell-deficient mice, Rodgers et al. (1996) showed that a single gavage dose of malathion (600 mg/kg bw) suppressed the generation of IgM and IgG antibodies to sheep erythrocytes on days 3 and 5 after immunization, but did not affect macrophage function. In male and female rats, a single subcutaneous dose of malathion (100 mg/kg bw) significantly decreased the humoral immune response defined as IgM-type (estimated from the number of antibody-producing cells in the spleen) by 4 days after dosing (Zabrodskii et al., 2008). The IgG-type response (estimated from the number of antibody-producing cells in the spleen) was also significantly decreased by 13 days after dosing.

Studies on the cell-mediated immune response showed adverse effects with malathion. Banerjee et al. (1998) reported that short-term treatment of rats and mice with malathion suppressed cell-mediated immunity (marked inhibition of leukocyte and macrophage migration). In BALB/c mice given a single oral dose of a 2% or 8% water solution of malathion, Relford et al. (1989) reported no significant effect on the cellular immune response by exposure of lympcytes to mitogens. In male and female rats given a single subcutaneous dose of malathion (100 mg/kg bw), blood concentrations of IFN-γ and IL-4 (interpreted as an indication of Th1 and Th2 function) were significantly decreased (Zabrodskii et al., 2008).

In vitro

Malathion and its metabolites stimulated rapid histamine release in cultured rat basophilic leukaemia (RBL-1) cells and rat peritoneal mast cells (Xiong & Rodgers, 1997). Direct suppression of nitrite production and inhibition of lipopolysaccharide-induced TNF-α production were observed in primary rat peritoneal macrophages treated with malathion (5, 10, or 20 µg/mL for 24 hours) (Ayub et al., 2003). As noted below, malathion is cytotoxic at concentrations of 75 µM and above to primary C57BL/6 mouse thymocytes (Olgun et al., 2004).

(iii) Non-mammalian experimental systems

Several studies investigated whether malathion causes immunotoxicity in wildlife toxicity models. Positive associations between exposure to malathion and various immunotoxic effects were observed in birds (Day et al., 1995; Nain et al., 2011), fish (Khalaf-Allah, 1999; Munshi et al., 1999; Yonar, 2013), and amphibians (Rumschlag et al., 2014).

4.2.4 Cell proliferation and death

(a) Thyroid gland

No data in humans were available to the Working Group.

In experiments in Osborne-Mendel rats given diets containing malathion, hyperplasia was observed in follicular and C-cells of the thyroid gland (NTP, 1978). A diffuse increase in the number of interfollicular cells was also observed in one or both lobes, with the cells positioned around and between thyroid follicles, seemingly encroaching on them and reducing their size. The follicular hyperplasia, detected microscopically, was described as unilateral and focal, with one or two foci consisting of several follicle of varying size occurring within the same lobe. [The nature of the C-cell hyperplasia was not described]. In an experiment in male and female Fischer rats given diets containing malaoxon for 103 weeks (NTP, 1979b), C-cell hyperplasia was significantly increased in each treatment group ($P \leq 0.025$, Fisher exact test) and in a dose-related fashion ($P < 0.0001$, by trend); however, a blinded re-evaluation of the histopathology by the National Toxicology Program (NTP) found that these results were not statistically significant (Huff et al., 1985). The re-evaluation found that the incidence of C-cell tumours (adenomas and carcinomas combined) was significantly increased in males and females at the highest dose ($P < 0.05$, Fisher exact), and with a dose-related trend.

Although proliferative lesions of thyroid cells were not reported in male and female Fischer 344 rats given feed containing malathion (NTP, 1979a), hyperplasia of the parathyroid occurred in 46% (16/35) of male rats at the lowest dose, compared with 11% (4/37) of the matched controls ($P < 0.001$, Fisher exact test). The incidence of hyperplasia was not increased in male rats at the highest dose. The NCI report contained no discussion of this observation, other than noting the lesion as being "NOS," i.e. not otherwise specified.

In a study in teleost fish (*Channa punctatus* Bloch) exposed to a malathion-based formulation (malathion, 50%) at a concentration of 2 or 4 ppm in aquaria water for 6 months, follicular cell hyperplasia of the pharyngeal thyroid and the complete degeneration of some follicles were reported. In subgroups of exposed fish or controls injected with radioiodine tracer, thyroid uptake of iodine decreased in a dose-dependent fashion. In contrast to controls, fish exposed to the malathion-based formulation had enlarged thyrotrophs, with large nuclei and vacuolation, indicative of thyroid dysfunction (Ram et al., 1989).

(b) Liver

No data in humans were available to the Working Group.

In experiments in male and female mice, macroscopic observations showed that liver mass, foci and nodules increased with dose, and were significantly elevated at 8000 and 16 000 ppm compared with controls (EPA, 1994). [Histological details were not available to the Working Group.]

In an assay in rats involving initiation by diethylnitrosamine followed by partial hepatectomy, exposure to malathion increased the number and size of foci that were positive for glutathione *S*-transferase placental form (GST-P) (Hoshiya et al., 1993). [Kinetic data to characterize proliferation and apoptosis rates were not collected.]

(c) Mammary gland

No studies in exposed humans were available to the Working Group.

In an in-vitro study using a human breast epithelial cell line (MCF-10F), Calaf & Roy (2008) reported an increased rate of proliferation in cells treated with malathion (100 ng/L) when compared with controls. Malathion was also associated with changes in the expression, mostly upregulation, of 44 of the 96 human cell-cycle genes involved in cell proliferation and metastasis in an array analysis (Human Cancer Microarray by Superarray).

In Sprague-Dawley rats (age, either 21 or 39 days), the growth of mammary-gland structures was evaluated following malathion by subcutaneous injection for 5 days (Cabello et al., 2001). The rats were killed 16 hours after the last injection, and whole mounts were made of mammary glands from the left side. In the mounts of rats exposed from age 21 days, malathion appeared to have no effect on terminal end bud (TEB) or alveolar bud (AB) density. In rats exposed from age 39 days (a period when active differentiation of TEBs into ABs normally occurs), the TEB density in rats treated with malathion was roughly four times that in the control animals (11.26 ± 0.48 versus 3.30 ± 0.27 TEBs/mm^2), and one ninth of the density of ABs (2.50 ± 0.56 versus 20.80 ± 1.68 ABs/mm^2). In contrast, in rats treated with malathion and the anticholinergic drug atropine, TEB or AB density did not differ significantly from that in controls. Histological examination of mammary glands excised from the right side showed a significant ($P < 0.05$) increase in the size of TEBs and the number of epithelial layers in malathion-treated rats, compared with controls.

In another set of experiments reported in three articles, female Sprague-Dawley rats (age 39 days) were exposed to malathion, and killed the rats at 30, 120, or 240 days after the last injection (Calaf & Garrido, 2011). Malathion inhibited normal differentiation and increased the proliferation of TEB epithelial cells. With time, the density of TEB decreased and the ducts markedly increased in size and cell number (per mm^2). The increase in number of these proliferating ducts was higher in rats treated with malathion than in rats co-treated with estrogen, estrogen alone, or the vehicle alone. Calaf & Echiburú-Chau (2012) reported increased protein expression of genes involved in cell proliferation (*c-myc*, *c-fos*) and tumour suppression (*p53*) in these female Sprague-Dawley rats. The rats exposed to malathion in this experiment were also reported to have an increased incidence of proliferative lesions of the lung (Calaf & Echiburú-Chau, 2012) and kidney (Alfaro-Lira et al., 2012).

(d) Haematopoietic cells

No data in humans were available to the Working Group.

In long-term studies in male Sprague-Dawley rats exposed to diets containing malathion, the incidence of reticuloendothelial hyperplasia increased with dose ($P < 0.05$, trend) and was elevated in rats given malathion at a dietary concentration of 5000 ppm (EPA, 1980). [The cell type of origin of mononuclear cell leukaemia observed in a study in Fischer 344 rats exposed to malathion (EPA, 2000b; described in Section

3.2.3) is thought to be reticuloendothelial (Abbott et al., 1983)]. The incidence of lymphoid hyperplasia was also significantly increased in the groups at 100 and 1000 ppm (P = 0.001, Fisher exact test). [The study reporting was very limited and further details on the lesions were not available to the Working Group.]

In an in-vitro study of C57BL/6 mouse thymocytes, malathion (37.5, 75, 150, or 300 µM) caused apoptotic and necrotic cell death in a dose-dependent fashion, with a significant response at all except the lowest dose (Olgun et al., 2004).

(e) Testis

No data in humans were available to the Working Group.

In juvenile rats given malathion at a dose of 20 mg/kg bw on postnatal days 4–24, the number of Sertoli and interstitial Leydig cells and A-spermatogonia per seminiferous tubular cross-section was reduced (Krause et al., 1975). In CF-1 mice (age, 10–12 weeks) injected intraperitoneally with malathion and killed 40 days after injection, epithelial height and tubular diameter were significantly reduced, indicative of tubule atrophy (Bustos-Obregón & González-Hormazabal, 2003). In NMRI-IVIC mice exposed intraperitoneally to malathion at a dose of 241 mg/kg bw, a decrease in the average diameter of seminiferous tubules was observed at days 8, 17, and 33 after injection when compared with control animals (Penna-Videau et al., 2012). This was accompanied by observations of increased percentage of seminiferous tubules with apoptotic cells and proliferation of the seminiferous epithelium.

In non-mammalian studies, malathion increased cell proliferation as measured by incorporation of bromodeoxyuridine in earthworm seminal vesicles (Espinoza-Navarro & Bustos-Obregón, 2005).

4.2.5 Other mechanisms

No data were available to the Working Group on the effects of malathion on DNA repair.

Few data were available on the effects of malathion on immortalization, genomic instability, and epigenetic alteration. Calaf et al. (2009) studied the effects of malathion alone (2 µg/mL) and in combination with 17β-estradiol (10^{-8} M) on a spontaneously immortalized human breast epithelial cell line (MCF-10F). In cells treated with malathion only, or malathion plus 17β-estradiol, there was positive, anchorage-independent growth, and formation of agar-positive clones; in contrast, cells treated with 17β-estradiol only, and control cells, were unable to form colonies. Cells treated with malathion only, or malathion plus estrogen, also exhibited invasive capacity (as measured by number of cells crossing a membrane), compared with untreated and 17β-estradiol-treated controls. In cells co-treated with malathion and 17β-estradiol, microsatellite instability was observed in markers for the *p53* tumour suppressor gene and for *c-Ha-ras*.

In genome-wide DNA methylation analyses in a human haematopoietic cell line (K562) exposed to malathion, Zhang et al. (2012) did not find an increased frequency of methylated gene-promoter CpG sites when compared with ethanol controls.

4.3 Data relevant to comparisons across agents and end-points

4.3.1 General description of the database

The analysis of the in-vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 112 (i.e. malathion, parathion, diazinon, and tetrachlorvinphos) was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) research programmes of the government of the USA

(Kavlock et al., 2012; Tice et al., 2013). At its meeting in 2014, the Advisory Group to the *IARC Monographs* programme encouraged inclusion of analysis of high-throughput and high-content data (including from curated government databases) (Straif et al., 2014.).

Diazinon, malathion, and parathion, as well as the oxon metabolites, malaoxon and diazoxon, are among the approximately 1000 chemicals tested across the full assay battery of the Tox21 and ToxCast research programmes as of 3 March 2015. This assay battery includes 342 assays, for which data on 821 assay end-points are publicly available on the web site of the ToxCast research programme (EPA, 2015a). Z-Tetrachlorvinphos (CAS No. 22 248-79-9; a structural isomer of tetrachlorvinphos), and the oxon metabolite of parathion, paraoxon, are among an additional 800 chemicals tested as part of an endocrine profiling effort using a subset of these assays. Glyphosate was not tested in any of the assays carried out by Tox21 or ToxCast research programmes.

Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is also publicly available (EPA, 2015b). It should be noted that the metabolic capacity of the cell-based assays is variable, and generally limited. [The Working Group noted that the limited activity of the oxon metabolites in in-vitro systems may be attributed to the high reactivity and short half-life of these compounds, hindering interpretation of the results of in-vitro assays.]

4.3.2 Aligning in-vitro assays to 10 "key characteristics" of known human carcinogens

To explore the bioactivity profiles of the agents being evaluated in *IARC Monographs* Volume 112 with respect to their potential impact on mechanisms of carcinogenesis, the Working Group first mapped the 821 available assay end-points in the ToxCast/Tox21 database to the key characteristics of known human carcinogens (IARC, 2014). Independent assignments were made by the Working Group members and *IARC Monographs* staff for each assay type to the one or more "key characteristics." The assignment was based on the biological target being probed by each assay. The consensus assignments comprised 263 assay end-points that mapped to 7 of the 10 "key characteristics" as shown below.

1. *Is electrophilic or can undergo metabolic activation (31 end-points)*: the 31 assay end-points that were mapped to this characteristic measure cytochrome p450 (CYP) inhibition (29 end-points) and aromatase inhibition (2 end-points). All 29 assays for CYP inhibition are cell-free. These assay end-points are not direct measures of electrophilicity or metabolic activation.
2. *Is genotoxic (9 end-points):* the only assay end-points that mapped to this characteristic measure TP53 activity. [The Working Group noted that while these assays are not direct measures of genotoxicity, they are an indicator of DNA damage.]
3. *Alters DNA repair or causes genomic instability (0 end-points)*: no assay end-points were mapped to this characteristic.
4. *Induces epigenetic alterations (11 end-points):* assay end-points mapped to this characteristic measure targets associated with DNA binding (4 end-points) and histone modification (7 end-points) (e.g. histone deacetylase, HDAC).
5. *Induces oxidative stress (18 end-points):* a diverse collection of assay end-points measure oxidative stress via cell imaging, and markers of oxidative stress (e.g. nuclear factor erythroid 2-related factor, NRF2). The 18 assay end-points that were mapped to this characteristic are in subcategories relating to metalloproteinase activity (5), oxidative stress (7), and oxidative-stress markers (6).

6. *Induces chronic inflammation* (45 end-points): the assay end-points that were mapped to this characteristic include inflammatory markers and are in subcategories of cell adhesion (14), cytokines (e.g. interleukin 8, IL8) (29), and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) activity (2).
7. *Is immunosuppressive (0 end-points):* no assay end-points were mapped to this characteristic.
8. *Modulates receptor-mediated effects (81 end-points):* a large and diverse collection of cell-free and cell-based nuclear and other receptor assays were mapped to this characteristic. The 81 assay end-points that were mapped to this characteristic are in subcategories of AhR (2), androgen receptor (11), estrogen receptor (18), farnesoid X receptor (FXR) (7), others (18), peroxisome proliferator-activated receptor (PPAR) (12), pregnane X receptor-vitamin D receptor (PXR-VDR) (7), and retinoic acid receptor (RAR) (6).
9. *Causes immortalization (0 end-points):* no assay end-points were mapped to this characteristic.
10. *Alters cell proliferation, cell death, or nutrient supply (68 end-points):* a collection of assay end-points was mapped to this characteristic in subcategories of cell cycle (16), cytotoxicity (41), mitochondrial toxicity (7), and cell proliferation (4).

Assay end-points were matched to a "key characteristic" to provide additional insights into the bioactivity profile of each chemical under evaluation with respect to their potential to interact with, or have an effect on, targets that may be associated with carcinogenesis. In addition, for each chemical, the results of the in-vitro assays that represent each "key characteristic" can be compared with the results for a larger compendium of substances with similar in-vitro data, so that particular chemical can be aligned with other chemicals with similar toxicological effects.

The Working Group then determined whether a chemical was "active" or "inactive" for each of the selected assay end-points. The decisions of the Working Group were based on raw data on the concentration–response relationship in the ToxCast database, using methods published previously (Sipes et al., 2013) and available online (EPA, 2015b). In the analysis by the Working Group, each "active" was given a value of 1, and each "inactive" was given a value of 0.

Next, to integrate the data across individual assay end-points into the cumulative score for each "key characteristic," the toxicological prioritization index (ToxPi) approach (Reif et al., 2010) and associated software (Reif et al., 2013) were used. In the analyses of the Working Group, the ToxPi score provides a measure of the potential for a chemical to be associated with a "key characteristic" relative to 178 other chemicals that have been previously evaluated by the *IARC Monographs* and that had been screened by ToxCast. Assay end-point data were available in ToxCast for these 178 chemicals, and not for other chemicals previously evaluated by the *IARC Monographs*. ToxPi is a dimensionless index score that integrates multiple different assay results and displays them visually. The overall score for a chemical takes into account the score for all other chemicals in the analysis. Different data are translated into ToxPi scores to derive slice-wise scores for all compounds as detailed below, and in the publications describing the approach and the associated software package (Reif et al., 2013). Within the individual slice, the values are normalized from 0 to 1 based on the range of responses across all chemicals that were included in the analysis by the Working Group.

The list of ToxCast/Tox21 assay end-points included in the analysis by the Working Group, description of the target and/or model system for each end-point (e.g. cell type, species, detection technology, etc.), their mapping to 7 of the 10

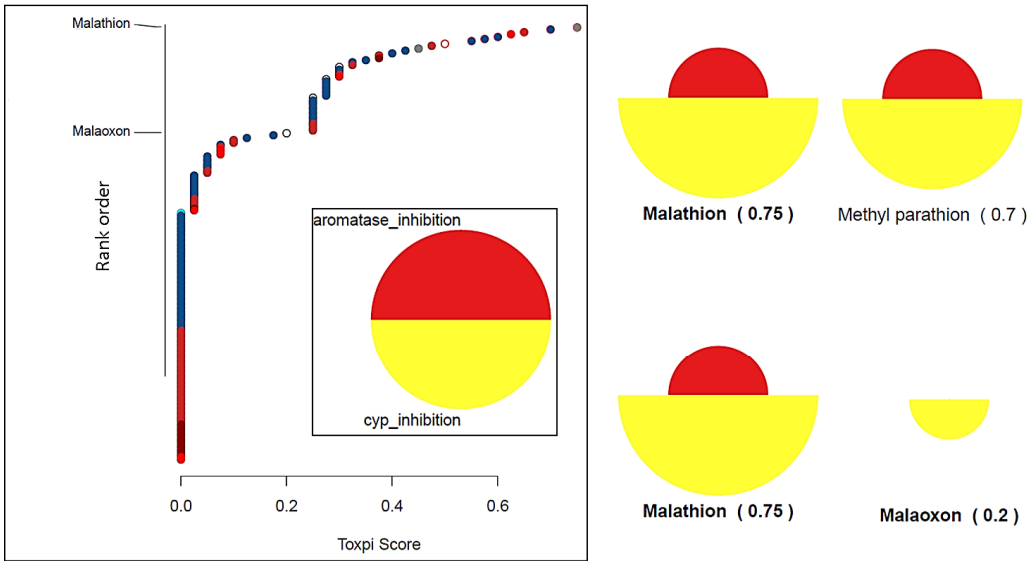
"key characteristics" of known human carcinogens, and the decision as to whether each chemical was "active" or "inactive" are available as supplemental material to *Monograph* Volume 112 (IARC, 2015). The output files generated for each "key characteristic" are also provided in the supplemental material, and can be opened using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

4.3.3 Specific effects across 7 of the 10 "key characteristics" based on data from high-throughput screening in vitro

The relative effects of malathion and malaoxon were compared with those of 178 chemicals selected from the more than 800 chemicals previously evaluated by the *IARC Monographs* and also screened by the ToxCast/Tox21 programmes, and with those of the other three compounds evaluated in the present volume of the *IARC Monographs* (Volume 112) and with three of their metabolites. Of these 178 chemicals previously evaluated by the *IARC Monographs* and screened in the ToxCast/Tox21 programmes, 8 are classified in Group 1 (*carcinogenic to humans*), 16 are in Group 2A (*probably carcinogenic to humans*), 58 are in Group 2B (*possibly carcinogenic to humans)*, 95 are in Group 3 (*not classifiable as to its carcinogenicity to humans*), and 1 is in Group 4 (*probably not carcinogenic to humans).* The results are presented as a rank order of all compounds in the analysis arranged in the order of their relative effect. The relative positions of malathion and malaoxon in the ranked list are also shown on the *y* axis. The inset in the scatter plot shows the components of the ToxPi chart as subcategories that comprise assay end-points in each characteristic, as well as their respective colour-coding. On the top part of the graph on the right-hand side, the two highest-ranked chemicals in each analysis are shown to represent the maximum ToxPi scores (with the scores in parentheses). At the bottom of the right-hand side, ToxPi images and scores (in parentheses) for malathion and malaoxon are shown.

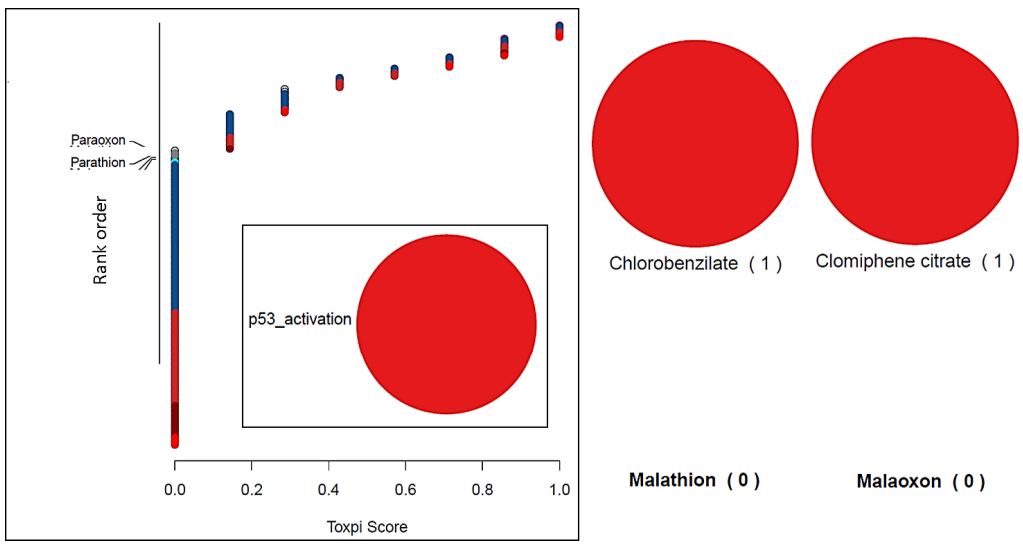
- Characteristic (1) *Is electrophilic or can undergo metabolic activation:* Malathion and malaoxon were tested for 31 assay end-points. Malathion was active for 20 of the 29 assay end-points related to CYP inhibition, and for 1 out of 2 assay end-points related to aromatase inhibition. Overall, malathion showed strong activity for this characteristic, being ranked highest of the 178 chemicals included in the comparison. Malaoxon demonstrated moderate CYP inhibition, being active for 7 of 29 assay end-points (Fig. 4.3).
- Characteristic (2) *Is genotoxic*: Malathion and malaoxon were inactive for all 9 assay end-points related to TP53 activity for which they were tested (Fig. 4.4).
- Characteristic (4) *Induces epigenetic alterations:* Malathion and malaoxon were tested for 11 assay end-points. Malathion showed activity for 1 out of 4 DNA-binding assay end-points. Malaoxon was inactive for all assay end-points. (Fig. 4.5)
- Characteristic (5) *Induces oxidative stress*: Malathion and malaoxon were tested for 18 assay end-points. Malathion was active for 3 out of 6 assay end-points relating to oxidative-stress markers, while malaoxon was active for 2 out of 6 of these end-points. Malathion and malaoxon exhibited intermediate activity for this characteristic relative to the 178 chemicals included in the comparison, the highest ranked chemicals being carbaryl and tannic acid (Fig. 4.6).
- Characteristic (6) *Induces chronic inflammation*: Malathion and malaoxon were tested for 45 assay end-points. Malathion showed no activity for any assay end-point. Malaoxon was ranked second of the 178 chemicals included in the comparison, largely on the

Fig. 4.3 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to metabolic activation



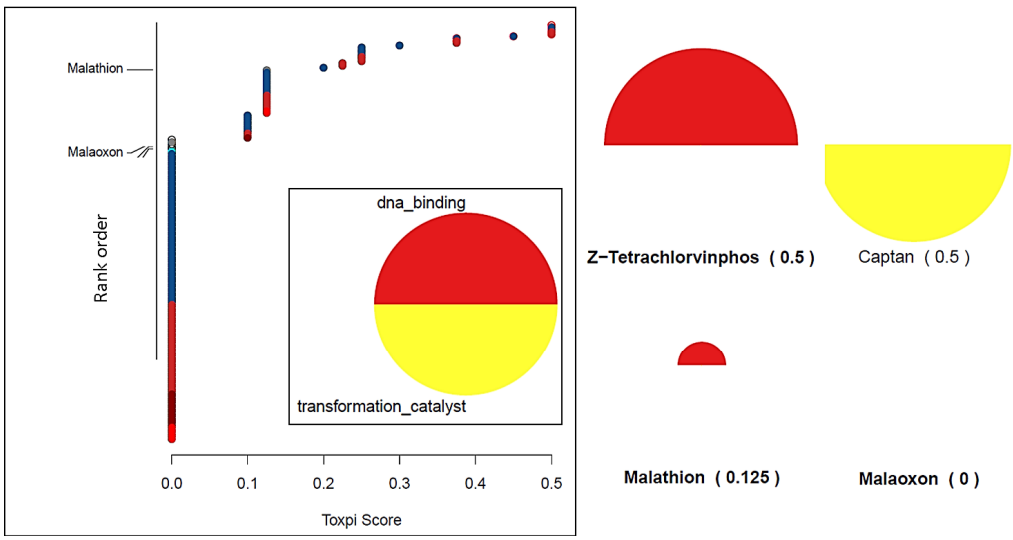
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis).The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, malathion and methyl parathion) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.4 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to genotoxic activity



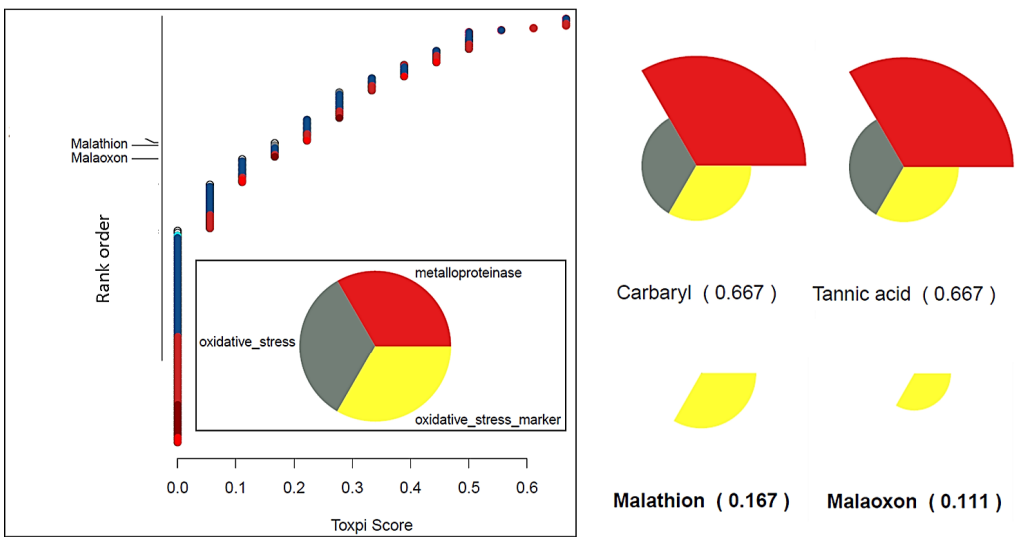
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, chlorobenzilate and clomiphene citrate) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.5 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to epigenetic alterations



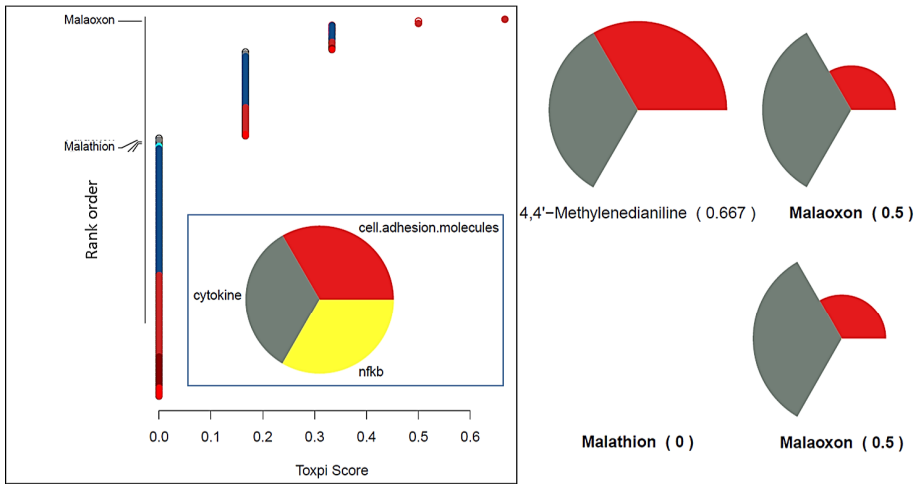
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi)score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, Z-tetrachlovinphos and captan) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.6 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to oxidative stress



On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their ToxPi score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, carbaryl and tannic acid) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.7 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to chronic inflammation



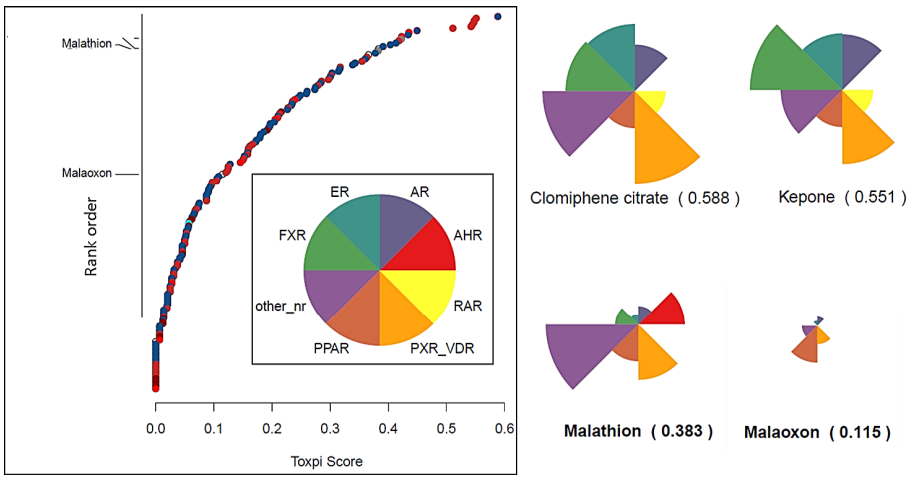
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their ToxPi score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, 4,4′-methylenedianiline and malaoxon) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

basis of its cytokine activity (active for 2 assay end-points) and cell-adhesion activity (active for 1 end-point). The highest ranked chemical in the comparison, 4,4′-methylenedianiline, was also only active for 2 out of 29 assay end-points relating to cytokine activity, and for 2 out of 14 assay end-points relating to cell-adhesion activity, demonstrating high selectivity in these assay end-points across this chemical set (Fig. 4.7).

- Characteristic (8) *Modulates receptor-mediated effects:* Malathion and malaoxon were tested for 81 assay end-points. Malathion was active for 17 assay end-points, while malaoxon was active for 6 assay end-points. Malathion was active for 3 assay end-points relating to the pregnane X receptor (PXR), and showed activity for other nuclear receptors, specifically the retinoid X receptor (RXR). Malaoxon was generally inactive for these assay end-points (Fig. 4.8).
- Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply:* Malathion was tested for all assay end-points; a single assay end-point was missing for malaoxon. Malathion and malaoxon both showed little to no activity (Fig. 4.9).

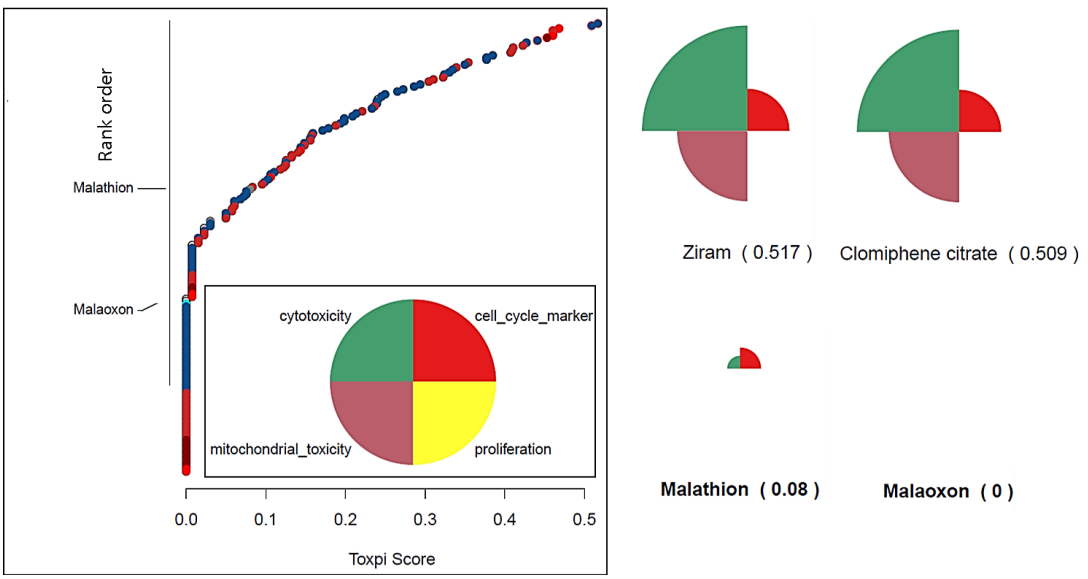
Overall, malathion demonstrated consistent activity in CYP inhibition and effects on nuclear receptors and related proteins, most notably PXR and AhR. Malaoxon showed a high ranking in activity related to chronic inflammation, but the assigned assay end-points were highly selective, with a maximum of 4 actives across all 45 assay end-points. Despite concerns about the stability of malaoxon in in-vitro systems, it was found to be active for several independent assay end-points, including in cell-free and cell-based assays.

Fig. 4.8 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to modulation of receptor-mediated effects



On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their ToxPi score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, clomiphene and kepone) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.9 ToxPi ranking for malathion, and its metabolite malaoxon, using ToxCast assay end-points mapped to cytotoxicity and cell proliferation



On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their ToxPi score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, ziram and clomiphene citrate) and the target chemicals (malathion and malaoxon) are shown with their respective ToxPi score in parentheses.

4.4 Susceptibility

Most studies of acute poisoning with malathion or other organophosphate pesticides have implicated polymorphism in metabolic enzymes as being responsible for inter-individual variability in effects (Buratti et al., 2005). [The Working Group noted that the relevance of these studies to cancer susceptibility in humans was uncertain].

A study described above in Section 2.3.1(b) evaluated single nucleotide polymorphism (SNP)–environment interactions between 30 confirmed prostate-cancer susceptibility loci and risk of cancer of the prostate associated with pesticide exposure (Koutros et al., 2013b). In men carrying two T alleles at rs2710647 SNP in Eps15 homology domain binding protein 1 (EHBP1), the risk of cancer of the prostate in men with high use of malathion was 3.43 times greater than in men with no use (95% CI, 1.44–8.15) (*P*-value for interaction, 0.003).

4.5 Other adverse effects

4.5.1 Humans

Limited epidemiological data on adverse effects other than cancer were available for malathion. A control-matched study on the latent effects of poisoning with organophosphate pesticides examined 100 matched pairs, including six cases of acute poisoning attributed to malathion (Savage et al., 1988). The study found no significant differences across several audiometric tests, ophthalmic tests, electroencephalograms, or clinical serum and blood chemistry evaluations, but did observe abnormalities in memory, abstraction, and mood among other neurological impairments. Accidental acute exposure to malathion and other organophosphate pesticides has been associated with severe aplastic anaemia in children, resulting in death (Reeves et al., 1981).

Malathion was found to be a weak contact sensitizer, inducing mild cutaneous reaction in high proportion of subjects (Gosselin et al., 1984). In another study in adult volunteers, malathion was found to have a relatively low acute toxicity, as indicated by the fact that a daily oral dosage of 24 mg given for more than 14 days was necessary to lower blood cholinesterase activity (Moeller & Rider, 1962; IARC, 1983). In an experiment in which four men were exposed to malathion at 84.8 mg/m^3 for 1 hour per day, for 42 days, moderate irritation of the nose and conjunctiva was observed, but there were no clinical signs or symptoms of inhibition of cholinesterase activity (NIOSH/OSHA, 1976).

4.5.2 Experimental systems

Malathion was tested in ten regulatory toxicity submissions included in the Toxicity Reference Database (ToxRefDB) and reviewed by the EPA (2015c). Specifically, study design, treatment group, and treatment-related effect information were captured for four long-term studies of toxicity or carcinogenicity, two studies of developmental toxicity, one multigenerational study of reproductive toxicity, and three studies of developmental neurotoxicity. Malathion and its metabolite, malaoxon, were tested in several strains of rats and mice in multiple bioassays by the National Cancer Institute (NCI) (NTP, 1978, 1979a, b). [The Working Group noted that although long-term studies on malathion were available, the ability to determine a full range of adverse effect potential is heavily confounded by sensitivity to the cholinergic effects of malathion, which limits the available dosing range.]

Cholinergic effects were observed in numerous studies, and included inhibition of plasma, erythrocyte, and brain cholinesterase activity at doses as low as 5 mg/kg bw per day (EPA, 1989, 1990a, 1994, 1996, 2000a, b, 2002a). Corresponding clinical signs were also observed at doses as low as 50 mg/kg bw per day, and

included increased salivation, abnormal gait, tremors, and reduced activity.

In liver, a long-term study in rats given malathion at a dose of 0, 4, 29, 359, or 739 mg/kg bw per day (males) and 0, 5, 35, 415, or 868 mg/kg bw per day (females) reported congestion and spongiosis hepatis at the two higher doses, with accompanying liver-weight increases at the highest dose (EPA 1996, 2000a). Fatty metamorphosis in the liver was also observed in female F344 rats exposed to malathion for 2 years (NTP, 1979a). In study of carcinogenicity in mice given malathion at a dose of 0, 17.4, 143, 1476, or 2978 mg/kg bw per day (males) and 0, 20.8, 167, 1707, or 3448 mg/kg bw per day (females), there were increases in liver weights and in the incidence of hypertrophy in males and females at the two higher doses. Foci and increased liver mass were also observed grossly in mice at the highest dose (EPA, 1994).

In the kidney, a long-term rat study reported inflammation in females at doses of 29 mg/kg bw per day and above, and in males at 359 mg/kg bw per day and above (EPA, 1996). Congestion, nephropathy, and irregular surface, as well as increases in kidney weights, were observed in males and females at the two higher doses. In a long-term study in mice, decreased renal tubule vacuolation was observed in males at 1476 or 2978 mg/kg bw per day, and increased mineralization was observed in females at 1707 or 3448 mg/kg bw per day (EPA, 1994).

Increased spleen weight was observed in males at the two higher doses of a long-term rat study (EPA, 1996). Atrophy and depletion in splenic lymphoid follicles was seen at the two higher doses in males and females. On the other hand, separate studies did not report effects in the spleen of mice or rats treated with malathion (NTP, 1978, 1979a), or malaoxon (NTP, 1979b), for 2 years.

In the forestomach, a long-term rat study reported congestion, oedema, hyperkeratosis, squamous and basal cell hyperplasia, inflammation and ulcers at the two higher doses in males and females the (EPA 1996). Similar findings of chronic inflammation and ulcers of the stomach were observed in F344 rats exposed to both malathion and its metabolite, malaoxon (NTP, 1979a, b).

In the testis, a long-term rat study reported atrophy, degeneration, oligospermia and arrested maturation at the highest dose, but only at the interim kill (EPA, 1996). Evidence for testicular toxicity also came from a study in which male rats were exposed to malathion at a dose of 0 or 27 mg/kg bw per day for 4 weeks, or to a combination of malathion with vitamins C and E (Uzun et al., 2009). Significantly lower sperm counts and motility and higher rates of abnormal sperm were observed across the treated groups compared with the untreated control group, with protective effects observed after co-treatment with vitamins C and E. Levels of follicle-stimulating hormone, luteinizing hormone, and testosterone were altered with and without co-treatment, and there were pathological changes to the seminiferous and interstitial tissues.

In the thyroid, a long-term study in rats reported congestion in males at the two intermediate doses, and in males and females at the highest dose, while cysts of the thyroid gland were observed in males and females at the highest dose. Thyroid weights were increased in males at 29, 359, or 739 mg/kg bw per day, but decreased in females at 415, or 868 mg/kg bw per day (EPA, 1996, 2000a). In the same study, increased vacuolization of the adrenal gland was reported in males at 359 or 739 mg/kg bw per day, while females at 415 or 868 mg/kg bw per day experienced early disappearance of the X-zone of the adrenal cortex EPA (1996).

Regarding other organs, a long-term rat study also reported parathyroid hyperplasia all doses, accompanied by increased parathyroid weights in males at the two higher doses (EPA, 1996). Sternal and femoral bone-marrow congestion was observed in males at 29 mg/kg bw per

day, and in males at 359, or 739 mg/kg bw per day and females at 415, or 868 mg/kg bw per day. In the lung, increased congestion was reported in males and females at the highest dose, and collapsed alveoli were observed in males at the two higher doses. In brain, congestion was increased in males at 29, 359, and 739 mg/kg bw per day, and in females at the highest dose. Pituitary glands were congested in males at 359 mg/kg bw per day, and in males and females at the highest dose. Depletion and atrophy of the mediastinal lymph nodes were observed in males at 29, 359, and 739 mg/kg bw per day, and in the mesenteric lymph nodes of males at the highest dose (EPA, 1996). Nasal hyperplasia, cysts, degeneration, dilation and inflammation were observed in males and females at the two higher doses. Unspecified lesions of the pharynx were observed in males and females at the two higher doses. Corneal mineralization and neutrophilic cellular infiltration of the eye were observed in males at 359 mg/kg bw per day, and in males and females at the highest dose. Lacrimal and Hardarian glands were congested for males and females at the two higher doses. Heart congestion was observed in males at 29 mg/kg bw per day, and in males and females at the two higher doses. In a study of carcinogenicity in mice given malathion at a dose of 0, 17.4, 143, 1476, or 2978 mg/kg bw per day (males) and 0, 20.8, 167, 1707, or 3448 mg/kg bw per day (females), fibrous osteodystrophy was observed in the femur and sternum of females at the two higher doses (EPA, 1994). Treatment with malathion at a dose of 0, 17, or 22 mg per 100 g bw, either alone or combined with estrogen, has also been associated with increased pathological proliferative responses in mammary-gland tissue, with effects ameliorated after treatment with atropine (an anticholinergic drug), suggesting that the cholinergic effects of malathion play a role in toxicity at the mammary gland (Cabello et al., 2001; Calaf & Echiburú-Chau, 2012).

Developmental and reproductive toxicity

In a two-generation study of reproductive toxicity in rats given malathion at a dose of 0, 51, 153, 451, or 703 mg/kg bw per day (males) and 0, 43, 131, 394, or 612 mg/kg bw per day (females), offspring weights were reduced at the two higher doses in males and females in multiple generations (EPA, 1990a). In a study of developmental toxicity in rabbits dosed given malathion at a dose of 25, 50, or 100 mg/kg bw per day, increased resorptions were observed in the maternal groups at the two higher doses (EPA, 1985). In a study of developmental neurotoxicity in rats given malathion at a dose of 5, 50 or 150 mg/kg bw per day, renal dilation and vacuolation in addition to hydronephrosis were observed in male offspring at the highest dose (EPA, 2002b). Increased thickness of the corpus callosum was also observed in males and females at the highest dose. Auditory reflexes were reduced at all doses in males and females. Decreased vertical rearing and horizontal locomotion were observed in females at the two higher doses.

5. Summary of Data Reported

5.1 Exposure data

Malathion is a non-systemic broad-spectrum organophosphate insecticide, which was first commercialized in the 1950s, and continues to be produced and used in substantial volumes in many countries. It is used for the control of insect pests of crops, pastures, and rangeland, in residential areas, for control of ectoparasites on animals, and in pest-eradication programmes. It is also used for disease-vector control, and as a pharmaceutical preparation to treat lice on humans.

Occupational exposure to malathion has been measured in farm and greenhouse workers and in pest- and vector-control workers. Dermal

contact has been found to be the most important route of occupational exposure. The general population may be exposed to malathion through residues in food, residence near sprayed areas, and home use of products containing malathion; however, measured concentrations of malathion in environmental media appear to be low. Urinary concentrations of the metabolite malathion dicarboxylic acid are generally below 1 μg/g creatinine in the general population.

5.2 Human carcinogenicity data

Since the last evaluation of malathion by the Working Group in 1987, several studies have been published on the association between malathion and cancer. Several studies provided useful information; in particular, one cohort study (the Agricultural Health Study, exploring 11 cancer sites in adults and childhood cancer) and two case–control studies nested in occupational cohorts (cancer of the lung in the Florida Pest Control Workers cohort; cancers of the haematopoietic system and breast in the United Farm Workers of America cohort). Four independent case–control studies, three of them in adults (in the midwest USA, Canada, and Sweden) and one in children (Costa Rica) have also estimated the association between exposure to malathion and haematological malignancies. Three additional case–control studies explored other cancer sites: prostate (Canada), soft-tissue sarcomas (Canada), colorectum (USA) and glioma (USA).

In these epidemiological studies, positive associations were observed between exposure to malathion and cancer at several sites, but associations were most consistent for non-Hodgkin lymphoma (NHL) and cancer of the prostate.

5.2.1 NHL

A pooled analysis of three case–control studies, a nested case–control study and one cohort study provided information on the association between exposure to malathion and NHL. Some studies presented analyses by subtype, but none of the studies provided information on the grading of tumours.

Evidence initially came from a large pooled analysis of case–control studies (748 cases) performed in the 1980s in the midwest USA, which found a statistically significant association between NHL and ever exposure to malathion (odds ratio, OR, 1.6; 95% CI, 1.2–2.2), higher in small lymphocytic leukaemia (OR, 1.9; 95% CI, 0.8–4.7), when exposure started 20 years ago or more (OR, 1.7; 95% CI, 1.1–2.9), but with no clear trend with the number of days of use per year. The magnitude of the relative risks from proxies was larger (OR, 3.7; 95% CI, 2.0–7.1) than those from direct interviews (OR, 1.2; 95% CI, 0.9–1.6). When this analysis was adjusted for use of multiple pesticides in a subsample of the initial data set, no association remained (OR, 1.1; 95% CI, 0.6–1.8).

A twofold increased risk of non-Hodgkin lymphoma associated with exposure to malathion was also found in a large case–control study in Canada (1.8; 95% CI, 1.3–2.5; 517 cases). No clear trend with the number of days of use was observed in this study. A further analysis of the use of malathion paired with other pesticides (2,4-D, mecoprop, carbaryl, glyphosate, and DDT) demonstrated that an increased risk of non-Hodgkin lymphoma associated with exposure to malathion remained. A nearly threefold increase in risk of non-Hodgkin lymphoma (OR, 2.81; 95% CI, 0.54–14.7) was also observed in individuals ever exposed to malathion in a case–control study in Sweden (910 cases), but it was based only on five exposed cases.

The case–control analysis nested in the United Farm Workers of America cohort found a twofold increase in risk of non-Hodgkin lymphoma (OR, 1.77, 95% CI, 0.99–3.17) but the total number of cases was limited (60 cases).

In the Agricultural Health Study, an analysis of 523 incident cases (follow-up until 2011) did

not find an increase in the relative risk of total non-Hodgkin lymphoma for ever versus never use of malathion (OR, 0.9; 95% CI, 0.8–1.1). Analysis by histological subtype showed an association only for the follicular B-cell subtype (OR, 1.3; 95% CI, 0.7–2.4). No trend was observed with days of lifetime exposure, nor for intensity-weighted days of exposure.

The Working Group noted that four case–control analyses found excesses of non-Hodgkin lymphoma associated with exposure to malathion in the USA, Canada, and Sweden, but no association with number of days of use was observed. In the Cross-Canada Case–control Study, there was an association with malathion, but in a pooled analysis of case–control studies in the USA there was little evidence of an association. No excess occurred in the Agricultural Health Study cohort.

5.2.2 Other haematological malignancies

For leukaemia in adults, information came from the large cohort of the Agricultural Health Study, one case–control study of leukaemia, and one case–control study nested in the United Farm Workers of America study. The Agricultural Health Study did not find an association overall, but there was a small increase in the high-exposure category that was not statistically significant for exposure to malathion and risk of leukaemia. The United Farm Workers of America study found a moderate increased risk, which was more pronounced in highly exposed participants and statistically significant when the analyses were restricted to women. The case–control study found no association for use of malathion on crops or on animals. For childhood leukaemia in Costa Rica, a positive association was found for paternal exposure to malathion before conception, but only in boys. A case–control study on multiple myeloma found no excess associated with use of malathion on animals, and a twofold excess for use of malathion on crops. Analysis on multiple myeloma in the Agricultural Health Study cohort did not demonstrate elevated risks associated with exposures to malathion. No association was found for Hodgkin lymphoma in the Cross-Canada Case–control Study.

5.2.3 Cancer of the breast

Two studies that reported on malathion and risk of cancer of the breast in women provided inconsistent results. The study nested in the United Farm Workers of America cohort found an increase in risk of cancer of the breast associated with exposure to malathion, but there was no clear exposure–response relationship. The Working Group noted that, in the Agricultural Health Study, no elevation in risk was observed when considering the wife's use of malathion, while a statistically significant increase was observed when considering the husband's use of malathion (OR, 1.4; 95% CI, 1.0–2.0), without an apparent exposure–response trend.

5.2.4 Cancer of the prostate

Two studies showed some evidence of an association between use of malathion and risk of cancer of the prostate. In a case–control study conducted in British Columbia, Canada, a significant excess risk for ever use of malathion was observed, with an exposure–response relationship. In this study, the exposure was assessed using a job-exposure matrix, which in this case limited the possibility of disentangling the effects of multiple pesticides.

In the Agricultural Health Study, no increase in the risk of cancer of the prostate was associated with lifetime exposure days for malathion, but a statistically significant trend was observed for aggressive cancers of the prostate after adjustment for some other pesticides; a complementary analysis from the Agricultural Health Study found a significant interaction between several

genetic polymorphisms related to susceptibility to cancer of the prostate and use of malathion.

The Working Group noted these findings on aggressive tumours of the prostate. Aggressive tumours are less prone to screening bias, but this is unlikely to have caused the difference in relative risk for aggressive and non-aggressive tumours. Furthermore, aggressive cancers are a disease entity that are better specified, more accurately separating cases and non-cases in the population, and are therefore less likely to be misclassified. Because cancer of the prostate is relatively common and can be asymptomatic, non-aggressive tumours of the prostate may be undiagnosed in subjects in the referent group, making total prostate cancers more prone to misclassification.

5.2.5 Cancer of the lung

Cancer of the lung was evaluated in two cohort studies. No excess was observed in the Agricultural Health Study cohort, while the other study observed an excess using deceased, but not living, controls.

5.2.6 Other cancers

No positive association was observed for other cancer sites studied: soft tissue sarcoma, glioma, colorectum, melanoma, bladder, or kidney, but only one study was available for each site.

5.3 Animal carcinogenicity data

Malathion was tested for carcinogenicity in two feeding studies in male and female mice and four feeding studies in male and female rats. In addition, a study in female rats examined the effect of subcutaneous injections of malathion during morphogenesis of the mammary gland. Malaoxon, a metabolite of malathion, was tested for carcinogenicity in one feeding study in male and female mice and two feeding studies in male and female rats.

Two feeding studies with malathion in male and female mice were reviewed. In the first study, malathion induced an increase in the incidence of hepatocellular adenoma with a significant positive trend in male mice. No significant increase in tumour incidence was reported in female mice. In the second study, a significant increase in the incidence of hepatocellular adenoma, with positive trends in males and females, and of hepatocellular adenoma or carcinoma (combined) with a positive trend in males was reported; however, there was no significant increase in the incidence of hepatocellular carcinoma only in any of the treated groups.

Four feeding studies on malathion in male and female rats were reviewed. In the first and second studies, no treatment-related tumours were reported in males or females. In the third study, two very rare tumours of the nasal pharyngeal cavity were identified in male rats; in addition, a rare tumour of the oral cavity was identified in two female rats. In female rats, the incidences of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased with a positive trend. In the fourth study, significant increases in the incidences of fibroadenomas of the mammary gland and of uterine polyps were noted in female rats; no significant increase in the incidence of treatment-related tumours was reported in males.

Subcutaneous injection of female rats with malathion during the period of ductal morphogenesis of the mammary gland resulted in a significant increase in the incidence of adenocarcinoma of the mammary gland.

Malaoxon was evaluated for carcinogenicity in male and female mice in one feeding study; no treatment-related tumours were reported.

Two feeding studies evaluated malaoxon in male and female rats. A significant increase in the incidence of thyroid gland C-cell adenoma or

carcinoma (combined) with a positive trend was reported in male and female rats in one study. In the second study in rats, there was an increase in the incidence of mononuclear cell leukaemia with a positive trend in males. This result may have been treatment related. No significant increase in tumour incidence was reported in females.

5.4 Mechanistic and other relevant data

Malathion is rapidly absorbed after oral exposure in humans and rodents, whereas absorption via the dermal route is less efficient. In humans, data are limited as to the amount of malathion that can be inhaled and absorbed. After absorption in humans, malathion is distributed systemically and residues are detected in the lungs, liver, kidneys, spleen, brain, heart, blood, muscles, urine, and gastric contents. Malathion is rapidly metabolized in humans and experimental animals due to the presence of two carboxylic acid ethyl ester moieties that are hydrolytically labile. Most of the metabolite excreted in urine is malathion monocarboxylic acid (MMA), which is the hydrolytic product of the reaction catalysed by carboxylesterases.

Malathion is not electrophilic, but its bioactive metabolite, malaoxon, can covalently modify B-esterases specifically at the active site serine residue; however, it is unknown whether electrophilicity of malaoxon plays a role in carcinogenesis.

The overall evidence for genotoxicity of malathion is strong. The potential for malathion to exert genotoxicity has been studied in a variety of assays and model systems. Various types of genotoxic damage have been evaluated in humans exposed to mixtures of pesticides containing malathion in occupational settings, and in cases of acute intoxication with malathion-containing formulations. The effects observed range from DNA damage to various types of chromosomal damage including micronucleus formation, chromosomal aberrations, and sister-chromatid exchanges. The majority of studies reported positive results that were consistent in terms of the types of end-point observed. These results in studies in humans are corroborated by multiple positive in studies in experimental animals in vivo, and in human and animal cells in vitro. The findings in standard tests for genotoxicity in bacteria were negative.

The overall evidence for receptor-mediated effects of malathion is strong. There is compelling evidence for the activity of malathion on thyroid-hormone receptor-mediated pathways. The evidence for this activity comes from studies in experimental animals in vivo, and some supporting studies in human and rodent cells in vitro. In addition, there is evidence for the disruption of sex hormones, primarily for the androgen pathway, from studies in rodents in vivo and studies in fish. In addition, malathion, primarily through the metabolism to malaoxon, is a strong inhibitor of several esterases. This effect causes neurotoxicity through the inhibition of acetylcholinesterase. This activity may be related to the cancer hazard of malathion because co-administration of atropine ameliorated carcinogenesis-related effects of malathion in one study.

There is strong evidence that malathion can induce oxidative stress. The database is rich and includes one study in humans in vivo (acute poisoning cases), multiple studies in rodents that showed oxidative stress in multiple organs, and for many target organs there are numerous studies replicating the findings. A large number of oxidative stress end-points has been evaluated and in some studies this mechanism was challenged experimentally by testing the protective action of antioxidants. The evidence for the ability of malathion to induce inflammation is strong. Inflammatory effects of exposures were demonstrated in several studies in rodents in vivo across several exposure scenarios.

The evidence for immunosuppression as an effect of exposure to malathion is moderate. Depending on the exposure dose and model system, many immunosuppressive effects have been observed in mammals and wildlife species. It has also been observed in most experimental models that acute exposure to malathion results in immunosuppression, while low doses may result in enhanced immune system activity.

There is strong evidence that cell proliferation is induced by malathion in the thyroid and mammary gland. This is likely a result of the hormonal effects that are not associated with cytotoxicity.

There were not enough data for evaluation of the other key characteristics of human carcinogens.

Several studies reported pathological non-cancer observations in various tissues after exposure to malathion. In humans, accidental exposure to malathion caused severe aplastic anaemia in children. In studies in rodents, in addition to cholinergic effects, malathion also caused non-neoplastic and pre-neoplastic lesions confirming liver as a target site of malathion. Malathion was also shown to cause a wide variety of organ-weight changes and pathological lesions, including in the thyroid gland, adrenal gland, spleen, stomach, lung, brain, testis, kidney, and mammary gland.

The evidence for cancer-related susceptibility to malathion is weak. While the metabolizing enzymes are known to be highly polymorphic, the relevance of these polymorphisms to cancer hazard of malathion is unknown. One study reported that a polymorphism in EH domain binding protein 1 (EHBP1) is associated with the risk of cancer of the prostate in the individuals with high use of malathion.

Overall, the mechanistic data provide strong support for carcinogenicity findings of malathion. This includes strong evidence for genotoxicity, hormone-mediated effects, oxidative stress, and cell proliferation. There is evidence that these effects can operate in humans.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of malathion. Positive associations have been observed with non-Hodgkin lymphoma and cancer of the prostate.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of malathion.

6.3 Overall evaluation

Malathion is *probably carcinogenic to humans (Group 2A)*.

6.4 Rationale

In making this overall evaluation, the Working Group noted that the mechanistic and other relevant data support the classification of malathion in Group 2A. There is strong evidence that malathion can operate through several key characteristics of human carcinogens, and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to malathion-based pesticides is genotoxic based on studies in humans, in experimental animals, and in human and animal cells in vitro. Assays for mutagenesis in bacteria gave negative results, indicating no direct pro-mutagenic activity.
- There is strong evidence that malathion modulates receptor-mediated effects and pathways relevant to tumour findings in the hormone-responsive tissues, the thyroid, and

mammary gland. There is concordant strong evidence for alteration of cell proliferation in response to malathion in these tissues.

- There is strong evidence that malathion induces oxidative stress and inflammation. The most extensive database is from in-vivo studies in experimental animals. In addition, oxidative stress was demonstrated in human cells in vitro and in a study of humans acutely poisoned with malathion-based pesticides.

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