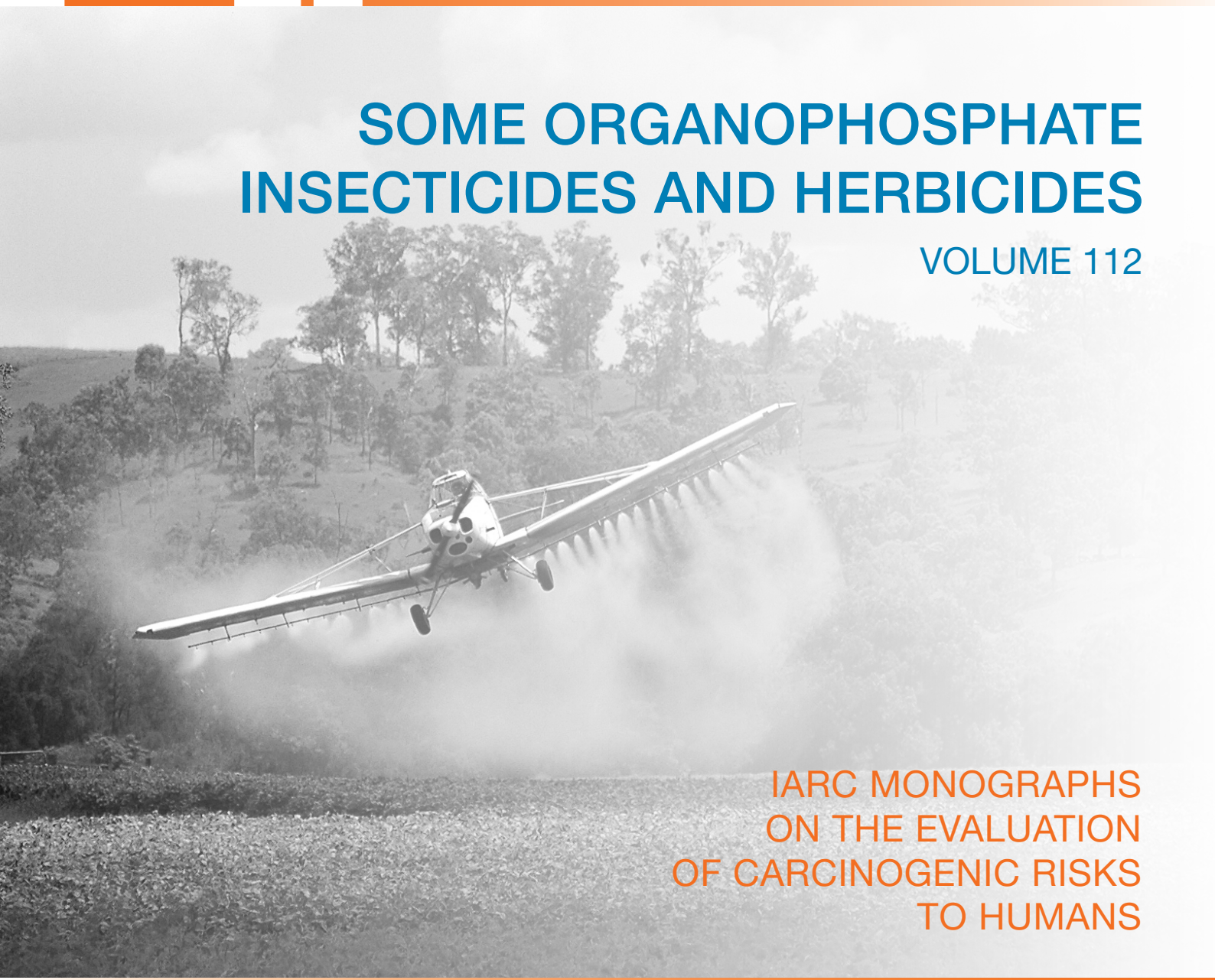
IARC MONOGRAPHS

SOME ORGANOPHOSPHATE INSECTICIDES AND HERBICIDES

VOLUME 112



IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

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This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 3–10 March 2015

LYON, FRANCE - 2017

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

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IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in carcinogenesis and related fields; and to indicate where additional research efforts are needed. The lists of IARC evaluations are regularly updated and are available on the Internet at http://monographs.iarc.fr/.

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission Directorate-General for Employment, Social Affairs, and Inclusion, initially by the Unit of Health, Safety and Hygiene at Work, and since 2014 by the European Union Programme for Employment and Social Innovation "EaSI" (2014–2020) (for further information please consult: http://ec.europa.eu/social/easi). Support has also been provided since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the United States National Cancer Institute, the United States National Institute of Environmental Health Sciences, the United States Department of Health and Human Services, or the European Commission.

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NOTE TO THE READER

The term 'carcinogenic risk' in the *IARC Monographs* series is taken to mean that an agent is capable of causing cancer. The *Monographs* evaluate cancer hazards, despite the historical presence of the word 'risks' in the title.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a *Monograph* does not mean that it is not carcinogenic. Similarly, identification of cancer sites with *sufficient evidence* or *limited evidence* in humans should not be viewed as precluding the possibility that an agent may cause cancer at other sites.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Section of IARC Monographs, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the *Monographs* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Section of IARC Monographs, so that corrections can be reported in future volumes.

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[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only. Invited Specialists do not serve as Meeting Chair or Subgroup Chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.
Each participant was asked to disclose pertinent research, employment, and financial interests. Current financial interests and research and employment interests during the past 4 years or anticipated in the future are identified here. Minor pertinent interests are not listed and include stock valued at no more than US$ 1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are listed as significant pertinent interests.

[2] Peter P. Egeghy received "in kind" support and reimbursement of travel expenses of on average less than US$ 2000 per year during the last 4 years from participation in meetings sponsored by the American Chemistry Council, an industry trade association for American chemical companies, and the Health and Environmental Sciences Institue (HESI), a nonprofit scientific research organization based in Washington and funded by corporate sponsors.

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[3] Christopher J. Portier receives a part-time salary from the Environmental Defense Fund, a United States–based non-profit environmental advocacy group.
[4] Amira Ben Amara attended as a representative of the National Agency of Sanitary and Environmental Control of Products, Tunisia.
[5] Catherine Eiden attended as a representative of the United States Environmental Protection Agency.
[6] Marie-Estelle Gouze attended as a representative of ANSES, France.
[7] Jesudoss Rowland attended as a representative of the United States Environmental Protection Agency.

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[8] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a *Monograph*, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.

[9] Mette Kirstine Boye Jensen attended as an observer for Cheminova A/S, Denmark. She is employed by Cheminova A/S, Denmark, a global company developing, producing and marketing crop protection products.

[10] Béatrice Fervers attended as an observer for the Centre Léon Bérard, France.

[11] Thomas Sorahan attended as an observer for Monsanto Company, USA. He is a member of the European Glyphosphate Toxicology Advisory Panel, and received reimbursement of travel cost from Monsanto to attend EuroTox 2012.

[12] Christian Strupp attended as an observer for the European Crop Protection Association (ECPA), Belgium. He is employed by ADAMA Agricultural Solutions Ltd, Israel, a producer of diazinon and glyphosphate.

[13] Patrice Sutton attended as an observer for the University of California, San Francisco, Program on Reproductive Health and the Environment. His attendance at this *Monographs* meeting was financed by the Clarence E. Heller Charitable Foundation, a philanthropic charity with a mission to protect and improve the quality of life through support of programmes in the environment, human health, education, and the arts.

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PREAMBLE

The Preamble to the IARC Monographs describes the objective and scope of the programme, the scientific principles and procedures used in developing a Monograph, the types of evidence considered and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a Monograph or list of evaluations.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to summarize adequately the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this topic. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended '... that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.' The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. In 1988, the phrase 'of chemicals' was dropped from the title, which assumed its present form, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Through the *Monographs* programme, IARC seeks to identify the causes of human cancer. This is the first step in cancer prevention, which is needed as much today as when IARC was established. The global burden of cancer is high and continues to increase: the annual number of new cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 (Stewart & Kleihues, 2003). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monographs* evaluations, national health agencies have been able, on scientific grounds, to take measures to reduce human exposure to carcinogens in the workplace and in the environment.

The criteria established in 1971 to evaluate carcinogenic risks to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad hoc Advisory Groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991; Vainio et al., 1992; IARC, 2005, 2006).

The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The procedures through which a Working Group implements these principles are not specified in detail. They usually involve operations that have been established as being effective during previous *Monograph* meetings but remain, predominantly, the prerogative of each individual Working Group.

2. Objective and scope

The objective of the programme is to prepare, with the help of international Working Groups of experts, and to publish in the form of *Monographs*, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

In this Preamble, the term 'agent' refers to any entity or circumstance that is subject to evaluation in a *Monograph*. As the scope of the programme has broadened, categories of agents now include specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioural practices, biological organisms and physical agents. This list of categories may expand as causation of, and susceptibility to, malignant disease become more fully understood.

A cancer 'hazard' is an agent that is capable of causing cancer under some circumstances, while a cancer 'risk' is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The *Monographs* are an exercise in evaluating cancer hazards, despite the historical presence of the word 'risks' in the title. The distinction between hazard and risk is important, and the *Monographs* identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

In the *Monographs*, an agent is termed 'carcinogenic' if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Part B, Section 3a) contribute to the judgement that the agent is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

The Preamble continues the previous usage of the phrase 'strength of evidence' as a matter of historical continuity, although it should be understood that *Monographs* evaluations consider studies that support a finding of a cancer hazard as well as studies that do not.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991; Vainio et al., 1992; IARC, 2005, 2006; see also Part B, Sections 4 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged

on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose–response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose–response relationship. A *Monograph* may undertake to estimate dose–response relationships within the range of the available epidemiological data, or it may compare the dose–response information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate Working Group with expertise in quantitative dose–response assessment.

The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning preventive measures, provide effective cancer control programmes and decide among alternative options for public health decisions. The evaluations of IARC Working Groups are scientific, qualitative judgements on the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country and relate to many factors, including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments or other international organizations.

3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme web site (http://monographs.iarc.fr). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data become available on an agent for which a *Monograph* exists, a re-evaluation may be made at a subsequent meeting, and a new *Monograph* published. In some cases it may be appropriate to review only the data published since a prior evaluation. This can be useful for updating a database, reviewing new data to resolve a previously open question or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Part B, Section 6) are more appropriately addressed by a full review.

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section 4). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to epidemiological studies, cancer bioassays, and mechanistic and other relevant data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government agency reports that are publicly available are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Exposure data and other information on an agent under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, published and unpublished sources of information may be considered.

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5. Meeting participants

Five categories of participant can be present at *Monograph* meetings.

(a) The Working Group

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

(b) Invited Specialists

Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues in the section on exposure, such as a general description of data on production and use (see Part B, Section 1). Invited Specialists do not serve as meeting chair

or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) Representatives of national and international health agencies

Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations.

(d) Observers with relevant scientific credentials

Observers with relevant scientific credentials may be admitted to a meeting by IARC in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings (available at http://monographs.iarc.fr).

(e) The IARC Secretariat

The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables and analyses.

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume (Cogliano et al., 2004).

The names and principal affiliations of participants are available on the *Monographs* programme web site (http://monographs.iarc.fr) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC (Cogliano et al., 2005).

All participants are listed, with their principal affiliations, at the beginning of each volume. Each participant who is a Member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year in advance of the meeting of a Working Group, the agents to be reviewed are announced on the *Monographs* programme web site (http://monographs.iarc.fr) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as PubMed. Meeting participants who are asked to prepare

preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, before the meeting, to Working Group Members and Invited Specialists for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the working papers, develop a joint subgroup draft and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days, the Working Group meets in plenary session to review the subgroup drafts and develop the evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections.

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

After the meeting, the master copy is verified by consulting the original literature, edited and prepared for publication. The aim is to publish the volume within six months of the Working Group meeting. A summary of the outcome is available on the *Monographs* programme web site soon after the meeting.

B. SCIENTIFIC REVIEW AND EVALUATION

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The scope of the *IARC Monographs* programme has expanded beyond chemicals to include complex mixtures, occupational exposures, physical and biological agents, lifestyle factors and other potentially carcinogenic exposures. Over time, the structure of a *Monograph* has evolved to include the following sections:

Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and some key issues the Working Group encountered during the meeting.

This part of the Preamble discusses the types of evidence considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations.

1. Exposure data

Each *Monograph* includes general information on the agent: this information may vary substantially between agents and must be adapted accordingly. Also included is information on production and use (when appropriate), methods of analysis and detection, occurrence, and sources and routes of human occupational and environmental exposures. Depending on the agent, regulations and guidelines for use may be presented.

(a) General information on the agent

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host response and clinical disease other than cancer are also presented.

For physical agents that are forms of radiation, energy and range of the radiation are included. For foreign bodies, fibres and respirable particles, size range and relative dimensions are indicated.

For agents such as mixtures, drugs or lifestyle factors, a description of the agent, including its composition, is given.

Whenever appropriate, other information, such as historical perspectives or the description of an industry or habit, may be included.

(b) Analysis and detection

An overview of methods of analysis and detection of the agent is presented, including their sensitivity, specificity and reproducibility. Methods widely used for regulatory purposes are emphasized. Methods for monitoring human exposure are also given. No critical evaluation or recommendation of any method is meant or implied.

(c) Production and use

The dates of first synthesis and of first commercial production of a chemical, mixture or other agent are provided when available; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided when available. In addition, methods of synthesis used in past and present commercial production and different methods of production,

which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d) Occurrence and exposure

Information on the occurrence of an agent in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, plants, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases.

Data that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with date and place. For biological agents, the epidemiology of infection is described.

(e) Regulations and guidelines

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccination and therapy, are described.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part A, Section 4). Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

(a) Types of study considered

Several types of epidemiological study contribute to the assessment of carcinogenicity in humans — cohort studies, case–control studies, correlation (or ecological) studies and intervention studies. Rarely, results from randomized trials may be available. Case reports and case series of cancer in humans may also be reviewed.

Cohort and case–control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the subsequent decrease in risk for lung cancer.

In correlation studies, the units of investigation are usually whole populations (e.g. in

particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports and case series usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure.

The uncertainties that surround the interpretation of case reports, case series and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, these types of study may add materially to the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an agent and disease. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to several aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

First, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Second, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than

those with national rates. Internal comparisons of frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

Third, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well conducted analyses can be considered. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis) and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) (Greenland, 1998).

The advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, procedures of data collection, methods of measurement and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about the potential carcinogenicity of agents.

IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monograph* (see Part A, Section 4). Additionally, as a means of gaining insight from the results of multiple individual studies, ad hoc calculations that combine data from different studies may be conducted by the Working Group during the course of a *Monograph* meeting. The results of such original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important that the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

(d) Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and

time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes (IARC, 1991; Vainio et al., 1992; Toniolo et al., 1997; Vineis et al., 1999; Buffler et al., 2004). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses, of individual susceptibility or host responses, and inference of a mechanism (see Part B, Section 4b). This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites that are thought to be the basis of susceptibility may provide evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons that arise from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f) Criteria for causality

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or that use different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in exposure), and results of studies that are judged to be of high quality are given more weight than those of studies that are judged to be methodologically less sound.

If the risk increases with the exposure, this is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of

multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first that the studies meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of effect of unity for any observed level of exposure, (b) when considered together, provide a pooled estimate of relative risk that is at or near to unity, and (c) have a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn et al., 1986; Tomatis et al., 1989). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio et al., 1995). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (see Part A, Section 4). In all experimental settings, the nature and extent of impurities or contaminants present in the agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, route of exposure, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported. Those studies in experimental animals that are judged to be irrelevant to the evaluation or judged to be inadequate

(e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. OECD, 2002).

Other studies considered may include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); studies in which the end-point was not cancer but a defined precancerous lesion; experiments on the carcinogenicity of known metabolites and derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For studies of mixtures, consideration is given to the possibility that changes in the physicochemical properties of the individual substances may occur during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose–response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar in structure or of a similar virus genus) to that being evaluated is also considered. Such results may provide biological and mechanistic information that is relevant to the understanding of the process of carcinogenesis in humans and may strengthen the biological plausibility that the agent being evaluated is carcinogenic to humans (see Part B, Section 2f).

(a) Qualitative aspects

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age and duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

Considerations of importance in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence (Huff et al., 1989). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo transition to malignancy, the agent

should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship (Hoel et al., 1983; Gart et al., 1986), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto et al., 1980; Gart et al., 1986; Portier & Bailer, 1989; Bieler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed (Sherman et al., 1994; Dunson et al., 2003).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from a given experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls, particularly

when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals (Haseman et al., 1984; Fung et al., 1996; Greim et al., 2003).

Although meta-analyses and combined analyses are conducted less frequently for animal experiments than for epidemiological studies due to differences in animal strains, they can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

4. Mechanistic and other relevant data

Mechanistic and other relevant data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of the mechanistic and other relevant data depends on the biological activity of the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important; thus, not every available study is cited. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive; thus, the same studies may be discussed in more than one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

(a) Toxicokinetic data

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect dose–response relationships include uptake, deposition, biopersistence and half-life in tissues, protein binding, metabolic activation and detoxification. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be important for the extrapolation of hazards between species and in clarifying the role of in-vitro findings.

(b) Data on mechanisms of carcinogenesis

To provide focus, the Working Group attempts to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to gaps in the data and to data that suggests that more than one mechanism may be operating. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems. Changes in the affected organs, tissues or cells can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of potentially adverse physiological changes include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal hormones and changes in immune surveillance.

(ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities of enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in cyclin-dependent kinases that govern cell cycle progression, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in the secretion of factors related to the stimulation of DNA replication and transcription and changes in gap–junction-mediated intercellular communication.

(iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis is given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal aberration/aneuploidy to carcinogenesis (Vainio et al., 1992; McGregor et al., 1999). The adequacy of the reporting of sample characterization is considered and, when necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The concentrations employed are given, and mention is made of whether the use of an exogenous metabolic system in vitro affected the test result. These data are listed in tabular form by phylogenetic classification.

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap–junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests have been published (Montesano et al., 1986; McGregor et al., 1999).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of

greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals in vivo indicates that it may have carcinogenic activity. Negative results in tests for mutagenicity in selected tissues from animals treated in vivo provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) (Vainio et al., 1992). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere (Montesano et al., 1986; McGregor et al., 1999).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression, and formation of calculi and other deposits that cause chronic irritation), that evidence is presented and reviewed critically in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. Capen et al., 1999).

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may include descriptions of the pathology of infection, integration and expression of viruses, and genetic alterations seen in human tumours. Other observations that might comprise cellular and tissue responses to infection, immune response and the presence of tumour markers are also considered.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. 'Physical agents' may also be considered to comprise foreign bodies, such as surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(c) Other data relevant to mechanisms

A description is provided of any structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from testing hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret changes in individual end-points (e.g. changes in expression in one gene) without considering the consistency of that finding in the broader context of the other end-points (e.g. other genes with linked transcriptional control). High-output data can be used in assessing mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data, where the number of observations far exceeds the number of end-points measured, their utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) Susceptibility data

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on toxicokinetics, mechanisms of carcinogenesis and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in-vivo and in-vitro laboratory studies to humans.

(e) Data on other adverse effects

Data on acute, subchronic and chronic adverse effects relevant to the cancer evaluation are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is judged by the same criteria as those applied to epidemiological studies of cancer, but fewer details are given.

5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be found on the *Monographs* programme web site (http://monographs.iarc.fr).

(a) Exposure data

Data are summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given to compare exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

(b) Cancer in humans

Results of epidemiological studies pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) in which an increase in cancer was observed is identified. Dose–response and other quantitative data may be summarized when available.

(c) Cancer in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species, study design and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also mentioned. Negative findings, inverse relationships, dose–response and other quantitative data are also summarized.

(d) Mechanistic and other relevant data

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and the possible mechanism(s) of carcinogenesis (e.g. genetic toxicity, epigenetic effects) are summarized. In addition, information on susceptible individuals, populations and life-stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data that are considered to be important.

6. Evaluation and rationale

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms. The strength of the mechanistic evidence is also characterized.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

An evaluation of the degree of evidence is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of the degree of evidence.

(a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:**

The Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

***Limited evidence of carcinogenicity*:**

A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence of carcinogenicity*:**

The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

***Evidence suggesting lack of carcinogenicity*:**

There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative

risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:**

The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

***Limited evidence of carcinogenicity*:**

The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

***Inadequate evidence of carcinogenicity*:**

The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

***Evidence suggesting lack of carcinogenicity*:**

Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) Mechanistic and other relevant data

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and toxicokinetics, physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is probably carcinogenic to humans.

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is possibly carcinogenic to humans.

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed,

especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is probably not carcinogenic to humans.

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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GENERAL REMARKS

This one-hundred-and-twelfth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of five pesticides: diazinon, glyphosate, malathion, parathion, and tetrachlorvinphos.

Most uses of the insecticide diazinon have been restricted in the USA, Canada, and the European Union, and parathion has been severely restricted globally since the 1980s. Tetrachlorvinphos is banned in the European Union, but continues to be used in the USA and elsewhere as an insecticide on animals, including in pet flea collars. Exposures to the insecticide malathion may occur through its continued use in agriculture, residential, or public-health applications, notably mosquito control. The herbicide glyphosate is structurally similar to other organophosphate pesticides, but is toxicologically distinct and does not inhibit cholinesterase activity. Glyphosate has the highest production volumes of all herbicides and is currently used worldwide in agriculture, forestry, urban, and home applications.

The organophosphate insecticides are part of the grouping of "non-arsenical insecticides," that in 1991 were classified as Group 2A (*probably carcinogenic to humans*) (IARC, 1991). This classification applies to the group of chemicals as a whole, and not necessarily to all individual chemicals within the group. Regarding the individual agents, malathion, parathion, and tetrachlorvinphos were previously evaluated by a Working Group in 1987 and were assigned to Group 3 (*not classifiable as to its carcinogenicity to humans*). The *IARC Monographs* programme had not previously evaluated glyphosate or diazinon. In light of the new data published since any prior evaluations, especially on cancer epidemiology and cancer mechanisms, organophosphate pesticides were accorded priority for evaluation by the *IARC Monographs* during 2015–2019 (Straif et al., 2014). A systematic and objective approach using chemoinformatics, database integration, and automated text mining (Guha et al., 2016) informed selection of agents evaluated in Volume 112. A summary of the findings of this volume appears in *The Lancet Oncology* (Guyton et al., 2015).

Use of systematic review approaches and tools

The principles for evaluating studies and integrating evidence for the *IARC Monographs* are outlined in the Preamble. An Advisory Group to recommend Priorities for *IARC Monographs* during 2015–2019 (Straif et al., 2014) endorsed these principles and encouraged the *Monographs* programme to explore use of new systematic review tools in a manner consistent with them, particularly with respect to the evaluation of mechanistic data. The Advisory Group's report noted "the need for systematic identification of mechanistic data with transparent selection of publications was recognized, in order to clarify mechanistic processes" (Straif et al.,

2014). Accordingly, several new practices were implemented starting from Volume 112 of the *Monographs*, as documented in the "Instructions for authors" of the *IARC Monographs*. In particular, the evaluation introduced a new approach for objectively and systematically collating and analysing mechanistic information based on 10 key characteristics of carcinogens. An expert Working Group convened by IARC concluded that carcinogens in Group 1 (*carcinogenic to humans*) commonly show one or more of these 10 key characteristics (Smith et al., 2016).

In addition, this volume of the *Monographs* made systematic use of large-scale toxicity screening data that are publicly available from government databases for the first time. Specifically, high-throughput screening (HTS) data generated by the Tox21 and ToxCast research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) were analysed to inform evaluations about the in-vitro bioactivity of the chemicals included in *IARC Monographs* Volume 112. Such data were used to provide supporting information and to fill data gaps in the determination on whether several of the chemicals under evaluation (diazinon, malathion, parathion, and tetrachlorvinphos) may act through the key characteristics of known human carcinogens (Smith et al., 2016).

Finally, the "Instructions to authors" (IARC, 2014) outline the literature search strategy, inclusion and exclusion criteria, databases, tools and other elements of the systematic reviews. These practices are also consistent with other authoritative recommendations on the conduct of systematic literature reviews, such as those from the United States National Research Council panels on formaldehyde (National Research Council, 2011) and Integrated Risk Information System (IRIS) process (National Research Council, 2014).

Critical review of exposure assessment methods

Section 1 of this volume includes a critical review of the exposure assessment methods used in the pertinent epidemiological studies (see Section 1.4.2 of Malathion). Assessment of exposure to the agents considered here is challenging due to the predominant role of dermal exposure in occupational settings, correlated exposures to multiple pesticides, and the lack of persistent biological markers of long-term exposure. The Working Group considered the strengths and limitations of the exposure assessment methods used in each study and took these into account in its evaluations.

Studies of cancer in humans

The epidemiological database for evaluating the carcinogenicity to humans of the agents considered here is relatively sparse, and there are no studies of workers manufacturing these pesticides. Several major studies, all conducted in North America, provided data for several of the agents evaluated in this volume (see the Monograph on Malathion, Table 2.1). All except one of the cohort studies investigated the occurrence of cancer among agricultural or pest-control workers or their families. Case–control studies in the USA, Canada, Sweden, and France, most population-based, also provided pertinent data. One of these studies assessed pesticide exposures using a job-exposure matrix, while the others used questionnaires. Although these studies investigated associations involving a diverse range of cancers, the largest body of data available for evaluation concerned non-Hodgkin lymphoma and other lympho-haematopoietic cancers. A meta-analysis of the associations between non-Hodgkin lymphoma and exposure to malathion, diazinon, or glyphosate was also taken into account in considering the evidence for those pesticides.

Studies in experimental systems

In the interests of transparency, IARC evaluations rely only on data that are in the public domain and available for independent scientific review. The evaluation of glyphosate by the Working Group included any industry studies that met these criteria. However, they did not include data from summary tables in online supplements to published articles, which did not provide enough detail for independent assessment. This was the case for some of the industry studies of cancer in experimental animals.

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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

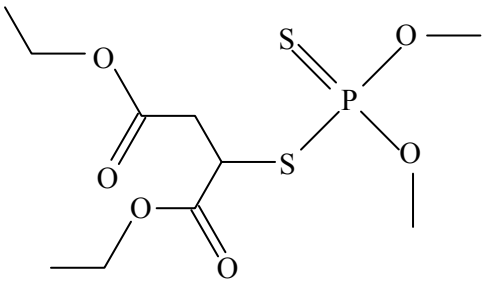
Chem. Abstr. Serv. Name: diethyl 2-[(dimethoxyphosphinothioyl)thio]butanedioate

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).

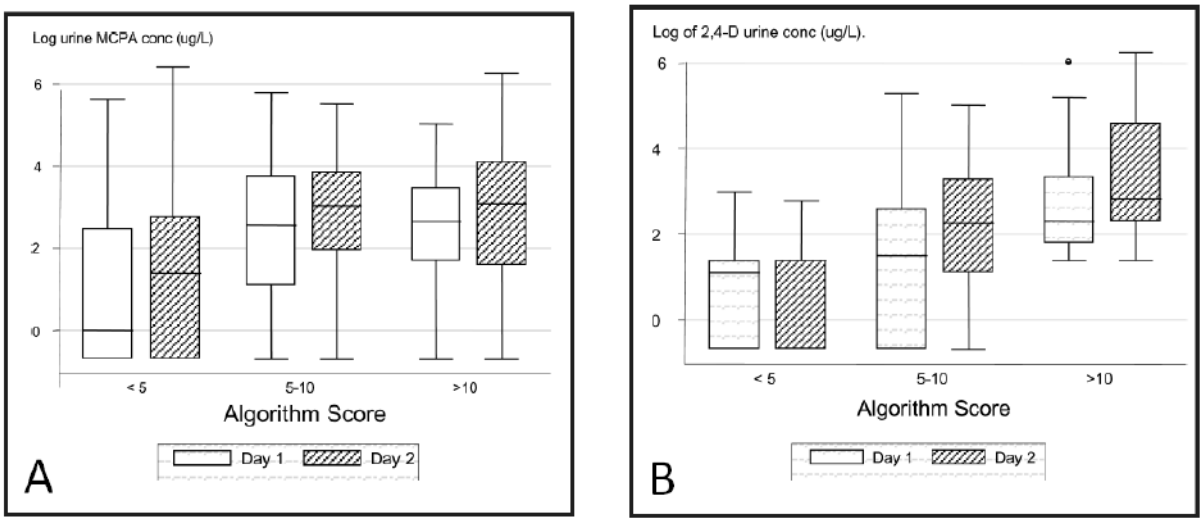
(a) Agricultural Health Study

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)	Ever use	14	0.8 (0.4–1.6)	Age, sex	[Limitations: many proxy-questionnaires; self-report of pesticide use; possible misclassification of exposures]
		Oesophagus (adeno-carcinoma)	Ever use	12	0.7 (0.4–1.5)	Age, sex	

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%)	*Males* 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 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%)	*Females* NS	*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%)
		C-cell carcinoma: 0/50, 0/49, 1/47 (2%)	NS	Gastric ulcers: 0/49, 1/49, 3/49
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.

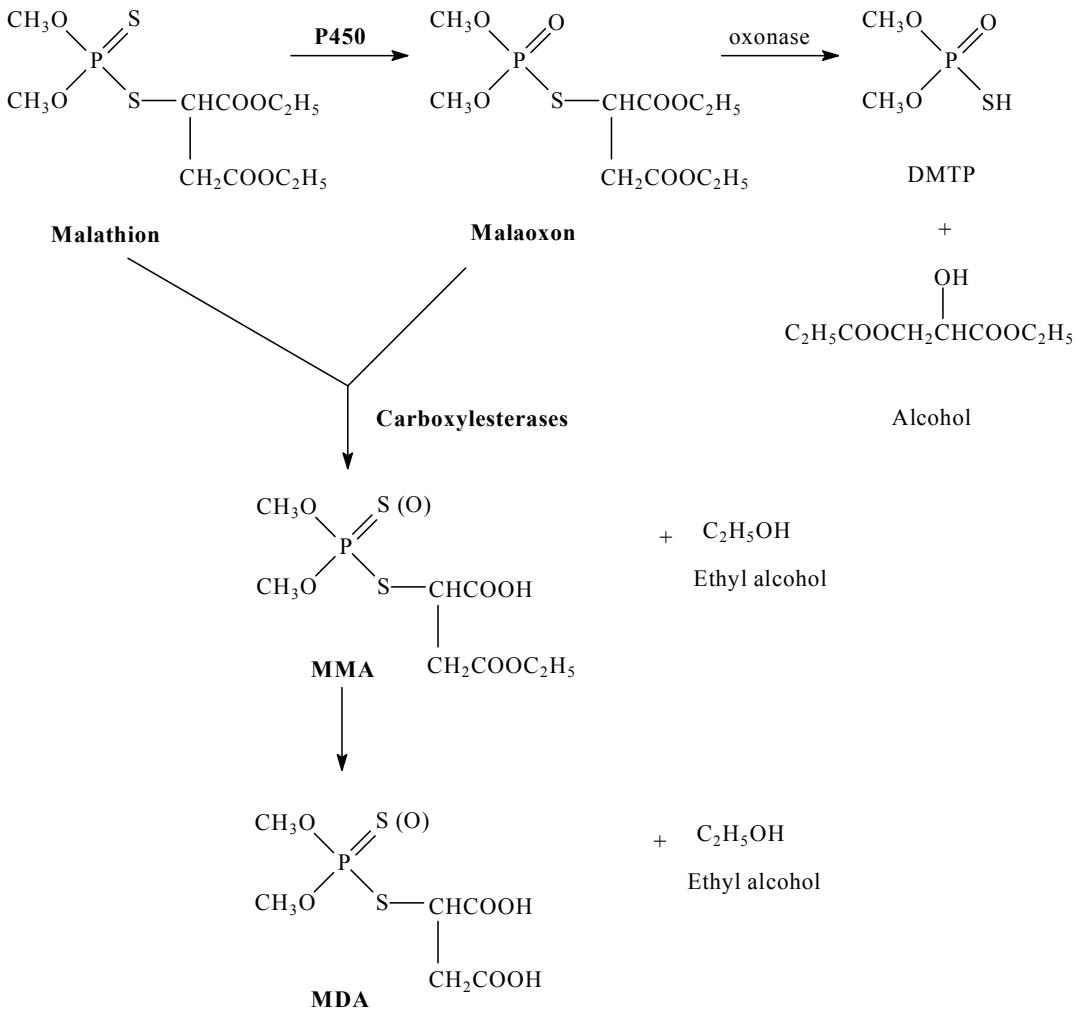
4.1.3 Metabolism

(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

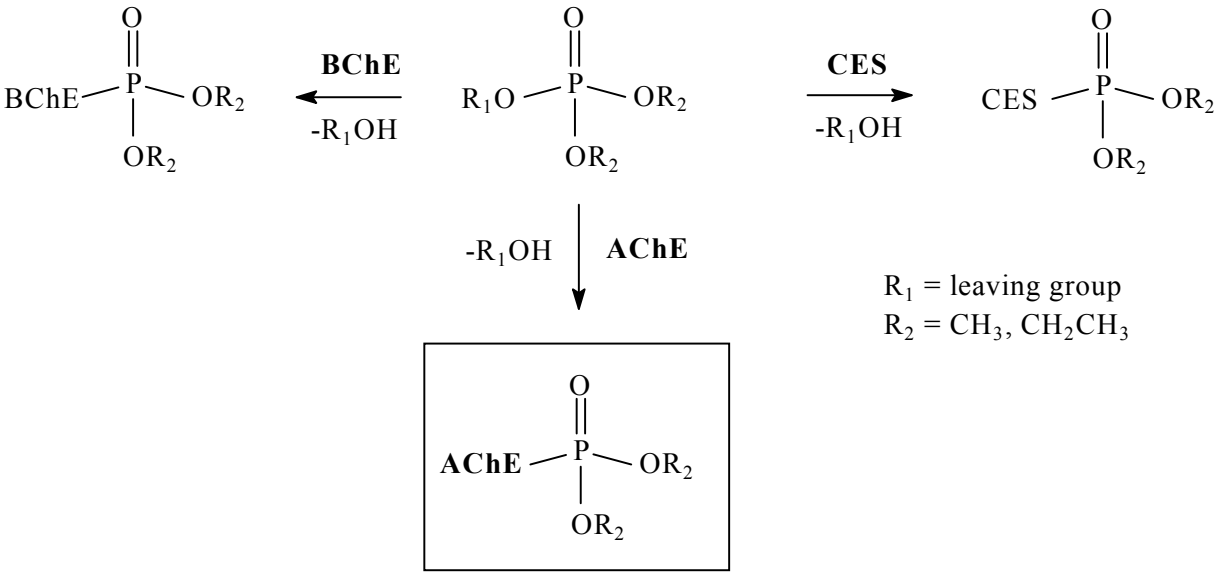
Fig. 4.1 Major pathways of biotransformation of malathion



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

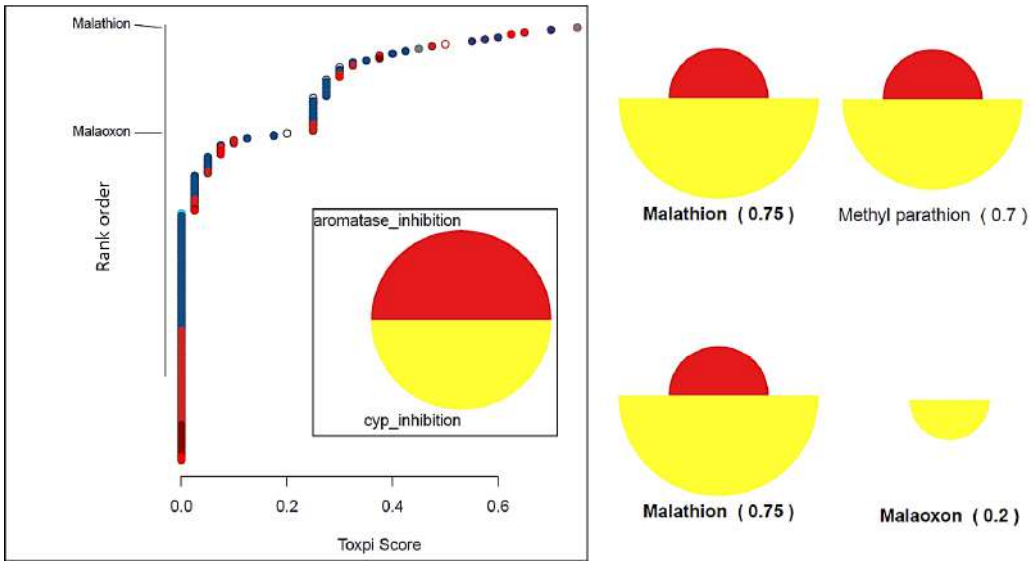
"key characteristics" of known human carcinogens, and the decision as to whether each chemical was "active" or "inactive" are available as supplemental material to *Monograph* Volume 112 (IARC, 2015). The output files generated for each "key characteristic" are also provided in the supplemental material, 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.

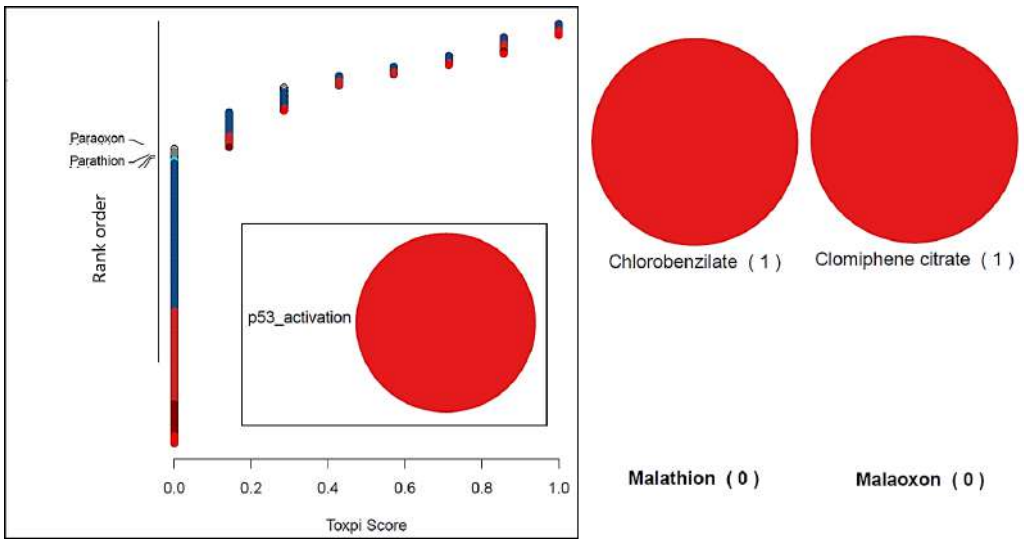
- Characteristic (1) *Is electrophilic or can undergo metabolic activation:* Malathion and malaoxon were tested for 31 assay end-points. Malathion was active for 20 of the 29 assay end-points related to CYP inhibition, and for 1 out of 2 assay end-points related to aromatase inhibition. Overall, malathion 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



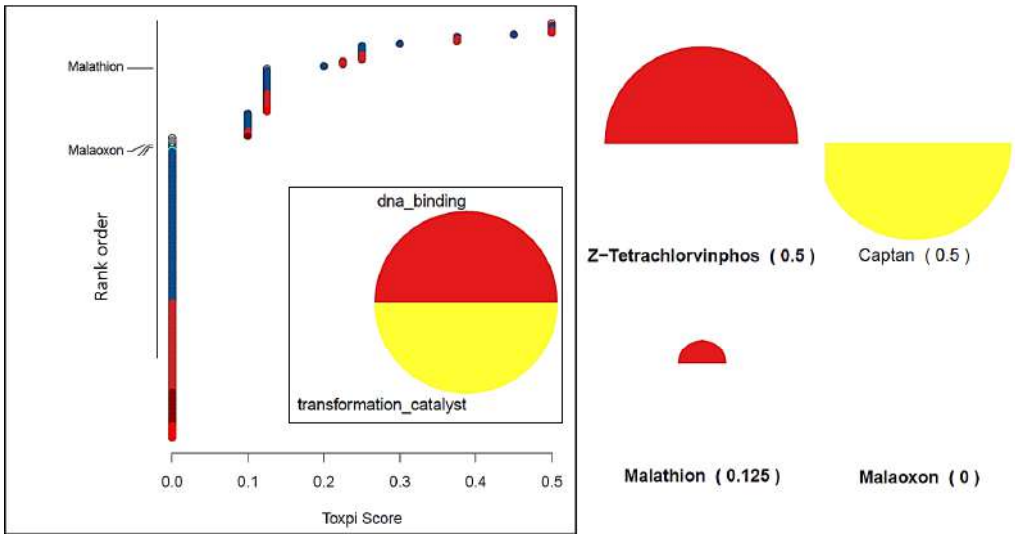
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis).The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the 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



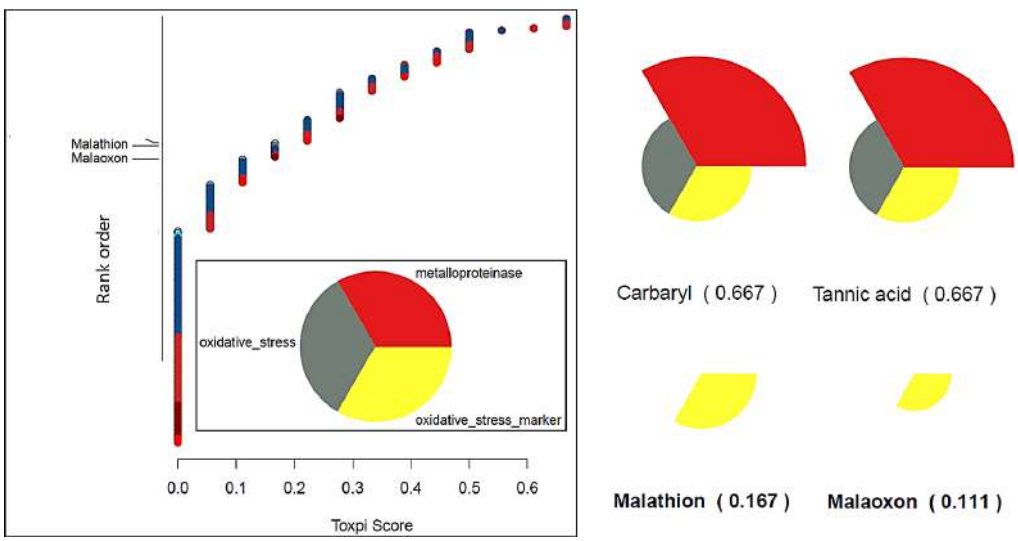
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the 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



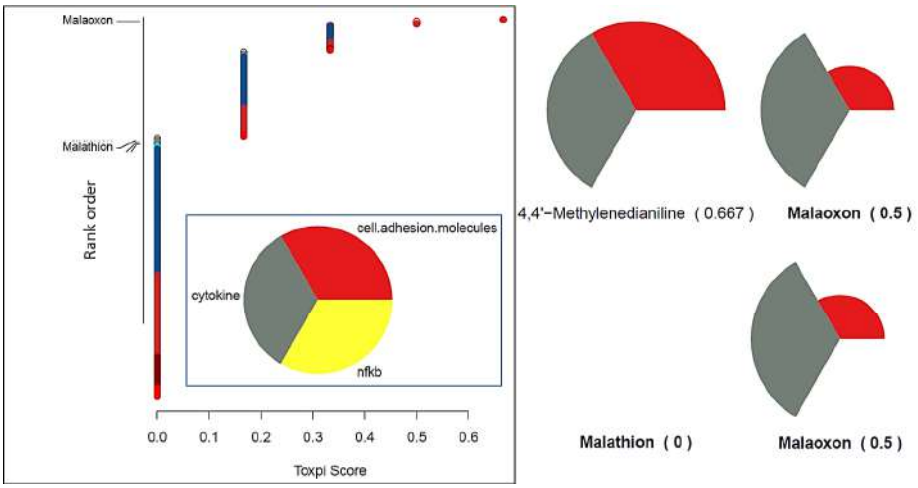
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi)score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the 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



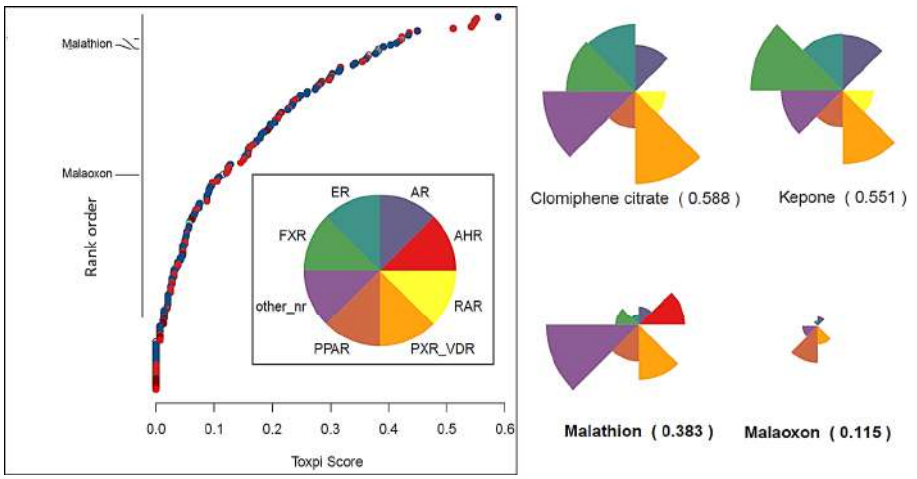
On the left-hand side, the relative ranks of malathion, and its metabolite malaoxon, are shown (*y* axis) with respect to their ToxPi score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals 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).

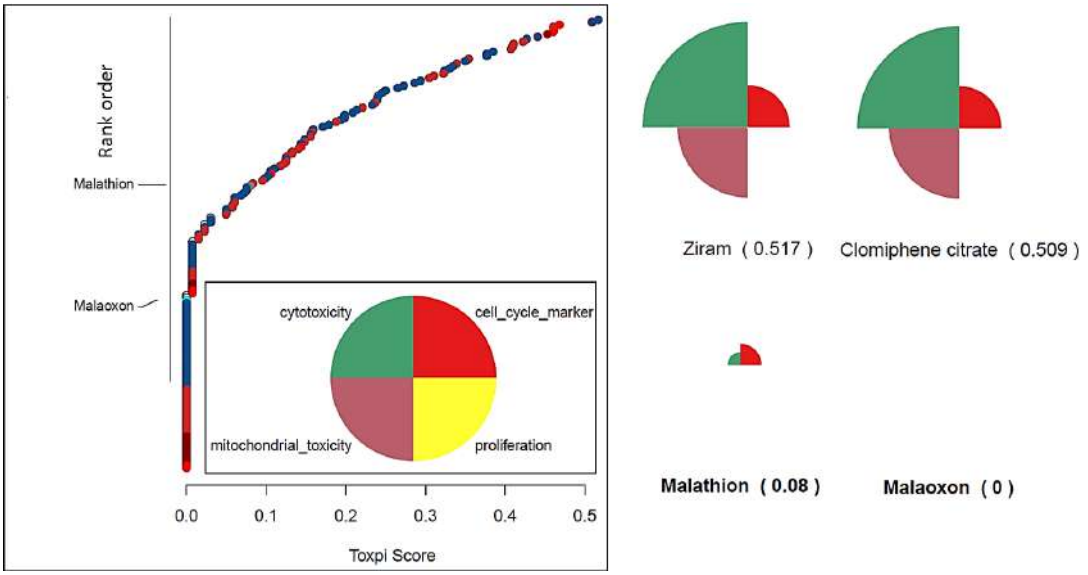
Overall, malathion demonstrated consistent activity in CYP inhibition and effects on nuclear receptors and related proteins, most notably PXR and AhR. Malaoxon showed a high ranking in activity related to chronic inflammation, but the assigned assay end-points were highly selective, with a maximum of 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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PARATHION

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 56-38-2

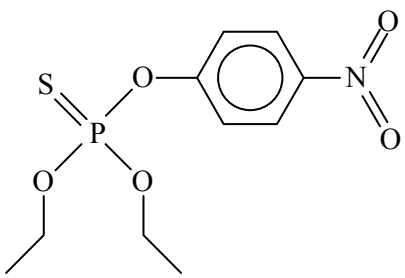
Chem. Abstr. Serv. Name: O,O-diethyl *O*-(4-nitrophenyl) phosphorothioate

Preferred IUPAC Name: O,O-diethyl *O*-(4-nitrophenyl) phosphorothioate

Synonyms: ethyl parathion; parathion-ethyl; thiophos

Selected Trade Names: Products containing parathion have been sold worldwide under several trade names, including Alkron; Alleron; Bladan; Bladan F; Corothion; Ethlon; Folidol; Fosfermo; Orthophos; Panthion; Paradust; Paraphos; Thiophos (IARC, 1983)

1.1.2 Structural and molecular formulae, and relative molecular mass



From NIST (2011)

Molecular formula: $C_{10}H_{14}NO_5PS$

Relative molecular mass: 291.26

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: Solid below 6.1 °C (43°F), otherwise pale-yellow to dark-brown liquid with a garlic-like or phenol-like odour (NCBI, 2015)

Solubility: Very slightly soluble in water (11 mg/L at 20 °C, 24 mg/L at 25 °C) (IARC, 1983; NCBI, 2015); soluble in chloroform (Weast, 1988); miscible with most organic solvents; slightly soluble in petroleum oils (IARC, 1983; NCBI, 2015)

Volatility: Vapour pressure, reported as 6.68×10^{-6} mm Hg (20 °C) (NCBI, 2015); little volatilization from moist and dry soil surfaces is expected

Stability: Hydrolyses very slowly in acidic media, more rapidly in alkaline media to diethylphosphorothioic acid and *para*-nitrophenol; slowly isomerizes on heating above 130 °C to the *O,S*-diethyl analogue (IARC, 1983); decomposes above 200 °C to produce toxic gases including carbon monoxide, nitrogen oxides, phosphorous oxides, and sulfur oxides (IPCS, 2004).

Reactivity: Readily reduced to *O,O*-diethyl *O*-*para*-aminophenyl phosphorothioate; oxidized with difficulty to diethyl *para*-nitrophenyl

phosphate (Metcalf, 1981); reacts with strong oxidants (IPCS, 2004); attacks some forms of plastic, rubber and coatings (IPCS, 2004).

Octanol/water partition coefficient (P): log K_{ow}, 3.83 (NCBI, 2015)

Henry's law: 2.98×10^{-7} atm m^3 $mole^{-1}$ at 25 °C (HSDB, 2016), little volatilization from water surfaces is expected

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 11.9 ppm (EPA, 2000b).

1.1.4 Technical products and impurities

Technical parathion is reported to be 96–98.5% active ingredient and 15% inert ingredients (IARC, 1983; HSDB, 2016). Observed impurities include diethyl and triethyl thiophosphates; nitrophenetole; nitrophenol; and the dithio analogue of parathion (Warner, 1975; IARC, 1983).

1.2 Production and use

1.2.1 Production

(a) Manufacturing

Parathion was introduced in 1947 and first registered in the USA in 1948 (IARC, 1983; EPA, 2000a). Ethyl parathion was only the second phenyl organophosphate introduced into agriculture, and the first to be used commercially (Ware & Whitacre, 2004).

Formulations including dusts (0.5–2% active ingredient); emulsifiable concentrates (2–8% active ingredient); granules (10% active ingredient); aerosols (10% active ingredient), and wettable powders (15–25% active ingredient) have been produced (IPCS, 1992).

(b) Production volume

Data on production volumes for parathion are very limited; however, it was listed as a chemical with a high production volume (> 1000 tonnes/year) in 2004 (OECD, 2004). Parathion is reported to be manufactured by seven producers worldwide: four in China, and one each in El Salvador, Germany, and the USA (AgriBusiness Global Sourcing Network, 2015). In the 1970s, parathion was manufactured in the USA by several companies, with an estimated total production of about 6000 tonnes per year, but only one company was still producing parathion in the 1990s (IARC, 1983; EPA, 2000a). Past production has also been reported in India in 1980–1981 at 1.2 tonnes, and around that same period annual production in western Europe was estimated to be in the range of 2000–5000 tonnes (IARC, 1983).

1.2.2 Uses

(a) Agriculture

Parathion is a broad spectrum, non-systemic, insecticide and miticide with contact, stomach, and some respiratory action (IPCS, 1992; EPA, 2000a). It has been used as a treatment for soil and foliage pre-harvest, and to control sucking and chewing insects, mites, and soil insects on a large variety of orchard, row, and field crops, including cereals, fruit, vines, vegetables, ornamentals, and cotton, both outdoors and in greenhouses (EPA, 2000a; IPCS, 1992; FAO/UNEP, 2005). When last used in the USA, parathion was restricted to nine crops: alfalfa, barley, rapeseed, corn, cotton, sorghum, soybean, sunflower, and wheat (EPA, 2000a).

(b) Regulation

Due to increasing concerns regarding hazards to wildlife and human health, the use of parathion as a pesticide has been banned, de-authorized or phased out by several counties including: Angola, Australia, Belize (1985), Bulgaria, China, Colombia (1991, except for on cotton using aerial equipment), Ecuador, El Salvador, Guatemala, Hungary, India (1974), Indonesia, Ireland, Japan (1955), Kuwait (1980), Malaysia, New Zealand (1987), Philippines, Portugal (1994), Russian

Table 1.1 Methods of analysis for parathion

Sample matrix	Analytical method	Limit of detection	Reference
Air	GC/FPD (phosphorus mode)	0.4 µg/m³	NIOSH (1994)
Water	GC/MS	0.15 µg/L	Munch et al. (2012)
Urine	Isotope dilution GC-MS/MS	9 µg/L (as 4-nitrophenol)	Fenske et al. (2002)
		0.2 µg/L (DEP) 0.1 µg/L (DETP)	Bravo et al. (2004)
Fruits and vegetables	GC/MS	0.03 mg/kg	Fillion et al. (2000)
Solids (soils, sediments, sludges)	GC/FPD (phosphorus mode)	NR	EPA (2007)
Dust	GC/MS (selected ion monitoring mode)	0.013-0.052 µg/g	Fenske et al. (2002)

DEP, diethylphosphate; DETP, diethylthiophosphate; FPD, flame photometric detector; GC, gas chromatography; MS, mass spectrometry

Federation, Sri Lanka (1984), Sweden (1971), the United Republic of Tanzania (1986), Thailand (1988), Turkey, United Kingdom, and the USA (2003) (IPCS, 1992; FAO, 1997; EPA, 2000a). In the European Community, all authorizations for plant protection products containing parathion were withdrawn by 2002; previously all formulations except capsule suspensions were included in Annex III of the Rotterdam Convention on international trade of hazardous chemicals (FAO/UNEP, 2005). In the USA, use sites and practices were restricted in 1991 to mitigate risk to workers; use was restricted to aerial equipment application of emulsifiable concentrates to nine specified crops, noted above, and all uses of parathion were terminated in 2003 (EPA, 2000a).

Limits for occupational exposure to parathion in air of 0.05–0.1 mg/m³ have been established in several countries (IFA, 2015). An acceptable daily intake of 0–0.005 mg/kg body weight (bw) from residues in food was established in 1967 (IPCS, 1992).

1.3 Measurement and analysis

Parathion is typically measured using "multi-residue" analytical techniques developed for the simultaneous measurement of a large number of organophosphate pesticides that might be present in a sample. Parathion can be measured in air, water, soil, dust, fruits and vegetables, and urine and faeces. The alkyl phosphate metabolites of parathion, diethylphosphate (DEP) and diethylthiophosphate (DETP), plus *para*-nitrophenol (also common to methyl-parathion) can be measured in urine. Representative chemical analysis methods for parathion and its metabolites are listed in Table 1.1.

In water and soil, most parathion degrades over several weeks but a small residual presence may remain in the soil for several months (HSDB, 2016).

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

The majority of exposure to workers is estimated to be via the dermal route (e.g. Cohen et al., 1979). Parathion poisoning has been reported in workers who had dermal contact with the foliage of treated fruit trees and vines (Quinby & Lemmon, 1958).

In the 1960s, dermal measurements of parathion during a range of different agricultural tasks were between 2.4 and 77.7 mg/hour, and respiratory levels were between 0.02 and 0.19 mg/hour (Wolfe et al., 1967). Exposure may vary considerably for a single task. For example, when spraying fruit trees, dermal exposure to parathion varied by up to 200-fold depending

on the environmental conditions (particularly wind), the method of application (upward spraying equipment gave more exposure than downward spraying equipment), rate of application, and operator technique (Wolfe et al., 1967).

A study of 57 workers in a plant manufacturing powdered parathion found mean dermal exposures of 67.3 mg/hour and mean respiratory exposures of 0.62 mg/hour (Wolfe et al., 1978). The highest exposures were found in those undertaking bagging tasks.

Farm workers hand-harvesting onions (n = 64) had a geometric mean dermal exposure of 0.84 µg/hour for the first day, and 0.36 µg/hour for the second day (Munn et al., 1985). There was no difference in exposure by age or sex of the worker.

A study of ambient parathion concentrations in aeroplane cockpits during aerial spraying have shown very high peak levels (up to 440 µg/mL) over short intervals (between 11 and 21 minutes). Spraying pilots and ground crews also showed reduced whole blood cholinesterase activity (Richter et al., 1980).

A study of 14 workers in cotton fields sprayed with parathion in the USA reported a small decline in plasma and erythrocyte cholinesterase activity in a group that entered a field 24 hours after treatment, and a larger decline among a group exposed 48 hours after treatment and following a light rain (Ware et al., 1974).

(b) Community exposures

The general population can be exposed to parathion from drinking-water, residues on food, spray drift from nearby farms, and para-occupational sources (EPA, 2000b).

(i) Drinking-water

Parathion has been rarely detected in groundwater or surface water in the USA (Gilliom et al., 2006). The concentration of ethyl parathion was reported as 0 ppb for all of the 410 measurements in surface water recorded in the Surface Water Protection Program Database of the California Department of Pesticide Regulation (2015). Data from other countries were not available to the Working Group.

(ii) Residues on food

Parathion residues are rarely detected on food in recent data from the USA, Canada, and the European Union (Rawn et al., 2004; EFSA, 2011; FDA, 2015). Parathion was not detected in 226 samples of 7 types of vegetables from Hebei Province, China (Li et al., 2014). In a study in Shaanxi, China, parathion was not detectable in 60 samples of cereals, or 60 samples of fruit; however, it was detected in 2 out of 80 samples of vegetables, and the mean concentrations of parathion exceeded the national maximum residue limit (Bai et al., 2006). Parathion residues were detected in 10–16% of sampled tomatoes, eggplant, and peppers purchased at a market in Ghana, with concentrations ranging from 0.061 to 0.089 mg/kg (Darko & Akoto, 2008).

(iii) House dust

In Washington state, USA, dust in the houses of 12 farmworkers and 49 pesticide applicators was tested for ethyl parathion (Fenske et al., 2002). It was found in 48% of houses, more often in the houses of applicators than in those of general farm workers; the arithmetic mean concentration was 0.06 µg/g with a range of 0 to 0.95 µg/g. Another study of 48 agricultural families and 11 reference families in Washington state detected parathion in dust in homes of 69% of agricultural families and 27% of reference families, with mean levels of 0.365 µg/g and 0.076 µg/g, respectively (Simcox et al., 1995). Among the agricultural workers, levels were higher in farmers and applicators than farmworkers.

1.4.2 Exposure assessment and biological markers

Exposure assessment methods in epidemiological studies on parathion and cancer are discussed in Section 1.4.2 and Section 2.1.2 of the *Monograph* on Malathion, in the present volume.

There are no biomarkers that are specific for parathion. Urinary and blood measures of breakdown products of parathion and suppression of acetylcholinesterase activity are only useful to measure parathion when exposure to any other organophosphate pesticide can be definitively ruled out.

2. Cancer in Humans

2.1 Introduction

In previous *IARC Monographs* (IARC, 1983, 1987), parathion was evaluated as *Group 3, unclassifiable as to carcinogenicity in humans*, as there was no evidence to evaluate direct exposure in humans. Although relevant reports have since been published, there is still relatively little epidemiological literature on whether there is an association between cancer and exposure to parathion. In contrast, the general class of organophosphate insecticides has been more heavily investigated, and while parathion is a member of this class, other members are used in greater frequency and amounts (e.g. diazinon, malathion, chlorpyrifos, etc.), which has resulted in their more frequent examination in published reports. The organophosphate insecticides are part of the grouping of "non-arsenical insecticides," which in 1991 were classified as *Group 2A, probably carcinogenic to humans* (IARC, 1991).

A general discussion of the epidemiological studies on agents considered in the present volume (Volume 112) of the *IARC Monographs* is presented in Section 2.2 of the *Monograph* on Malathion. The scope of the available epidemiological studies is discussed in Section 2.1 of the *Monograph* on Malathion, and includes a consideration of chance, bias and confounding, and exposure assessment.

2.2 Cohort studies

2.2.1 Agricultural Health Study

Epidemiological evidence regarding parathion derived from cohort studies (Table 2.1) has been largely from the Agricultural Health Study (AHS). The AHS is a prospective cohort of licensed pesticide applicators enrolled in 1993–1997 in Iowa and North Carolina, USA (Alavanja et al., 1996; see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study).

Engel et al. (2005) examined whether exposure to pesticides was associated with incidence of cancer of the breast among farmers' wives in the AHS cohort, as this cancer occurred frequently enough to be studied after a minimum of only 3 years of follow-up. The study included 30 454 women with no history of cancer of the breast before cohort enrolment in 1993–1997. Parathion was one of 24 specific pesticides for which results were reported. Personal use of parathion was reported for fewer than three women, which was too few for a relative risk estimate to be calculated. The relative risk of cancer of the breast among women whose husbands used parathion was not significant overall, but statistically significant associations were detected with stratification by state or family history of breast cancer (and there was also an elevated but not significant relative risk (RR) for postmenopausal breast cancer). Husband's use of parathion was reported for 18 (13%) cases and 1385 (11%) controls, yielding a relative risk of 1.3 (95% CI, 0.8–2.1). Stratified analyses suggested that the association with husband's parathion use was stronger in Iowa (RR, 2.0; 95% CI, 1.0–4.1) than in North Carolina (RR, 0.9; 95% CI, 0.5–1.8); and may be higher with postmenopausal (RR, 1.4; 95% CI, 0.8–2.5)

Table 2.1 Cohort studies of cancer and exposure to parathion

Study name, reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled
Florida Pest Control Worker Study 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 (living controls)	6	3.2 (0.5–20.7)	Age, smoking
AHS Engel et al. (2005) IA and NC, USA Enrolment, 1993–1997; follow-up to 2000	30 454 wives of male licensed pesticide applicators, with no history of breast cancer at enrolment Exposure assessment method: questionnaire	Breast	Husband's use (indirect exposure)	18	1.3 (0.8–2.1)	Age, race (white/other), state of residence
			By state – IA	8	2.0 (1.0–4.1)	
			By state – NC	10	0.9 (0.5–1.8)	
			Premenopausal	3	0.9 (0.3–3.0)	
			Postmenopausal	13	1.4 (0.8–2.5)	
			No family history of breast cancer	11	0.9 (0.5–1.8)	
			Family history of breast cancer	7	4.2 (1.6–10.6)	
AHS Lee et al. (2007) IA and NC, USA Enrolment, 1993–1997; follow-up to 2002	56 813 licensed pesticide applicators with no prior history of colorectal cancer (97% males) Exposure assessment method: questionnaire	Colorectum	Ever use	46	0.9 (0.6–1.3)	Age, smoking, state, total days of pesticide use
		Colon	Ever use	31	0.9 (0.6–1.5)	
		Rectum	Ever use	15	0.9 (0.5–1.7)	
AHS Dennis et al. (2010) IA and NC, USA 1993–2005	25 291 licensed pesticide applicators (mostly farmers) (24 704 in analysis) Exposure assessment method: questionnaire	Melanoma	Ever use	21	1.9 (1.2–3.0)	Age, sex, tendency to burn, red hair, sun exposure duration, BMI Prevalence of parathion use in whole cohort = 11% (7% for non-cases; 15% for cases)
			Not exposed to lead arsenate	13	1.5 (0.8–2.7)	
			Exposed to lead arsenate	8	7.3 (1.5–34.6)	
			Low exposure (< 56 days)	10	1.6 (0.8–3.1)	
			High exposure (≥ 56 days)	11	2.4 (1.3–4.4)	
			Trend-test *P* value: 0.003			

Table 2.1 (continued)

Study name, reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled
AHS Alavanja et al. (2014) IA and NC, USA Enrolment, 1993–1997; follow up 2010 in NC, and 2011 in IA	54 306 licensed pesticide applicators (523 incident cases of NHL) with no prevalent cancer at baseline, not living outside the catchment area of IA and NC cancer registries, and with complete data on potential confounders Exposure assessment method: questionnaire	NHL (including multiple myeloma)	Ever used	69	1.1 (0.8–1.4)	Age, state, race (white/black), total days of herbicide use
			Low (LED, ≤ 8.75)	9	1.0 (0.5–2.0)	
			Medium (LED, > 8.75–24.5)	6	1.4 (0.6–3.2)	
			High (LED, > 24.5)	6	0.8 (0.3–1.8)	
			Trend-test *P* value: 0.64			
		NHL (including multiple myeloma)	IW-LED			
			Low (≤ 8.75)	7	0.9 (0.4–2.0)	
			Medium (> 8.75–24.5)	8	1.4 (0.7–2.9)	
			High (> 24.5)	6	0.8 (0.4–1.9)	
			Trend-test *P* value: 0.74			
AHS Koutros et al. (2013) IA and NC, USA Enrolment, 1993–1997; follow-up to 2007	54 412 licensed private pesticide applicators (IA and NC) and 4916 licensed commercial applicators (IA); 1962 incident cases including 919 aggressive cancers Exposure assessment method: questionnaire	Prostate, total cancers	IW-LED:			Age, state, race, smoking, fruit servings, family history of prostate cancer, and physical activity A prior AHS publication already reported on diazinon and prostate cancer (Beane Freeman et al., 2004), but here 5 years additional follow-up were included
			Quartile 1 (low use)	25	1.21 (0.81–1.81)	
			Quartile 2	25	1.37 (0.92–2.05)	
			Quartile 3	25	1.21 (0.81–1.81)	
			Quartile 4 (high use)	24	0.85 (0.56–1.28)	
			Trend-test *P* value: 0.51			

Table 2.1 (continued)

Study name, reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled
AHS Koutros et al. (2013) IA and NC, USA Enrolment, 1993–1997; follow-up to 2007 (cont.)		Prostate, aggressive cancers	Quartile 1 (low use)	12	1.96 (1.1–3.5)	
			Quartile 2	12	1.04 (0.58–1.86)	
			Quartile 3	12	1.5 (0.82–2.77)	
			Quartile 4 (high use)	11	0.98 (0.53–1.79)	
			Trend-test *P* value: 0.97			
		Prostate (no family history)	Quartile 1 (low use)	16	1.14 (0.69–1.87)	
			Quartile 2	18	1.36 (0.85–2.19)	
			Quartile 3	16	1.08 (0.66–1.79)	
			Quartile 4 (high use)	20	0.99 (0.63–1.55)	
			Trend-test *P* value: 0.98			
		Prostate (with family history)	Quartile 1 (low use)	5	1.32 (0.54–3.23)	
			Quartile 2	5	1.54 (0.63–3.8)	
			Quartile 3	6	1.58 (0.65–3.84)	
			Quartile 4 (high use)	3	–	
			Trend-test *P* value: 0.88			
			GC rs7041			Age, state
			Low exposure – CC	12	2.58 (1.07–6.25)	
			High exposure – CC	10	3.09 (1.1–8.68)	
			Trend-test *P* value: $P < 0.01$			

Table 2.1 (continued)

Study name, reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled
AHS Koutros et al. (2013) IA and NC, USA Enrolment, 1993–1997; follow-up to 2007 (cont.)		Prostate	Never vs IW-LED			Age, state
			Ever used	102	1.02 (0.78–1.33)	
			Low level	30	1.28 (0.79–2.06)	
			High level	22	0.9 (0.53–1.54)	
			Trend-test *P* value: 0.91			
		Prostate	*RXRB* rs1547387			Age, state
			Low exposure – CC	22	1.12 (0.65–1.94)	
			High exposure – CC	12	0.54 (0.28–1.04)	
			Low exposure – CG + GG	8	1.82 (0.68–4.89)	
			High exposure – CG + GG	10	4.27 (1.32–13.78)	
			Trend test *P* value, < 0.01			
		Prostate	*GC* rs222040			Age, state
			Low exposure – AA	11	2.14 (0.89–5.12)	
			High exposure – AA	11	3.39 (1.23–9.36)	

AHS, Agricultural Health Study; CI, confidence interval; *GC*, Group specific Component gene; IA, Iowa; IW-LED, intensity-weighted lifetime days of use; NC, North Carolina; NR, not reported; *RXRB*, Retinoid-X-Receptor-β gene

than premenopausal (RR, 0.9; 95% CI, 0.3–3.0) breast cancer. The effect varied by family history (*P* value for interaction = 0.04): among women with a family history of breast cancer there was a relative risk of 4.2 (95% CI, 1.6–10.6; 7 exposed cases; exposure prevalence, 19%) associated with exposure to parathion, while among those who did not have a family history, the relative risk was 0.9 (95% CI, 0.5–1.8; 11 (9%) exposed cases). [The strengths of this study included its large sample size, comprehensive exposure assessment, extent of potential confounder control, and exploration of potential interactions, such as by family history. To date, this is the only study to have reported on whether parathion is associated with cancer in women.]

Cancer of the colorectum was studied by Lee et al. (2007) in the AHS, with a total of 305 incident cases of cancer of the colorectum (colon, 212; rectum, 93) diagnosed during the study period, 1993–2002. Among the 50 pesticides examined, use of parathion was reported in 46 (20%) cases of cancer of the colorectum, with a relative risk of 0.9 (95% CI, 0.6–1.3); use of parathion varied very little according to whether the cancer was of the colon or rectum. Given that no association was seen for parathion in the ever versus never analysis, and that there were no a-priori hypotheses or previous results related to parathion, there was no further analysis of exposure–response relationships. [The Working Group noted that the large sample size provided a relatively precise null result, and that among the many potential confounders considered, the final models included an indicator of exposure to other pesticides.]

The incidence of cutaneous melanoma was studied within the AHS by Dennis et al. (2010), with an average length of follow-up of 10.3 years until 2005. This study focused on the AHS subset for which data on arsenical pesticides were available, that is, the 24 704 pesticide applicators (43% of the full AHS cohort) who completed the more detailed take-home questionnaire in addition to the baseline questionnaire. Of the 50 specific pesticides assessed, 4 were found to be associated with risk of melanoma (parathion, benomyl, carbaryl, maneb/mancozeb), and these 4 were further analysed to assess whether results varied with use of lead arsenate. Dennis et al. also assessed whether the observed relationship between exposure to parathion and risk of melanoma was modified by exposure to arsenic compounds; previous reports had suggested that arsenic exposure may be related to melanoma (Beane Freeman et al., 2004), that arsenic may interact with certain pesticides and sun exposure in causing skin lesions (Chen et al., 2006), and that sunscreen may increase absorption of parathion (Brand et al., 2003). A total of 150 incident cases of cutaneous melanoma were detected, and use of parathion was reported by 11% of the whole cohort, with 21 (15%) exposed cases. The odds ratio for ever versus never use of parathion was 1.9 (95% CI, 1.2–3.0), and a monotonic trend was found with increasing level of exposure: the odds ratio was 1.6 (95% CI, 0.8–3.1) for < 56 exposure-days, compared with 2.4 (95% CI, 1.3–4.4) for ≥ 56 lifetime exposure-days (*P* value for trend = 0.003). Both these analyses were based on models that adjusted for major potential confounders, including age, sex, burn tendency, red hair, duration of sun exposure, and body mass index. There was no effect modification of the association with pesticides by sun exposure [stated by authors, data not presented]. A possible statistical interaction was detected between use of parathion and exposure to lead arsenate (*P* value for interaction = 0.065), since among workers who had used lead arsenate there was a significant association (OR, 7.3; 95% CI, 1.5–34.6; 8 exposed cases), compared with those who did not use lead arsenate (OR, 1.5; 95% CI, 0.8–2.7; 13 exposed cases). [There was potentially plausible effect modification, with risk increased among those who also applied lead arsenate. Although Dennis et al. (2010) controlled for the potential effects of established risk factors for melanoma,

sun exposure and duration of pesticide use are likely to be correlated so there was potential for residual confounding in the effect estimates for each pesticide. Also, results arising from the testing of multiple exposures and interactions must be interpreted with caution; however, the combination of main effect, gradient of effect, and potentially plausible effect modification provided support for the hypothesis that exposure to parathion and other agricultural chemicals may be an additional source of risk beyond established risk factors for melanoma (e.g. host factors, susceptibility, and sun exposure).]

Cancer of the prostate was assessed in the AHS by Koutros et al. (2013), with follow-up to 2007, which resulted in 1962 incident cases among the full cohort of 54 412 pesticide applicators. For persons who did not respond to the questionnaire regarding parathion use, values were imputed. [The Working Group noted that Heltshe et al. (2012) demonstrated there was a very high level of agreement between observed and imputed values, in part due to the rarity of exposure to parathion.] The relationship between exposure and incidence of cancer of the prostate was assessed for 48 pesticides, plus stratified analyses assessed whether associations varied according to the aggressiveness of the tumour, or family history of prostate cancer. Aggressive cancer of the prostate was defined as having one or more of the following features: distant stage, poorly differentiated grade, Gleason score ≥ 7, or fatality. Due to updates in grade classification by pathologists, Gleason scores for cases diagnosed before 2003 were re-abstracted and analyses were repeated for alternative definitions of aggressiveness. Results for parathion demonstrated that in general there was neither a statistically significant increase in risk, nor a trend for all cancers of the prostate (*P* value for trend = 0.51) or aggressive cancers of the prostate (*P* value for trend = 0.97); with the exception of a significantly increased risk of aggressive cancer of the prostate in the lowest quintile of parathion exposure (OR for Q1, 1.96; 95% CI, 1.1–3.5). Stratification by family history of cancer of the prostate did not result in statistically significant associations or trends. Although the odds ratio estimates for all quartiles of exposure were > 1.0 for men with a family history of cancer of the prostate, the estimates were imprecise due to small numbers (i.e. there were 6 or fewer exposed cases in each quartile). [The Working Group noted 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 for aggressive prostate cancers, but not for all prostate cancers.]

A case–control study on cancer of the prostate, nested within the AHS, was reported by Karami et al. (2013); the unique contribution of this study was the exploration of whether certain pesticides may be linked to cancer of the prostate via an interaction with vitamin D-related genetic variants. The motivation for this study was stated to be that anti-carcinogenic effects of vitamin D and its metabolites (e.g. by stimulating cell differentiation, inhibiting cell proliferation or inducing apoptosis) may be reduced by certain pesticides. Karami et al. (2013) compared 776 cases of cancer of the prostate and 1444 controls, who were white, male, pesticide applicators. Interactions were evaluated between 41 pesticides and 152 single-nucleotide polymorphisms (SNPs) in nine genes involved in vitamin D pathways, after adjusting for false discovery rate, to account for multiple comparisons. Parathion use was not associated with cancer of the prostate (OR for ever use, 1.02; 95% CI, 0.78–1.33; *P* value for trend = 0.91). However, statistical interactions were detected between use of parathion and two vitamin D-related genes: the strongest interaction observed was between the *RXRB* gene variant rs1547387 and parathion [(*RXRB* is the Retinoid-X-Receptor-beta gene that is involved in binding vitamin D to vitamin D receptors).

No previously published study has evaluated the association between this specific SNP and cancer.] Significant interactions were also observed between parathion and the *GC* gene (Group specific Component, which is a binding protein that carries vitamin D in blood) variants rs7041 and rs222040. [Ahn et al. (2009) previously showed that the presence of the variant form of the *GC* gene was associated with reduced levels of circulating vitamin D (25-OH-D) in the Prostate, Lung, Colon and Ovary (PLCO) Screening Trial.] The exposure–response pattern among participants with increasing use of parathion and the variant form (G) of the rs1547387 SNP of the *RXRB* gene and the homozygote CC genotype for the *GC* gene in the rs7041 SNP (which alters circulating vitamin D levels) was noteworthy when compared with unexposed participants. [The Working Group noted that this result was not independent from that of the previous study of prostate cancer within the AHS, and confirmed that overall there was no association between exposure to parathion and prostate cancer. However, the contribution of this study was the analysis of potential modification of pesticide effects by genetic variation involving the vitamin D pathway. This study was large enough to allow examination and detection of trends with exposure level in subsets defined by the genetic variants.]

Alavanja et al. (2014) investigated whether exposure to pesticides influenced the risk of non-Hodgkin lymphoma (NHL) and its subtypes in the AHS. Ever having used parathion was not associated with NHL overall (RR, 1.1; 95% CI, 0.8–1.4) or by subtype (small lymphocytic lymphoma/chronic lymphocytic leukaemia/mantle cell lymphoma; diffuse large B-cell lymphoma; follicular B-cell lymphoma; multiple myeloma), and there was no evidence of heterogeneity across subtypes (e.g. relative risk estimates were 1.0 or 1.1 for each subtype). There was no monotonic trend with categories of total days of lifetime use (*P* value for trend = 0.64) or intensity-weighted lifetime days of use (*P* value for trend = 0.74). [The strengths of this analysis were that the comprehensive data permitted controls for multiple confounders, including indicators of total use of other pesticides, and that the large sample size enabled separate analyses of the heterogeneous subtypes of NHL.]

2.2.2 Other cohort studies

A nested case–control study derived from a previous occupational cohort study was reported by Pesatori et al. (1994) (Table 2.1). This was based on a cohort of Florida pest control workers whose licensing records were linked with mortality files (e.g. national death index, death certificates, social security mortality files) (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Parathion use was reported for 2 (3%) cases, 0 deceased controls, and 6 (3%) of living controls, which for the latter resulted in an odds ratio of 3.2 (95% CI, 0.5–20.7) with adjustment for age and smoking [The Working Group noted that the report stated that adjustments for diet, other occupations and other factors did not alter risk estimates. This study was limited by its small size (with 65 deceased cases), and the potential for exposure misclassification by collecting pesticide exposure by interviewing next of kin. The wide confidence interval for the odds ratio demonstrated the imprecision of this estimate due to the modest size of the cohort and the rarity of parathion use.]

2.3 Case–control studies

2.3.1 Case–control studies on lympho-haematopoietic cancers

A single case–control study reported on whether exposure to parathion was associated with risk of lymphoma (Table 2.2). Waddell et al. (2001) pooled data from three case–control studies of NHL among white men in the USA

Table 2.2 Case–control study of lympho-haematopoietic cancers and exposure to parathion

Reference, location follow-up/enrolment period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 748 (response rate, 83%) from tumour registries, clinical groups, and hospitals Controls: 2236 (response rate, 86%) living cases from health service administration records; deceased cases from mortality records Exposure assessment method: questionnaire; Iowa & Minnesota: see Cantor et al. (1992); Kansas: telephone interview, days/year of pesticide use and years of use were asked about herbicides and insecticides overall, not by specific pesticide; subjects were asked to volunteer the pesticides they used; Nebraska: telephone interview days per year of use and years of use were asked for each pesticide used; asked about a predetermined list of about 90 pesticides	NHL	Ever use	5	2.9 (0.9–9.7)	Age, state, proxy/direct respondent	Studies in midwest USA (pooled)

NHL, non-Hodgkin lymphoma

(Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) to evaluate organophosphate pesticides, including parathion, as used by farmers. The three studies were population-based and yielded 748 cases of NHL and 2236 controls (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Detailed subset analyses (e.g. by histological type, state) were done for five pesticides, but this could not be done for parathion due to the rarity with which it was used. Comparing farmers using parathion to non-farmers yielded an odds ratio of 2.9 (95% CI, 0.9–9.7; 5 exposed cases; 8 exposed controls) adjusted for age, state and respondent type (direct versus proxy). [The strengths of this report included the large sample size, which enabled assessment of infrequent exposure to parathion; however, the study was not sufficiently large to detect a gradient of effect. While several potential confounders were considered, the result must be interpreted with caution since the effect of parathion could be confounded by other pesticides that were not controlled for in the analysis.]

2.3.2 Case–control studies on other cancers

Band et al. (2011) reported on a case–control study of cancer of the prostate, for which all male cancer patients identified in the population-based cancer registry for British Columbia, Canada, from 1983 to 1990 were invited to complete a self-administered occupational history and questionnaire, and for whom a job-exposure matrix (JEM) was developed (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Results for 100 pesticides were presented in the report, and it was estimated that 30 (2%) cases and 63 (1%) controls had used parathion, for an odds ratio of 1.51 (95% CI, 0.94–2.41), after adjusting for alcohol, smoking, education, and type of respondent (proxy/direct). With exposure levels defined as above or below the median number of lifetime days on which parathion was used, compared with never users, the odds ratios for low and high use were 1.29 (95% CI, 0.66–2.50) and 1.82 (95% CI, 0.94–3.53), respectively, with a *P* value for the trend of 0.06. [While strengthened by the large number of cases, the results of this study should 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 parathion.]

2.4 Meta-analyses

No data were available to the Working Group.

3. Cancer in Experimental Animals

Studies of carcinogenicity previously assessed by the Working Group (IARC, 1983), and leading to the previous evaluation of *inadequate evidence* in experimental animals for the carcinogenicity of parathion (IARC, 1987), were also included in the present *Monograph*.

3.1 Mouse

See Table 3.1

Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 5 weeks) were fed diets containing parathion (purity, 99.5%; impurities unspecified) at a concentration of 80 or 160 ppm for 71 and 62 weeks, respectively (males), and for 80 weeks (females). Male mice were then observed for 18 and 28 weeks, respectively, while female mice were observed for 9 and 10 weeks, respectively. A matched control group of 10 males and 10 females was observed for 90 weeks. Since the numbers of mice in the matched control groups were small, pooled control groups of 140 males and 130 females were also used for statistical

Table 3.1 Studies of carcinogenicity with parathion in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
B6C3F$_1$ (M, F) 89–90 wk NTP (1979)	Diets containing parathion (purity, 99.5%) at 0 ppm (matched control), 80 ppm, or 160 ppm, ad libitum, 7 days/wk: lower-dose males for 71 wk and then held untreated for an additional 18 wk; higher-dose males for 62 wk and then held untreated for an additional 28 wk; lower- and higher-dose females for 80 wk and then held untreated for an additional 9–10 wk 50 M and 50 F/treated group Since the numbers of mice in the matched-control groups were small, pooled-control groups also were used for statistical comparisons 10 M and 10 F/matched –control group 140 M and 130 F/pooled-control group	No tumours occurred in either sex at incidences that were significantly higher in the dosed groups than in the corresponding matched or pooled control groups	NS	Short duration of treatment, and small number of matched controls Pooled controls: matched controls from study on parathion were combined with matched controls from long-term studies on azinphosmethyl, chlordane, dieldrin, dimethoate, heptachlor, lindane, malathion, phosphamidon, photodieldrin, and tetrachlorvinphos
B6C3F$_1$ (M, F) 18 mo EPA (1991a)	Diets containing parathion (purity, 96.7%) at 0 (control), 60, 100, or 140 ppm for 18 mo 50 M and 50 F/group [age, NR]	*Males* Bronchiolo-alveolar adenoma[a]: 5/50 (10%), 13/50 (26%)*, 6/50 (12%), 4/50 (8%) Bronchiolo-alveolar carcinoma[b]: 0/50, 1/50 (2%), 0/50, 0/50 Bronchiolo-alveolar adenoma or carcinoma (combined): 5/50 (10%), 14/50 (28%)**, 6/50 (12%), 4/50 (8%) *Females* Malignant lymphoma[c]: 0/50, 5/50 (10%)***, 3/50 (6%), 2/50 (4%) Histiocytic sarcoma[d]: 0/50, 1/50 (2%), 0/50, 2/50 (4%)	*P = 0.033 **P = 0.020 ***P = 0.028	Lowest-dose mice were incorrectly dosed with parathion at 500 ppm on days 300–307. These mice were switched to control diet for 17 days to recover, and then returned to the correct dose level. Six males and two females at the lowest dose died within 14 days of the misdosing There was a dose-related decrease in body weight in males and females without treatment-related increase in mortality

[a] Historical controls at testing laboratory: 16/150 (11%); range, 10–12%
[b] Historical controls at testing laboratory: 10/150 (7%); range, 2–12%
[c] Historical controls at testing laboratory: 41/150 (27%); range, 24–32%
[d] Historical controls at testing laboratory: 0/150
F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week

comparisons. Matched controls from the study on parathion were combined with matched controls from other long-term studies performed at the same laboratory on azinphosmethyl, chlordane, dieldrin, dimethoate, heptachlor, lindane, malathion, phosphamidon, photodieldrin, and tetrachlorvinphos. By the end of the experiment (89 weeks), 80% of males at the highest dose, 92% of females at the highest dose, 92% of males and females at the lowest dose, 100% of matched-control males, and 80% of matched-control females were still alive. Full histopathology was performed. There was no significant increase in tumour incidence observed in any of the tissues examined compared with matched or pooled controls (NTP, 1979). [The Working Group noted the short duration of treatment and the small number of matched controls.]

A report by the United States Environmental Protection Agency (EPA, 1991a) provided information on a study in which groups of 50 male and 50 female B6C3F$_1$ mice [age not specified] were fed diets containing parathion (purity, 96.7%) at a concentration of 0 ppm, 60 ppm, 100 ppm, or 140 ppm, ad libitum, 7 days per week for 18 months. Mice at the lowest dose were mistakenly dosed with parathion at 500 ppm between days 300 and 307 of the study. These mice were switched to control diet for 17 days to recover and then returned to the proper dose level. Six males at the lowest dose and two females at the lowest dose died within 14 days of the misdosing. There was a dose-related decrease in body weight in males and females without treatment-induced increase in mortality. The only increases in tumour incidence that were statistically significant were observed in the groups at 60 ppm. The incidences were: 5/50 (10%, control), 13/50 (26%, $P = 0.033$), 6/50 (12%), 4/50 (8%) for bronchiolo-alveolar adenoma in males; 5/50 (10%, control), 14/50 (28%, $P = 0.020$), 6/50 (12%), 4/50 (8%) for bronchiolo-alveolar adenoma or carcinoma (combined) in males; and 0/50 (0%, control), 5/50 (10%, $P = 0.028$), 3/50 (6%), 2/50 (4.0%) for malignant lymphoma in females. At 60 ppm, the incidence of bronchiolo-alveolar adenoma in males (13/50; 26%) exceeded the upper bound of the range reported for historical controls at the testing laboratory (16/150; 11%; range, 10–12%); the incidence of bronchiolo-alveolar carcinoma in males (1/50; 2%) was within the range for historical controls (10/150; 7%; range, 2–12%); and the incidence of malignant lymphoma in females (5/50; 10%) was below the lower bound of the range for historical controls (41/150; 27%; range, 24–32%). [The Working Group noted that tumour incidences were significantly increased only in the group receiving the lowest dose (60 ppm), which had been misdosed.]

3.2 Rat

See Table 3.2

3.2.1 Oral administration

Hazleton & Holland (1950) reported two studies in albino rats [strain and age at start not reported; body weight, 60–70 g], fed diets containing parathion (purity, 95–97%; impurities unspecified) at different concentrations. Two groups of 20 male rats received parathion at a concentration of 10 or 25 ppm for 88 weeks. Two groups of male rats received parathion at a concentration of 50 (10 rats) or 100 ppm (8 rats) for 104 weeks. There were two control groups of 10 and 20 male rats, respectively. In addition, groups of 8–9 female rats received parathion at a concentration of 10 or 50 ppm for 64 weeks, and 6 females served as controls. Survival of males was 69% at 10 ppm, 87% at 25 ppm, and 60% in the first control group; 80% at 50 ppm, 62% at 100 ppm, and 70% in the second control group. Survival of females was 100% at 10 ppm, 62% at 50 ppm, and 67% in controls. Macroscopic examination of the rats, and microscopic examination of a limited number of tissues from males in the groups at 50 ppm and 100 ppm, did not reveal

Table 3.2 Studies of carcinogenicity with parathion in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Albino [strain, NR] (M, F) 64–104 wk Hazleton & Holland (1950)	Diets containing parathion (purity, 95–97%) at 0, 10, 25, 50, or 100 ppm for 64–104 wk 8–20 M and 6–9 F/group (age at start, NR)	Macroscopic examination of the rats and microscopic examination of a limited number of tissues from males at 50 ppm or 100 ppm (exposed for 104 wk) revealed no tumours	NS	Small number of tested rats, and small number of organs used for histopathological examination; limited reporting of the study
Albino [strain, NR] (M, F) ≤ 12 mo Barnes & Denz (1951)	Diets containing parathion (purity, 76.8%) in ethanol at 0, 10, 20 or 50 ppm, for 6 days/wk for 12 mo; or 75 or 100 ppm for 27 or 19 days, respectively, and these rats were observed for up to 12 mo 36 M and 36 F (age, 6 wk)/treated group 30 M and 30 F (age, 6 wk)/control group	No evidence of tumours, with the exception of one spindle cell sarcoma of the mediastinum in one rat at 20 ppm	NS	Short duration of exposure and observation periods, and small number of rats undergoing histopathological examination; limited reporting of the study

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Osborne-Mendel (M, F) ≤ 113 wk NTP (1979)	Diets containing parathion (purity, 99.5%), ad libitum, 7days/wk, at 0 ppm (M and F; matched controls); 32 ppm TWA (lowest-dose M – 40 ppm for 13 wk then lowered to 30 ppm for 67 wk); 63 ppm TWA (highest-dose M – 80 ppm for 13 wk then lowered to 60 ppm for 67 wk); 23 ppm TWA (lowest-dose F – 20 ppm for 13 wk, then increased to 30 ppm for 21 wk, and then lowered to 20 ppm for 46 wk); or 45 ppm TWA (highest-dose F – 40 ppm for 13 wk, then increased to 60 ppm for 21 wk, and then lowered to 40 ppm for 46 wk). The rats were held untreated until experimental wk 112–113 Since the numbers of rats in the matched control groups were small, pooled control groups also were used for statistical comparisons 50 M and 50 F/treated group 10 M, 10 F/matched-control group 90 M, 90 F/pooled-control group	*Males* Adrenal cortical adenoma: 0/9 (matched control), 2/80 (3%, pooled control), 5/49 (10%), 9/46 (20%) Adrenal cortical adenoma or carcinoma (combined): 0/9 (matched control), 3/80 (4%, pooled control), 7/49 (14%), 11/46 (24%) Thyroid follicular-cell adenoma: 3/10 (30%, matched control), 5/76 (7%, pooled control), 2/46 (4%), 8/43 (19%) Pancreatic islet cell carcinoma: 0/9 (matched control), 0/79 (pooled control), 1/49 (2%), 3/46 (7%) *Females* Adrenal cortical adenoma: 1/10 (10%, matched control), 4/78 (5%, pooled control), 4/47 (9%), 11/42 (26%) Adrenal cortical adenoma or carcinoma (combined): 1/10 (10%, matched control), 4/78 (5%, pooled control), 6/47 (13%), 13/42 (31%) Mammary gland fibroadenoma: 2/10 (20%, matched control), 9/85 (11%, pooled control), 16/50 (32%), 8/50 (16%)	$P = 0.001$ (trend, vs pooled) $P = 0.002$ (highest dose vs pooled) $P < 0.001$ (trend, vs pooled) $P = 0.048$ (trend, vs matched) $P = 0.035$ (lowest dose vs pooled) $P < 0.001$ (highest dose vs pooled) $P = 0.037$ (trend, vs pooled) $P = 0.046$ (highest dose vs pooled) $P = 0.024$ (trend, vs pooled) $P = 0.048$ (highest dose vs pooled) $P = 0.037$ (trend, vs matched) $P = 0.001$ (trend, vs pooled) $P = 0.001$ (highest dose vs pooled) $P = 0.028$ (trend, vs matched) $P < 0.001$ (trend, vs pooled) $P < 0.001$ (highest dose vs pooled) $P = 0.002$ (lowest dose vs pooled) Cochran-Armitage trend test and Fisher exact test (for pairwise comparison)	Study limited by adaptation of dose levels to observed toxicity during study, and the use of a small number of matched controls Pooled controls: matched controls from study on parathion were combined with matched controls from long-term studies on azinphosmethyl, captan, chloramben, chlordane, dimethoate, heptachlor, malathion, and pichloram

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Sprague-Dawley (M, F) ≤ 120 wk EPA (1984)	Diets containing parathion (purity, 95.11%) at 0 (control), 0.5, 5.0, or 50 ppm for up to 120 wk 60 M and 60 F weanling rats/group (age at start, NR)	*Males* Thyroid follicular cell adenoma[a]: 1/59 (2%), 1/58 (2%), 2/58 (3%), 5/58 (9%) [4/58, 6.9%]	[NS] (see comments)	A histopathological re-evaluation of thyroid and parathyroid glands performed 2 years after the original report noted only four follicular cell adenomas at the highest dose (EPA, 1986a) as opposed to five identified in the first histopathological evaluation (EPA, 1984). In addition, no increase in the incidence of thyroid gland hyperplasia, and no carcinomas were reported
Wistar (M, F) 26 mo EPA (1989a, b)	Parathion (purity, 96.7%) in the feed at concentrations of 0 (control), 2, 8, and 32 ppm to give doses of 0, 0.1, 0.42, and 1.75 mg/kg bw (M) and 0, 0.14, 0.53 and 2.47 mg/kg bw (F) for 26 mo 50 M and 50 F rats (age, 5–6 wk)/group	*Males* Pancreas:[b] Exocrine adenoma: 0/50*, 0/50, 1/49 (2%), 3/50 (6%) Exocrine carcinoma: 0/50, 0/50, 0/49, 1/50 (2%) Exocrine adenoma or carcinoma (combined): 0/50**, 0/50, 1/49 (2%), 4/50 (8%) Islet cell adenoma: 0/50***, 0/50, 1/49 (2%), 3/50 (6%) *Females* No increase in tumour incidence	*P = 0.002 (trend, Cochran Armitage) **P = 0.0022 (trend, Peto test) ***P = 0.007 (trend, Cochran Armitage)	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Sprague-Dawley (F) ≤ 28 mo Cabello et al. (2001)	Subcutaneous injection of parathion at a dose of 0 (saline control), or 250 µg/100 g bw, injected twice per day for 5 days, and then observed for 28 mo 70 (age, 39 days)/group	Mammary gland adenocarcinoma: 0/70, 10/70 (14%)	[$P < 0.002$, Fisher exact test]	Body weight and survival, NR Rats developing mammary tumours were killed 1 mo after first mammary tumour detected by palpation Tumour latency, 490–619 days The examination of lungs, heart, intestinal tract, ovaries, and uterus did not show any tumour s [the authors did not report how these tissues were examined]

[a] Historical controls: 3.9% (range, 0–8.0%)

[b] Historical controls in male Wistar rats: pancreatic islet cell tumours, 0–6%; exocrine adenomas, 0–6%

bw, body weight; F, female; M, male; mo, month; NR, not reported; NS, not significant; TWA, time-weighted average; wk, week

any tumours. [The Working Group noted the small number of rats tested, the limited number of organs examined by histopathology, and the limited reporting of the study.]

Barnes & Denz (1951) described a study in which three groups of 36 male and 36 female albino rats [strain not reported] (age, 6 weeks) were given diets containing parathion (purity, 76.8%) at a concentration of 10, 20, or 50 ppm for 6 days per week for up to 12 months. Two additional groups of 36 male and 36 female rats were given parathion at 75 or 100 ppm for 27 and 19 days, respectively; these animals were observed for up to 12 months. A control group of 30 males and 30 females was observed for 12 months. The survival rates were 98%, 97%, 97%, and 61% in the groups at 0, 10, 20, and 50 ppm, respectively. Mortality rates during the dosing period were 82% in the group at 75 ppm and 90% in the group at 100 ppm. Histopathological examination was performed on all rats at 75 or 100 ppm, and on 20% of rats in the groups at 0, 10, 20, or 50 ppm, and that were still alive after 12 months. With the exception of a spindle cell sarcoma of the mediastinum in one rat at 20 ppm, no tumours were observed. [The Working Group noted the high mortality in the two groups at the higher doses, the short duration of the exposure and observation periods, the small number of rats undergoing histopathological examination, and the limited reporting of the study.]

In a study by the United States National Toxicology Program, groups of 50 male and 50 female Osborne-Mendel rats (age, 5 weeks), were fed diets containing parathion (purity, 99.5%; impurities unspecified) (NTP, 1979). Male rats initially received parathion at 40 ppm (lower dose) or 80 ppm (higher dose) for 13 weeks, then doses were lowered to 30 ppm (lower dose) and 60 ppm (higher dose) for 67 weeks, resulting in time-weighted average doses of 32 ppm (lower dose) and 63 ppm (higher dose). Female rats initially received parathion at 20 ppm (lower dose) or 40 ppm (higher dose) for 13 weeks, then doses were increased to 30 ppm (lower dose) and 60 ppm (higher dose) for 21 weeks (to be consistent with the doses for male rats); but then lowered to 20 ppm (low dose) and 40 ppm (high dose) for 46 weeks (due to generalized tremors among females at the higher dose after 33 weeks), resulting in time-weighted average doses of 23 ppm (lower dose) and 45 ppm (higher dose). All rats were subsequently observed for 32–33 weeks. A matched control group of 10 males and 10 females was observed for 112 weeks, while a pooled group of 90 males and 90 females served as controls for the statistical analysis. Matched controls from the study on parathion were combined with matched controls from long-term studies performed at the same laboratory on azinphosmethyl, captan, chloramben, chlordane, dimethoate, heptachlor, malathion, and picloram. At the end of the study, survival in the groups at the higher dose was 72% in males and 68% in females, while survival in the groups at the lower dose was 62% in males and 72% in females. In the matched control group, a survival rate of 70% was recorded for males and females. Full histopathology was performed.

In males, the incidence of adrenal cortical adenoma or carcinoma (combined) was 3/80 (4%) in pooled controls, 0/9 in matched controls, 7/49 (14%) in the group at the lower dose (two rats developed carcinoma), and 11/46 (24%) in the group at the higher dose (two rats developed carcinoma) (Cochran-Armitage test for positive trend: $P < 0.001$ using pooled controls, $P = 0.048$ using matched controls; Fisher exact test: high-dose group versus pooled controls, $P < 0.001$, and low-dose group versus pooled controls, $P = 0.035$). The incidence of adrenal cortical adenoma was 2/80 (3%) in pooled controls, 0/9 in matched controls, 5/49 (10%) in the group at the lower dose and 9/46 (20%) in the group at the higher dose (Cochran-Armitage test for positive trend: $P = 0.001$ using pooled controls; Fisher exact test: higher-dose versus pooled controls, $P = 0.002$).

In females, the incidence of adrenal cortical adenoma or carcinoma (combined) was 4/78 (5%) in pooled controls, 1/10 (10%) in matched controls, 6/47 (13%) in the group at the lower dose (two rats developed carcinoma), and 13/42 (31%) in the group at the higher dose (two rats developed carcinoma) (Cochran-Armitage test for positive trend: $P < 0.001$ using pooled controls, $P = 0.028$ using matched controls; Fisher exact test: high-dose group versus pooled controls, $P < 0.001$).

In males, the incidence of islet cell carcinoma of the pancreas was 0/79 in pooled controls, 0/9 in matched controls, 1/49 (2%) in the group at the lower dose, and 3/46 (7%) in the group at the higher dose (Cochran-Armitage test for positive trend: 0.024 using pooled controls; Fisher exact test: high-dose group versus pooled controls, $P = 0.048$). Follicular cell adenoma of the thyroid gland was also observed, with incidences of 5/76 (7%) in pooled controls, 3/10 (30%) in matched controls, 2/46 (4%) in the group at the lower dose, and 8/43 (19%) in the group at the higher dose (Cochran-Armitage test for positive trend: $P = 0.037$ using pooled controls; Fisher exact test: higher-dose group versus pooled controls, $P = 0.046$). In females, there was a significant increase ($P = 0.002$) in the incidence of fibroadenoma of the mammary gland in the group at the lower dose (16/50; 32%) compared with pooled controls (9/85; 11%) (NTP, 1979). [The Working Group noted the adaptation of dose levels because of observed toxicity, and the use of small numbers of matched controls.]

A report by the EPA (1984) provided information on a study in which diets containing parathion (purity, 95.11%) were given to groups of 60 male and 60 female weanling Sprague-Dawley rats [age at start, not reported] at a concentration of 0 (control), 0.5, 5, or 50 ppm for up to 120 weeks. Mortality in all groups was similar by the end of the study. Body-weight gain was decreased in males and females in the group at the highest dose. Follicular cell adenoma of the thyroid gland was observed at a [non-significantly] higher incidence in the groups of treated males compared with controls: 1/59 (2%, control), 1/58 (2%), 2/58 (3%), 5/58 (9%) [4/58; 6.9%]. The EPA (1986a) indicated that the historical incidence for this tumour in male Sprague-Dawley rats at this laboratory ranged from 0% to 8.0% (mean, 3.9%). No other increase in tumour incidence was reported. Two years after the original report, a re-evaluation of the histopathology of the thyroid and parathyroid glands was performed and published (EPA, 1986a). The re-evaluation was considered necessary owing to the lack of increase in the incidence of hyperplasia of the thyroid gland reported in the group at the highest dose. [Such an increase may precede the appearance of neoplastic changes.] A re-evaluation of the histology slides by an expert in endocrine pathology reported only four follicular cell adenomas of the thyroid in the group at the highest dose, as opposed to five as identified in the original histological evaluation. No follicular carcinomas of the thyroid were reported.

The EPA (1989a, b) also provided information on a study in which groups of 50 male and 50 female Wistar rats (age, 5–6 weeks) were given diets containing parathion (purity, 96.7%) at a concentration of 0 (control), 2, 8, or 32 ppm for 26 months. There was a treatment-related increase in mortality in females at the highest dose, while mortality in all other groups was similar at termination of the study. A decrease in body-weight gain was observed in males and females at the highest dose. There was a significant positive trend in the incidence of tumours of the pancreas in male rats; the incidences of exocrine adenoma were: 0/50, 0/50, 1/49 (2%), 3/50 (6%) ($P = 0.002$, Cochran Armitage test); the incidences of exocrine adenoma or carcinoma (combined) were: 0/50, 0/50, 1/49 (2%), 4/50 (8%) ($P = 0.0022$, Peto test); and the incidences of islet cell adenoma were: 0/50, 0/50, 1/49 (2%), 3/50 (6%) ($P = 0.007$, Cochran Armitage test). No other increases in tumour incidence were reported.

An additional study in rats treated by gavage was found to be inadequate for the evaluation of parathion by the Working Group because a mixture of 15 pesticides (including only 0.70% parathion) was studied (Pasquini et al., 1994).

3.2.2 Subcutaneous administration

Cabello et al. (2001) carried out an experiment on 140 virgin female Sprague-Dawley rats (age, 39 days); 70 rats were treated subcutaneously with saline, while an additional 70 rats were treated with parathion (250 µg/100 g bw) twice per day for 5 days, and observed for 28 months. Changes in body weight and survival were not reported. Rats with tumours of the mammary gland were killed at 1 month after detection of the tumour by palpation. Tumours were examined by light microscopy. At termination of the experiment, rats in the control group did not develop any type of tumour, while 10 out of 70 (14%) rats treated with parathion developed adenocarcinoma of the mammary gland [$P = 0.002$]. Tumour latency was 490–619 days.

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

Extensive literature was available on the toxicokinetics of parathion in humans and experimental animals.

4.1.1 Absorption

(a) Humans

The evidence for absorption and internal exposure to organophosphate pesticides, such as parathion, has been documented in a large number of biomonitoring studies in humans (e.g. Arcury et al., 2007). For example, *para*-nitrophenol, a metabolic by-product of parathion, was detectable in the urine of children aged ≤ 6 years in a central Washington State agricultural community (Fenske et al., 2002).

Acute poisoning episodes in humans also confirm that parathion can be absorbed from the gastrointestinal tract (Hoffmann & Papendorf, 2006).

Specific data on rates of oral absorption or fractional uptake in humans were not available but on the basis of depressed blood cholinesterase activities and the detection of urine metabolites of parathion in intoxicated patients, absorption of parathion does occur via the gastrointestinal tract (Areekul et al., 1981; Olsson et al., 2003). [The Working Group noted that, because of its lipophilicity, parathion is expected to be absorbed via passive diffusion.] On the basis of biomonitoring studies of parathion in humans, dermal and oral exposures during occupational practices and diet are important routes of exposure, whereas inhalation appears to be less important (Alavanja et al., 2013).

Several studies were identified that examined dermal penetration of parathion in a variety of different model systems. Parathion was not efficiently absorbed into the body after dermal contact under controlled experimental settings (Qiao et al., 1994; Wester et al., 2000; van der Merwe & Riviere, 2005). Only a small fraction of the dermally applied parathion dose to human skin was absorbed and bioavailable. The rate-limiting step during percutaneous absorption appeared to be the partitioning of parathion into the stratum corneum (Qiao et al., 1994).

Dermal uptake can be affected by parathion formulation, ambient temperature, relative humidity, and airflow across the exposed skin (Durham et al., 1972). The extent of absorption of parathion after dermal exposure, assessed by measurements of parathion on pads worn by workers on clothing near bare skin, was significantly influenced by the ambient temperature. The excretion of *para*-nitrophenol (parathion metabolite) in urine increased as a function of

the ambient temperature, indicating enhanced dermal absorption of parathion.

In controlled experiments to separately assess respiratory and dermal absorption among orchard workers engaged in applying parathion using power airblast spray equipment, and wearing either protective clothing or a respirator during exposure, dermal absorption proved to be much greater (0.497–0.666 mg of the absorbed dose) than respiratory absorption (0.006–0.088 mg of the absorbed dose), based on excretion of the parathion metabolite *para*-nitrophenol (Durham et al., 1972).

Clinical reports of severe intoxication with parathion indicated that there were large differences in plasma levels of parathion and paraoxon between the patients, suggesting inter-individual differences in absorption, metabolism, or excretion (Eyer et al., 2003). The estimated amount of parathion that was absorbed varied widely (range, 0.12–4.4 g).

(b) Experimental systems

There was rapid absorption of parathion in male Wistar rats given parathion orally (at one third of the median lethal dose, LD_{50}), as shown by detection of parathion in the blood within minutes after administration (Garcia-Repetto et al., 1995).

The peak serum concentrations in six mongrel dogs treated orally with parathion at a dose of 10 mg/kg bw varied from 0.02 to 0.41 µg/mL, while time to peak concentration ranged from 30 minutes to 5 hours, indicating substantial inter-individual variability in oral absorption (Braeckman et al., 1983).

A toxicokinetic study of parathion in rabbits given a single oral exposure of parathion (3 mg/kg bw) showed that the first-order rate constant of oral parathion absorption was 33 h^{-1} (Peña-Egido et al., 1988a), which indicates that absorption from the gastrointestinal compartment is rapid and that parathion in this compartment has a half-life of 1.3 minutes. In rabbits, the rates of dermal absorption per unit area were estimated to be 0.059 µg/minute per cm^2 of skin for parathion and 0.32 µg/minute per cm^2 for paraoxon; these are much slower than rates of uptake after oral absorption (Nabb et al., 1966).

In pigs, the rate of dermal absorption was significantly influenced by the vehicle used. Absorption of parathion ranged from 15% to 30% of the applied dose when administered in dimethylsulfoxide or octanol, while only 4–5% of the applied dose was absorbed when administered in macrogol. The type of surfactant in the formulation under consideration also significantly influences rates of dermal absorption (Gyrd-Hansen et al., 1993).

The extent of absorption after dermal application of parathion using a porcine skin in-vitro model was 1–3% of the applied dermal dose (van der Merwe & Riviere, 2005).

4.1.2 Distribution

(a) Humans

No data on tissue distribution beyond blood concentrations of parathion in humans were available to the Working Group. After intoxication with parathion, measurement of plasma concentrations of parathion indicated that the volume of distribution at steady-state (V_{dss}) for parathion was around 20 L/kg, suggesting a wide distribution (Eyer et al., 2003). Several studies have suggested that 94–99% of parathion is protein-bound at equilibrium, mostly to serum albumin (Braeckman et al., 1983; Nielsen et al., 1991; Foxenberg et al., 2011). In-vitro equilibrium dialysis experiments indicated that once equilibrium had been reached (in about 60 minutes), affinity for human serum albumin was greater for parathion (~94% bound) than for paraoxon (~60% bound) (Foxenberg et al., 2011).

(b) Experimental systems

After absorption, parathion is uniformly distributed systemically in rodents, with no evidence of long-term accumulation in any particular tissue, including fat (Hazleton & Holland, 1950). After absorption in rats injected subcutaneously with [^{32}P]-labelled parathion, radioactive material is readily taken up by the liver, kidney, and fat (Fredriksson & Bigelow, 1961), and metabolized. Available in-vivo data in rats show that parathion has an affinity for adipose tissue and the liver (Poore & Neal, 1972; Garcia-Repetto et al., 1995), which is supported by studies in rat and mouse tissues in vitro (Sultatos et al., 1990; Jepson et al., 1994). In male Sprague-Dawley rats given a single oral dose of [^{35}S]-labelled parathion (29 mg/kg bw), the tissue levels of radiolabel 35 minutes after dosing followed the rank order: liver > intestine > kidney > muscle > lung (Poore & Neal, 1972). [The Working Group noted that adipose tissue was not examined in this particular study.]

In rats, the time-course for parathion in blood after administration of an intravenous dose of parathion showed a rapid distribution phase, followed by a slower elimination phase (Eigenberg et al., 1983). The time-course of parathion levels in liver and brain followed the same kinetic profile as in blood. Rapid metabolism of parathion in liver was evident. The elimination half-life of parathion in the blood was 3.4 hours after an intravenous dose (3 mg/kg bw) in rats. Three to four times higher levels of paraoxon were found in weanling rat brain than in adult rat brain after intravenous administration of parathion to immature (age, 23 days) and adult (age, 60–75 days) rats (Gagné & Brodeur, 1972).

4.1.3 Metabolism

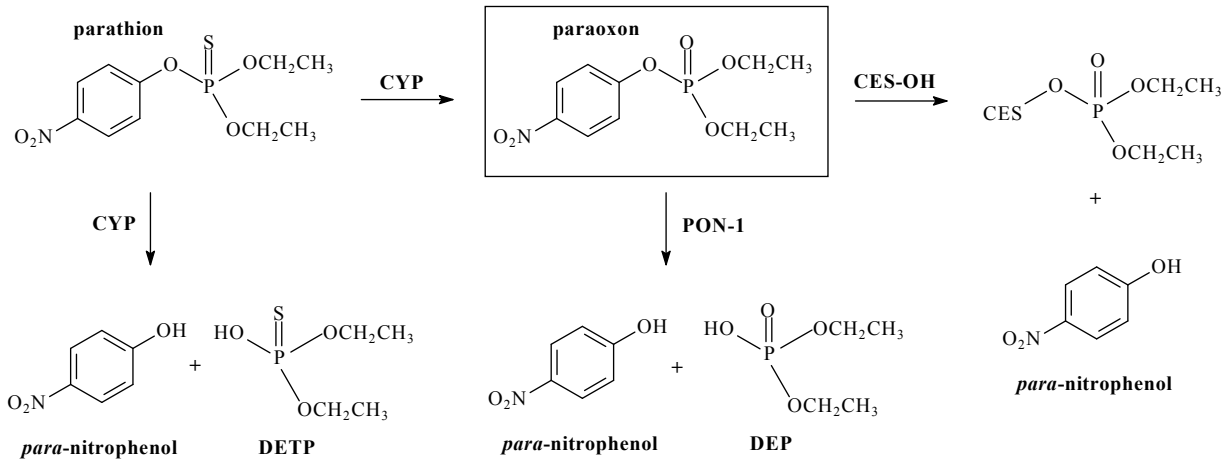
(a) Overview of the metabolism of parathion

Cytochrome P450s (CYPs) are important enzymes for the bioactivation and detoxification of parathion, as are paraoxonase-1 and carboxylesterase for the detoxification of the active paraoxon metabolite (see the pathways of metabolism of parathion outlined in Fig. 4.1). The bioactivation and detoxification pathways controlled by CYPs share a common phosphooxythiran intermediate (see Fig. 4.2; Neal & Halpert, 1982). In general, a complex picture emerges regarding the metabolism of organophosphorothioates. Multiple CYP isoforms are involved in their oxidation. Human CYP3A4/5, CYP2C8, and CYP1A2 have been identified as being involved in the metabolism of parathion (Mutch & Williams, 2006). During oxidative metabolism of parathion by CYP, the release of the sulfur atom from parathion leads to covalent modification of cysteine residues and a resulting loss of the haem moiety, thereby inactivating the CYP (Halpert et al., 1980).

Carboxylesterases (which are abundant esterases and members of the serine hydrolase superfamily) and paraoxonase-1 are found in the liver and plasma, and are important enzymes involved in paraoxon detoxification in several species, including humans (Ross et al., 2012; Costa et al., 2013), mice and rats (Crow et al., 2007), and rainbow trout (Abbas & Hayton, 1997). It is notable that humans express abundant amounts of carboxylesterase in the liver, but do not express carboxylesterase in the plasma as do rodents (Li et al., 2005). Paraoxonase-1 can catalytically hydrolyse the oxon (Costa et al., 2013), while carboxylesterases are 1:1 stoichiometric scavengers of oxons, which do not catalytically hydrolyse the substrate (Crow et al., 2012).

The oxon metabolite can also escape the scavenging function of carboxylesterase and instead covalently modify (and inhibit) various serine hydrolase enzymes, including the B-esterase

Fig. 4.1 Biotransformation of parathion



Cytochrome P450 (CYP)-catalysed reactions produce the desulfuration metabolite (oxon) or aryl alcohol and dialkylthiophosphate products. Paraoxonase-1 (PON-1) and carboxylesterase (CES) contribute to parathion detoxification reactions. The bioactive paraoxon metabolite is indicated by the box. 4-Nitrophenol is the dearylation product and the major metabolite of parathion. CES-OH, indicates carboxylesterase with –OH being the functionality of the active-site serine residue that is covalently modified by oxon metabolite.
DEP, diethyl phosphate; DETP, diethylthiophosphate
Compiled by the Working Group using information from Eaton (2000) and Poet et al. (2004)

targets butyrylcholinesterase, acetylcholinesterase, and carboxylesterase (Casida & Quistad, 2004; see Fig. 4.3). In general, analytical measurement of oxons in blood is difficult due to the low levels and relative instability of the metabolite formed (Timchalk et al., 2007). The most important target with respect to the insecticidal action of the oxon is acetylcholinesterase, the esterase responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems.

(b) Humans

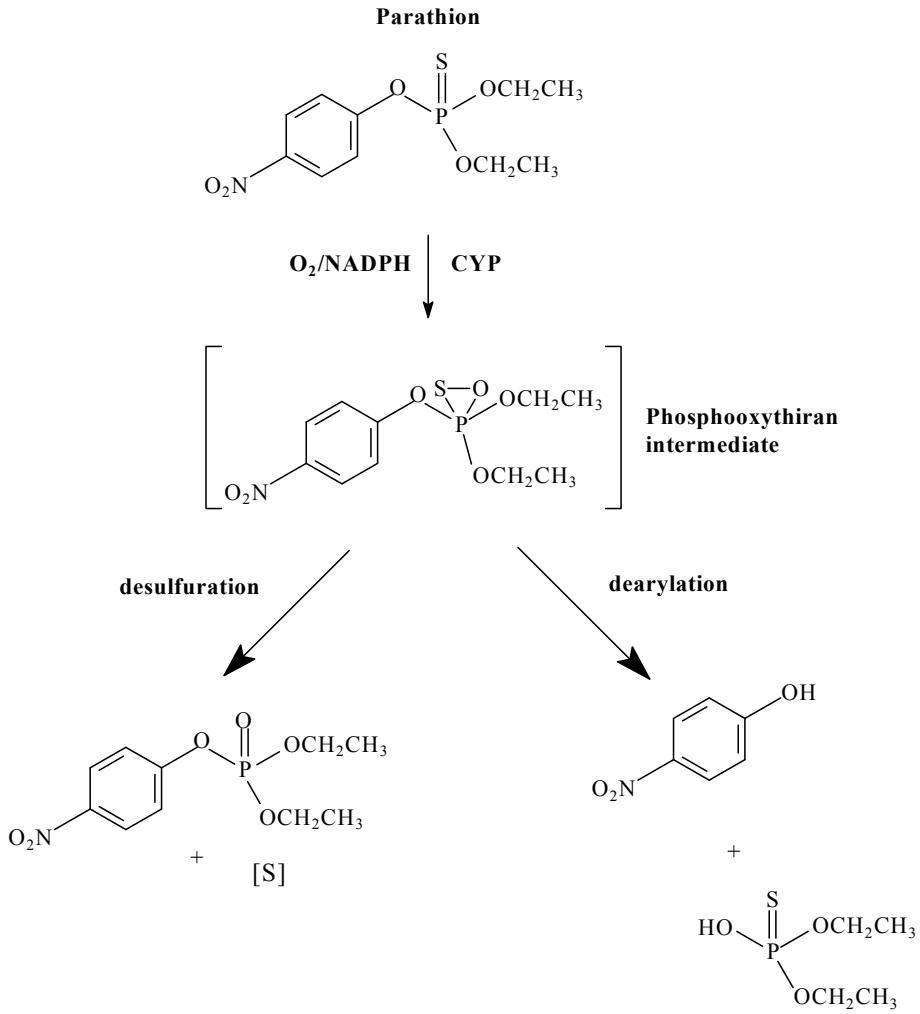
The metabolism of parathion in humans follows the pathways outlined in Fig. 4.1. Rates of parathion oxidation varied about 10-fold in human liver microsomes from 23 individuals (1.72–18.33 nmol total metabolites/mg protein per minute) (Butler & Murray, 1997). CYP3A4 was implicated as a major CYP isoform responsible for the oxidation of parathion. Desulfuration of parathion can result in substantial inhibition of CYP due to transfer of the phosphorothioate thionosulfur atom to the CYP apoprotein, resulting in amino acid modification and enzyme inactivation (Butler & Murray, 1997).

(c) Experimental systems

Metabolism by cytochrome P450s in liver is an important pathway of parathion detoxification in rodents. In-vivo inhibition of CYP3A in rat liver by neostigmine or physostigmine significantly increased the area under the curve (AUC) for parathion in blood, while substantially reducing its clearance (Hurh et al., 2000a, b). Braeckman et al. (1983) estimated an 82–97% hepatic extraction ratio in anaesthetized dogs given an intravenous dose of parathion in the foreleg vein, which emphasizes the efficient metabolism of parathion by the liver.

Compared with adult male rats, adult female rats exhibited a reduced capacity to metabolize parathion through the bioactivation and dearylation pathways (Gagné & Brodeur, 1972). In the same study, weanling rats were less capable of detoxifying parathion and paraoxon than were adults.

Fig. 4.2 Common CYP-derived phosphooxythiran intermediate of parathion



Phosphooxythiran can decompose to paraoxon and a sulfur atom [S] (desulfuration pathway) or to *para*-nitrophenol and diethylthiophosphate (dearylation pathway)
CYP, cytochrome P450
Compiled by the Working Group using information from Neal & Halpert (1982)

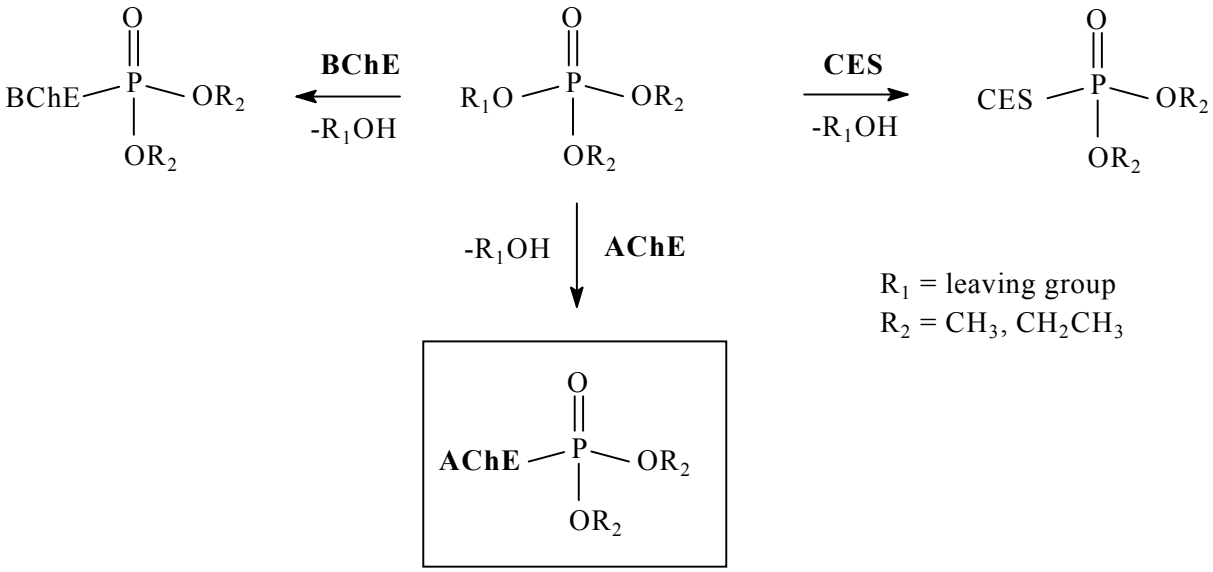
Extrahepatic metabolism of parathion has been demonstrated in two studies. Isolated perfused lungs from guinea-pigs and rabbits were shown to efficiently extract parathion and paraoxon from the perfusate solution, enabling biotransformation of the compounds in the lung tissue (Lessire et al., 1996). There was also evidence for first-pass metabolism of parathion by isolated porcine skin after topical application (Chang et al., 1994). Conversion to paraoxon and *para*-nitrophenol was noted.

4.1.4 Excretion

(a) Humans

The polar metabolites of parathion are excreted primarily via the kidney into the urine. For example, *para*-nitrophenol, DEP, and DETP are found in human urine after exposure to parathion, and have been used for biomonitoring purposes (Arterberry et al., 1961; Wolfe et al., 1970; Morgan et al., 1977). *Para*-Nitrophenol is excreted as glucuronide or sulfate conjugates in

Fig. 4.3 Reactions of a generic oxon metabolite with esterases



Reaction product with the canonical toxicological target of organophosphate responsible for neurotoxicity is shown in box; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CES, carboxylesterase
Adapted with permission from Casida & Quistad (2004); copyright (2004) American Chemical Society

the urine (Elliott et al., 1960). Larger amounts of parathion metabolites (DEP and DETP) were detectable in the urine of children of farmworkers in North Carolina when compared with reference data for the USA (Arcury et al., 2006). Metabolic degradates of parathion have also been detected in amniotic fluid (Bradman et al., 2003).

(b) Experimental systems

DETP, DEP, and *para*-nitrophenol were detected in the urine of male Sprague-Dawley rats given parathion by oral gavage (0.032 or 0.32 mg/rat per day) once per day for 3 days (Bradway et al., 1977). The dialkyl(thio)phosphate degradates of parathion, DEP and DETP, can also be readily absorbed after oral exposure in rats and are rapidly excreted unchanged in the urine (Timchalk et al., 2007). When DEP or DETP were administered orally by gavage to male Sprague-Dawley rats, peak plasma concentrations were reached 1–3 hours after administration. By 72 hours after dosing, essentially all DEP was recovered in the urine, suggesting minimal metabolism, while 50% of the administered dose of DETP was recovered in the urine (Timchalk et al., 2007).

The urinary excretion kinetics of the metabolite *para*-nitrophenol were studied in rabbits given parathion as an oral dose of 3 mg/kg bw (Peña-Egido et al., 1988b). Elimination of *para*-nitrophenol began rapidly and, of the total amount excreted during the study period, 46% was excreted in the first 3 hours; 85% was excreted 6 hours after administration of parathion. After topical application of [^{14}C]-labelled parathion (200 µg) to weanling Yorkshire sows, > 80% of the absorbed radiolabel was eliminated in the urine (Carver & Riviere, 1989). In another study in pigs, intravenous administration of [^{14}C]-labelled parathion at 0.5 mg/kg bw resulted in urinary excretion of 18%, 48%, and 82% of the administered dose within 3 hours in newborn, 1-week-old, and 8-week-old piglets, respectively, suggesting age-dependent excretion of parathion (Nielsen et al., 1991). The main metabolite

detected was *para*-nitrophenyl-glucuronide, which comprised 85% of the [^{14}C]-labelled material in the urine.

4.2 Mechanisms of carcinogenesis

This section summarizes evidence for the key characteristics of carcinogens (IARC, 2014) for which there were adequate data for evaluation, concerning whether parathion is genotoxic; modulates receptor-mediated effects; induces oxidative stress; induces chronic inflammation; and alters cell proliferation, death or nutrient supply.

4.2.1 Genotoxicity and related effects

Parathion has been studied in several assays for genotoxicity in different test systems. Table 4.1, Table 4.2, Table 4.3, Table 4.4 and Table 4.5 summarize the studies carried out in exposed humans, in human cells in vitro, in non-human mammals in vivo and in vitro, and in non-mammalian systems in vitro, respectively.

(a) Humans

See Table 4.1 and Table 4.2

In 25 male vegetable-garden workers exposed occupationally to seven pesticides, including parathion, the frequency of chromosomal aberration and sister-chromatid exchange was increased in peripheral lymphocytes when compared with controls (Rupa et al., 1988).

In human liver HepG2 cell cultures, parathion induced DNA damage as measured by the comet assay (Edwards et al., 2013). Parathion caused sister-chromatid exchange in the lymphoid cell line LAZ-007, with or without metabolic activation (Sobti et al., 1982), but not in cultured human lymphocytes with or without metabolic activation (Kevekordes et al., 1996). Parathion did not cause unscheduled DNA synthesis in human fetal lung fibroblasts, WI-38 (Waters et al., 1980).

Paraoxon, a metabolite of parathion, induced DNA strand breaks in lymphocytes from adult peripheral blood and from newborn umbilical cord blood, with a dose–response relationship; induction was greater in newborns than in adults. Paraoxon also increased the frequency of micronucleus formation in human lymphocytes from adults and newborns (Islas-González et al., 2005; Rojas-García et al. 2009).

(b) Experimental systems

See Table 4.3, Table 4.4, Table 4.5

Parathion did not cause dominant lethal mutation in mice after oral administration (Waters et al., 1980). Parathion also failed to induce micronucleus formation in mouse bone marrow after a single oral (Kevekordes et al., 1996) or intraperitoneal (EPA, 1988) dose; however, micronucleus formation was induced by repeated intraperitoneal doses (Ni et al., 1993).

Parathion induced micronucleus formation in Chinese hamster lung cells (Ni et al., 1993). Parathion also induced sister-chromatid exchange in Chinese hamster ovary cells; the metabolite paraoxon also induced sister-chromatid exchange, with a stronger effect (Nishio & Uyeki, 1981). Parathion did not cause sister-chromatid exchange in rat primary hepatocytes, nor did it show a clear mutagenic effect in the *Hprt* test in Chinese hamster ovary cells (EPA, 1988).

Parathion did not cause mutations in *Drosophila melanogaster* (Waters et al., 1980).

Parathion did not induce mutations in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, and TA1538 (Bartsch et al., 1980; EPA, 1988). Paraoxon, a metabolite of parathion, did not induce mutation in *Salmonella typhimurium* YG1024 with metabolic activation (Wagner et al., 1997), but caused forward mutation in *Schizosaccharomyces pombe* (ade6) without metabolic activation (Gilot-Delhalle et al., 1983).

Table 4.1 Genetic and related effects of parathion in exposed humans

End-point	Test	Tissue	Cell type (if specified)	Description of exposed and controls	Response[a], significance	Comments	Reference
Chromosomal damage	Chromosomal aberrations	Blood	Lymphocytes	25 male workers in vegetable gardens, smokers and alcohol consumers, exposed to 7 pesticides, including parathion 30 controls, healthy males from the same age group and socioeconomic class (control I, 20 non-smokers and non-consumers of alcohol; control II, 10 smokers and alcohol consumers)	(+), $P < 0.05$	Exposure to several pesticides; P value for exposed workers, irrespective of the duration of exposure, compared with control group I or II	Rupa et al. (1988)
Chromosomal damage	Sister-chromatid exchanges	Blood	Lymphocytes	25 male workers in vegetable gardens, smokers and alcohol consumers, exposed to 7 pesticides, including parathion 30 controls, healthy males from the same age group and socioeconomic class (control I, 20 non-smokers and non-consumers of alcohol; control II, 10 smokers and alcohol consumers)	(+), $P < 0.05$	Exposure to several pesticides; P value for exposed workers, irrespective of the duration of exposure, compared with control group I or II	Rupa et al. (1988)

[a] +, positive

Table 4.2 Genetic and related effects of parathion and paraoxon 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			
Parathion							
Liver, HepG2	DNA damage	DNA strand break Comet assay	+	NT	12 mM [5242 µg/mL]		Edwards et al. (2013)
Fetal lung fibroblasts (WI-38)	DNA damage	Unscheduled DNA synthesis	–	–	NR		Waters et al. (1980)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	–	–	100 µM [29 µg/mL]		Kevekordes et al. (1996)
Lymphoid cell line (LAZ-007)	Chromosomal damage	Sister-chromatid exchange	+	+	0.2 µg/mL without, and 20 µg/mL with metabolic activation	Only one concentration tested [20 µg/mL] with metabolic activation	Sobti et al. (1982)
Paraoxon							
Lymphocytes (adult peripheral blood or newborn umbilical cord blood)	DNA damage	DNA strand breaks, comet assay	+	NT	0.075 µg/mL	No statistical calculations	Islas-González et al. (2005)
Lymphocytes (blood)	Chromosomal damage	Micronucleus formation	+	NT	1 µM [0.29 µg/mL]	Positive dose–response relationship (1–25 µM)	Rojas-García et al. (2009)
Lymphocytes (adult peripheral blood or newborn umbilical cord blood)	Chromosomal damage	Micronucleus formation	+	NT	0.2 µg/mL		Islas-González et al. (2005)

[a] +, positive; –, negative

HepG2, human hepatocellular carcinoma cell line; HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; NR, not reported

Table 4.3 Genetic and related effects of parathion in non-human mammalian experimental systems in vivo

Species	End-point	Test	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse	Mutation	Dominant lethal test	Ovary/uterus after mating	(–)	10 mg/kg	NA	Only one dose tested; no detailed data available (abstract only)	Degraeve et al. (1979)
Mouse	Mutation	Dominant lethal test	Ovary/uterus after mating	–	250 mg/kg diet	Three doses tested: 62.5, 125, 250 mg/kg diet, p.o. ×1	The index of dead implants per total implants was evaluated after mating	Waters et al. (1980)
Mouse	Chromosomal damage	Micronucleus formation	Bone marrow	–	2.2 mg/kg bw (male), 1.5 mg/kg bw (female)	p.o. ×1	Only one dose tested per sex: highest tolerated dose	Kevekordes et al. (1996)
Mouse	Chromosomal damage	Micronucleus formation	Bone marrow	+	0.1, 0.2, 0.4, 0.8 × LD_{50}	i.p. 1×/day, ×4	LD_{50} not given; LED not specified	Ni et al. (1993)
Mouse	Chromosomal damage	Micronucleus formation	Bone marrow	–	26 mg/kg bw	i.p. ×1		EPA (1988)

[a] +, positive; –, negative; (–), negative, no detailed data available

HID, highest ineffective dose; i.p., intraperitoneal; LD_{50}, median lethal dose; LED, lowest effective dose, NA, not available; NT, not tested; p.o., oral

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

Species	Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC/HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Parathion								
Hamster	Chinese hamster ovary cells	Mutation	*Hprt*	+/–	+/–	0.03 µL/mL	No effect at a higher dose (0.3 µL/mL); equivocal results	EPA (1988)
Hamster	Chinese hamster ovary cells	Chromosomal damage	Sister-chromatid exchange	+	NT	0.3 mM [87.5 µg/mL]		Nishio & Uyeki (1981)
Rat	Primary hepatocytes	DNA damage	Unscheduled DNA synthesis	–	NT	0.003 µL/mL		EPA (1988)
Hamster	Chinese hamster lung	Chromosomal damage	Micronucleus formation	+	NT	NR	Only one dose tested: highest dose that induced 50% of cell death [50% toxicity], NR	Ni et al. (1993)
Paraoxon								
Hamster	Chinese hamster ovary cells	Chromosomal damage	Sister-chromatid exchange	+	NT	0.1 mM [27.5 µg/mL]	Produced a higher frequency of exchange than parathion	Nishio & Uyeki (1981)

[a] +, positive; –, negative; +/–, equivocal

HIC, highest ineffective concentration; Hprt, hypoxanthine-guanine phosphoribosyltransferase; LEC, lowest effective concentration; NR, not reported; NT, not tested

Table 4.5 Genetic and related effects of parathion and paraoxon in non-mammalian systems

Phylogenetic class	Test system	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Parathion								
Insect	*Drosophila melanogaster*	Mutation	Sex-linked recessive lethal	–	NA	0.5 ppm [0.5 µg/mL]		Waters et al. (1980)
Prokaryote (bacteria)	*Salmonella typhimurium* TA98, TA100	Mutation	Reverse mutation	–	–	1.35 µmol/plate [372 µg/plate]		Bartsch et al. (1980)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Mutation	Reverse mutation	–	–	10 000 µg/plate		EPA (1988)
Paraoxon								
Prokaryote (bacteria)	*Salmonella typhimurium* YG1024	Mutation	Reverse mutation	NT	–	1 mM [275 µg/mL]		Wagner et al. (1997)
Lower eukaryote (yeast)	*Schizosaccharomyces pombe* (ade6)	Mutation	forward mutation	+	–	12 mM [3300 µg/mL]		Gilot-Delhalle et al. (1983)

[a] +, positive; –, negative

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

4.2.2 Receptor-mediated mechanisms

(a) Neurotoxicity-pathway receptors

Parathion is bioactivated to paraoxon in insects and mammals (Section 4.1.3; Casida & Quistad, 2004). Paraoxon can covalently modify the catalytic serine residue of several B-esterases and inhibit their catalytic activity, including the canonical target acetylcholinesterase, resulting in acute neurotoxicity (see Fig. 4.3). Additional receptor targets of parathion and paraoxon that can affect neurotoxicity include butyrylcholinesterase, neuropathy target esterase, and cannabinoid receptor (Quistad et al., 2002). Some studies reviewed in Sections 4.2.4 and 4.2.5 showed that some mechanistic effects of relevance to the carcinogenicity of parathion are blocked or mitigated by co-administration of the anticholinergic drug atropine, and may be at least in part be related to inhibition of acetylcholinesterase activity.

(b) Sex-hormone pathway disruption

(i) Humans

No data from exposed humans were available to the Working Group.

In an in-vitro human androgen-receptor reporter-gene assay using a transfected African monkey kidney cell line (CV-1), parathion (0.1–10 μM) showed significant inhibitory effects on transcriptional activity induced by 5α-dihydrotestosterone (Xu et al., 2008). The concentration for 50% inhibition (IC_{50}) of 5α-dihydrotestosterone-induced chloramphenicol acetyltransferase activity was 0.20 ± 0.04 μM. Parathion did not exhibit androgenic activity. Similarly, in a human androgen-receptor reporter-gene assay in a Chinese hamster ovary cell line (CHO-K1), parathion was an androgen receptor antagonist, and did not exhibit androgen agonist activity (Kojima et al., 2004, 2010).

Parathion was neither an agonist nor an antagonist for human estrogen receptors α or β in similarly constructed transactivation assays in CHO-K1 cells (Kojima et al., 2010). Parathion tested negative for estrogenicity in an estrogen receptor-positive human breast-cancer cell line (MCF-7 BUS), and did not show estrogenic activity in an estrogen receptor-negative breast-cancer cell line (MDA MB 231) (Oh et al., 2007).

(ii) Experimental systems

In vivo

In CF-1 mice, serum testosterone levels were dramatically reduced 1 and 8 days after an intraperitoneal injection of either commercial-grade (9 mg/kg bw) or pure (300 mg/kg bw) parathion (Contreras et al., 2006). In the group receiving commercial-grade parathion levels were still very low at 40 days after injection. Pathological changes in the testes and teratozoospermia were also observed at days 8 and 40.

In castrated immature male Wistar-Imamichi rats treated with testosterone, daily subcutaneous injections of a metabolite of parathion, 4-nitrophenol (see Section 4.1.1), elevated levels of follicle-stimulating hormone and luteinizing hormone in the Hershberger assay at a dose of 0.1 mg/kg for 5 days; there were no effects with 4-nitrophenol at doses of 0.01 or 1.0 mg/kg (Li et al., 2006). There were no observed effects on levels of follicle-stimulating hormone and luteinizing hormone in ovariectomized immature female injected subcutaneously with 4-nitrophenol at a dose of 1, 10, or 100 mg/kg per day for 7 days. In follow-up studies, levels of luteinizing hormone were significantly lowered, while levels of corticosterone were significantly elevated in male rats injected subcutaneously with 4-nitrophenol for 14 days at daily doses 0.01, 0.1, 1 or 10 mg/kg, and levels of follicle-stimulating hormone were low in all groups except at the lowest dose (Li et al., 2009). Plasma levels of inhibin, an inhibitor of follicle-stimulating hormone, were also increased in all groups except at the lowest dose. Levels of testosterone

were elevated above those of controls in all treatment groups, but the increase was statistically significant only at the highest dose.

Early studies explored the potential impact of parathion on steroid metabolism Thomas & Schein (1974). In adult male mice, neither uptake nor metabolism of [^{3}H]-labelled testosterone was significantly affected by prior treatment with parathion. However, levels of [^{3}H]-labelled testosterone were elevated compared with controls (239 ± 37, 260 ± 38, 471 ± 51, and 421 ± 87 dpm/mg in the control group, and groups receiving parathion at 1.3, 2.6, or 5.3 mg/kg, respectively).

In vitro

Parathion (0.01 to 10 μM) significantly inhibited, in a dose-dependent manner, the binding of dihydrotestosterone to cytosol androgen-binding components from prostate, seminal vesicle, kidney, and liver, but not from the intestine (Schein et al., 1980). Parathion (0.4, 4, or 20 μM) also significantly reduced the formation of [^{3}H]-labelled dihydrotestosterone in mouse but not rat prostate gland in vitro (Thomas & Schein, 1974). However, formation of [^{3}H]androstanediol and [^{3}H]androstenedione was strongly affected by exposure to parathion in the rat under the same in-vitro conditions. Using hepatic microsomes from mice treated with parathion, formation of [^{3}H]androstanediol in vitro was elevated for the group at the highest dose. In a later experiment, the in-vitro metabolism of [1,2-^{3}H]testosterone by anterior prostate gland from mice treated with parathion was not altered by this treatment (Thomas et al., 1977).

Production of testosterone in vitro was not significantly altered in Leydig cells harvested 1, 8 or 40 days from CF-1 mice injected intraperitoneally with a single dose of commercial or pure parathion (Contreras et al., 2006), in contrast to findings in vivo (see above). [The Working Group noted that levels of testosterone after 8 and 40 days for treated animals were markedly lower than for controls, but did not meet the authors' significance cut-off of $P < 0.01$.]

Welch et al. (1967) reported that parathion (10 and 100 μM) inhibited hydroxylation of testosterone in rat microsomes.

In fresh liver microsomes from adult male Swiss Webster mice, incubated with added testosterone-4-[^{3}H], parathion at a concentration of 0.1 mM, but not at 0.01 mM, significantly reduced testosterone metabolism (Stevens, 1973). Parathion did not alter the production of progesterone in primary granulosa cells harvested from pig ovaries and cultured in vitro (Haney et al., 1984).

(c) Other receptors

(i) Humans

No data from exposed humans were available to the Working Group.

Parathion acted as an agonist in a human pregnane X receptor (PXR) reporter-gene assay in a CHO-K1 cell line (Kojima et al., 2010).

(ii) Experimental systems

Growth hormone was significantly elevated in the pituitary of male and female rats that received paraoxon at a dose of 0.124 mg/kg bw by intraperitoneal injection daily for 14 days (Cehovic et al., 1972). The same effect on growth hormone was seen with high near-lethal exposures (600 μg/mg kg, daily intraperitoneal injection) over a 3-day period, and prolactin levels were elevated in females.

A series of experiments in rats studied the effects of parathion on melatonin synthesis. In a study by Attia et al. (1991), morning administration of parathion by oral gavage for 6 days significantly elevated nocturnal levels of melatonin in serum and in the pineal gland; levels of *N*-acetyltransferase, which acetylates serotonin, were also elevated, but not levels of hydroxyindole-*O*-methyltransferase, which converts *N*-acetylserotonin to melatonin. In a subsequent study, the *β*-adrenergic receptor antagonist

propranolol abrogated the effects of parathion on *N*-acetyltransferase and on nocturnal levels of serum melatonin (levels of pineal melatonin were not significantly increased by parathion) (Attia et al., 1995). Parathion also significantly reduced nocturnal levels of serotonin, and this was also reversed by propranolol. Levels of hydroxyindole-*O*-methyltransferase, *S*-hydroxytryptophan, and hydroxyindole acetic acid were unaffected by treatment with parathion or propranolol. Attia (2000) concluded that parathion affects serotonin metabolism either by effects on sympathetic innervation to the pineal gland, or on the β-adrenergic receptors in the pinealocyte membrane.

Parathion was not an agonist for the aryl hydrocarbon receptor (AhR) in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing copies of a dioxin-responsive element (Takeuchi et al., 2008; Kojima et al., 2010).

Parathion was not an agonist for mouse peroxisome proliferator-activated receptors α or γ (PPAR α or γ) 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

No data from exposed humans were available to the Working Group.

In human salivary-gland cells exposed in vitro, paraoxon at 10 μM (a non-cytotoxic concentration) induced superoxide formation as determined by dihydroethidium fluorescence (Prins et al., 2014). In addition, paraoxon at the same concentration induced DNA fragmentation, and expression of glutathione synthetase (*GSS*), superoxide dismutase 2 (*SOD2*), and glutathione *S*-transferases m2 and t2 (*GSTM2* and *GSTT2*) genes. [The Working Group noted the recognized limitations of using dichlorodihydrofluorescein as a marker of oxidative stress (e.g. 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.] In human liver-derived HepG2 cells, parathion induced a significant increase in cellular accumulation of malondialdehyde at concentrations equal to or below those that affected the viability of HepG2 cells (Edwards et al., 2013). The results of comet assays were consistent with the findings for malondialdehyde.

(ii) Experimental systems

In female Wistar and Norway rats, intraperitoneal injection of paraoxon (0.3, 0.7, 1, or 1.5 mg/kg) lead to a decrease in glutathione levels and in the activity of catalase and glutathione-*S*-transferase in various tissues (Jafari et al., 2012). An increase in superoxide dismutase activity and malondialdehyde levels was also found. The extent of induction of oxidative stress by paraoxon was in the following order: brain > liver > heart > kidney > spleen.

Two studies examined parathion-associated markers of oxidative stress in the hippocampus area of the brain. In a study of female Wistar rats exposed to parathion by inhalation (dose not stated; exposure consisted of four consecutive cycles of 15 minutes exposure/45 minutes clean air) 5 days per week for 2 months, significant elevation in levels of malondialdehyde in the hippocampus (determined by *N*-methyl-2-phenylindol colorimetric assay) was reported (Canales-Aguirre et al., 2012). In male Wistar rats given a single subcutaneous dose of parathion at 15 mg/kg, induction of pro-inflammatory and lipid peroxidation biomarkers was observed in the hippocampus (López-Granero et al., 2013).

In pheochromocytoma PC12 cells, an increase in levels of thiobarbituric-acid reactive

substances was observed when cells were treated with parathion at 30 μM (Slotkin et al., 2007).

(b) Inflammation and immunomodulation

The ability of paraoxon and other organophosphate pesticides to act on nicotinic and muscarinic receptors is well documented, and has been proposed as a mechanism of toxicity that is independent of the inhibition of acetylcholinesterase activity (Pope, 1999). Cholinergic signalling may play an important role in the immune system (Verbout & Jacoby, 2012). Evidence for acetylcholine synthesis, storage, release and breakdown – all elements indicative of a potential signalling role – have been demonstrated in various immune cells, including lymphocytes (Kawashima & Fujii, 2004). The association between exposure to parathion and immunomodulation (e.g. lung hypersensitivity and asthma) has been examined in studies detailed below, and it has been hypothesized that such effects are attributable to the action of organophosphates (i.e. paraoxon) on non-neuronal signalling events involving cholinergic systems in cells of the immune system, and the inhibition of acetylcholinesterase activity (Banks & Lein, 2012).

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

In vivo

Pathological effects of parathion (16 mg/kg) on the spleen were reported in C57Bl/6 mice; a significant decrease in spleen weight was observed 2 days after a single oral dose (Casale et al., 1983). Long-term studies conducted by the United States National Toxicology Program did not find increases in non-neoplastic pathology in the spleen or bone marrow of mice or rats treated with parathion for up to 2 years (NTP, 1979). No effect on spleen weight was observed in a study in BALB/c mice given daily intraperitoneal injections of paraoxon at doses of 30 or 40 nmol for 6 weeks (Fernandez-Cabezudo et al., 2008).

Immunosuppressive effects of parathion in mice were first reported by Wiltrout et al. (1978). Subsequent studies of hypersensitivity demonstrated that exposure of mice to parathion led to the following effects in response to immunogenic challenge with picryl chloride: increases in the severity of dermatitis, serum IgE and IgG2a levels, numbers of helper T-cells and IgE-positive B-cells, production of Th1 and Th2 cytokines, and production of IgE in auricular lymph-node cells; and a marked decrease in numbers of splenic regulatory T-cells (Fukuyama et al., 2012). Another study by the same group showed that pretreatment with parathion before allergic challenge in mice caused a marked increase in numbers of helper and cytotoxic T-cells, and levels of Th1 and Th2 cytokines (Fukuyama et al., 2011). Altered host resistance to viral (Selgrade et al., 1984) and bacterial (Fernandez-Cabezudo et al., 2010) infections upon exposure to parathion or paraoxon has also been reported in mice.

Suppression of the humoral immune response by parathion has been reported in studies in mice. Numbers of IgM plaque-forming cells were reduced by 65% in C57Bl/6 mice given parathion (16 mg/kg, per os) 2 days after immunization with sheep erythrocytes (Casale et al., 1984); however, the immunosuppressive dose also caused signs of cholinergic poisoning and 20% mortality. Non-poisonous doses of parathion (4 mg/kg, per os) had no effect on markers of humoral immunity. Effects on the cell-mediated immune system were demonstrated in studies of exposure to parathion in mice. In C57Bl/6 mice treated with parathion (4 mg/kg, per os) for 14 days, leukocyte counts were elevated on days 2 and 5, and effects on haematopoietic stem cells in the bone marrow were also observed (Gallicchio et al., 1987a). In a study of ovalbumin-induced allergic immune response in mice, oral exposure to parathion led to marked increases in serum IgE levels, the number of IgE-positive B cells, and

also levels of IgE and cytokines in lymph nodes, and eosinophils and chemokines in broncho-alveolar lavage fluid, and interleukin IL-10 and IL-17A in the lung (Nishino et al., 2013). Similar effects were observed in studies in guinea-pigs. Ovalbumin sensitization of guinea-pigs increased the vulnerability to parathion-induced airway hyper-reactivity (Proskocil et al., 2008, 2013).

In vitro

Casale et al. (1993) found that exposure of mouse T-cell lymphoma lines CTLL2 to paraoxon produced marked concentration-dependent inhibition of interleukin IL2-driven cell proliferation.

4.2.4 Cell proliferation and death

(a) Humans

No data from exposed humans were available to the Working Group.

The Working Group identified several studies examining effects of parathion on MCF-10F cells, a breast epithelial cell line spontaneously immortalized from non-malignant cells. In the first study, proliferation was increased in MCF-10F cells treated with parathion at 100 ng/mL, when compared with controls (Calaf & Roy, 2007a). Expression of the following proteins was enhanced in treated cells: EGFR, NOTCH-4, DVL-2, EZRIN, RAC 3, RHO-A, trio, c-kit, β catenin, and mutant p53. This increase in expression was significantly inhibited by atropine. Purified mRNAs from treated cells were used to synthesize cDNA probes, which were then studied in a human cell-cycle array of 96 genes (GE Assay Q Series Human DNA cell cycle cDNA expression array membranes). Treatment with parathion was associated with the elevated expression of 12 genes, including cyclins and cyclin-dependent kinases. In a second study with the same design, Calaf & Roy (2008a) studied the effect of parathion on a human cell-cycle array of 96 genes involved in cell proliferation and metastasis (Human Cancer Microarray by SuperArray). Parathion modulated the expression of 44 of the 96 genes involved in cell proliferation, including insulin-like growth factor binding proteins (IGFBP), cyclins, and cyclin-dependent kinase 4. In a third similar study, Calaf & Roy (2008b) found increased protein expression of NOTCH-4, DVL-2, CD146 and β catenin, also indicative of cell proliferation and adhesion potential.

In a study on the apoptotic effects of parathion and other chemicals on the human acute T-cell leukaemia cell line J45.01, parathion (0.03, 0.1, and 0.3 μM) caused a dose-dependent decrease in the percentage of viable cells and increased the percentage of apoptotic cells after 4 and 8 hours (Fukuyama et al., 2010). Co-incubation with the caspase inhibitor Z-VAD-fmk (tested on cells receiving parathion at 0.3 μM) was protective, while the caspase-3 inhibitor Ac-DEVD-CHO was not. There was a dose-dependent increase in the proportion of caspase 3/7 (but not caspase-8 or 9) activity, and in levels of DNA fragmentation, which was blocked by one or more of the caspase inhibitors.

Erythrocyte and granulocyte–macrophage progenitor cells, cloned from human bone marrow taken from healthy volunteers or heart surgery patients, were exposed to paraoxon (Gallicchio et al., 1987b). Erythroid as well as granulocyte colony formation and burst-forming erythroid units were inhibited in a strongly dose-dependent fashion, with sensitivity as low as 0.001 μM for burst erythroid and granulocyte colony formation.

Paraoxon or parathion at 1 mM induced time-dependent increases in apoptosis in human neuroblastoma cells (Carlson et al., 2000). Cyclosporin A, an inhibitor of the mitochondrial permeability transition pore, was protective. Paraoxon (1 mM) and parathion (100 μM, 1 mM) induced significant time-dependent increases in caspase-3 activation, which was modulated by pretreatment with cyclosporin

A. In a study on non-cholinergic neurotoxic effects, neuroblastoma cells exposed to paraoxon showed two upregulated genes (one of which was thyroid hormone receptor-associated protein 5), and thirteen downregulated genes, four (*APC*, *FAS*, *MDM4,* and *PTEN*) of which are involved in cell proliferation or apoptosis regulation (Qian et al., 2007). Pomeroy-Black & Ehrich (2012) also found that paraoxon upregulated the mitogen-activated protein kinase (MAPK) pathway in SY5Y cells, and caused significant activation of protein kinase B (Akt) in the phosphatidylinositol PI3K cell-survival pathway.

(b) Experimental systems

(i) In vivo

Cabello et al. (2001) investigated the impact on the structure of the mammary gland of subacute exposure to parathion (2500 µg/kg bw, subcutaneous injection, twice per day for 5 days) in Sprague Dawley rats (age 16 days or 39 days). The rats were killed 16 hours after the last injection. In whole mounts of mammary glands from the left side of rats exposed from age 21 days, parathion had no effect on terminal end bud or alveolar bud density. In rats exposed from age 39 days (normally a period of active differentiation of terminal end buds into alveolar buds), the density of terminal end buds was markedly increased compared with control animals (terminal end bud density, 12.04 ± 1.77/mm^2 versus 3.30 ± 0.27/mm^2), and a markedly lower density of alveolar buds (alveolar bud density, 1.28 ± 0.52/mm^2 versus 20.80 ± 1.68/mm^2). Histological examination of mammary glands excised from the right side showed a significant ($P < 0.05$) increase in the size of terminal end buds and the number of epithelial layers.

The apoptotic effect of parathion on sperm was studied in young mice (onset of spermatogenesis) and in adult mice (full spermatogenesis) (Bustos-Obregón et al., 2001). Parathion increased the proportion of cells undergoing apoptosis in young animals and adults, affecting spermatocytes at the beginning of the meiotic process, and spermatids at the elongation period.

(ii) In vitro

In the study by Fukuyama et al. (2010) in primary mouse thymocytes discussed above, parathion had a strong adverse, dose-dependent, effect on cell viability, and increased the proportion of cells undergoing apoptosis. Caspase 3/7 (but not caspase 8 or 9) activity was increased by parathion, and reduced by caspase 3/7 inhibitors (Z-VAD-fmk and Ac-DEVD-CHO) in these cells. Neither caspase-3/7 inhibitor had any significant measurable effect on cell viability, but Z-VAD-fmk reduced the proportion of apoptotic mouse thymocytes affected by parathion.

Paraoxon (0.001–0.01 µM) increased the activity of caspase-3 and induced apoptosis in a concentration-dependent manner in the mouse lymphocytic leukaemia T-cell line, EL4 (Saleh et al., 2003a). Parathion had a similar effect, but at higher concentrations of 0.05–10 µM. In a follow-up study, a caspase-9 inhibitor (zLEHD-fmk) attenuated apoptosis, and blocked the activation of caspases 3, 8, and 9 by paraoxon, implicating caspase 9-dependent mitochondrial pathways in paraoxon-induced apoptosis (Saleh et al., 2003b). In EL4 T-cells, Li et al. (2010) demonstrated attenuation of parathion-induced apoptosis, and inhibition of paraoxon-induced increased expression of caspase-12, by calcium-channel receptor antagonists or by calcium chelation.

Seminiferous tubules harvested from CF1 mice (age, 90 days) exposed to parathion or paraoxon (0.8 mM) showed a substantial reduction in cell replication, compared with controls (Rodriguez & Bustos Obregon, 2000; Rodriguez et al., 2006).

Paraoxon induced apoptosis and inhibited cell replication in a neuronal cell line, differentiated PC12 cells derived from rat adrenal medulla pheochromocytoma, in several studies (Flaskos

et al., 1994; Slotkin et al., 2007; Sadri et al., 2010). In hippocampal cells harvested from Wistar rat neonates, paraoxon reduced cell viability (Yousefpour et al., 2006). Neurotoxicity and activation of rat primary glial cells in response to exposure to parathion in vitro has also been demonstrated (Zurich et al., 2004).

A positive association between exposure to parathion and cytotoxicity was reported in a fish-derived cell line FG-9307 (Li & Zhang, 2001).

4.2.5 Other mechanisms

Calaf & Roy (2007b) studied the effects of parathion on cell transformation and gene expression in the immortalized human breast epithelial cells MCF-10F. Cells treated with parathion (100 ng/mL) exhibited anchorage-independent growth and invasiveness, measured 20 passages after treatment. Protein expression in treated cells was enhanced for mutant p53 protein, and other proteins that play a role in the cell cycle (see Section 4.2.4).

In a genome-wide DNA methylation study in a human haematopoietic cell line derived from erythroblastic leukaemia (K562), parathion elevated the methylation of gene-promoter CpG sites, including for genes involved in cell differentiation, DNA dealkylation involved in DNA repair, and regulation of apoptosis and cell proliferation (Zhang et al., 2012).

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 website of the ToxCast research programme (EPA, 2016a). *Z*-Tetrachlorvinphos (CAS No. 22248-79-9; 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 the Tox21 or ToxCast research programmes.

Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is also publicly available (EPA, 2016b). 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

In order to explore the bioactivity profiles of the compounds under evaluation 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 Tox21/ToxCast 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 comprise 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).
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" in order 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, 2016b). 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 Working Group's analyses, 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 in 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 *IARC Monographs*. ToxPi is a dimensionless index score that integrates of multiple different assay results and displays them visually. The overall score for a chemical takes into account 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 Volume 112 (see Annex I) 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 parathion and paraoxon 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 the other three compounds evaluated in the present volume of the *IARC Monographs* (Volume 112) and 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 parathion and paraoxon 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 parathion and paraoxon are shown.

Characteristic (1) *Is electrophilic or can undergo metabolic activation:* Parathion was tested for all 31 end-points. It was active in 18 of the 29 CYP-inhibition assay end-points (all cell-free). The highest ranked of the 178 chemicals included in the comparison was malathion, which was active for 20 out of 29 assay end-points. Parathion was inactive for the two aromatase-inhibition assay end-points. Paraoxon was only tested for the two aromatase-inhibition assay end-points and was active for both (Fig. 4.4).

Characteristic (2) *Is genotoxic*: Parathion and paraoxon were tested and found inactive in 9 and 6, respectively, of the 9 available TP53 assay end-points. In comparison, top-ranked chemicals chlorobenzilate and clomiphene citrate were found to be active for 7 out of the 9 assay end-points for which they were tested (Fig. 4.5).

Characteristic (4) *Induces epigenetic alterations:* Parathion paraoxon were tested and found inactive in 11 and 4, respectively, of the 11 available assay end-points. In comparison, the highest-ranked chemical *Z*-tetrachlorvinphos was active in all 4 of the DNA binding assay end-points, but was not tested in any of the 7 transformation-assay end-points (Fig. 4.6).

Characteristic (5) *Induces oxidative stress*: Parathion was tested in all 18 assays, and was active in 2 out of the 6 oxidative-stress marker assay end-points. Paraoxon was inactive for the 7 assay end-points for which it was tested. In comparison to the two highest-ranked chemicals, carbaryl and tannic acid, parathion was moderately active in assays with metalloproteinases and oxidative-stress markers. The metalloproteinase assay end-points were highly selective with the maximal responder (i.e. carbaryl) only activating 2 out of 5 end-points. Parathion displayed activity in a single assay (BSK_hDFCGF_MMP1_up). Parathion also induced transcription-factor activation of NRF2 and the metal response element (MRE) (Fig. 4.7).

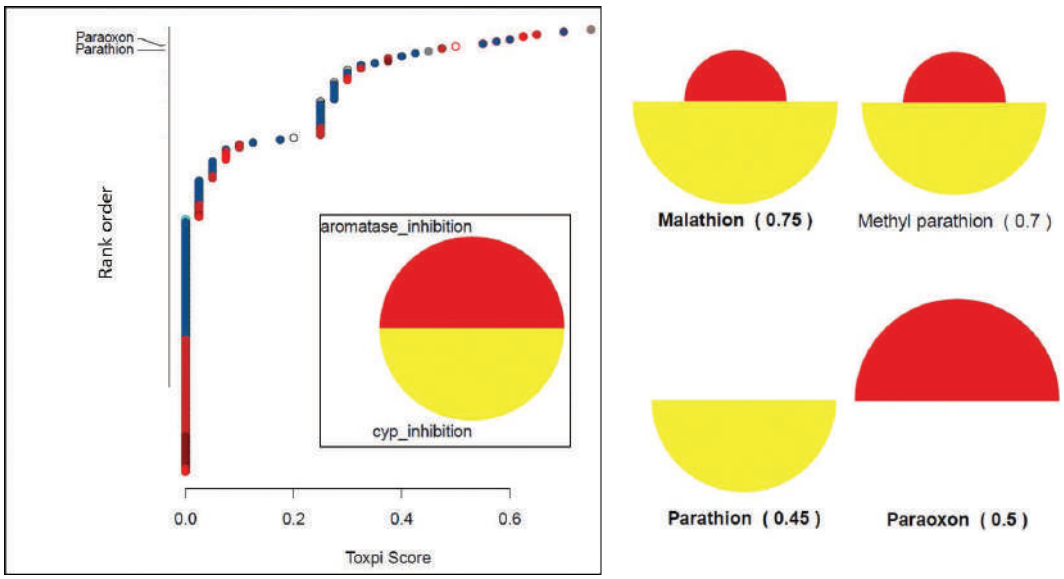
Characteristic (6) *Induces chronic inflammation*: Parathion was tested for all 45 assay-end-points, while paraoxon was tested for 2 (both NFkB); both chemicals showed weak to no activity across assay end-points associated with chronic inflammation when compared with the highest-ranked compounds 4,4′-methylenedianiline and malaoxon (Fig. 4.8).

Characteristic (8) *Modulates receptor-mediated effects:* Parathion and paraoxon were tested for all 81 assay end-points in this group. In comparison to the two highest-ranked chemicals, clomiphene citrate and kepone, parathion selectively activated both AhR assay end-points. In addition, parathion showed appreciable activity in 14 "other nuclear receptor" assay end-points, making it one of the most highly active chemicals overall. Paraoxon showed relatively weak receptor activity (Fig. 4.9).

Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply:* Parathion and paraoxon were tested in 67 and 27, respectively, of the 68 assay end-points, but showed almost no activity for end-points associated with cytotoxicity or cellular proliferation (Fig. 4.10).

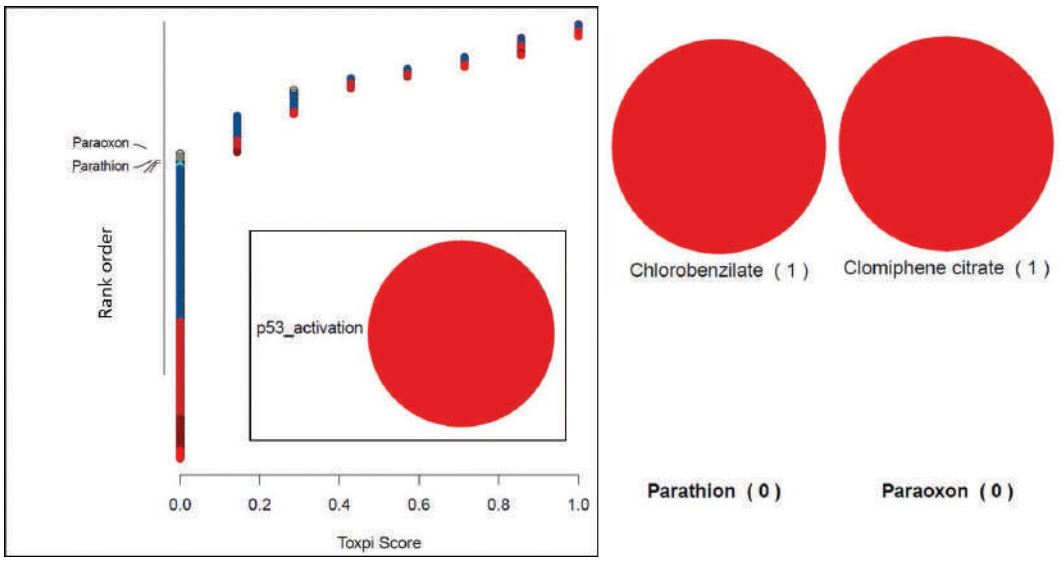
Overall, parathion was active in 42 out of 263 assay end-points for which it was tested. The analysis of the ToxCast/Tox21 data for parathion corroborates findings in other model systems as described in Section 4.2. Its oxon metabolite, paraoxon, showed little bioactivity under the conditions of these assay end-points, with activity for only 7 assay end-points of the 137 tested. The limited activity of paraoxon may be attributed to the high reactivity and short half-life of this

Fig. 4.4 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to enzyme inhibition



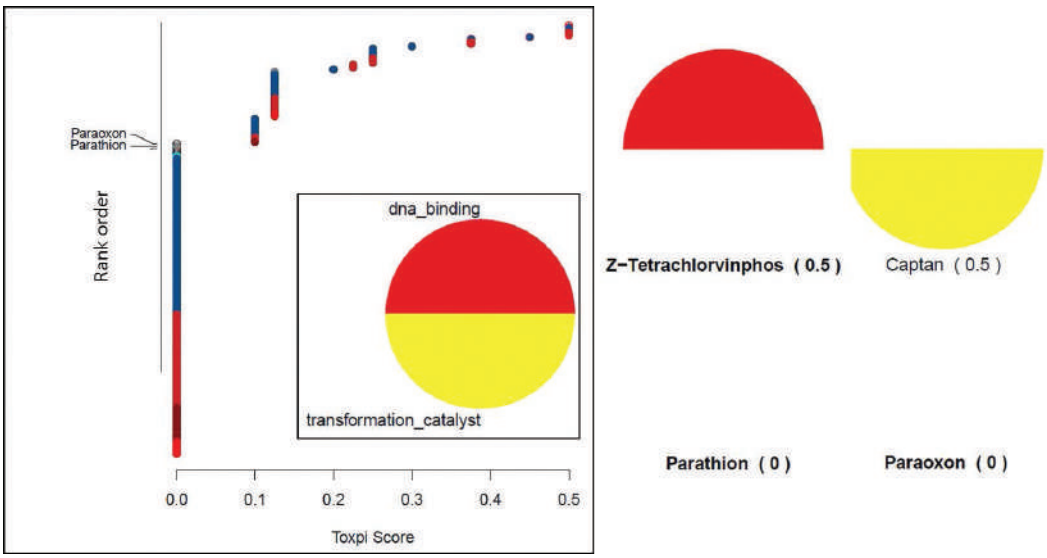
On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 178 chemicals 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 (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.5 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to genotoxicity



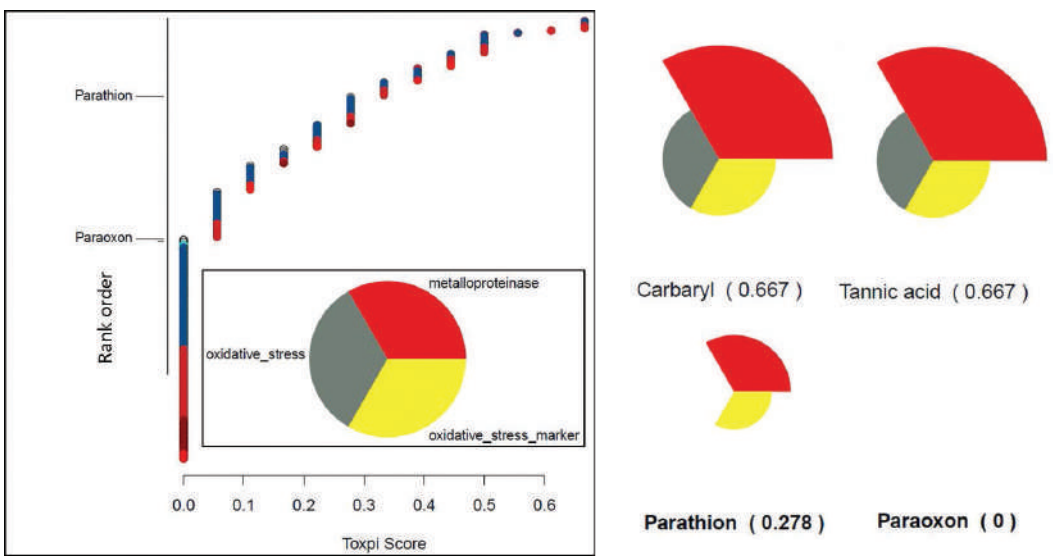
On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 178 chemicals 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 (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.6 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to epigenetic alterations



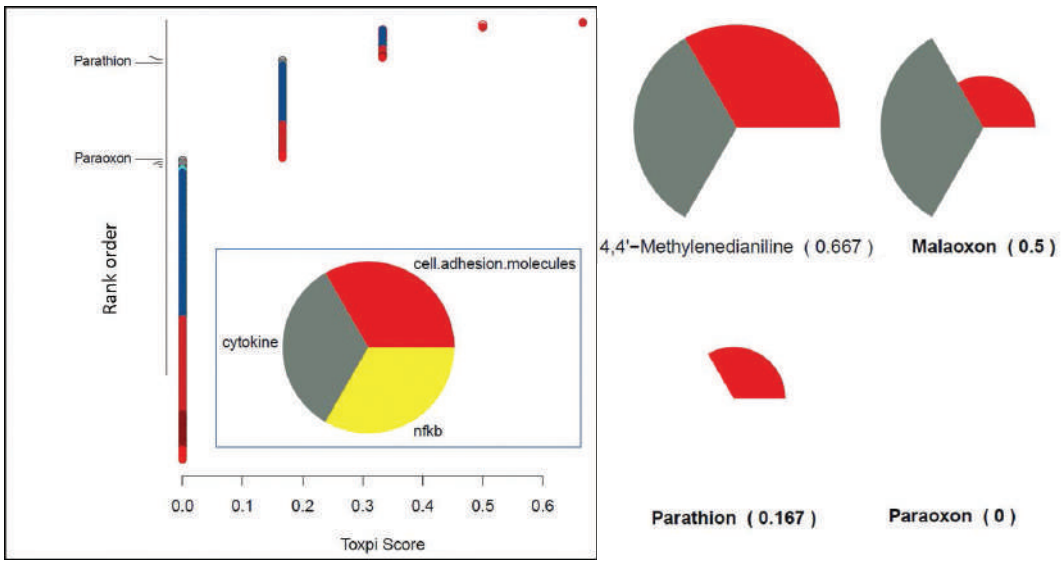
On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 178 chemicals 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-tetrachlorvinphos and captan) and the target chemicals (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.7 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to oxidative stress



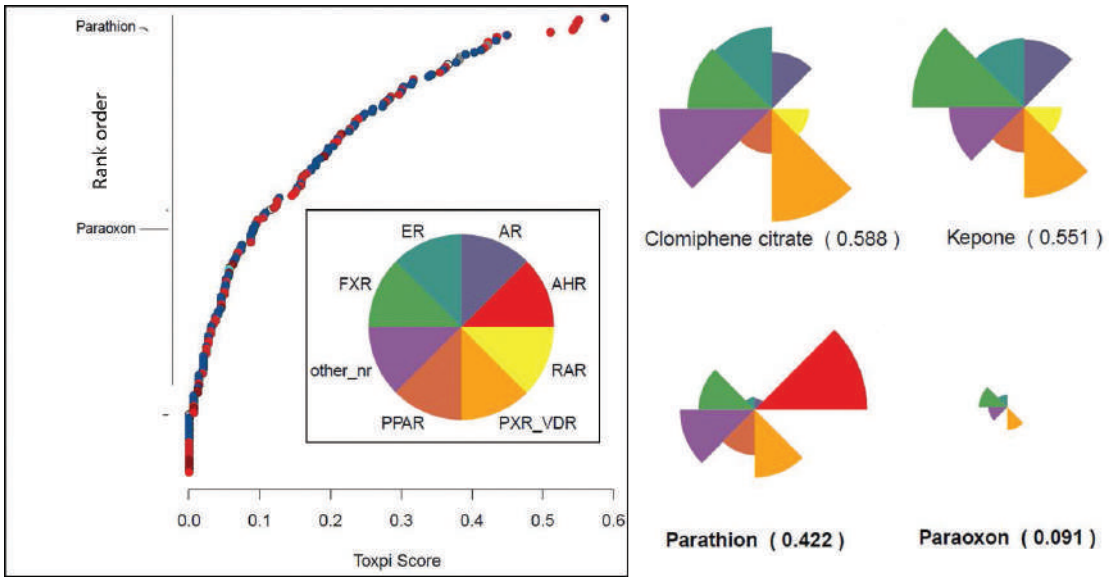
On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 178 chemicals 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 (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.8 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to chronic inflammation



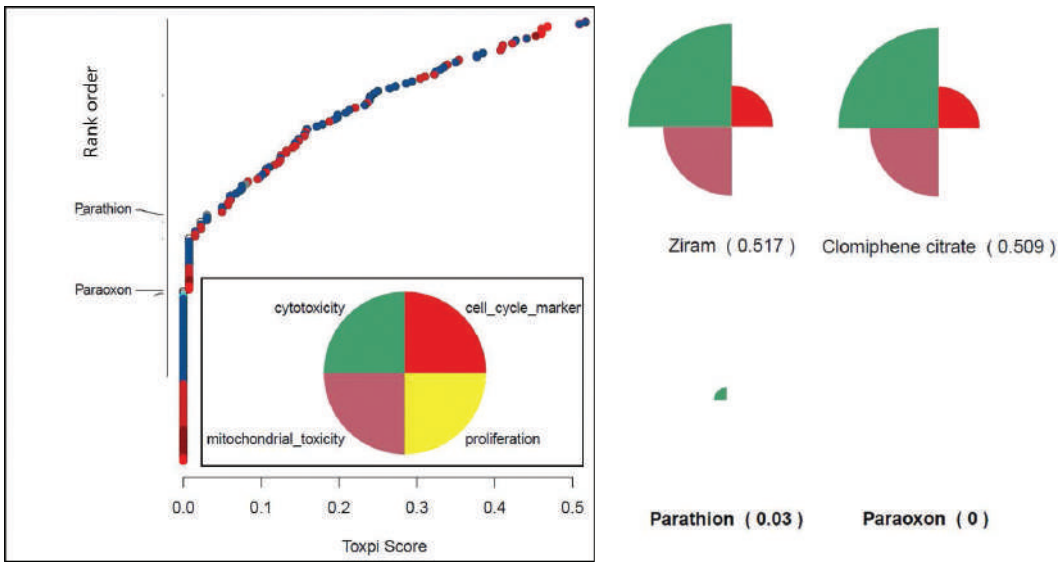
On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 178 chemicals 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 (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.9 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to receptor-mediated effects



On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 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 citrate and kepone) and the target chemicals (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.10 ToxPi ranking for parathion and its metabolite paraoxon using ToxCast assay end-points mapped to cytotoxicity and cell proliferation



On the left-hand side, the relative ranks of parathion, and its metabolite paraoxon, are shown (*y*-axis) with respect to their toxicological prioritization index (ToxPi) score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* 112) and with 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 citrate and ziram) and the target chemicals (parathion and paraoxon) are shown with their respective ToxPi score in parentheses.

compound, which hampers interpretation of the results of the in-vitro assay end-points.

4.4 Susceptibility

A nested case–control study of Caucasian pesticide applicators within the AHS examined the interactions between exposure to 41 pesticides and 152 single-nucleotide polymorphisms (SNP) in nine genes involved in the vitamin D pathway among 776 cases of cancer of the prostate and 1444 controls (Karami et al., 2013; see Section 2.2.1). The strongest interaction observed in this study was between the *RXRB* (Retinoid-X-Receptor β) gene variant rs1547387 and parathion exposure. In addition, significant interactions were observed between *GC* (Group specific Component vitamin D-binding protein) gene variants rs7041 and rs222040, prostate cancer, and use of parathion.

Paraoxonase 1 (PON1) is an enzyme involved in metabolism of parathion and other organophosphate pesticides (see Section 4.1). It is a polymorphic enzyme, and several well-established common genetic variants that markedly affect its activity and protein levels have been identified in humans (Humbert et al., 1993; Costa et al., 2013). No study has examined cancer outcomes as a function of PON1 polymorphism. Two studies (Lee et al., 2003; Singh et al., 2011a) were conducted in populations of agricultural workers who were exposed to uncharacterized mixtures of pesticides, and demonstrated a significant association between *PON1* polymorphisms (*PON1* 192QQ) and markers of genotoxicity (DNA damage measured by comet assay in circulated lymphocytes). The follow-up studies in some of these populations demonstrated that genetic variants in several other enzymes involved in metabolism such as CYP2D6, CYP2D9, GSTM1, and NAT2 also had a significant effect on markers for

genotoxicity (DNA damage) (Singh et al., 2011b, 2012). One study found a significant association between exposure to organophosphates (not exclusive to parathion), sperm quality parameters, and *PON1* 192RR genotype (Pérez-Herrera et al., 2008).

The greater sensitivity of weanling rodents of either sex and of adult females, compared with adult males, to acute toxicity of parathion (Gagné & Brodeur, 1972; Harbison, 1975; Deskin et al., 1978) is attributed to age- and sex-related differences in the toxicokinetics of the parent compound and its metabolites. Embryo and fetus lethality in studies was seen in rats exposed to parathion during gestation, in the absence of severe maternal toxicity (Harbison, 1975). Other studies of neonatal exposure to parathion indicated that female rats were more sensitive than male rats to the later alterations in response to high-fat diet in adulthood (Lassiter et al., 2008; Slotkin, 2011).

4.5 Other adverse effects

4.5.1 Humans

Although currently unusual in industrialized countries such as the USA, toxicity caused by exposure to parathion is a common source of severe poisoning in low- and middle-income countries (Rumack, 2015). Epidemiological evidence, including evidence of hospitalization and death due to accidental dermal exposure and ingestion, indicates that parathion is more toxic to children than to adults (Hayes & Laws, 1991). In several studies of exposure in humans, parathion was shown to be an inhibitor of erythrocyte and plasma cholinesterase activity (NIOSH, 1976). Acute and long-term exposure to parathion have been associated with various clinical signs including nausea, vomiting, abdominal cramps, diarrhoea, excessive salivation, headache, weakness, difficulty in breathing, vision impairment, convulsions, central nervous system depression, paralysis, coma, and respiratory failure (IARC, 1983; O'Neil et al., 2013).

4.5.2 Experimental systems

In numerous studies, parathion induced cholinergic effects, including inhibition of plasma, erythrocyte, and brain cholinesterase activity at doses as low as 0.0024 mg/kg bw per day, and corresponding clinical signs (abnormal gait, tremors, and reduced activity) at doses as low as 1.75 mg/kg bw per day (EPA, 1986b, c, 1991b; Atkinson et al., 1994). In the 2-year study of toxicity and carcinogenicity in female rats, the inhibition of cholinesterase activity was accompanied by clinical signs including tremors, abnormal gait, and increased mortality (EPA, 1984, 1986b).

Other effects in long-term studies were decreased body-weight gain in rats (EPA, 1986c). Effects on the eye were also reported in the combined study of chronic toxicity and carcinogenicity in rats. Parathion induced gross retinal abnormalities in males and females, in addition to cataracts and turbid lenses in females, and epithelium, optic nerve, and ciliary body degeneration, as well as retinal atrophy in males (EPA, 1984, 1986b, c).

A study of developmental neurotoxicity reported reductions in motor activity, and in the density of muscarinic receptor binding in the cerebral cortex (Stamper et al., 1988). In another study of developmental neurotoxicity in rats given parathion at a dose of 0.1 or 0.2 mg/kg per day on postnatal days 1–4, learning and memory impairment when tested with a maze and decreased reflexes were observed in males and females at the highest dose (Timofeeva et al., 2008).

5. Summary of Data Reported

5.1 Exposure data

Parathion is a broad-spectrum organophosphate insecticide that is effective against a wide range of insects on crops. It was first used in 1947, but because of its toxicity to wildlife and human health, use of parathion has been banned or severely restricted throughout the world. Most countries banned parathion in the 1980s and 1990s, and all authorizations for use in the European Union and USA were banned by 2003. Most exposure to workers is via the dermal route in both manufacturing and use of parathion. Exposure can vary considerably depending on the task, the method of application, the environmental conditions, the rate of application, and the operator technique. The available data indicated that general population exposures to parathion are low subsequent to restrictions on its use.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological data on parathion, the Working Group identified reports from two cohort studies, plus two additional case–control studies, all in the USA or Canada. The Agricultural Health Study (AHS) is the major source of evidence from cohort studies, with reports on non-Hodgkin lymphoma (NHL), melanoma, and cancers of the prostate, breast, and colorectum. The Florida pest-control worker cohort reported on a nested case–control study of cancer of the lung. Case–control studies were also reported on NHL and cancer of the prostate. The Working Group observed that evidence regarding parathion remains sparse, that several studies reported elevated odds ratios that did not reach statistical significance, and the few associations that have been detected have not been replicated in separate studies.

5.2.1 Non-Hodgkin lymphoma

The relationship between exposure to parathion and NHL was examined in two studies. The case–control report was from the pooled analysis of three case–control studies of farmers in the mid-western USA, and yielded a multivariable-adjusted (but not for other pesticides) odds ratio (OR) of 2.9 (95% CI, 0.9–9.7). In a recent report from the AHS, there was no association between parathion and NHL; the relative risk of ever having used parathion was 1.1 (95% CI, 0.8–1.4), and there was no evidence of heterogeneity across histological subtypes, or a trend with increasing number of days of use. The Working Group noted the inconsistency of these results and concluded that there was no strong evidence of an association between exposure to parathion and NHL.

5.2.2 Cancer of the prostate

Three publications reported on the relationship between exposure to parathion and cancer of the prostate. The first was a case–control study in Canada that estimated exposure to parathion from a locally derived job-exposure matrix (OR for ever use, 1.51; 95% CI, 0.94–2.41) and there was a suggestion of trend (P = 0.06) with lifetime-days of parathion use. From the AHS, two nested case–control studies have been reported, with a large study that included 1962 cases finding that overall there was no significant association or trend across quartiles of cumulative lifetime exposure; however, when restricted to aggressive tumours of the prostate, risk was elevated (OR, 1.96; 95% CI, 1.10–3.50) in the subset with the lowest quartile of exposure. A further analysis of cancer of the prostate in the AHS was in a nested case–control study that included a smaller number of subjects (e.g. there were 776 cases of cancer of the prostate) for whom biospecimens were available for genetic analysis. Overall, there was no association with ever having used

parathion (OR, 1.02; 95% CI, 0.78–1.33); however, effect modification was detected such that significant elevations in risk were seen in subgroups defined by the presence of variants in two vitamin-D pathway genes. The Working Group noted that while there is no consistent evidence of an association with cancer of the prostate overall, recent results from a large and comprehensive cohort study have revealed possible increases in risk for subgroups defined on the basis of variation in vitamin-D pathway genes.

5.2.3 Melanoma

A statistically significant association between parathion and cutaneous melanoma was detected in a single case–control study nested within the AHS (OR for any use, 1.9; 95% CI, 1.2–3.0). There was also a statistically significant monotonic trend in increasing risk with more frequent use, and a plausible effect modification among those who also applied lead arsenate; users of parathion who were exposed to lead arsenate had a much higher risk of developing melanoma than those who were not exposed to lead arsenate. The Working Group recognized that there may be residual confounding with established risk factors for melanoma, and noted the lack of replication in other settings.

5.2.4 Other cancer sites

A single report from the AHS examined risk of cancer of the breast among women, and although there was no significant relationship overall with whether husbands used parathion (RR, 1.3; 95% CI, 0.8–2.1), significantly increased risk was seen for those who had a family history of breast cancer, and for those who lived in one of the two states investigated. Also within the AHS, a study on cancer of the colorectum found that it was not associated with parathion use. Finally, the single study that assessed cancer of the lung also reported a non-significant increase in risk but owing to its limitations, this study did not contribute substantially to the conclusions of the Working Group.

5.3 Animal carcinogenicity data

Parathion was tested for carcinogenicity in male and female mice in two feeding studies, in male and female rats in five feeding studies, and in female rats in one study with subcutaneous injection.

In one feeding study in mice, parathion produced a significant increase in the incidence of bronchiolo-alveolar adenoma, and bronchiolo-alveolar adenoma or carcinoma (combined) in treated males. In treated females, there was an increase in the incidence of malignant lymphoma. In the other feeding study, there was no significant increase in tumour incidence in male or female treated mice.

In a first feeding study in rats, there was a significant increase in the incidence of adrenal cortical adenoma, adrenal cortical adenoma or carcinoma (combined), thyroid follicular cell adenoma, and pancreatic islet cell carcinoma in treated males. Also significant was the increase in the incidence of adrenal cortical adenoma, adrenal cortical adenoma or carcinoma (combined), and mammary gland fibroadenoma observed in treated females. In a second feeding study, a significant increase in the incidence of pancreatic exocrine adenoma, exocrine adenoma or carcinoma (combined), and islet cell adenoma was observed in treated males only. In a third feeding study, parathion non-significantly increased the incidence of follicular cell adenoma of the thyroid gland in males only. The two other feeding studies with parathion gave negative results. In the study with parathion given by subcutaneous injection, there was a significant increase in the incidence of adenocarcinoma of the mammary gland in female rats.

5.4 Mechanistic and other relevant data

Rapid absorption of parathion from the gastrointestinal tract occurs in humans and experimental species, but dermal absorption is less efficient. Data are limited on how much compound is absorbed through inhalation in humans and experimental animals. Parathion is rapidly distributed in the blood after absorption in humans; however, no data on distribution to other tissues in humans were available. Most (94–99%) of the absorbed parathion is bound to proteins, mostly serum albumin, in the blood. After absorption in rats, parathion is readily taken up by liver, kidney, and fat.

The metabolism of parathion is similar in humans and experimental species. The bioactive metabolite, paraoxon, is formed via cytochrome P450 (CYP)-catalysed oxidation, and is then degraded by carboxylesterase and paraoxonase 1, liberating *para*-nitrophenol. Dearylation of parathion is another pathway catalysed by CYP. In humans, the major pathway of oxidation for parathion is via CYP3A4 for both paraoxon and *para*-nitrophenol.

The polar metabolites of parathion are excreted mainly in the urine in humans and experimental species. Several studies indicated that the remaining [^{14}C]-derived residues were negligible in experimental animal models within hours to days after administration of [^{14}C]-labelled parathion.

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

With respect to whether parathion is genotoxic, the evidence is *moderate*. In humans exposed to parathion and other pesticides in an occupational setting, chromosomal damage and sister-chromatid exchange were observed in one study. DNA and chromosomal damage were found in several studies in human cells (mostly lymphocytes) in vitro. Studies in experimental animals in vivo gave predominantly negative results for dominant lethal mutation and micronucleus formation in bone marrow. There were two in-vitro studies that gave positive results for chromosomal damage in rodent cells, although there were also studies that gave negative results. Studies of gene mutation in bacteria gave negative results for parathion, with or without metabolic activation.

The evidence is *weak* that parathion modulates receptor-mediated effects. Inhibition of acetylcholinesterase activity by paraoxon causes acute neurotoxicity in insects and mammalian species. Whether this is related to hyperplastic disease is unknown. No studies were identified in exposed humans. Studies using cultured human cells in vitro showed that parathion could antagonize the human androgen receptor. Parathion did not have nuclear receptor activity in one series of experiments. In Toxicity Forecaster (ToxCast™) assays, parathion showed appreciable activity in several assays for activity regarding nuclear and other receptors, including the aryl hydrocarbon receptor.

The evidence is *weak* that parathion induces oxidative stress, induces chronic inflammation, and is immunosuppressive. No studies in exposed humans were available to the Working Group. There were some studies showing positive effects in assays in vitro and in vivo; however, the database was too small to draw any firm conclusions. Several immune parameters in animal models in vivo, such as serum immunoglobulin levels, number of helper T cells and regulatory T cells, number of immunoglobulin E (IgE)-positive B cells, and cytokine levels were shown to be modulated after exposure to parathion.

The evidence is *strong* that parathion alters cell proliferation, cell death or nutrient supply. No studies in exposed humans were available to the Working Group. Sprague Dawley rats

(age, 39 days) treated with parathion exhibited a markedly increased density of terminal end buds compared with controls, at this time of active differentiation of terminal end bud into alveolar buds in the mammary gland. Studies using cultured human MCF-10F cells indicated that parathion could alter gene expression and cell proliferation. Treatment of human breast epithelial cell line MCF-10F with parathion resulted in increased levels of proliferating cell nuclear antigen and mutant TP53, an effect that was mitigated by atropine. In addition, several studies in cultured human and other mammalian cell lines indicated that treatment with parathion (or paraoxon) leads to the induction of apoptosis and cell death.

For the other key characteristics of human carcinogens, data were too few to allow evaluation.

There were no data on cancer-related susceptibility after exposure to parathion.

Overall, the mechanistic data provide some additional support for carcinogenicity findings of parathion.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of parathion.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Parathion is *possibly carcinogenic to humans* (*Group 2B*).

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DIAZINON

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 333-41-5

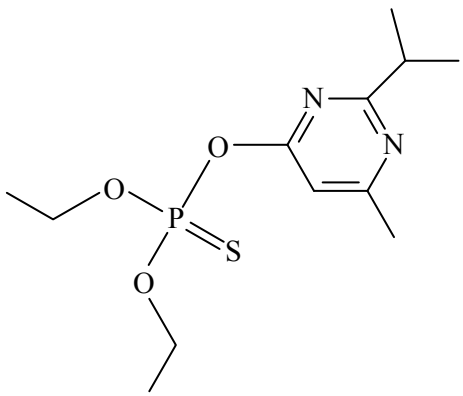
Chem. Abstr. Serv. Name: O,O-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate

Preferred IUPAC Name: O,O-diethyl *O*-[6-methyl-2-(propan-2-yl)pyrimidin-4-yl] phosphorothioate

Synonyms: Bazudine, Diazinon, Dimpylate, Neocidol, Neotsidol

Trade Names: Diazinon products have been sold in various countries under numerous trade names, including, for example, Basudin; Cekuzinon; Dianon; Diazol; Dragon; Kayazinon; Knox Out; Neocidol; Spectracide; Terminator (Farm Chemicals International, 2014; NCBI, 2015)

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_{12}H_{21}N_2O_3PS$

Relative molecular mass: 304.35

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: The pure form is a colourless oily liquid. The technical grade is light amber to dark brown in colour, and the insecticide formulation is a colourless liquid with a faint ester-like odour (NIOSH, 2010; NCBI, 2015).

Solubility: Slightly soluble in water at 60 mg/L (NCBI, 2015) at 20 °C. Completely miscible with common organic solvents, e.g. ethers, alcohols, benzene, toluene, hexane, cyclohexane, dichloromethane, acetone, petroleum oils (NCBI, 2015)

Volatility: Vapour pressure, 9.01×10^{-5} mm Hg (25 °C); low vapour pressure suggests that little volatilization from soil would be expected (NCBI, 2015).

Stability: More stable in alkaline formulations than at neutral or acid pH (NCBI, 2015)

Reactivity: Susceptible to oxidation above 100 °C (Tomlin, 2000)

Octanol/water partition coefficient (P): log K_{ow} 3.81 (NCBI, 2015)

Henry's law: 1.13×10^{-7} atm m^3 mol^{-1}; the low Henry's law constant suggests that little volatilization from water surfaces would be expected (NCBI, 2015).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), $mg/m^3 = 12.4 \times ppm$.

1.1.4 Technical products and impurities

Concentrations of *O*,*O*,*O*',*O*'-tetraethyl thiopyrophosphate (*O*,*S*-TEPP) and *O*,*O*,*O*',*O*'-tetraethyl dithiopyrophosphate (*S*,*S*-TEPP) are limited to 0.2 and 2.5 g/kg, respectively (WHO, 1999). Some diazinon formulations may contain other pesticides such as pyrethrins, lindane (gamma-hexachlorocyclohexane), and disulfoton (EXTOXNET, 2015).

1.2 Production and use

1.2.1 Production

Production and usage figures for diazinon are not available for most parts of the world. In the USA, the production volume of diazinon in 1990 was 4670 tonnes (Davies et al., 1996). The USA exported an estimated 2600 tonnes of diazinon between 1997 and 2000 (ATSDR, 2008). From 1987 until 1997, annual usage of diazinon in the USA was more than 5900 tonnes, with about 70% for outdoor residential uses (ATSDR, 2008). Total use of diazinon in the USA decreased from 2000–3000 tonnes in 2001 (diazinon was ranked third among organophosphate insecticides) to < 500 tonnes in 2007 (diazinon was ranked eighth) as a result of regulatory action (EPA, 2011).

Diazinon is reported to be manufactured by 46 producers in 11 countries, including 22 in China, six in India, five in the USA, four in Singapore, three in the United Kingdom, and one each in Canada, Israel, Japan, Mexico, Taiwan (China), and Thailand (Farm Chemicals International, 2015).

1.2.2 Uses

Diazinon is a wide-ranging non-systemic insecticide, miticide, and nematicide with contact, stomach, and respiratory action. It is effective against flying insects, crawling insects, mites, ticks, and spiders (IPCS, 1998). It has been employed since the early 1950s (IPCS, 1998) for uses including control of sucking and chewing insects and mites on a wide range of fruit, vegetables, and forage and field crops; on ranges, pastures, grasslands, and ornamentals; against ticks on cattle, blowflies and mites on sheep, and flies in greenhouses and mushroom houses; against grubs and nematodes in turf, and in seed treatment (Tomlin, 2000; EPA, 2006). Diazinon has also been used for general-purpose gardening and for indoor pest control against cockroaches, silverfish, ants, and scorpions, and in flea collars for pets (IPCS, 1998).

Diazinon has been produced in various commercial formulations, including liquids and concentrates, wettable powders, granules, dusts, and impregnated materials (EPA, 2006). Liquid formulations of diazinon can be sprayed by several application methods, including backpack and hand-held sprayers, and by aircraft; granular diazinon can be applied using manual or mechanized spreaders or grinders (EPA, 2006).

(a) Agriculture

Important agricultural applications of diazinon have been in rice, fruit, vineyards, sugar cane, corn, tobacco, potatoes, horticultural crops, and dips and sprays fror animals (IPCS, 1998). Diazinon has been used as the active pesticide ingredient topically applied (e.g. as aerosols, sprays, dips, ear tags) on livestock to control biting insects or ectoparasites (ATSDR, 2008). In the United Kingdom, dipping of sheep in baths containing diazinon to control a mite that causes sheep scab was compulsory until 1992 (Watterson, 1999; HSE, 2010). Diazinon has also been registered for incorporation into compost to control flies in mushroom cultivation (Shamshad, 2010).

(b) Residential use

Diazinon has been widely employed in residential settings, with such uses representing about 70% of total use of diazinon in the USA in 1987–1997 (ATSDR, 2008) Diazinon reportedly represented about 30% of all the homeowner-related insecticide use in the USA before 2004, when all remaining authorized indoor and outdoor residential uses of diazinon were cancelled (Stone et al., 2009). Diazinon was used for the control of household insects, lawn and garden insects, and insects on pets. Residential application methods included aerosol cans, spray equipment, and granular spreaders (ATSDR, 2008).

(c) Public health

In the USA, diazinon is currently permitted for the control of fire ants, and for the control of plague-infected fleas on squirrels (EPA, 2004).

(d) Regulation

In the 1980s, both the USA and Canada suspended the use of diazinon for control of grubs and nematodes on golf courses and sod farms, due to deaths of migratory waterfowl (ATSDR, 2008). In the USA, about 30% of agricultural uses (including most granular, aerial, and foliar applications) were cancelled at the end of 2002, and remaining uses were restricted to trained, certified applicators (EPA, 2001). All indoor residential and non-residential uses of diazinon, as well as outdoor residential lawn and garden products, were phased out of use in the USA by 2004 (EPA, 2006).

Withdrawal of authorizations for use of diazinon-containing products on crops and animals was finalized by the Health and Consumer Protection Directorate General of the European Commission in 2006 (European Commission, 2006). In France in 2012, the Agence Nationale du Médicament Vétérinaire withdrew permission to sell flea collars that contain organophosphates, including diazinon and tetrachlorvinphos (ANSES, 2012).

Occupational exposure limits for diazinon ranging from 0.01 mg/m^3 to 0.3 mg/m^3 have been been established in several countries (IFA, 2015).

1.3 Measurement and analysis

Representative methods of chemical analysis for diazinon and its specific metabolite 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) are listed in Table 1.1.

1.4 Occurrence and exposure

1.4.1 Exposures

(a) Occupational exposure

Occupational exposure may occur in workers involved in the manufacture of diazinon and formulations containing diazinon, applicators who spray or mix diazinon, farm workers engaged in re-entry tasks, sheep farmers and other livestock workers, vector-control workers, and veterinarians.

Table 1.1 Methods for the analysis of diazinon

Sample matrix	Assay procedure	Limit of detection	Reference
Air	GC-MS	0.3 ng/m³	Elflein et al. (2003)
Water	GC-FPD (with 526 nm filter)	0.01 µg/L	EPA (1992b)
	GC-MS (selected-ion monitoring mode)	0.01 µg/L	Zaugg et al. (1995)
Urine	GC-MS-ECNI-SIM	1 µg/L (as IMPY)	Bouchard et al. (2006)
Fruits and vegetables	GC-MS	0.02 mg/kg	Fillion et al. (2000)
Dust	GC-MS	2 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; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine

No data on exposure of workers involved in the production of diazinon were available to the Working Group.

(i) Air

Concentrations of diazinon in air were measured in a greenhouse during and after spraying and cold fogging operations (Lenhart & Kawamoto, 1994). The personal exposure of an applicator during spraying was 226 µg/m³, resulting in an 8-hour, time-weighted average (TWA) exposure of 25 µg/m³. Area measurements of diazinon concentrations were similar during spraying, but considerably higher (up to 3030 µg/m³) during cold fogging. TWA concentrations declined after both types of operation, but diazinon was still detectable after 4 days (Lenhart & Kawamoto, 1994).

(ii) Skin

In agricultural workers and pesticide applicators, skin contact is the most important route of exposure. Davis et al. (1983) estimated that dermal exposure in applicators spraying diazinon was 5500–29 000 µg/hour, depending on the activity, spraying method, and type of clothing worn, while exposure by the respiratory route was 1.9–7.4 µg/hour.

(iii) Biological markers

Several studies have reported metabolites of diazinon in the urine of exposed workers (Table 1.2). The highest mean urinary concentration of IMPY was reported in banana-plantation workers from Nicaragua, and was related to the volume of diazinon used, inappropriate application methods, and poor protection and hygiene of the workers (Rodríguez et al., 2006).

Diazinon has also been detected in saliva and blood of banana-plantation workers (Lu et al., 2006).

(b) Community exposure

(i) Air and dust

Diazinon and its metabolite, diazoxon, have been detected in urban and agricultural settings in the USA in the past, but levels are expected to have been reduced due to the implementation of regulations (EPA, 2004).

Available reports of diazinon concentrations in outdoor air ranged from not detected to a mean of 0.42 µg/m³ (Carey & Kutz 1985; Zabik & Seiber 1993; Whitmore et al. 1994; Majewski et al., 1998; Morgan et al., 2014). In indoor air, mean concentrations of diazinon ranged from 0.001 to 6 µg/m³, with the highest concentrations reported in studies in homes of pregnant women in New York, USA (Whitmore et al., 1994; Whyatt et al., 2005; Morgan et al., 2014). Diazinon may be transported in the atmosphere, with concentrations declining with distance from the source (Aggarwal et al., 2013).

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

Country, year	Job/process	Results	Comments/additional data	Reference
Canada, 2003	Greenhouse; 18 workers	IMPY, < LOD	IMPY not detected in 54 samples from 18 workers at an horticultural greenhouse (LOD, 1 µg/L)	Bouchard et al. (2006)
Nicaragua, 2003		IMPY: Geometric mean, 1.3–168 µg/L for two plantations Range for individual workers, ND to 412 µg/L	IMPY was detected in 79% of samples. Concentrations declined 45–75% after 24 hours	Rodríguez et al. (2006)
USA, 2002	Flea-control operations; 5 workers	DEP range, < LOD to 16.2 µg/L DETP range, < LOD to 44.6 µg/L	DEP and DETP are non-specific metabolites of organophosphate pesticides, but only diazinon was used by the workers	Gerry et al. (2005)
USA, 2010	Migrant farmworkers; 371 men	IMPY, ≥ LOD in 15% of samples	Geometric mean, NR	Raymer et al. (2014)

DEP, diethyl phosphate; DETP, diethyl thiophosphate; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine (specific metabolite of diazinon); LOD, limit of detection; ND, not detected; NR, not reported

Residues of diazinon in domestic dust ranged from not detected to 11 µg/g in urban and agricultural settings, with higher maximum concentrations in urban areas (Gunier et al., 2011; Quirós-Alcalá et al., 2011; Morgan et al., 2014).

(ii) Water

Diazinon is released into water directly by drift during application and runoff from rural and urban areas (ATSDR, 2008). It is moderately mobile in some soil types, and therefore has the potential to leach into groundwater (Fenlon et al., 2011). Diazinon has been reported in groundwater, drinking-water, main streams, and rural ponds in regions close to cultivation areas. Table 1.3 summarizes concentrations of diazinon reported in surface water in largely agricultural areas in the USA, Canada, and the Islamic Republic of Iran; concentrations ranged from not detected to 491.6 µg/L (Carey & Kutz, 1985; Frank & Logan, 1988; Frank et al., 1990; Maguire & Tkacz, 1993; Hall, 2003; Banks et al., 2005; Shayeghi et al., 2007; Zhang et al., 2012).

(iii) Soil

Morgan et al. (2014) reported detectable concentrations of diazinon in soil samples from 18% of 129 homes with children (range, not detected to 5.5 µg/g), and none of 13 day-care centres sampled in North Carolina, USA.

Diazinon is considered to be moderately mobile in soil. Microbiological degradation in soil and water is the main manner by which diazinon dissipates in the environment. In microbially active soils, diazinon is degraded rapidly (Bondarenko et al., 2004; Fenlon et al., 2011).

(iv) Household exposure

In a survey of 259 households in California, USA, 12% were found to be storing a product containing diazinon (Guha et al., 2013).

(v) Residues in food, and dietary intake

Several studies have reported small amounts of diazinon in a variety of food items, including fruits, vegetables, grains, meat, milk, and oils sold to consumers in several countries (Túri et al. 2000; Quintero et al., 2008; Zhang et al., 2008; Cho et al., 2009; Fuentes et al., 2010; Riederer

Table 1.3 Concentration of diazinon in surface water

Country Year of sampling	Number of samples/setting	Results	Comments/additional data	Reference
Ontario, Canada 1981–1985	446 samples from three rivers in agricultural areas	Detected in 1 out of 446 samples Concentration, 0.21 µg/L		Frank & Logan (1988)
Quebec, Canada 1986–1987	Number, NR; surface water	Range, 0.002–0.027 µg/L		Maguire & Tkacz (1993)
Islamic Republic of Iran Year, NR	Four stations near agricultural areas; samples were taken 1 day, 1 week, 2 weeks, 1 month, 2 months, and 3 months after spraying	Range, ND–491.6 µg/L	Maximum concentrations detected 1 day after application Diazinon residues decreased with increasing distance and time since spraying	Shayeghi et al. (2007)
USA 1976–1980	Number, NR; surface water	Detected in 1.2% of samples		Carey & Kutz (1985)
California, USA 1991–2001	27 sites; surface water	Range, ND–6.84 µg/L	90th percentile range, 0.01–14.90 µg/L	Hall (2003)
Texas, USA 2001–2004	1243 samples from 70 sites in agricultural areas	Range of mean concentrations, 0.04–0.32 µg/L	Concentrations decreased significantly between 2001 and 2004	Banks et al. (2005)
California, USA 2005–2010	3638 samples from 251 sites in 5 agricultural areas	Diazinon detection frequencies ranged from 10% to 90% Range of maximum concentrations, 1.0–24 µg/L		Zhang et al. (2012)
Washington, USA Year, NR	211 rural ponds in agricultural areas	Mean, 1.2 µg/L Range, < 0.002 to 1.7 µg/L	Found in two ponds contaminated by spill	Frank et al. (1990)

ND, not detected; NR, not reported

et al., 2010; EFSA, 2011; Srivastava et al., 2011; USDA, 2014). The highest concentration reported (3.8 mg/kg) was found in vegetables in the Republic of Korea (Cho et al., 2009). Many of the concentrations recorded in industrialized countries were below the reported limit of detection. [The Working Group noted the wide range of detection limits reported.]

(vi) Biological markers

Exposure to diazinon in the general population has been assessed by the presence of IMPY in urine samples, and diazinon in blood and saliva. IMPY was detected in 55% of urine samples from 60 farmworkers' children in North Carolina, USA, with a creatinine-adjusted geometric mean of 0.70 µg/g (Arcury et al., 2007). IMPY was detected in 5% of urine samples, and diazinon was found in 41% of saliva samples from 10 children of banana-plantation workers in Nicaragua (Lu et al., 2006; Rodríguez et al., 2006).

1.4.2 Exposure assessment

Exposure assessment methods in epidemiological studies on diazinon and cancer are discussed in Section 1.4.2 and Section 2.1.2 of the *Monograph* on Malathion, in the present volume.

2. Cancer in Humans

2.1 Summary of frequently cited epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.2 of the *Monograph* on Malathion in the present volume. The scope of the available epidemiological studies is discussed in Section 2.1 of the *Monograph* on Malathion, and includes a consideration of chance, bias and confounding, and exposure assessment.

2.2 Cohort studies

Three cohort studies were identified that reported relative risk estimates for the association between diazinon exposure and cancer outcomes: the Florida Pest Control Worker Study (Section 2.2.1), the United Farm Workers of America cohort study (Section 2.2.2), and the Agricultural Health Study (AHS) (Section 2.2.3). The studies were conducted among farm workers (United Farm Workers of America), and professional pesticide users (Florida Pest Control Worker Study; AHS) and their spouses (AHS) in the USA (see Table 2.1).

2.2.1 Florida Pest Control Worker Study

Pesatori et al. (1994) conducted a case–control study nested within the cohort of the Florida Pest Control Worker Study cohort and included 65 deceased cases of cancer of the lung and 294 controls (deceased, 122; living, 172) (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Proxy interviews were completed for 65 cases deceased between 1965–1982, and for 122 deceased and 172 living controls randomly selected from cohort members matched on year of birth and death. Telephone interviews covered tobacco use, diet, and occupations. For each occupation involving pesticide use, information on specific chemicals used was collected. Ever versus never use of diazinon was associated with an odds ratio of 2.0 (95% CI, 0.7–5.5) when comparing with deceased controls, and 1.3 (95% CI, 0.6–3.1) when comparing with living controls, after adjusting for age and smoking (see Table 2.1). [The Working Group noted substantial limitations to the pesticide exposure assessment based on proxy interviews, and the potential for differential exposure misclassification.]

Table 2.1 Cohort studies of cancer and exposure to diazinon

Reference, location, enrolment/ follow-up period, study-design	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%; percentage of surrogate respondents that could be located and interviewed) Controls: 294 (122 deceased, 172 living) (response rates: deceased controls, 80%, living controls, 75%); 16 living controls were interviewed directly because next-of-kin was not located Exposure assessment method: questionnaire; information collected from proxies at time of interview	Lung	Diazinon (using deceased controls) Diazinon (using living controls)	17 17	2 (0.7–5.5) 1.3 (0.6–3.1)	Age, smoking Age, smoking	Florida Pest Control Worker Study [Strengths: population of pesticide applicators with high exposure prevalence. Use of both deceased and living controls. Limitations: exposure assessment to specific pesticides based on interview with proxies (mostly wives) (possible information bias); small number of cases in the cohort; possible healthy worker effect]

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	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. (2005a) California, USA 1988–2001 Nested case–control study	Cases: 131 (response rate, NR) identified by linking the cohort to the California Cancer Registry for 1988–2001 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 vs low	NR	1.32 (0.65–2.65)	Age, sex, length of union affiliation, date of first union affiliation	United Farm Workers of America [Strengths: the study was conducted among farm workers (as opposed to pesticide applicators); the study included women; objective exposure assessment using a historical databank of pesticide use in the region – this method reduced recall bias. Limitations: number of cases was small; number of cases and controls exposed was not reported; the exposure assessment was based on regional pesticide use data and does not take personal use of pesticides or tasks into account, leading to possible exposure misclassification]
			High vs low (men)	NR	0.9 (0.37–2.19)		
			High vs low (women)	NR	2.7 (0.8–9.13)		
		Total NHL	High vs low	NR	1.39 (0.76–2.53)		
			High vs low (men)	NR	1.97 (0.97–4.00)		
			High vs low (women)	NR	0.8 (0.23–2.81)		
		Lymphocytic leukaemia	High vs low	NR	1.42 (0.46–4.43)		
		Granulocytic leukaemia	High vs low	NR	1.94 (0.66–5.72)		
		NHL, nodal	High vs low	NR	1.26 (0.60–2.66)		
		NHL, extranodal	High vs low	NR	1.57 (0.57–4.32)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mills & Yang (2005b) California, USA 1988–2001 Nested case–control study	Cases: 128 (response rate, NR); identified by linking the cohort to the California Cancer Registry for 1988–2001 Controls: 640 (response rate, NR); five controls for each case from the cohort who had not been diagnosed with any cancer and matched on sex, and ±1 yr of birth Exposure assessment method: 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-yr period before cancer diagnosis; classified "high exposure" can be interpreted as having worked in an area with high use	Breast (diagnosed 1988–1994)	No diazinon use (ref.)	12	1	Age, date of first union affiliation, fertility, socioeconomic level	United Farm Workers of America cohort [Strengths: the study was conducted among farm workers (as opposed to pesticide applicators); the study included women; objective exposure assessment using a historical databank of pesticide use in the region – this method reduced recall bias. Limitations: number of cases was small; number of cases and controls exposed was not reported; the exposure assessment was based on regional pesticide use data and does not take personal use of pesticides or tasks into account, leading to possible exposure misclassification; surrogate variables for reproductive histories: county level measures of fertility and socioeconomic status]
			Low	9	0.78 (0.12–4.84)		
			Medium	17	1.54 (0.22–10.68)		
			High	10	1.50 (0.18–12.35)		
		Breast (diagnosed 1995–2001)	No diazinon use (ref.)	26	1		
			Low	20	1.18 (0.27–5.20)		
			Medium	21	1.42 (0.30–6.81)		
			High	13	0.76 (0.15–3.92)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	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. (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 31 December 2001	57 284 licensed pesticide applicators and 32 333 spouses with no history of lung cancer at enrolment Incident cancers were identified from enrolment (i.e. 1993–1997) until 31 December 2001. Study subjects alive but no longer residing in Iowa or North Carolina (n = 875) were identified through personal contacts with the study subject, motor vehicle records, pesticide registration records, and the current address records of the Internal Revenue Service, and they were censored in the year they left the state Exposure assessment method: questionnaire	Lung	None (ref.) LED < 20.0 LED 20.0–108.5 LED > 108.5 Trend-test P value: 0.008	65 10 11 7	1 0.93 (0.50–1.80) 1.40 (0.70–2.70) 2.70 (1.20–6.10)	Age, sex, smoking, total days of any pesticide application	AHS [Strengths: large study of highly exposed workers. Limitations: very low study power for female cohort members (only 3 cases of cancer of the lung)]

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Beane Freeman et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, until 2002	23 106 male applicators who completed the take-home questionnaire that included questions on duration and frequency of diazinon use Exposure assessment method: take-home self-administered questionnaire	All neoplasms (ICD-9 140–208)	No diazinon (ref.)	722	1	Age, smoking, education, family history of cancer, state of residence, total days of any pesticide application	AHS This publication from the AHS is on cancer risk associated with diazinon specifically. Exposure metrics were LED and IW-LED, with analyses using either unexposed referents or the lowest exposure category as referents, essentially showing the same results. Results were reported for the cancer sites: colorectum, lung, prostate, melanoma, lympho-haematopoietic, NHL, and leukaemia. This paper overlaps with Lee et al. (2007) [Strengths: large size; licensed pesticide applicators only, resulting in high exposure prevalence (21.5% for diazinon) and good quality reporting of use of specific pesticides; complete follow-up; detailed exposure assessment based on questionnaire completed at time of enrolment, before disease outcome; different approaches for quantification of lifetime exposure enabling dose–response analyses. Limitations: results are for men only]
			LED < 20	106	1.12 (0.91–1.38)		
			LED 20.0–38.8	64	1.08 (0.83–1.40)		
			LED > 38.8	77	1.39 (1.09–1.78)		
			Trend-test *P* value: 0.009				
		All neoplasms	No diazinon (ref.)	722	1		
			LED < 20	106	1.12 (0.91–1.38)		
			LED 20.0–38.8	64	1.08 (0.83–1.39)		
			LED 38.9–108.8	45	1.28 (0.93–1.73)		
			> 108.8	32	1.58 (1.10–2.28)		
			Trend-test *P* value: 0.007				
		All neoplasms	IW-LED				
			No diazinon (ref.)	722	1		
			Tertile 1	85	1.10 (0.95–1.49)		
			Tertile 2	81	1.09 (0.86–1.38)		
			Tertile 3, low	39	1.16 (0.84–1.62)		
			Tertile 3, high	42	1.41 (1.03–1.95)		
			Trend-test *P* value: 0.033				
		All neoplasms	IW-LED				
			No diazinon (ref.)	722	1		
			Tertile 1	85	1.19 (0.95–1.50)		
			Tertile 2	81	1.09 (0.86–1.38)		
			Tertile 3	81	1.28 (1.01–1.63)		
			Trend-test *P* value: 0.05				
		Lung	No diazinon (ref.)	57	1		
			LED < 20	9	1.01 (0.48–2.15)		
			LED 20.0–38.8	3	0.54 (0.17–1.75)		
			LED > 38.8	15	2.41 (1.31–4.43)		
			Trend-test *P* value: 0.005				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Beane Freeman et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, until 2002 (cont.)		Prostate	No diazinon (ref.)	299	1		
			LED < 20	56	1.41 (1.05–1.88)		
			LED 20.0–38.8	32	1.28 (0.88–1.85)		
			LED > 38.8	26	1.19 (0.79–1.81)		
			Trend-test *P* value: 0.34				
		Lympho-haematopoietic (ICD-9 200–208)	No diazinon (ref.)	67	1		
			LED < 20	10	1.17 (0.60–2.29)		
			LED 20.0–38.8	7	1.31 (0.60–2.90)		
			LED > 38.8	9	1.84 (0.89–3.82)		
			Trend-test *P* value: 0.094				
		NHL (ICD-9 200 & 202)	No diazinon (ref.)	26	1		
			LED < 20	6	1.76 (0.72–4.35)		
			LED 20.0–38.8	3	1.36 (0.40–4.56)		
			LED > 38.8	2	0.92 (0.21–4.05)		
			Trend-test *P* value: 0.95				
		Leukaemia (ICD-9 204–208)	No diazinon (ref.)	21	1		
			LED < 20	3	1.1 (0.32–3.72)		
			LED 20.0–38.8	4	2.62 (0.88–7.82)		
			LED > 38.8	4	3.36 (1.08–10.49)		
			Trend-test *P* value: 0.026				
		Colorectum	No diazinon (ref.)	57	1		
			LED < 20	6	0.92 (0.39–2.15)		
			LED 20.0–38.8	6	1.53 (0.65–3.59)		
			LED > 38.8	4	1.21 (0.43–3.45)		
			Trend-test *P* value: 0.61				
		Melanoma	No diazinon (ref.)	31	1		
			LED < 20	7	1.67 (0.73–3.87)		
			LED 20.0–38.8	2	0.75 (0.18–3.15)		
			LED > 38.8	2	0.71 (0.16–3.04)		
			Trend-test *P* value: 0.59				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	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 Exposure assessment method: questionnaire	Breast	Wife's use (direct exposure)	31	1.0 (0.7–1.5)	Age, race (white/ other), state of residence	AHS [Strengths: large study; female study population; mostly farmers' wives with high exposure prevalence (10% of all wives used diazinon); focus on direct and indirect exposure (24% of wives who never used pesticides themselves were indirectly exposed to diazinon); collection of detailed exposure information at enrolment, before disease outcome. Limitations: few cases who used diazinon themselves based on self-reported exposure]
			Wife's use (direct exposure), Iowa	18	0.9 (0.6–1.5)		
			Wife's use (direct exposure), North Carolina	13	1.2 (0.7–2.1)		
			Wife's use (direct exposure), premenopausal women	8	0.8 (0.4–1.6)		
			Wife's use (direct exposure), postmenopausal women	19	1.1 (0.7–1.8)		
			Wife's use (direct exposure), family history of breast cancer	13	1.7 (0.9–3.2)		
			Wife's use (direct exposure), no family history of breast cancer	17	0.8 (0.5–1.2)		
			Husband's use (indirect exposure)	39	1.4 (0.9–2.0)		
			Husband's use (indirect exposure), Iowa	19	1.6 (0.9–2.6)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	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 (cont.)			Husband's use (indirect exposure), North Carolina	20	1.2 (0.7–20)		
			Husband's use (indirect exposure), premenopausal women	10	1.5 (0.7–3.2)		
			Husband's use (indirect exposure), postmenopausal women	28	1.5 (0.9–2.3)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2013) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to December 31 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, total cancers	Not exposed to diazinon (ref.)	727	1	Age, state, family history of prostate cancer, smoking, fruit servings, leisure time physical activity in winter, race	AHS A prior AHS publication already reported on diazinon and prostate cancer (Beane Freeman et al., 2005), but here 5 years additional follow-up were included [Strengths: large cohort study in agricultural population with high exposure prevalence, good exposure assessment; large number of prostate cancers, subanalysis of aggressive tumours, defined on histological and clinical parameters; adjustments for other pesticides]
			LED quartile 1	66	1.30 (1.01–1.68)		
			LED quartile 2	63	1.15 (0.88–1.49)		
			LED quartile 3	66	1.04 (0.81–1.35)		
			LED quartile 4	63	0.94 (0.72–1.24)		
			Trend-test *P* value: 0.59				
		Prostate, aggressive cancers	Not exposed to diazinon (ref.)	343	1	Age, state, family history of prostate cancer, smoking, fruit servings, leisure time physical activity in winter, race	
			LED quartile 1	31	1.24 (0.84–1.85)		
			LED quartile 2	29	1.00 (0.67–1.48)		
			LED quartile 3	30	0.89 (0.59–1.34)		
			LED quartile 4	30	1.31 (0.87–1.96)		
			Trend-test *P* value: 0.27				
		Prostate (no family history)	Not exposed to diazinon (ref.)	531	1	Age, state, smoking, fruit servings, leisure time physical activity in winter, race	
			LED quartile 1	51	1.34 (1.00–1.79)		
			LED quartile 2	49	1.20 (0.89–1.61)		
			LED quartile 3	45	0.96 (0.71–1.31)		
			LED quartile 4	48	1.08 (0.79–1.47)		
			Trend-test *P* value: 0.78				
		Prostate (family history)	Not exposed to diazinon (ref.)	121	1	Age, state, smoking, fruit servings, leisure time physical activity in winter, race	
			LED quartile 1	11	1.15 (0.62–2.14)		
			LED quartile 2	9	0.93 (0.46–1.86)		
			LED quartile 3	15	1.26 (0.72–2.20)		
			LED quartile 4	8	0.88 (0.42–1.83)		
			Trend-test *P* value: 0.82				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	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. (2014a) Iowa and North Carolina, USA Enrolment, 1993–1997; follow up until 31 December 2010 in North Carolina, and 31 December 2011 in Iowa	54 306 licensed pesticide applicators (523 incident cases of NHL) with no prevalent cancer at baseline, not living outside the catchment area of Iowa and North Carolina cancer registries, and with complete data on potential confounders Exposure assessment method: questionnaire	NHL	Ever vs never	144	1.0 (0.8–1.3)	Age, state, race (white/black), tertiles of total herbicide use days (for all except diazinon and lindane for follicular lymphoma)	AHS Additional 8–9 yr of follow-up since Beane Freeman et al. (2005). This paper overlaps with Alavanja et al. (2014b) [Strengths: prospective design; adjustment for other pesticides. Limitations: missing data on specific pesticides were imputed (validation on a subsample)]
			No diazinon (ref.)	187	1		
			Low (LED ≤ 8.75)	28	1.1 (0.7–1.6)		
			Medium (LED > 8.75–25)	19	1 (0.6–1.8)		
			High (LED > 25–457.25)	23	1.2 (0.7–1.9)		
			Trend-test *P* value: 0.52				
		NHL	No diazinon (ref.)	187	1		
			IWED tertile 1	23	1.1 (0.7–1.8)		
			IWED tertile 2	24	0.9 (0.5–1.5)		
			IWED tertile 3	22	1.3 (0.8–2.1)		
			Trend-test *P* value: 0.33				
		SLL, CLL, MCL	Ever vs never	46	1.3 (0.9–1.9)		
		SLL, CLL, MCL	No diazinon (ref.)	53	1		
			Low (LED)	14	1.4 (0.7–2.7)		
			High (LED)	12	1.9 (0.98–3.6)		
			Trend-test *P* value: 0.06				
		DLBC	Ever vs never	30	0.9 (0.6–1.4)		
		DLBC	No diazinon (ref.)	40	1		
			Low (LED)	9	1.5 (0.7–3.2)		
			High (LED)	8	1.1 (0.5–2.4)		
			Trend-test *P* value: 0.72				
		Follicular	Ever use	22	1.3 (0.7–2.3)		
		Follicular	No diazinon (ref.)	15	1		
			Low (LED)	8	2.2 (0.9–5.4)		
			High (LED)	7	3.8 (1.2–11.4)		
			Trend-test *P* value: 0.02				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	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. (2014a) (cont.)		Follicular	Diazinon & lindane in model concurrently			Age, state, race (white/black), tertiles of total herbicide use days, lindane use (tertiles of LED)	
			No diazinon (ref.)	15	1		
			Low (LED)	8	4.1 (1.5–11.1)		
			High (LED)	7	2.5 (0.9–7.2)		
			Trend-test *P* value: 0.09				
		Multiple myeloma	Ever vs never	27	1 (0.6–1.6)		
		Multiple myeloma	No diazinon (ref.)	41	1		
			Low (LED)	4	0.4 (0.1–1.2)		
			High (LED)	3	0.5 (0.2–1.7)		
			Trend-test *P* value: 0.35				
		Other B-cell	Ever vs never	12	0.8 (0.4–1.6)		
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa)	22 830 male applicators who completed the take home questionnaire and with complete information for LEDs (reported or imputed). Excluded were individuals with prevalent cancer at baseline (n = 622) or who were missing follow-up information (n = 145) and women due to the small number of female applicators (n = 663; 188 of whom reported using diazinon at follow-up)	Lung	LED < 20	32	1.11 (0.75–1.65)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	AHS [Strengths: prospective design; adjustment for other pesticides. Nearly 15 years of follow-up; triple the number of exposed cases of lung cancer since previous AHS report on lung cancer and diazinon; first report on bladder and kidney cancer and diazinon from AHS; separate results for colon and rectal cancer. Limitations: missing data on specific pesticides were imputed (validation on a subsample)]
			LED 20.0–38.8	16	0.76 (0.44–1.3)		
			LED > 38.8	36	1.6 (1.11–2.31)		
			Trend-test *P* value: 0.02				
		Lung, adenocarcinoma	LED < median	9	1.21 (0.57–2.57)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	14	1.37 (0.75–2.51)		
			Trend-test *P* value: 0.02				
		Lung, squamous cell carcinoma	LED < median	11	1.31 (0.69–2.53)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	8	0.65 (0.31–1.38)		
			Trend-test *P* value: 0.3				
		Lung, small cell carcinoma	LED < median	4	0.71 (0.25–2.02)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	11	1.23 (0.62–2.43)		
			Trend-test *P* value: 0.1				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)	Exposure assessment method: questionnaire; lifetime use of diazinon was collected in the take-home survey, and updated during the telephone follow-up interview (phase 2); multiple imputation for applicators who did not participate in follow-up (28%) Linkage to state cancer registries from enrolment until 31 December 2010 for North Carolina and 31 December 2011 for Iowa	Lung, other carcinomas	LED < median LED ≥ median Trend-test *P* value: 0.09	8 19	0.96 (0.42–2.19) 1.53 (0.88–2.66)	Age, smoking, family history of cancer, state of residence	
		Lung	IW-LED < 368 IW-LED 369–1800 IW-LED > 1800 Trend-test *P* value: 0.08	22 25 37	1.09 (0.61–1.53) 0.99 (0.66–1.52) 1.41 (0.98–2.04)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
		Lung, adenocarcinoma	IW-LED < median IW-LED ≥ median Trend-test *P* value: 0.14	10 13	1.17 (0.57–2.39) 1.43 (0.76–2.69)	Age, smoking, family history of cancer, state of residence	
		Lung, squamous cell carcinoma	IW-LED < median IW-LED ≥ median Trend-test *P* value: 0.54	9 10	0.98 (0.48–1.98) 0.89 (0.45–1.76)	Age, smoking, family history of cancer, state of residence	
		Lung, small cell carcinoma	IW-LED < median IWED ≥ median Trend-test *P* value: 0.18	4 11	0.63 (0.22–1.76) 1.36 (0.68–2.71)	Age, smoking, family history of cancer, state of residence	
		Lung, other carcinomas	IW-LED < median IW-LED ≥ median Trend-test *P* value: 0.19	10 17	1.06 (0.49–2.29) 1.50 (0.84–2.69)	Age, smoking, family history of cancer, state of residence	
		Bladder	LED < 20 LED 20.0–38.8 LED > 38.8 Trend-test *P* value: 0.77	13 8 11	0.69 (0.37–1.28) 0.72 (0.35–1.48) 0.93 (0.49–1.74)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)		Bladder	IW-LED < 368	8	0.58 (0.27–1.24)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	11	0.70 (0.37–1.32)		
			IW-LED > 1800	13	1.05 (0.59–1.90)		
			Trend-test *P* value: 0.96				
		Kidney	LED < 20	5	0.53 (0.21–1.31)	Age, smoking, state of residence	
			LED 20.0–38.8	6	0.89 (0.36–2.22)		
			LED > 38.8	10	1.77 (0.9–3.51)		
			Trend-test *P* value: 0.09				
		Kidney	IW-LED < 368	6	0.85 (0.37–1.86)	Age, smoking, state of residence	
			IW-LED 369–1800	6	0.77 (0.33–1.78)		
			IW-LED > 1800	9	1.37 (0.64–2.92)		
			Trend-test *P* value: 0.45				
		Prostate	LED < 20	148	1.10 (0.91–1.32)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			LED 20.0–38.8	70	0.89 (0.69–1.17)		
			LED > 38.8	79	1.01 (0.79–1.30)		
			Trend-test *P* value: 0.84				
		Prostate	IW-LED <368	111	1.16 (0.95–1.43)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			IW-LED 369–1800	102	0.89 (0.72–1.12)		
			IW-LED > 1800	83	0.99 (0.77–1.28)		
			Trend-test *P* value: 0.64				
		Prostate, aggressive cancers	LED < 20	71	1.08 (0.82–1.41)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			LED 20.0–38.8	36	0.98 (0.69–1.39)		
			LED > 38.8	44	1.16 (0.83–1.63)		
			Trend-test *P* value: 0.44				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)		Prostate, aggressive cancers	IW-LED < 368	54	1.11 (0.82–1.50)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			IW-LED 369–1800	47	0.90 (0.66–1.23)		
			IW-LED > 1800	50	1.29 (0.93–1.79)		
			Trend-test *P* value: 0.22				
		Colon	LED < 20	16	0.84 (0.50–1.41)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			LED 20.0–38.8	14	1.03 (0.57–1.86)		
			LED > 38.8	16	1.12 (0.63–1.99)		
			Trend-test *P* value: 0.67				
		Colon	IW-LED < 368	9	0.63 (0.32–1.24)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	19	1.21 (0.75–1.97)		
			IW-LED > 1800	18	1.03 (0.56–1.88)		
			Trend-test *P* value: 0.7				
		Rectum	LED < 20	5	0.51 (0.18–1.40)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			LED 20.0–38.8	5	0.88 (0.32–2.44)		
			LED > 38.8	4	0.94 (0.33–2.66)		
			Trend-test *P* value: 0.94				
		Rectum	IW-LED < 368	5	0.67 (0.24–1.85)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	2	0.18 (0.02–1.33)		
			IW-LED > 1800	7	1.62 (0.71–3.66)		
			Trend-test *P* value: 0.49				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study-design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)		Melanoma	LED < 20	15	0.96 (0.53–1.71)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			LED 20.0–38.8	11	1.22 (0.63–2.36)		
			LED > 38.8	6	0.58 (0.24–1.45)		
			Trend-test *P* value: 0.33				
		Melanoma	IW-LED < 368	14	1.27 (0.71–2.28)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	8	0.55 (0.24–1.26)		
			IW-LED > 1800	10	1.00 (0.49–2.02)		
			Trend-test *P* value: 0.69				

IW-LED, intensity-weighted lifetime exposure days; LED, lifetime exposure days; NHL, non-Hodgkin lymphoma

2.2.2 United Farm Workers of America

Mills et al. (2005a) reported on a case–control study of lympho-haematopoietic cancers nested within the United Farm Workers of America cohort (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). The cohort was drawn from the 139 000 ever members of a largely Hispanic farm-workers' union in California between 1973 and 1998 (Mills & Kwong, 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. For the 15 most commonly used pesticides (including diazinon), odds ratios for high versus low use were reported. Odds ratios for high versus low diazinon and total leukaemia (51 cases), lymphocytic leukaemia (23 cases), granulocytic leukaemia (20 cases), total non-Hodgkin lymphoma (NHL) (60 cases), nodal NHL (38 cases), and extranodal NHL (22 cases) were reported. Odds ratios were not reported for multiple myeloma (20 cases). Odds ratios were also reported by sex for all leukaemias (35 males, 16 females) and all NHL (45 males, 15 females). None of the odds ratios reported for diazinon reached statistical significance (see Table 2.1). Results were similar when the odds ratio for each chemical was adjusted for the other 15 chemicals. [The Working Group noted that although some elevated relative risks were observed (see Table 2.1), these were difficult to interpret because the number of exposed cases on which these estimates were based was not reported. The method of exposure assessment 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.]

Mills & Yang (2005b) reported on a case–control study that was nested in the United Farm Workers of America cohort and followed the same methodology as the study of lympho-haematopoietic cancers described above (Mills et al., 2005a), and included 128 cases of cancer of the breast in women. The association between estimated exposure to diazinon (low/medium/high versus no exposure) was presented separately for cases diagnosed in 1988–1994 (n = 48) and in 1995–2001 (n = 80); some increased risks were observed but they were not statistically significant (see Table 2.1).

2.2.3 Agricultural Health Study

The Agricultural Health Study (AHS) is a prospective cohort of licensed pesticide applicators enrolled in 1993–1997 in Iowa and North Carolina, USA (Alavanja et al., 1996; see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study).

Alavanja et al. (2004) reported on pesticide use and incidence of cancer of the lung in the AHS; 240 incident cases of cancer of the lung were identified. For 22 of the 50 pesticides evaluated (including diazinon, malathion, and parathion), the exposure index "lifetime exposure days" (LEDs) was based on the take-home questionnaire and computed as application days per year × total years of exposure. Unconditional multivariate logistic regression was used to compare cases of cancer of the lung with non-cases for the 50 specific pesticides, adjusting for smoking, age, sex, and total days of any pesticide application. For 7 out of 50 pesticides (including diazinon), LEDs showed some evidence of an exposure–response relationship and were reported. Compared with participants with no exposure to diazinon, odds ratios were 0.93 (95% CI, 0.5–1.8) for < 20 LEDs; 1.4 (95% CI, 0.7–2.7) for 20–108.5 LEDs, and 2.7 (95% CI, 1.2–6.1) for > 108.5 LEDs (P for trend, 0.008) (see Table 2.1). This statistically significant trend remained when the low-exposure category of < 20 LEDs was used as the reference group

(*P* for trend, 0.04). The odds ratios for cancer of the lung did not vary by more than 10% after additional adjustment for non-farm occupational exposures, regular recreational physical activity, alcohol consumption, fruit and vegetable intake, body mass index, medical conditions, medical conditions in a first-degree relative including a history of cancer of the lung, race, state of residence, license type, and education.

Beane Freeman et al. (2005) explored the associations between exposure to diazinon and cancer at multiple sites in the AHS. Results were reported for the following cancers: colorectum, lung, prostate, melanoma, lympho-haematopoietic system, NHL, and leukaemia. Analyses included only male pesticide applicators who had completed the take-home questionnaire that included questions on duration and frequency of diazinon use. Of the 23 106 applicators included in the study, 4961 had reported using diazinon (21%). During the follow-up period ending in December 2002 (approximately 7 years of follow-up), 1269 incident cases of cancer were diagnosed. Poisson regression was used to calculate rate ratios for LEDs and IW-LEDs. For LEDs (categories: none; < 20; 20.0–38.8; > 38.8), increased risks for the highest tertile of exposure (> 38.8 LEDs) and significant trend tests were observed for all neoplasms [OR, 1.39 (95% CI, 1.09–1.78)]; for cancer of the lung [already reported by Alavanja et al. (2004) based on 1 year shorter follow-up]; and for leukaemia [OR, 3.36 (95% CI, 1.08–10.49)] (see Table 2.1). Additional adjustment for use of pesticides most highly correlated with diazinon (ethylene dibromide, aluminium phosphide, metalaxyl, chlordane, and dieldrin), pesticides for which the AHS had reported an increased risk of lympho-haematopoietic cancers and leukaemia (alachlor) (Lee et al., 2004), or cancer of the lung (chlorpyrifos, metolachlor, pendimethalin, and carbofuran) (Alavanja et al., 2004; Bonner et al., 2005), did not markedly alter the results. The exposure–response relationship for IW-LEDs was not as strong as for the reported LEDs. [The intensity index used gave particular weight to dermal exposure and not to the potentially more relevant respiratory exposure, and therefore may have introduced more random error.] No other reported cancer site (including colorectum, prostate, melanoma, and NHL) showed an association with diazinon for the highest tertile of exposure (see Table 2.1).

Engel et al. (2005) examined the association between use of pesticides and incidence of cancer of the breast among farmers' wives in the AHS. Participants were 30 454 women with no history of cancer of the breast before enrolment and excluded licensed pesticide users. Until 2000 (average follow-up, 4.8 years), 309 incident cases of cancer of the breast were identified. Analyses were repeated for two groups: all farmers' wives (n = 30 454), and farmers' wives who had never used pesticides (n = 13 449). For all farmers' wives, exposure was based on a spouse take-home questionnaire, including a question on never versus ever use of diazinon (potential direct exposure). For farmers' wives who had never used pesticides, exposure was based on the husband's enrolment questionnaire, including a question on never versus ever diazinon use (potential indirect exposure). Rate ratios were calculated for individual pesticides using Poisson regression. The relative risk for potential direct exposure to diazinon within the group of all farmers' wives (exposure prevalence, 10%) was 1.0 (95% CI, 0.7–1.5). Potential indirect (husband's) exposure to diazinon within the group of farmers' wives who had never used pesticides (exposure prevalence, 24%) was associated with an odds ratio of 1.4 (95% CI, 0.9–2.0). There was no apparent trend in relation to the husbands' cumulative use of diazinon and risk of cancer of the breast (relative risks not reported). Relative risks were also presented by state and by menopausal status (see Table 2.1), and none reached statistical significance. [The Working Group noted that an increased risk was only observed for indirect (husband's) exposure to diazinon, and not for women's personal (direct) use of diazinon, although the latter was

based on smaller numbers. The strengths of this study included the large sample size, comprehensive exposure assessment, control for potential confounders, and exploration of potential interactions such as family history.]

Lee et al. (2007) studied the risk of cancer of the colorectum associated with exposure to specific pesticides among 56 813 pesticide applicators (women, 2.7%) within the AHS, who were followed up until 31 December 2002, and included 212 incident cases of cancer of the colon and 93 incident cases of cancer of the rectum. Odds ratios for ever use of diazinon were 0.7 (95% CI, 0.5–1.0) for cancer of the colon, and 1.3 (95% CI, 0.8–2.2) for cancer of the rectum. [The Working Group noted that because the follow-up period for this report was the same as that for Beane Freeman et al. (2005), and Beane Freeman et al. had already reported on cancer of the colorectum specifically in relation to exposure to diazinon in the AHS, including detailed dose–response analyses, the results from Lee et al. were not included in Table 2.1. It should be noted, however, that Beane Freeman et al. (2005) reported only on pesticide applicators who completed the take-home questionnaire and for whom LEDs for diazinon could be calculated, while Lee et al. (2007) reported on ever exposure to diazinon based on double the number of study participants. Also, Lee et al. (2007) reported relative risks for cancer of the colon and rectum separately, while Beane Freeman et al. (2005) did not.]

Koutros et al. (2013) studied the risk of cancer of the prostate associated with exposure to specific pesticides among 54 412 male pesticide applicators within the AHS, who were followed up from 1993 to 2007 (approximately 12 years). A total of 1962 incident cases were identified, including 919 aggressive cancers of the prostate. Rate ratios were calculated by Poisson regression to evaluate lifetime use of 48 pesticides for which there were 15 or more exposed cases (incuding diazinon) and cancer of the prostate. Exposure assessment (quartiles of IW-LEDs based on the distribution of exposed cases) included exposure data from data collection phases 1 (1993–1997) and phase 2 (1999–2003 for private applicants in spouses, and 2003–2005 for commercial applicators) of the study. Relative risks were presented for diazinon, but did not show a dose–response association (see Table 2.1). [The Working Group noted that Beane Freeman et al. (2005) had already reported on the association between exposure to diazinon and cancer of the prostate in the AHS, but the study by Koutros et al. (2013) presented analyses that included an additional 5 years of follow-up and relative risk estimates for all cancers of the prostate, as well as aggressive prostate cancers specifically. Because this constituted additional information, the results are reported here and included in the tables.]

Alavanja et al. (2014a, b) reported on an update of the AHS to 31 December 2010 in North Carolina, and 31 December 2011 in Iowa (approximately 15–16 years of follow-up), with a focus on NHL and its subtypes. Analyses included 54 306 male pesticide applicators, among whom there were 523 incident cases of NHL classified into six subtypes using the Surveillance Epidemiology and End Results (SEER) coding scheme (i.e. 148 small B-cell lymphocytic lymphomas (SLL)/chronic B-cell lymphocytic lymphomas (CLL)/mantle cell lymphomas (MCL); 117 diffuse large B-cell lymphomas; 67 follicular lymphomas; 53 other B-cell lymphomas; 97 multiple myelomas; and 19 T-cell NHL and 22 undefined cell types, which were not analysed due to small numbers). Assessment of exposure to diazinon was based on the enrolment questionnaire (never versus ever), take-home applicator questionnaire (LEDs), and the phase 2 follow-up questionnaire. For participants who did not complete the phase 2 questionnaire, use of specific pesticides in phase 2 was imputed. Information on pesticide use from phase 1, phase 2, and imputation for phase 2 was used to construct three cumulative exposure metrics: (i) LEDs (i.e. the product of years of use

of a specific pesticide and the number of days used per year); (ii) IW-LEDs (i.e. the product of lifetime days of use and a measure of exposure intensity); and (iii) data on ever versus never use for each pesticide. Intensity was derived from an exposure algorithm (Coble et al., 2011). [The Working Group noted that these exposure-intensity estimates are not the same as those used in the AHS publications on cancer of the lung (Alavanja et al., 2004; Beane Freeman et al., 2005), the limitations of which were reported in Section 2.2.3.] Poisson models were fitted to estimate rate ratios for tertiles of exposure indices based on the distribution of all exposed cases of NHL, and compared with unexposed cases, for all NHLs, and for the five NHL subtypes. Only the pesticides for which there were 15 or more exposed cases of total NHL were evaluated (26 out of 50 pesticides, including diazinon). Of all cases of NHL, 28% were ever exposed to diazinon, with a rate ratio of 1.0 (95% CI, 0.8–1.3). Rate ratios for ever exposure to diazinon by NHL subtype were also reported, and showed no statistically significant associations (see Table 2.1). LEDs for diazinon were not associated with all NHL (see Table 2.1), but an exposure–response relationship was observed for follicular lymphoma (*P* for trend, 0.02) and suggestive for SLL/CLL/MCL (*P* for trend, 0.06). An exposure–response association was not observed for diffuse large B-cell lymphoma (*P* for trend, 0.72). Polytomous logit models indicated some heterogeneity across subtypes for diazinon, although this did not reach statistical significance ($P = 0.09$). The pattern of increased risk of follicular lymphoma with diazinon use remained after adjusting for tertiles of LEDs of lindane (which was the only other pesticide showing an exposure–response relationship for follicular lymphoma; $P = 0.04$), although the trend was not statistically significant (none: rate ratio, 1.0 (ref.); low: rate ratio, 4.1 (95% CI, 1.5–11.1); high: rate ratio, 2.5 (95% CI, 0.9–7.2); *P* for trend, 0.09).

Jones et al. (2015) reported on the association between exposure to diazinon and seven solid cancers, based on 15–16 years of follow-up of the AHS cohort [an additional 8–9 years of follow-up after the Beane Freeman et al. (2005) report on diazinon]. Included were 22 830 male pesticide applicators who completed the take-home questionnaire and for whom there was complete information for LEDs of diazinon based on exposure data from both data collection phases 2 (1999–2003 for private applicants in spouses, and 2003–2005 for commercial applicators) and phase 3 (2005–2010) of the study. For 28% of the cohort, exposure data from phase 2 were not available and were therefore imputed. Rate ratios were calculated through Poisson regression for tertiles of LED and IW-LED, for cancers of the lung, bladder, kidney, prostate, colon, rectum, and for melanoma. [This was the first report from the AHS on associations between exposure to diazinon and cancers of the bladder, kidney, and lung subtypes.] For cancers of the bladder, prostate, colon, rectum and melanoma, there was no evidence of a dose–response relationship (see Table 2.1). The positive dose–response relationship for cancer of the lung was consistent with previous AHS reports (see Table 2.1), and analyses by subtype suggested an association for adenocarcinoma (rate ratio, LED < median = 1.21, 95% CI, 0.57–2.57; rate ratio, LED ≥ median = 1.37, 95% CI, 0.75–2.51), but not for squamous cell carcinoma (see Table 2.1). For aggressive cancer of the prostate, the highest rate ratios were observed for the highest exposure tertile, without reaching statistical significance (see Table 2.1). For cancer of the kidney, the highest tertile of LEDs for diazinon was associated with a borderline increased risk (rate ratio, 1.77; 95% CI, 0.90–3.51). There was no substantive evidence that dieldrin or five additional most strongly correlated pesticide exposures (from among those with available usage information) were confounders in the reported key analyses for diazinon.

2.3 Case–control studies on lympho-haematopoietic cancers

Two large multicentre case–control studies were identified that reported on the association between specific pesticides, including diazinon, and lympho-haematopoietic cancers: the combined case–control studies in the midwest USA (Section 2.2.1), and the Cross-Canada Case–control Study (Section 2.2.2; see the *Monograph* on Malathion, Section 2.2, for a detailed description of these studies). The case–control studies in the Midwest USA were conducted in the 1980s, initially as three autonomous case–control studies in Iowa and Minnesota (Cantor et al., 1992), Kansas (Hoar et al., 1986), and Nebraska (Hoar Zahm et al., 1990). The study in Iowa and Minnesota included leukaemia and NHL, the study in Nebraska included NHL, Hodgkin lymphoma, multiple myeloma, and CLL, and the study in Kansas included NHL, soft tissue sarcoma, and Hodgkin lymphoma. The data on NHL from these studies were subsequently pooled, which increased the power enabling analyses for specific pesticides.

The Cross-Canada Case-control Study was conducted in the early 1990s, and included NHL, Hodgkin lymphoma, and multiple myeloma (and soft tissue sarcoma, which is covered in the next section) (see Table 2.2).

2.3.1 Studies in the midwest USA

(a) Leukaemia

Brown et al. (1990) reported on the leukaemia component of the case–control study in Iowa and Minnesota. [The analysis included CLL, now a recognized subtype of NHL.] During 1981–1984, all newly diagnosed cases of leukaemia among white men aged ≥ 30 years were ascertained from tumour registry or hospital records. Controls were a population-based stratified sample of white men without lymphatic or haematopoietic cancer, frequency-matched to the leukaemia and NHL cases by 5-year age group, vital status at time of interview, and state of residence. In-person interviews were conducted with the subjects or with close relatives if the subjects were deceased or unable to be interviewed. The questions regarding farming covered farm locations and the number and type of animals raised and crops cultivated. Information concerning the use of 24 animal insecticides, 34 crop insecticides, 38 herbicides, and 16 fungicides on the farm was also obtained, including the first and last year used, and whether the subject personally mixed or applied the pesticide. The number of days per year that each pesticide was used was not collected in the initial interview, but in a supplemental interview in 1987 (only Iowa) for 86 cases (23 living, 63 deceased) and 203 controls (146 living, 57 deceased). The total study population consisted of 578 cases (340 living, 238 deceased; 293 from Iowa, 285 from Minnesota) and 1245 controls (820 living, 425 deceased). The odds ratio comparing farmers who had mixed, handled, or applied diazinon as a crop insecticide to non-farmers (243 cases, 547 controls), was 1.2 (95% CI, 0.6–2.1). Odds ratios according to the number of days per year diazinon was handled were 2.1 (95% CI, 0.8–5.6) for 1–4 days, and 0.5 (95% CI, 0.1–2.4) for 5–9 days; there were no cases exposed for ≥ 10 days (see Table 2.2).

(b) NHL

Cantor et al. (1992) reported relative risks for NHL specifically for diazinon based on case–control studies in the midwest USA, including only the Iowa and Minnesota component (Brown et al., 1990). Between 1980 and 1983, a total of 622 newly diagnosed cases of NHL (white men aged ≥ 30 years) and 1245 population controls (frequency-matched by 5-year age group, vital status, state) were interviewed in-person (the questionnaire was completed by a proxy for 30% of cases and 34% of controls). Exposure to diazinon was defined as having ever personally handled, mixed, or applied diazinon on crops. The odds

Table 2.2 Case–control studies on lympho-haematopoietic cancers and exposure to diazinon

Reference, location follow-up/ enrolment period, 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
Brown et al. (1990) Iowa and Minnesota, USA 1981–1984	Cases: 578 (response rate, 86%); white men, newly diagnosed, age ≥ 30 yr Controls: 1245 (response rate, 77–79%); white men, population-based; frequency matched on 5 year age group, vital status, state of residence Exposure assessment method: questionnaire; in-person interview with subject or proxy; farming and pesticide use history for subjects who worked on farm, listing 23 animal insecticides, 34 crop insecticides, 38 herbicides, 16 fungicides. Exposure defined as ever personally handled, mixed or applied; ORs for diazinon refer to use on crops	Leukaemia (including myelo-dysplasia)	Ever vs never use on crops Use (days/yr) 1–4 days/yr 5–9 days/yr ≥ 10 days/yr	17 8 2 0	1.2 (0.6–2.1) 2.1 (0.8–5.6) 0.5 (0.1–2.4) –	Vital status, age, state, tobacco use, family history of lympho-haematopoietic cancer, high-risk occupations, high-risk exposures	Studies in midwest USA Overlaps with Cantor et al. (1992) [Strengths: large study in farming area with high exposure prevalence; detailed questionnaire using list of specific pesticides and quantification of exposure; information on other potential risk factors collected. Limitations: for 41% of cases and 34% of controls the questionnaire was completed by a proxy, for whom recall of specific pesticide use will be problematic and subject to recall bias which may be different for cases and controls; multiple comparisons; self-reported pesticide use]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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
Cantor et al. (1992) Iowa and Minnesota, USA 1980–1983	Cases: 622 (response rate, 89%); white men newly diagnosed, age ≥ 30 yr Controls: 1245 (response rate, 77–79%); white men; population-based; frequency matched on: 5-year age group, vital status, state of residence Exposure assessment method: questionnaire; in-person interview with subject or proxy; farming and pesticide use history for subjects who worked on farm, listing 23 animal insecticides, 34 crop insecticides, 38 herbicides, 16 fungicides; exposure defined as ever personally handled, mixed or applied; ORs for diazinon refer to use on crops	NHL	Ever vs never As crop insecticide No personal protective equipment Before 1965 Before 1965, Iowa Before 1965, Minnesota	 27 17 14 10 4	 1.5 (0.9–2.5) 1.7 (0.9–3.2) 2.6 (1.2–5.9) 2.4 (0.9–6.2) 3.8 (0.7–22)	Vital status, age, state, smoking status, family history lymphopoietic cancer, high risk occupations, high risk exposures other than farming	Studies in midwest USA Overlaps with Brown et al. (1990) [Strengths: large study; in rural population; questionnaire using list of specific pesticides. Limitations: white men only; for 30% of cases and 34% of controls, the questionnaire was completed by a proxy, for whom recall of specific pesticide use would be problematic and subject to recall bias, which may be different for cases and controls]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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
Hoar Zahm et al. (1993) Nebraska, USA 1983–1986	Cases: 184 (response rate, 89%); Histologically confirmed cases of NHL diagnosed age ≥ 21 yr and identified through the Nebraska Lymphoma Study Group and area hospitals Controls: 707 (response rate, 86%); residents of the same area 3 : 1 frequency-matched by race, sex, vital status, age (5 yr) (matched to the four cancer sites included in the study i.e. NHL, HD, MM, CCL). For controls aged ≤ 65 yr: random-digit dialling. For living controls age ≥65 yr: Health Care Financing Administration (Medicare) records. Controls for deceased cases: Nebraska state mortality files matched on year of death (excluding causes of death: NHL, HD, MM, leukaemia, malignancy of unknown site, aplastic anaemia, suicide, homicide, legal intervention) Exposure assessment method: questionnaire	NHL	Exposed to diazinon Personally handled diazinon	7 2	1.9 4.1 (0.4–43.2)	Age	Studies in midwest USA [Strengths: the study included women exposed to pesticides. Limitations: relatively small size; number of proxy interviews not stated]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 748 (response rate, NR); white men, newly diagnosed age ≥ 21 yr (Iowa & Minnesota: 462; Kansas: 150; Nebraska: 136) Controls: 2236 (response rate, NR); white men, population-based, frequency matched on: 5-yr age group, vital status, state of residence (Iowa & Minnesota: 927; Kansas: 823; Nebraska: 486) Exposure assessment method: questionnaire; Iowa & Minnesota: see Cantor et al. (1992); Kansas: telephone interview, days/yr of pesticide use and years of use were asked about herbicides and insecticides overall, not by specific pesticide; subjects were asked to volunteer the pesticides they used; Nebraska: telephone interview days per year of use and years of use were asked for each pesticide used; asked about a predetermined list of about 90 pesticides	NHL	Ever use (incl. proxies)	60	1.7 (1.2–2.5)	Age, state of residence, respondent type (proxy/direct), except where otherwise stated	Studies in midwest USA (pooled) Iowa & Minnesota cases and controls overlap those in Cantor et al. (1992). Smaller numbers because of exclusions of those with missing data and those who did not know whether pesticides used [Strengths: large pooled study population; focus of pesticide exposure assessment; risk estimates excluding all proxy respondents are presented; analysis of subtype; cases were pathologically confirmed. Limitations: white men only. Pooled analyses of studies using different questionnaires (days/yr for each active ingredient only available in Iowa & Minnesota and Kansas); no list of pesticides in Kansas); proxies for 33% of cases and 41% of controls (however, risk estimates were presented excluding all proxies)]
			Ever use (excl. proxies)	44	1.3 (0.8–2.0)	Age, state of residence	
			Ever use, Iowa (excl. proxies)	22	1.1 (0.6–2.1)	Age	
			Ever use, Minnesota (excl. proxies)	5	1.3 (0.4–4.0)		
			Ever use, Kansas (excl. proxies)	1	13.0 (0.7–230.0)		
			Ever use, Nebraska (excl. proxies)	16	1.4 (0.7–2.9)		
			First use (excl. proxies): < 20 yr ago	20	1.1 (0.6–2.0)	Age, state of residence	
			First use (excl. proxies): ≥ 20 yr ago	16	1.4 (0.4–2.7)		
			Duration of use (excl. proxies): < 10 yr	20	0.9 (0.5–1.7)	Age, state of residence	
			Duration of use (excl. proxies): 10–19 yr	10	1.8 (0.7–4.4)		

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986 (cont.)			Duration of use (excl. proxies): ≥ 20 yr	1	1.9 (0.1–31.6)		
			Days/yr used (excl. proxies): < 5 days	6	1.3 (0.5–3.9)	Age	
			Days/yr used (excl. proxies): ≥ 5 days	6	2.4 (0.7–8.0)		
			Protective gear used (excl. proxies): yes	12	0.9 (0.4–1.9)	Age, state of residence	
			Protective gear used (excl. proxies): no	17	1.4 (0.7–2.8)		
		Follicular NHL	Ever use (excl. proxies)	17	1.3 (0.7–2.3)	Age, state of residence	
		Diffuse NHL	Ever use (excl. proxies)	13	1.2 (0.6–2.4)	Age, state of residence	
		SLL	Ever use (excl. proxies)	9	2.8 (1.1–7.3)	Age, state of residence	
		Other type NHL	Ever use (excl. proxies)	5	0.7 (0.3–2.0)	Age, state of residence	

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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
De Roos et al. (2003) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records; white men Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files; white men Exposure assessment method: questionnaire and interview (direct or next-of-kin); analyses focused on 47 pesticides to which ≥ 20 persons were exposed; any subject with a missing or "don't know" response for any of the 47 pesticides was excluded from all analyses	NHL	Ever use Logistic regression Hierarchical regression Joint effects of diazinon & atrazine Neither (ref.) Diazinon only Atrazine only Both diazinon and atrazine	 40 40 551 9 59 31	 1.9 (1.1–3.6) 1.7 (1.0–2.8) 1 1.2 (0.5–3.1) 1.5 (1.0–2.3) 3.9 (1.7–8.8)	Age, study site, all the other 46 pesticides to which 20 or more persons were exposed	Studies in midwest USA (pooled). Included participants from Cantor et al. (1992), Hoar Zahm et al. (1990), Hoar et al. (1986), and Brown et al. (1990) Included the same study population of Waddell et al. (2001). Analyses presented are different, with focus on exposure to multiple pesticides and whether there is a more than additive effect. Smaller numbers due to further exclusions (see exposure assessment notes) [Strengths: in addition to the strengths of Waddell et al. (2001), the strength of this analysis was the focus on exposure to multiple pesticides (realistic exposure scenarios), and adjustment of risk estimates for other pesticides. Limitations: In addition to the limitations of Waddell et al. (2001), a limitation of this analysis was that results excluding proxy respondents were not presented]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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
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; males newly diagnosed, age ≥ 19 yr Controls: 1506 (response rate, 48%); random sample from health insurance and voting records; males age ≥ 19 yr, frequency-matched on province and ± 2 yr to the age distribution of entire case group (which also included soft tissue sarcoma, Hodgkin lymphoma, multiple myeloma) Exposure assessment method: questionnaire; self-administered postal questionnaire, followed by telephone interview for subjects with ≥ 10 hours per year 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 used it at work, in home garden or as hobby	NHL	Ever use	18	1.69 (0.88–3.24)	Age, province of residence, medical variables	Cross-Canada Case–control Study [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]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, 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. (2012) Six provinces in Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, Saskatchewan) 1991–1994	Cases: 342 (response rate, 58%); men newly diagnosed (age ≥ 19 yr) Controls: 1506 (response rate, 48%); males (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, postal and telephone interview	Multiple myeloma (ICD-O M 9732/3)	Ever use	9	1.33 (0.59–3.01)	Age, province of residence, medical variables	Cross-Canada Case–control Study [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders; cases confirmed by pathology review. Limitations: men only; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides; no quantitative exposure data]
Karunanayake et al. (2012) Six provinces in Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia) 1991–1994	Cases: 316 (response rate, 68.4%); men, newly diagnosed, age ≥ 19 yr Controls: 1506 (response rate, 48%); males (age ≥ 19 yr), frequency-matched to province and ± 2 yr to the age distribution of entire case group (which also included soft tissue sarcoma, multiple myeloma, NHL) Exposure assessment method: questionnaire, postal and telephone interview	Hodgkin lymphoma	Ever use	10	2.08 (0.91–4.77)	Age, province of residence, medical variables	Cross-Canada Case–control Study [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders; cases confirmed by pathology review. Limitations: men only; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides; no quantitative exposure data]

CLL, chronic B-cell lymphocytic lymphoma; IW-LED, intensity-weighted lifetime exposure days; LED, lifetime exposure days; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; SLL; small B-cell lymphocytic NHL; yr, year

ratios for ever use of diazinon was 1.5 (95% CI, 0.9–2.5), and 2.6 (95% CI, 1.2–5.9) for diazinon use before 1965 (see Table 2.2). Adjustment for pesticides from other families of agents did not alter the results. [Odds ratios by days per year of diazinon handling were not presented.]

Hoar Zahm et al. (1993) reported on the female component of the case–control study on NHL in Nebraska, which included 184 women diagnosed with NHL (1983–1986) and 707 controls (from multiple sources; see Table 2.2). For those reporting exposure to diazinon (7 cases, 16 controls) the odds ratio of 1.9 was not statistically significant [95% CI, not reported.] Only 2 cases and 2 controls reported personally handling diazinon (OR, 4.1; 95% CI, 0.4–43.2). [The Working Group noted that this was the only case–control study identified that reported relative risk estimates for cancer in women exposed to diazinon.]

Waddell et al. (2001) reported on the association between exposure to diazinon and NHL based on the pooled database of case–control studies in the midwest USA, including Iowa and Minnesota, Kansas, and Nebraska (see the *Monograph* on Malathion, Section 2.2, for a detailed description of these studies). The odds ratio for ever use of diazinon was 1.7 (95% CI, 1.2–2.5). After excluding all proxy interviews, the odds ratio was 1.3 (95% CI, 0.8–2.0). All subsequent analyses were conducted excluding proxy interviews. As indicated in the table, odds ratios were greater for higher number of years of use, higher number of days of use per year, and for use of diazinon without protective equipment, but none reached statistical significance. Results for ever use of diazinon were also presented by major subtype of NHL (follicular, diffuse, small lymphocytic, other), with SLL associated with an odds ratio of 2.8 (95% CI, 1.1–7.3). After adjusting for fonofos, the odds ratio was 2.5 (95% CI, 0.8–7.6), and after adjusting for malathion, the odds ratio was 2.7 (95% CI, 0.7–10.7). [The Working Group noted that pesticide-specific relative risks have been reported for the Iowa and Minnesota component of the study population (Cantor et al., 1992). Odds ratios were reported by Waddell et al. (2001) by study centre, and were also elevated for the centres not included in the publication by Cantor et al. (1992). The elevated odds ratios reported by Waddell et al. (2001) were thus not entirely attributable to the Iowa and Minnesota component of the study.]

De Roos et al. (2003) also reported on risk estimates for NHL and exposure to diazinon in the pooled case–control studies from the midwest USA, but the focus of analysis was on exposure to multiple pesticides. The odds ratio for ever exposure to diazinon, fully adjusted for exposure to 46 other pesticides, was 1.9 (95% CI, 1.1–3.6). [The Working Group noted that an odds ratio for ever use of diazinon in this study population had already been reported in Waddell et al. (2001). The odds ratio reported in the article by De Roos et al. (2003) suggested that it was not likely to be attributable to confounding by other pesticides, considering the detailed adjustment made for other pesticides. A limitation of this analysis was that results excluding proxy respondents were not presented, although it can be assumed that this analysis probably eliminated many of the proxy interviews because it excluded individuals with missing and "don't know" responses.] Of 48 pesticide combinations, joint effects were more than additive for carbofuran and atrazine; alachlor and atrazine; and diazinon and atrazine. With those never having used diazinon or atrazine as the reference group, the odds ratio for using diazinon and not atrazine was 1.2 (95% CI, 0.5–3.1; 9 exposed cases), the odds ratio for using atrazine was 1.5 (95% CI, 1.0–2.3; 59 exposed cases), and the odds ratio for using both diazinon and atrazine was 3.9 (95% CI, 1.7–8.8; 31 exposed case), indicative of a more than additive effect.

2.3.2 Cross-Canada Case–control Study of Pesticides and Health

(a) NHL

McDuffie et al. (2001) reported the results for NHL (517 incident cases, 1506 population controls) in the Cross-Canada Case–control Study (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Exposure, defined as use of diazinon at work, in the home garden or as a hobby, was associated with an odds ratio of 1.69 (95% CI, 0.88–3.24).

(b) Multiple myeloma

Pahwa et al. (2012) reported the results for multiple myeloma (342 cases, 1506 controls) in the Cross-Canada Case–control Study (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Ever use of diazinon was associated with an odds ratio of 1.33 (95% CI, 0.59–3.01).

(c) Hodgkin lymphoma

Karunanayake et al. (2012) reported the results for Hodgkin lymphoma (315 cases, 1506 controls) in the Cross-Canada Case–control Study (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Ever use of diazinon was associated with an odds ratio of 2.08 (95% CI, 0.91–4.77).

2.4 Case–control studies on other cancers

Estimates of risk associated with exposure to diazinon based on a case–control study have been reported for cancers other than lympho-haematopoietic cancers, including soft tissue sarcoma, cancer of the prostate, and cancer of the brain in childhood and in adults (see Table 2.3).

2.4.1 Soft tissue sarcoma

Pahwa et al. (2011) reported the results for soft tissue sarcoma in the Cross-Canada Case–control Study (357 cases, 1506 population controls). Exposure, defined as used diazinon at work, in the home garden or as a hobby, was associated with an odds ratio of 3.31 (95% CI, 1.78–6.23). Aldrin was the only other agent for which a statistically significant association with soft tissue sarcoma was observed and the odds ratio for diazinon did not change substantially after adjustment for use of aldrin (OR, 3.19; 95% CI, 1.69–6.01).

2.4.2 Cancer of the prostate

Band et al. (2011) reported the results of a case–control study that included 1516 patients with cancer of the prostate and 4994 age-matched controls comprising patients with cancer at any other site except lung and cancers of unknown primary site (1153 cases and 3999 controls were included in the final analysis). A total of 47 cases (3.1%) and 109 controls (2.2%) was assessed as being exposed to diazinon (OR, 1.43; 95% CI, 0.99–2.07). By exposure index, the association reached statistical significance for the group with highest exposure (low exposure: OR, 0.91; 95% CI, 0.50–1.68; high exposure: 1.93; 95% CI, 1.21–3.08; *P* for trend, 0.02) compared with never exposed. Similar dose–response relationships were observed for 6 out of 15 fungicides, 3 out of 6 herbicides, and 6 other insecticides out of the total of 19 insecticides. [The Working Group noted that this paper reported high correlation between specific pesticides as assessed through a job-exposure matrix. This, together with the large number of pesticides showing dose–response relationships similar to diazinon, suggested that associations for specific pesticides may have been due to intercorrelation with other pesticides.]

Table 2.3 Case-control studies of other cancers and exposure to diazinon

Reference, location follow-up/ enrolment period, 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 age ≥ 19 yr, frequency matched to province and ± 2 yr 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 use Ever use	20 20	3.31 (1.78–6.23) 3.19 (1.69–6.01)	Age, province of residence, medical variables Age, province of residence, whooping cough, first-degree relative with cancer, aldrin user	Cross-Canada Case-control Study Results presented by soft tissue sarcoma subtype [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders. Limitations: men only; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides]

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, 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	Cases: 1153 (response rate, NR); 941 (response rate, 82%) direct respondents; British Columbia cancer registry; with histological confirmation Controls: 3999 (response rate, NR); male cancer patients from the same registry with cancers other than prostate, excluding lung cancer and cancer of unknown primary site Exposure assessment method: JEM; lifetime occupational history was obtained through a self-administered questionnaire and used in conjunction with a JEM to estimate the participants' lifetime cumulative exposure to approximately 180 active compounds in pesticides	Prostate	Ever use	47	1.43 (0.99–2.07)	Age, alcohol consumption, cigarette years, respondent (direct/proxy), education	[Strengths: large number of cases and controls; histologically confirmed incident cancer cases; use of cancer controls which may have limited differential recall; use of JEM limiting differential exposure misclassification; study was conducted before the period of early detection of prostate cancer. Limitations: use of cancer controls; included cancers that may be associated with pesticide exposure; lack of information on family history; potential exposure misclassification; multiple comparisons; use of JEM to assess pesticide exposure resulting in high correlations between specific pesticides; associations for specific pesticides may be due to intercorrelations with other pesticides]
			By exposure index				
			Never use (ref.)	1106	1		
			Low	15	0.91 (0.5–1.68)		
			High	32	1.93 (1.21–3.08)		
			Trend-test *P* value: 0.02				

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, 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
Davis et al. (1993) Missouri, USA 1985–1989	Cases: 45 (response rate, NR); cases aged 0–10 yr identified through the population-based Missouri cancer registry Controls: 85 friend controls, 108 cancer controls (response rate, NR) Exposure assessment method: questionnaire; during telephone interviews the biological mothers of cases and controls were asked about the number of times that pesticides had been used for nuisance pests in the home, garden or on pets, during pregnancy, during the interval from birth to age 6 mo, and since age 7 mo, and age of diagnosis; respondents were also asked whether several specific pesticide products had been used at any time from pregnancy to diagnosis	Brain, childhood	In garden or orchard (friend controls) In garden or orchard (cancer controls)	7 7	4.6 (1.2–17.9) 1.4 (0.4–4.7)	Age, environmental tobacco smoke, family income, father's education, mother's education, family member in construction industry, time between diagnosis and interview	[Strengths: study focused on home use of pesticides; during the relevant exposure period, diazinon was widely used as a garden and in-house insecticide; use of individual pesticides, including diazinon, in home and garden was assessed; use of both friend controls and cancer controls. Limitations: very small size; high risk estimates using friend controls (when compared with cancer controls) were likely due to differential recall of parents' use of pesticides between those with sick and healthy children]

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, 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 1 January 1995 – 31 January 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 diazinon – and collecting lifetime pesticide use in farming and non-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 (incl. proxies) Ever use (excl. proxies)	18 13	1.3 (0.7–2.5) 1.9 (0.9–4.1)	Age, 10-yr age group, education, other pesticides	Upper Midwest Health Study [Strengths: large size for a brain cancer study; first study to look at the association between farm pesticide exposure and glioma in adult women; extensive questionnaire on farm and rural risk factors and pesticide use; cases histologically confirmed and limited to glioma. Limitations: self-reported ever use of specific pesticides; controls older than cases; large proportion of proxy respondents (43% of cases, 2% of controls)]

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, 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
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 centres for Medicare services Exposure assessment method: questionnaire; based on self-report	Brain, intracranial glioma (ICD-O 938–948)	Ever use			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)]
			In non-farm job (incl. proxies)	10	0.61 (0.29–1.29)		
			In non-farm job (excl. proxies)	8	0.81 (0.35–1.87)		
			In house and garden (incl. proxies)	57	0.66 (0.47–0.92)		
			In house and garden (excl. proxies)	36	0.75 (0.50–1.12)		

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, 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, USA 1988–1993	Cases: 170 stomach and 137 oesophageal (response rates, 79%, 88%); identified from Nebraska cancer registry and discharge diagnosis and pathology records at 14 hospitals, incident cases age ≥ 21 yr Controls: 502 (response rate, 72%); population 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: questionnaire; used a list of 16 major insecticides and 14 herbicides used on Nebraska crops over the previous 40 yr including diazinon	Stomach Oesophagus	Ever use Ever use	6 10	0.5 (0.2–1.2) 0.8 (0.4–1.8)	Age, sex	[Strengths: high response rate; use of set list of pesticides in interview. Set in rural area and therefore reasonable exposure prevalence. Limitations: high percentage next-of-kin interviews for whom recall of specific pesticides used will be problematic; self-reported pesticide use; possible misclassification of exposures]

excl., excluding; incl., including; IW-LED, intensity-weighted lifetime exposure days; LED, lifetime exposure days; mo, month; NHL, non-Hodgkin lymphoma; yr, year

2.4.3 Cancer of the brain in childhood

Davis et al. (1993) reported the results of a case–control study that included 45 cases of childhood cancer of the brain (age, 0–10 years), 85 friend controls and 108 cancer controls (predominantly acute lymphoblastic leukaemia), diagnosed in 1985–1989, and interviews were conducted in 1989–1990. During telephone interviews, the biological mothers of cases and controls were asked about the number of times that pesticides had been used for nuisance pests in the home, garden, or on pets, during pregnancy, during the interval from birth to age 6 months, and since age 7 months, and age of diagnosis. Respondents were also asked whether several specific pesticide products had been used at any time between pregnancy and diagnosis. Of the 45 mothers of cases, 7 reported the use of diazinon in the garden or orchard at any time between pregnancy and diagnosis. When compared with friend controls, this yielded an odds ratio of 4.6 (95% CI, 1.2–17.9), and an odds ratio of 1.4 (95% CI, 0.4–4.7) when compared with cancer controls. [The Working Group noted that this was a very small study, but was conducted at a time when diazinon was still widely used in and around the home. The high risk estimate using friend controls as compared with cancer controls suggested differential recall of parents' use of pesticides for sick or healthy children.]

Leiss & Savitz (1995) reported on a case–control study on home pesticide use and childhood cancer. Results specifically for diazinon were not presented, and an association between treatment of the yard (lawn/garden) and cancer of the brain was not observed in this study.

Pogoda & Preston-Martin (1997) reported on a population-based case–control study of childhood tumours of the brain in Los Angeles County, California, USA, that involved 224 cases (diagnosed 1984–1991) and 218 controls; however, the exposure prevalence of diazinon as a garden insecticide was low, and risk estimates for diazinon were not reported.

2.4.4 Cancer of the brain in adults

The association between exposure to farm pesticides and risk of intracranial glioma in adults was studied in the Upper Midwest Health Study (UMHS) (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study).

Ruder et al. (2004) reported on the UMHS and included 457 male incident cases of intracranial glioma and 648 population controls aged 18–80 years. Proxy interviews were completed for 47% of the cases. Diazinon was among the 14 individual farm pesticides to which the most participants were exposed. Statistically significant associations were not observed for any of these pesticides, either with or without proxy respondents, and the pesticide-specific results were not reported.

Carreón et al. (2005) reported on the UMHS and included 341 female incident cases of intracranial glioma and 528 controls. Reported agricultural use of diazinon was associated with an odds ratio of 1.3 (95% CI, 0.7–2.5), and 1.9 (95% CI, 0.9–4.1) if all proxy interviews (43% of cases and 2% of controls) were excluded from analyses, adjusting for age, education, and any other pesticide exposure.

Yiin et al. (2012) reported on the UMHS and included men and women (798 cases and 1175 controls), aiming to improve on the pesticide exposure assessment to yield a quantitative estimated lifetime cumulative exposure (gram-years), and also investigating non-farm use of pesticides. Positive associations between risk of glioma and estimated quantitive exposure to any of the individual pesticides were not observed and odds ratios were not reported. Ever non-farm occupational use of diazinon was not associated with an increase in risk of glioma (see Table 2.3), nor was house and garden use of diazinon (see Table 2.3).

Table 3.1 Studies of carcinogenicity with diazinon in mice

Species, strain (sex) Duration Reference	Dosing regimen Animal/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 105–106 wk NTP (1979)	Diet containing diazinon at concentrations of 0 (vehicle control), 100, or 200 ppm, ad libitum, for 103 wk 50 M and 50 F/treated group, and 25 M and 25 F/matched-control group (age, 6 wk)	*Males* Hepatocellular adenoma: 1/21 (5%), 0/46, 3/48 (6%) Hepatocellular carcinoma: 4/21 (19%), 20/46 (43%)*, 10/48 (21%) Hepatocellular adenoma or carcinoma (combined): 5/21 (24%), 20/46 (43%), 13/48 (27%) *Females* No exposure-related increase in tumour incidence	*Males* *P = 0.046 (Fisher exact test) *Females* NS	Purity, 98% No significant increase in mortality in treated mice. The occurrence of hepatocellular carcinoma could not clearly be related to the administration of diazinon Incidence of hepatocellular carcinoma in historical controls, males: 498/2334 (21.3%); range, 8–36% (Haseman et al., 1984)

F, female; M, male; NS, not significant; wk, week

2.4.5 Cancer of the stomach and oesophagus

Lee et al. (2004) reported on a case–control study of incident cases of cancer of the stomach (n = 170) and oesophagus (n = 137) from Nebraska (1988–93) and 502 population controls. Compared with non-farmers, self-reported ever use of diazinon was associated with an odds ratio of 0.5 (95% CI, 0.2–1.2; 6 exposed cases) for cancer of the stomach, and 0.8 (95% CI, 0.4–1.8; 10 exposed cases) for cancer of the oesophagus.

2.5 Meta-analysis

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including diazinon. The meta-analysis for diazinon included three studies (McDuffie et al., 2001; Waddell et al., 2001; Mills et al., 2005a), and yielded a meta risk-ratio of 1.6 (95% CI, 1.2–2.2) with an I^2 value of 0% [indicating no inconsistency between studies].

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were given diets containing diazinon (purity, 98%; dissolved in acetone) at a concentration of 100 or 200 ppm, ad libitum, for 103 weeks, and then held for an additional 2–3 weeks for observation; a group of 25 male and 25 female B6C3F$_1$ mice served as matched controls (NTP, 1979). Survival was 98% (49/50), 90% (45/50), and 84% (21/25) among the males, and 98% (49/50), 100% (50/50), and 96% (24/25) among the females in the groups at the higher and lower dose, and control group, respectively, at week 78. Mean body weights of the treated male and female mice were essentially the same as those of the corresponding controls except for the last 20 weeks of the bioassay, when the mean body weights of the treated females were lower than those of the controls. In males, there was an increase in the incidence of hepatocellular carcinoma, with the incidence at the lower dose (20/46; 43%) being significantly increased (P = 0.046, Fisher exact test) compared with the controls

Table 3.2 Studies of carcinogenicity with diazinon in rats

Species, strain (sex) Duration Reference	Dosing regimen Animal/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 104–105 wk NTP (1979)	Diet containing diazinon at concentrations of 0 (vehicle control), 400, or 800 ppm, ad libitum, for 103 wk 50 M and 50 F/treated group, and 25 M and 25 F/matched-control group (age, 7 wk)	*Males* Leukaemia or lymphoma (combined): 5/25 (20% [all leukaemias]), 25/50 (50%)* [leukaemia, 24/50, lymphoma, 1/50], 12/50 (24%) *Females* No exposure-related increase in tumour incidence	*Males* *P = 0.011 (Fisher exact test) *Females* NS	Purity, 98% No significant increase in mortality in treated animals The occurrence of haematopoietic malignancies could not clearly be related to the administration of diazinon Historical control incidence, leukaemia or lymphoma (combined), males: 699/2320 (30.1%); range, 0–46% (Haseman et al., 1984)
Rat, Sprague-Dawley (M, F) 98 wk EPA (1993)	Diet containing diazinon at concentrations of 0 (vehicle control), 0.1, 1.5, 125, or 250 ppm, ad libitum, for 98 wk 20 M and 20 F/group (age, 9 wk)	No exposure-related increase in the incidence of any neoplasm	NS	Purity, 87.7% (impurities not reported) At 97 wk, survival in males was 45%, 30%, 50%, 35%, and 58%, respectively; and survival in females was 58%, 40%, 44%, 68%, and 58%, respectively. Because mortality was higher in the groups at low doses than in the controls, the study was terminated at wk 97

F, female; M, male; NS, not significant; wk, week

(4/21; 19%). [The Working Group concluded that the increase in the incidence of hepatocellular carcinoma could not clearly be related to the administration of diazinon because it was only observed in males at the lower dose, and the incidence was slightly above the upper limit of the range for historical controls in this strain of mice (incidence of hepatocellular carcinoma in historical controls, 498/2334; 21.3%; range, 8–36%; Haseman et al., 1984).] In females, there was no exposure-related increase in tumour incidence.

3.2 Rat

See Table 3.2

Groups of 50 male and 50 female F344 rats (age, 7 weeks) were given diets containing diazinon (purity, 98%; dissolved in acetone) at a concentration of 100 or 200 ppm, ad libitum, for 103 weeks, and then held for an additional 2–3 weeks for observation; a group of 25 male and 25 female F344 rats served as matched controls (NTP, 1979). Survival in male rats was 49/50 (98%) in each treated group, and 24/25 (96%) in the control group at week 78. Survival in female rats was 44/50 (88%) in of each treated group, and 23/25 (92%) in the control group at week 78. Mean body weights of the treated groups of males and females were essentially the same as those of the corresponding controls. In males, there was a significant increase (P = 0.011, Fisher exact test) in the incidence of leukaemia or lymphoma (combined) in rats at the lower dose: 25/50; 50% (leukaemia, 24/50; lymphoma, 1/50) versus 5/25 (all leukaemias) in controls. [The Working Group concluded that the increase in the incidence of

haematopoietic malignancies could not clearly be related to the administration of diazinon because it was observed only in males at the lower dose, and the incidence was slightly above the upper limit of the range for historical controls in this strain of rats (incidence of haematopoietic malignancies in historical controls, 699/2320; 30.1%; range, 0–46%; Haseman et al., 1984).] In females, there was no exposure-related increase in tumour incidence.

The United States Environmental Protection Agency (EPA) provided information on a long-term study in which groups of 20 male and 20 female Sprague-Dawley rats (age, 9 weeks), were given diets containing diazinon (purity, 87.7%; impurities not reported; dissolved in acetone) at a concentration of 0 (control), 0.1, 1.5, 125, or 250 ppm, ad libitum, for 98 weeks (EPA, 1993). There was no adverse effect on body weight in treated rats. At week 97, survival in males was 45% (controls), 30%, 50%, 35%, and 58% in each group, respectively; while survival in females was 58% (controls), 40%, 44%, 68%, and 58%, respectively. Because mortality was higher at the low doses than in the controls, the study was terminated at week 97. There was no exposure-related increase in the incidence of any neoplasm in groups of treated rats compared with controls (EPA, 1993). [The Working Group noted that mortality was higher in rats treated with low doses than in controls, and that the duration of the study was only 97 weeks.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

An extensive literature was available on the toxicokinetics of diazinon in humans and in experimental animals.

4.1.1 Absorption

(a) Humans

Dermal exposures resulting from occupational practices and oral exposures from diet are important in humans; there were limited data on exposure to diazinon by inhalation (Knaak et al., 2004; Alavanja et al., 2013). The evidence for absorption of organophosphate pesticides, such as diazinon, has been documented in a large number of biomonitoring studies (Cocker et al., 2002). To cite one example, a cohort of pregnant women belonging to urban minorities in New York City, USA, was evaluated for diazinon exposure by measuring the diazinon levels in personal air samples, and in maternal and umbilical cord sera (Whyatt et al., 2005). Diazinon was detected in 100% of the personal air samples, and in 45% and 44% of the maternal blood and cord blood samples, respectively, with average (± standard deviation) concentrations of 1.3 ± 1.8 pg/g and 1.2 ± 1.4 pg/g, respectively, as assessed by gas chromatography-mass spectrometry (GC-MS) analysis. [The Working Group noted that these data indicated that absorption of diazinon and subsequent internal exposures can occur in humans, and that the developing fetus might also be exposed.]

Diazinon can be absorbed from the gastrointestinal tract by mammals, including humans, via passive diffusion (Poet et al., 2004). Rapid absorption of diazinon was observed after an oral dose of 0.011 mg/kg bw in five volunteers, as shown by the excretion of approximately 60% of the administered dose as dialkylphosphate metabolites in the urine. Most of the administered dose was recovered within 14 hours after dosing (Garfitt et al., 2002). In a woman who intentionally consumed a lethal amount of diazinon (estimated dose, 293 mg/kg bw), diazinon was detected in several tissues (Poklis et al., 1980).

Diazinon was not absorbed very efficiently into the body after dermal exposure; only ~4% of the administered dose of [^{14}C]-labelled diazinon

(vehicle, acetone) was absorbed through the skin of the ventral forearm of volunteers during a 24-hour exposure period (Wester et al., 1993). One possible reason for the poor rate of dermal absorption was that the experimentally determined dermal permeability coefficient for diazinon in human skin ($K_p \approx 1 \times 10^{-9}$ cm/s) was similar to the desquamation rate of skin (Sugino et al., 2014), thus reducing the rate of penetration by diazinon.

The number of studies of dermal absorption in vitro with diazinon was limited. One study in vitro indicated that the absorption of diazinon though human skin was 20% of the applied dermal dose (Moody & Nadeau, 1994).

Other studies evaluated biomarkers of exposure and indicators of absorption, including plasma cholinesterase activity (and decrements thereof) (Poet et al., 2004) and urinary organophosphate metabolites. After oral (11 µg/kg bw) and dermal (100 mg, occluded dermal dose) exposures of human volunteers to diazinon, peak urinary concentrations of diethylphosphate occurred at 2 hours and 12 hours, respectively (Garfitt et al., 2002). Under acidic conditions (pH 1), similar to those in the stomach, diazinon steadily decreased in concentration due to acid-catalysed hydrolysis, exhibiting a half-life of ~90 minutes (Garfitt et al., 2002). [The Working Group noted that this suggested that some degradation of diazinon would occur in the stomach after oral exposures, and that a fraction of the diethylphosphate and IMPY generated in the body might be formed in the stomach.] These metabolites can be readily absorbed from the gastrointestinal tract in rats (Timchalk et al., 2007).

Using the human Caco-2 cell line, a widely used cell model to study intestinal absorption and transport, the levels of P-glycoprotein, which is a xenobiotic transporter that is expressed on the cell surface, were found to be upregulated by diazinon at low concentrations (Lecoeur et al., 2006). [The Working Group noted this suggested that intestinal absorption of diazinon might be reduced after long-term oral exposure to diazinon as a result of enhanced efflux from enterocytes, thus limiting systemic exposure.]

(b) Experimental systems

In male Sprague-Dawley rats exposed orally, diazinon (100 mg/kg bw) was well absorbed from the gastrointestinal tract, as shown by the marked reduction (< 20% of the control values) in plasma cholinesterase activity at 6 hours after dosing (Poet et al., 2004). When male and female Wistar rats were given [^{14}C]-labelled diazinon either as a single oral dose of 4 mg/kg bw or as daily doses of 0.5 mg/kg bw for 10 consecutive days, the rapid absorption of diazinon was shown by the excretion of a large amount of radiolabel in the urine (Mücke et al., 1970). Similar results were obtained in female beagle dogs given a single oral dose of [^{14}C]-labelled diazinon at 4.0 mg/kg bw; absorption was ~85% of the administered radiolabelled dose (Iverson et al., 1975). Toxicokinetic studies in rats (Sprague-Dawley or Wistar strains) and mice (ddy strain) indicated that maximum concentrations of diazinon in blood are reached 1–2 hours after oral and intraperitoneal dosing (Tomokuni et al., 1985; Poet et al., 2004). The oral bioavailability of diazinon in the rat was relatively low (~36%), which was determined by comparing the area under the curve from time-course levels of diazinon in blood after oral and intravenous dosing (Wu et al., 1996).

Rates of dermal absorption of [^{14}C]-labelled diazinon in rats and hairless guinea-pigs in vivo were 56% and 28% of the applied radiolabelled dose, respectively (Moody & Nadeau, 1994); these values are noticeably higher than those for humans (Wester et al., 1993).

4.1.2 Distribution

(a) Humans

Poklis et al. (1980) detected diazinon in tissues (blood, bile, adipose, liver, brain, and kidney) after intentional oral ingestion of diazinon. No other data on tissue distribution of diazinon in humans were available to the Working Group.

(b) Experimental systems

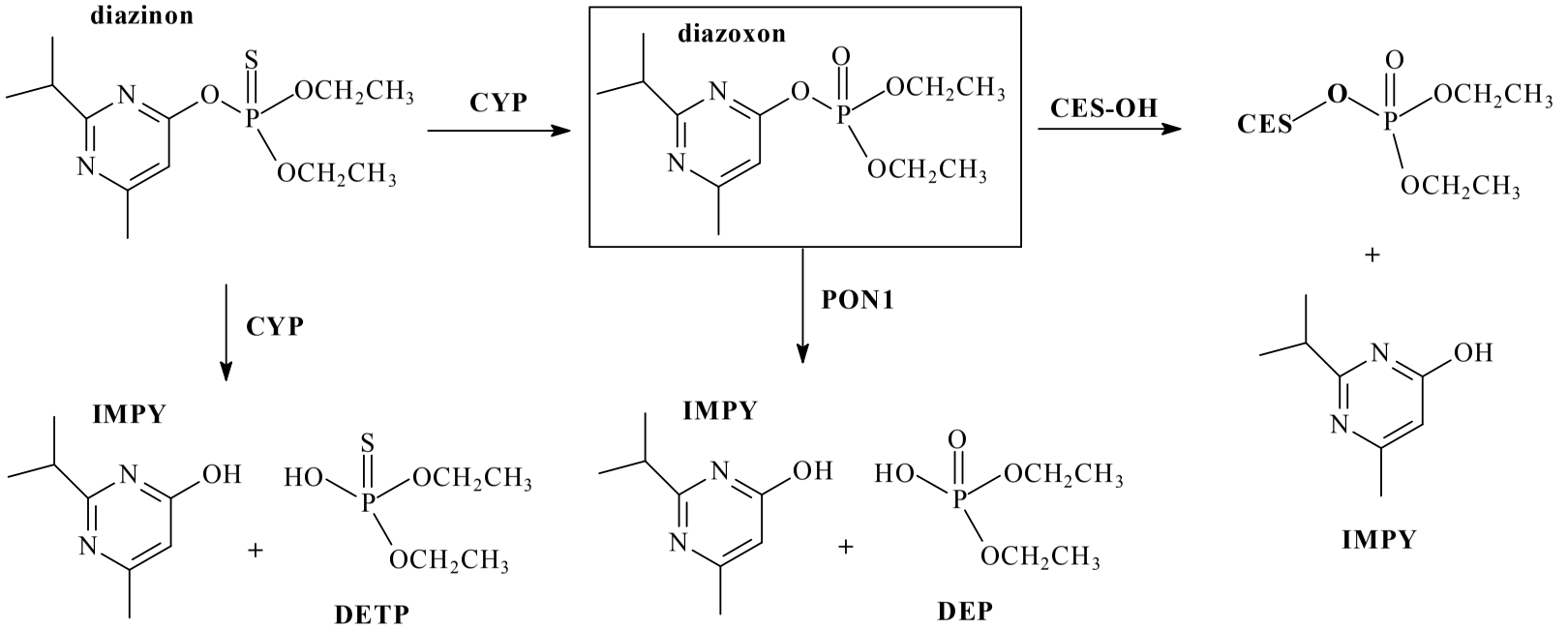
In experimental animals, diazinon is widely distributed to tissues after absorption. The elimination half-life of diazinon in the blood of male Wistar rats given intraperitoneal doses of 20 mg/kg bw or 100 mg/kg bw was estimated to be 4 hours and 6 hours, respectively (Tomokuni et al., 1985). Similarly, immediately after administration of intravenous (10 mg/kg bw) and oral (80 mg/kg bw) doses in rats, plasma concentrations of diazinon indicated half-lives of 4.7 and 2.9 hours, respectively (Wu et al., 1996). Most diazinon in the plasma (89%) is bound non-covalently to albumin and other plasma proteins (Wu et al., 1996; Poet et al., 2004). By 8 hours after intravenous administration (20 mg/kg bw) to rats, the concentration of diazinon was significantly higher in the kidney than in the liver, or brain (Tomokuni et al., 1985). After intravenous dosing (1 or 10 mg/kg bw), diazinon was distributed and eliminated rapidly in male Sprague-Dawley rats, and concentrations of diazinon in saliva were comparable to plasma concentrations of non-protein-bound diazinon (Lu et al., 2003).

4.1.3 Metabolism

(a) Overview of metabolism of diazinon

Organophosphate pesticides are subject to similar metabolic pathways in humans and experimental animals in vivo (Casida & Quistad, 2004); see also Section 4.1.3 of the *Monograph* on Malathion in the present volume. Biotransformation of organophosphates occurs primarily in the liver, and to a lesser extent in the small intestine, after oral exposure (Barr & Angerer, 2006). After absorption by the dermal or oral route, diazinon is rapidly biotransformed by several enzymes – including cytochrome P450 (CYP), paraoxonases, and carboxylesterases (CES) – to water-soluble metabolites that are rapidly eliminated (see Fig. 4.1). Both desulfuration and dearylation of diazinon are mediated by CYP. The bioactive diazoxon metabolite can be detoxified by paraoxonase (PON1)-catalysed reactions (Costa et al., 2013), yielding alcohol and diethylphosphate products. Alternatively, diazoxon can be subject to inhibition of CES function (Crow et al., 2012; Fig. 4.1). The oxon metabolite can escape detoxication by CES or PON1 in the liver and instead covalently modify (and inhibit) various serine hydrolase enzymes, including the B-esterase targets butyrylcholinesterase, acetylcholinesterase, and CES (Casida & Quistad, 2004; see Fig. 4.2). The bioactive oxon metabolite is generated by CYP-catalysed desulfuration (Buratti et al., 2005; Barr & Angerer, 2006). If the oxon 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 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 the oxon is acetylcholinesterase, the

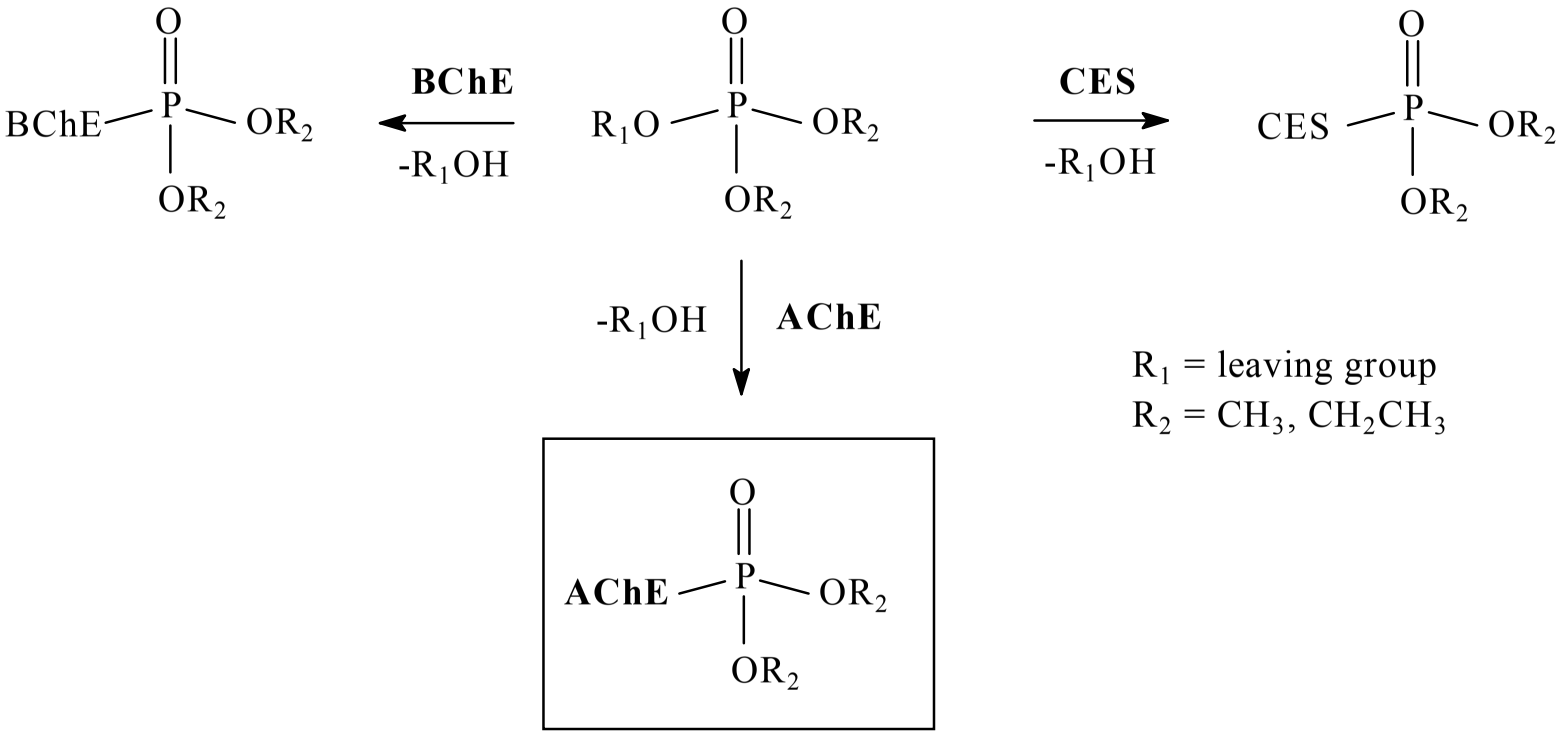
Fig. 4.1 Biotransformation of diazinon



From Poet et al. (2004); copyright (2004), modified with permission from Elsevier

Reactions catalysed by cytochrome P450 (CYP) produce the desulfuration metabolite (oxon) or aryl alcohol and dialkylthiophosphate products. Paraoxonase-1 (PON1) and carboxylesterase (CES) contribute to diazoxon metabolism reactions. The bioactive diazoxon metabolite is indicated by the box. 2-Isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) is the dearylation product and the major metabolite of diazinon. CES-OH indicates carboxylesterase with –OH being the functionality of the active-site serine residue that is covalently modified by the oxon metabolite.

Fig. 4.2 Reactions of a generic oxon metabolite with esterases



Adapted with permission from Casida & Quistad (2004); copyright (2004) American Chemical Society
The reaction of the oxon metabolite common to several organophosphate pesticides (in this case, diazinon, diazoxon) 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

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).

(b) Humans

Multiple human CYPs are implicated in diazinon metabolism. The major human CYP isoforms involved in the metabolism of diazinon to diazoxon are CYP1A1, CYP2C19, and CYP2B6, while CYP2C19 is also responsible for the dearylation (detoxification) of diazinon (Ellison et al., 2012). One study showed that recombinant CYPs 2D6, 2C19, 3A4, and 3A5 were also efficient at producing diazoxon or IMPY and diethylthiophosphate from diazinon (Mutch & Williams, 2006). Most of these biotransformation reactions take place in the liver where CYPs are most abundant. Using heterologously expressed CYP proteins, human CYP2C19 was identified to be the major isoform responsible for diazinon metabolism in liver, while other enzymes including CYP1A2 had a minor role (Kappers et al., 2001). On the basis of intrinsic clearance rates ($Cl_{int} = V_{max}/K_m$), the dearylation metabolism rate for diazinon was 2.5-fold that of the desulfuration metabolism rate in human liver microsomes (Sams et al., 2004). Desulfuration and dearylation reactions of diazinon were catalysed by individual CYP isoforms at roughly similar rates, in the following rank order: CYP2C 19 > CYP1A2 > CYP2B6 > CYP3A4 (Sams et al., 2004).

[The Working Group noted that CYP-mediated biotransformation of diazinon is an important metabolic pathway. The Working Group also noted the variation in organophosphate substrate specificity and rates of oxidation for individual CYP isoforms.]

PON1 is also an important detoxication enzyme of diazoxon. PON1 catalyses the hydrolytic degradation of diazoxon and possesses polymorphic variants (Costa et al., 2013). Coding region polymorphisms in human *PON1*, specifically the glutamine/arginine substitution at position 192 (192 Q/R) alloforms, can affect the catalytic efficiency of oxon hydrolysis for certain organophosphates (Povey, 2010). For example, when pure recombinant PON1 enzymes were examined, the $PON1_{R192}$ polymorphic isoform hydrolysed chlorpyrifos oxon more efficiently than the $PON1_{Q192}$ isoform, while both alloforms hydrolysed diazoxon with the same catalytic efficiency (Li et al., 2000). It was hypothesized that the PON1 Q192R polymorphism can influence susceptibility to organophosphates (Povey, 2010). In a cross-sectional study, farmers with ill health who had reportedly mixed and applied pesticides were more likely to possess a 192R allele than a 192Q allele when compared with healthy farmers (OR, 1.93; 95% CI, 1.24–3.01) (Cherry et al., 2002). In support of this notion, Davies and co-workers (Davies et al., 1996) showed using plasma samples that individuals who were 192QQ homozygotes were more efficient at hydrolysing diazoxon than 192RR homozygotes (Davies et al., 1996). However, another study showed opposite results: individuals with the RR genotype had the highest serum activity of diazoxonase, while activity was slightly reduced in individuals with the QR genotype, and reduced even further in those with the QQ genotype (O'Leary et al., 2005). The contrast in the results reported by the two studies was attributed to the different reaction conditions employed. High salt conditions (NaCl, 2 M; pH 8.5) were used in the study by Davies et al. (1996), while more physiologically relevant buffer conditions (NaCl, 150 mM; pH 7.4) were used in the study by O'Leary et al. (2005). [The Working Group noted that associations between the different polymorphisms at position 192 and PON1 activity towards diazoxon are unclear.]

It has also been suggested that protection or susceptibility to diazinon-induced toxicity is primarily determined by the expression level of PON1 protein and is not dependent on the Q192R genotype (Costa et al., 2013). Injection of *PON1*$^{-/-}$ mice with either recombinant human $PON1_{R192}$

or recombinant $PON1_{Q192}$ proteins afforded equal measures of protection against diazinon-induced toxicity (Li et al., 2000; Stevens et al., 2008).

When another human genetic polymorphism in PON1 was examined – leucine (L)/methionine (M) at codon 55, 55 L/M alloforms – there were also significant differences in enzyme activity towards diazoxon, with the following rank order: LL > LM > MM genotypes (O'Leary et al., 2005). Thus individuals exhibiting haplotypes combining 192Q and 55M alleles might have a reduced capacity to detoxify diazoxon, which suggests they would have a greater susceptibility to toxicity associated with diazinon (O'Leary et al., 2005).

In insects, glutathione transferases (GSTs) play an important role in resistance to organophosphates, and limited data suggested that GST-mediated *O*-dealkylation might also occur in humans. For example, when glutathione (1 mM) and methyl parathion (300 µM) are incubated together with recombinant GST enzymes, human GSTs hGSTT1-1 and hGSTA1-1 exhibited significant *O*-dealkylation activity: 546 and 65 nmol/min per mg, respectively (Abel et al., 2004). When expression level and enzymatic activity were considered, it was estimated that hGSTA1-1 was responsible for the majority of *O*-dealkylation of methyl parathion in human hepatic cytosol. [The Working Group noted that although no specific GST-mediated metabolism data for diazinon could be identified, it could be speculated that in organs such as brain and skeletal muscle, where hGSTT1-1 is expressed, hGSTT1-1-mediated biotransformation of organophosphate pesticides might be an important extrahepatic detoxication mechanism.] Furthermore, organophosphate pesticides have been shown to induce GSTα (GSTA1) in a human HepG2 cell line, which might aid their own detoxication (Medina-Díaz et al., 2011).

(c) Experimental systems

IMPY (also called pyrimidinol) is the dearylation product of diazinon (see Fig. 4.1) and a major metabolite of diazinon in vivo. CYP2C11, CYP3A2, and CYP2B1/2 are rat P450 isoforms responsible for oxidative dearylation of diazinon, affording IMPY (Fabrizi et al., 1999). Plasma concentrations of IMPY were ~20-fold those of diazinon at 3 hours after a single oral dose of diazinon of 100 mg/kg bw in Sprague-Dawley rats (Poet et al., 2004). These data demonstrate the rapid metabolism of diazinon that occurs in vivo in rats. [The Working Group noted that very few toxicological data concerning IMPY were available in the peer-reviewed and published literature.]

In a metabolomics study using a liquid chromatography–quadrupole–time-of-flight instrument, a novel metabolite (1-hydroxyisopropyl diazinon), was detectable in the plasma of male Sprague-Dawley rats given diazinon by intraperitoneal administration, or when diazinon was incubated with rat liver microsomes supplemented with reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Ibáñez et al., 2006). Absolute concentrations of this novel metabolite were not determined.

An important species difference is that human plasma contains no carboxylesterase 1c (CES1c), in contrast to the robust expression in experimental animals (such as mice, rats, and rabbits) (Li et al., 2005). This could potentially have an impact on the ability of humans to detoxify the bioactive diazoxon metabolite. However, it was demonstrated that *Ces1c*$^{-/-}$ knockout mice (which do not have Ces1c in plasma) were no more sensitive to the toxic effects of diazinon, delivered subcutaneously at 50 mg/kg bw, than were wildtype mice (Duysen et al. 2012). This was because the Ces1c present in the plasma of wildtype mice was insufficient to detoxify the diazoxon produced in vivo.

4.1.4 Excretion

(a) Humans

Because of its rapid metabolism in vivo, unchanged diazinon is not detected in the urine in humans. The metabolites and their glucuronide or sulfate conjugates are mainly excreted in the urine. However, the major metabolite of diazinon, IMPY, can be readily excreted from the body via urine and was detected in 29% of the population of the USA in urine samples collected for the National Health and Nutrition Examination Survey (NHANES, 1990–2000) in 1997, before residential use of diazinon was banned (Barr et al., 2005). In this study, the 95th percentile for IMPY concentration was 3.7 µg/L (3.4 µg/g creatinine). Dialkylphosphate metabolites are also found in human urine; after oral administration of diazinon, 66 ± 12% of an administered dose of 11 µg/kg bw was recovered, in contrast to only 0.5 ± 0.2% of a dermal dose (100 mg for 8 hours) (Garfitt et al., 2002). Unmetabolized diazinon was not detectable in the urine in either exposure scenario, nor was plasma cholinesterase activity reduced, indicating that measurement of urinary dialkylphosphate metabolites is a more sensitive biomarker of exposure than decreased plasma cholinesterase activity for biological monitoring purposes.

(b) Experimental systems

In female rhesus monkeys given [^{14}C]-labelled diazinon by intravenous administration, the cumulative level of ^{14}C residue in the urine after 7 days was 56% of the administered dose, while 23% was eliminated in the faeces (Wester et al., 1993). Similar findings with regard to excretion have been found in toxicokinetic studies in rodents (Poet et al., 2004). Thus experimental animals, like humans, absorb and metabolize diazinon very efficiently, and rapidly excrete the metabolites via the urine, with lesser amounts in the faeces. There was no evidence on the accumulation of diazinon and its metabolites in the body in either humans or experimental animals.

4.2 Mechanisms of carcinogenesis

4.2.1 Genotoxicity and related effects

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

(a) Humans

(i) Studies in exposed humans

See Table 4.1

In peripheral blood lymphocytes from 34 workers engaged in the production of diazinon, a significant increase in the frequency of stable chromosomal aberrations was found, compared with a control group (Király et al., 1979). [The Working Group noted that diazinon was not the only chemical to which these individuals may have been exposed.] A significant increase in the frequency of sister-chromatid exchange was observed in peripheral blood lymphocytes of subjects after exposure to a sheep dip containing diazinon, compared with before exposure; however, the formulation also contained other unspecified ingredients (Hatjian et al., 2000).

Other studies showed that long-term occupational exposure to multiple insecticides, including diazinon, is associated with an increase in the frequency of chromosomal aberration and sister-chromatid exchange in peripheral blood lymphocytes, compared with non-exposed populations (De Ferrari et al., 1991).

Table 4.1 Genetic and related effects of diazinon in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Peripheral blood	Lymphocytes	Chromosomal damage	Chromosomal aberration	34 workers engaged in diazinon production 49 controls, mainly males, Genetic Counselling Clinic of the National Institute of Hygiene	+ [no *P* calculation]	Significant increase in stable chromosomal aberrations in workers vs controls	Király et al. (1979)
Peripheral blood	Lymphocytes	Chromosomal damage	Chromosomal aberration	32 floriculturists exposed diazinon and other pesticides[b] 31 controls living in the same area, and with no history of occupational exposure to pesticides	(+) [see Comments]	Exposure to numerous pesticides, including diazinon Significant increase in structural ($P < 0.01$) and numerical ($P < 0.001$) chromosomal aberrations in exposed group vs controls	De Ferrari et al. (1991)
Peripheral blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	32 floriculturists exposed to diazinon and other pesticides[b] 31 controls living in the same area, and with no history of occupational exposure to pesticides	(+) $P < 0.01$	Exposure to numerous pesticides, including diazinon Significant increase in sister-chromatid exchange in exposed group vs controls	De Ferrari et al. (1991)
Peripheral blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	8 volunteer agricultural college students exposed to sheep dip containing approximately 45% diazinon 8 age-and ethnicity-matched controls, non-smoking male university research staff	+ $P < 0.001$	Diazinon formulation contained other unspecified ingredients Significant increase after, compared with before, exposure; no difference between groups before dipping	Hatjian et al. (2000)

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

[b] Other pesticides included 18 nitro-organic herbicides/fungicides, 9 nitro-organic fungicides, 12 organophosphate and organochlorophosphate insecticides, 4 hydrocarbon-derivative herbicides and 5 inorganic fungicides and insecticides

vs, versus

(ii) Humans cells in vitro

See Table 4.2

There was more evidence for diazinon-induced genotoxicity in human cells than in other mammalian cells. Diazinon induced genotoxicity in all studies in human cells in vitro, except in one quite old study. Diazinon induced DNA damage (comet assay) in human mucosal cells from the nose (Tisch et al., 2002), and from the tonsils (Tisch et al., 2007), as well as sister-chromatid exchange in lymphocytes (Sobti et al. 1982; Hatjian et al., 2000). DNA damage was also induced in spermatozoa (Salazar-Arredondo et al. 2008). Micronuclei were formed in blood lymphocytes exposed to diazinon (Colović et al., 2010; Karamian et al., 2013; Shokrzadeh et al., 2014), in skin fibroblasts (Colović et al., 2010), and in breast cancer (MCF-7) cells (Ukpebor et al., 2011).

Diazoxon was more active than diazinon in inducing DNA damage in spermatozoa (Salazar-Arredondo et al., 2008), while diethylthiophosphate (DETP), another diazinon metabolite, induced DNA damage in human hepatic cell lines (Vega et al., 2009). The metabolite IMPY induced formation of micronuclei in blood lymphocytes, skin fibroblasts, and MCF-7 cells (Colović et al., 2010; Ukpebor et al., 2011).

(b) Experimental animals

(i) Non-human mammals in vivo

See Table 4.3

Diazinon caused oxidative DNA damage (shown by increases in apurinic/apyrimidinic or abasic sites) in liver and kidney of rabbits given repeated oral doses over several months (Tsitsimpikou et al., 2013). Micronucleus formation was observed in peripheral blood lymphocytes of rats treated by intraperitoneal doses for 30 days (Shadboorestan et al., 2013; Shokrzadeh et al., 2013), and in bone-marrow cells in mice given repeated doses (Ni et al., 1993). Diazinon also induced micronucleus formation in blood cells of rats given repeated oral doses for 4 weeks (Hariri et al., 2011). Diazinon failed to induce sister-chromatid exchange in bone-marrow cells of mice treated by gavage (EPA, 1992a). A diazinon-based formulation also induced DNA damage in the testicular germinal epithelium and micronucleaus formation in bone marrow of mice given a single intraperitoneal dose (Sarabia et al., 2009a).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Conflicting results were obtained in the mouse lymphoma assay: McGregor et al. (1988) showed that diazinon induced mutation without metabolic activation, while the EPA (1989a) reported that diazinon did not induce mutation with or without metabolic activation. In Chinese hamster lung cells, diazinon caused chromosomal aberration in the presence of metabolic activation (Matsuoka et al., 1979). Diazinon did not cause micronucleus formation in rat hepatocytes (Frölichsthal & Piatti, 1996), or in Chinese hamster ovary cells (Kirpnick et al., 2005). Moreover, diazinon did not induce sister-chromatid exchange in Chinese hamster lung (V79) cells (Chen et al., 1981, 1982; Kuroda et al., 1992), or in Chinese hamster ovary cells (Nishio & Uyeki, 1981). Diazoxon caused sister-chromatid exchange in Chinese hamster ovary cells (Nishio & Uyeki, 1981).

In Chinese hamster ovary cells, there was an increase in the frequency of chromatid aberration after exposure to urine collected during spraying from non-smoking, male orchardists (n = 22) using 16 pesticides including diazinon, when compared with urine from the same individuals before spraying ($P < 0.001$) (See et al., 1990).

(iii) Non-mammalian systems in vivo

See Table 4.3

Diazinon induced sister-chromatid exchange in fish, *Umbra limi* (Vigfusson et al., 1983). DNA

Table 4.2 Genetic and related effects of diazinon, diazoxon, diethylthiophosphate, and IMPY 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			
Diazinon							
Primary nasal mucosal cells	DNA damage	DNA strand break, comet assay	+	NT	500 µM [152 µg/mL]	Positive for both cell types tested (middle and inferior turbinate)	Tisch et al. (2002)
Mucosal epithelial cells from human tonsil tissue	DNA damage	DNA strand break, comet assay	+	NT	50 µM [15.2 µg/mL]		Tisch et al. (2007)
Spermatozoa	DNA damage	Sperm-chromatin structure assay	+	NT	500 µM [152 µg/mL]		Salazar-Arredondo et al. (2008)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	20 µg/mL		Hatjian et al. (2000)
Lymphoid cell line (LAZ-007)	Chromosomal damage	Sister-chromatid exchange	–	+	20 µg/mL	Only one concentration tested with metabolic activation [20 µg/mL]	Sobti et al. (1982)
Peripheral blood lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	30 µg/mL		Lopez et al. (1986)
Blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	750 µM [228 µg/mL]	Only one concentration tested	Shokrzadeh et al. (2014)
Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	750 µM [228 µg/mL]	Only one concentration tested	Karamian et al. (2013)
Breast adenocarcinoma cell line (MCF-7)	Chromosomal aberration	Micronucleus formation	+	NT	10^{-6} µM [0.3×10^{-6} µg/mL]		Ukpebor et al. (2011)
Blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [6×10^{-3} µg/mL]		Colović et al. (2010)
Skin fibroblasts	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [6×10^{-3} µg/mL]		Colović et al. (2010)
Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	(+)	NT	4 µg/mL		Bianchi-Santamaria et al. (1997)

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Diazoxon							
Spermatozoa	DNA damage	Sperm chromatin structure assay	+	NT	300 µM [86.5 µg/mL]	Diazoxon was more active than diazinon	Salazar-Arredondo et al. (2008)
IMPY							
MCF-7, breast adenocarcinoma cell line	Chromosomal damage	Micronucleus formation	+	NT	10^{-6} µM [0.152 × 10^{-6} µg/mL]		Ukpebor et al. (2011)
Blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [3 × 10^{-3} µg/mL]	IMPY was more active than diazinon	Colović et al. (2010)
Skin fibroblasts	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [3 × 10^{-3} µg/mL]		Colović et al. (2010)
DETP							
HepG2, hepatocellular carcinoma cell line	DNA damage	DNA strand break Comet assay	+	NT	1 µM [0.17 µg/mL]		Vega et al. (2009)
WRL68, embryonic hepatic non-transformed cell line	DNA damage	DNA strand break Comet assay	+	NT	1 µM [0.17 µg/mL]	Positive effect linked to CYP450 enzymes: addition of sulconazole, a CYP450 inhibitor, inhibited the DNA damage	Vega et al. (2009)
HeLa, cervical adenocarcinoma cell line	DNA damage	DNA strand break, comet assay	–	NT	500 µM [85 µg/mL]		Vega et al. (2009)
Peripheral blood mononucleated cells	DNA damage	DNA strand break, comet assay	–	NT	500 µM [85 µg/mL]		Vega et al. (2009)
Diazinon-based formulation							
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	Diazinon, 45% NR		Hatjian et al. (2000)

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

DETP, diethyl thiophosphate; HIC, highest ineffective concentration; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine; LEC, lowest effective concentration; NR, not reported; NT, not tested

Table 4.3 Genetic and related effects of diazinon 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
Diazinon								
Rabbit, New Zealand White, F	Liver	DNA damage	Oxidative DNA damage AP sites	+	2.64 mg/kg bw per day	p.o., 12 mo (every 2 days for 3 mo, 8 mo without treatment, then 1 mo of treatment every 2 days)	Significant increase in apurinic/apyrimidinic or abasic (AP) sites with both doses tested (2.64 and 5.28 mg/kg bw) compared with controls Higher effect in liver than kidney $P < 0.001$	Tsitsimpikou et al. (2013)
Rabbit, New Zealand White, F	Kidney	DNA damage	Oxidative DNA damage AP sites	+	2.64 mg/kg bw per day	Gavage, 12 mo (every 2 days during 3 mo, 8 mo without treatment, then 1 mo of treatment every 2 days)	Significant increase in AP sites with both doses tested (2.64 and 5.28 mg/kg bw) compared with controls Higher effect in liver than kidney $P < 0.001$	Tsitsimpikou et al. (2013)
Rat, Wistar, M	Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	+	20 mg/kg bw per day	i.p. × 30 days	Only one dose tested, $P < 0.0001$; L-carnitine had antigenotoxic effect	Shadboorestan et al. (2013)
Rat, Wistar, M	Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	+	20 mg/kg bw per day	i.p. × 30 days	Only one dose tested; $P < 0.0001$; selenium had antigenotoxic effect	Shokrzadeh et al. (2013)
Rat	Blood Cells not specified	Chromosomal damage	Micronucleus formation	+	20 mg/kg bw per day	p.o., 1×/day, ×4 wk	One dose tested; $P < 0.001$	Hariri et al. (2011)
Mouse	Bone marrow Polychromatic erythrocytes	Chromosomal damage	Micronucleus formation	+	0.1, 0.2, 0.4, 0.6 and 0.8 × LD_{50}	i.p. 1×/day, ×4 days	LD_{50}, NR; LED, NR	Ni et al. (1993)
Mouse, ICR	Bone marrow	Chromosomal damage	Sister-chromatid exchange	–	100 mg/kg bw	Gavage, × 1		EPA (1992a)

Table 4.3 (continued)

Species, strain, sex	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Insect, *Drosophila melanogaster*		Mutation	Somatic mutation and recombination test (SMART)	+	1 ppm [1 µg/mL] feeding			Çakir & Sarikaya (2005)
Insect, *Drosophila melanogaster*		Chromosomal damage	Complete and partial chromosome losses	–	100 ppb [0.1 µg/mL]			Woodruff et al. (1983)
Diazinon-based formulation								
Mouse, CF-1, M	Germinal epithelium of testis, spermatocytes	DNA damage	DNA strand breaks, comet assay	+	43.33 mg/kg bw	i.p. × 1	Diazinon, 60% Two doses tested corresponding to 1/3 and 2/3 of the LD_{50} (65 mg/kg bw) Significant increase at higher dose (43.33 mg/kg bw); $P < 0.001$; melatonin prevented DNA damage $P < 0.001$	Sarabia et al. (2009a)
Mouse, CF-1, M	Bone marrow	Chromosomal damage	Micronucleus formation	+	21.66 mg/kg bw	i.p. × 1	Diazinon, 60% Significant increase in micronucleus formation with the two doses tested, 21.66 and 43.33 mg/kg bw: $P < 0.01$ pre-treatment with melatonin prevented micronucleus formation	Sarabia et al. (2009a)
Fish, *Umbra limi*		Chromosomal damage	Sister-chromatid exchange	+	5.4×10^{-10} M [0.164 µg/L]		Diazinon, 48.72% The highest concentration tolerated by fish was 5.4×10^{-9} M	Vigfusson et al. (1983)
Freshwater mussel, *Utterbackia imbecilis*	Glochidia	DNA damage	DNA strand break, comet assay	+	0.28 µg/mL		Diazinon, 22.4% 0.28 µg/mL corresponds to 1/4 NOAC, positive response at level below the NOAEC	Conners & Black (2004)

[a] +, positive; –, negative

AP, apurinic, apyrimidinic or abasic sites; F, female; HID, highest ineffective dose; i.p., intraperitoneal; ; LD50, median lethal dose LED, lowest effective dose (units as reported); M, male; mo, month; NOAEC, no-observed-adverse-effect concentration; NT, not tested; p.o., oral

Table 4.4 Genetic and related effects of diazinon 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			
Urine from exposed humans								
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Chromatid aberrations	(+)	NT	1–8 mg/mL creatine equivalent	Extracts of urine from 22 non-smoker male orchardists using 16 pesticides including diazinon 21 subjects non-smoking males and females Urine samples collected during spraying period had increased chromatid aberration frequency compared with urine before spraying ($P > 0.001$). (before use of pesticide, urine of orchardists caused same level of chromatid aberrations as urine of control group)	See et al. (1990)
Diazinon								
Mouse	Mouse lymphoma L5178Y cells	Mutation	*Tk*$^{+/-}$	+	NT	60 µg/mL		McGregor et al. (1988)
Mouse	Mouse lymphoma L5178Y	Mutation	*Tk*$^{+/-}$	–	–	108 µg/mL		EPA (1989a)
Rat	Hepatocytes	Chromosomal damage	Micronucleus formation	–	NT	54 µg/mL		Frölichsthal & Piatti (1996)
Hamster	Chinese hamster lung cells	Chromosomal damage	Chromosomal aberration	Toxic	+	100 µg/mL	– S9, 100 µg/mL was cytotoxic	Matsuoka et al. (1979)
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Micronucleus formation	–	–	94 µg/mL		Kirpnick et al. (2005)
Hamster	Chinese hamster lung cells	Chromosomal damage	Micronucleus formation	–	NT	NR	Only one dose tested: highest dose that induced 50% cell death (NR)	Ni et al. (1993)

Table 4.4 (continued)

Species	Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Hamster	Chinese hamster lung fibroblast V79 cells	Chromosomal damage	Sister-chromatid exchange	–	NT	0.4 µg/mL		Kuroda et al. (1992)
Hamster	Chinese hamster lung fibroblast V79 cells	Chromosomal damage	Sister chromatid exchange	–	–	80 µg/mL		Chen et al. (1981, 1982)
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Sister-chromatid exchange	–	NT	1 mM [304 µg/mL]		Nishio & Uyeki (1981)
Diazoxon								
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Sister-chromatid exchange	+	NT	1 mM [288 µg/mL]		Nishio & Uyeki (1981)

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

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

damage was induced in freshwater mussels exposed to diazinon, as shown by the comet assay (Conners & Black, 2004). In *Drosophila melanogaster,* diazinon induced mutation in the somatic mutation and recombination test (Çakir & Sarikaya, 2005), but did not cause complete or partial chromosome losses (Woodruff et al., 1983).

(iv) Non-mammalian systems in vitro

See Table 4.5

Diazinon did not induce chromosomal damage in *Saccharomyces cerevisiae* strain *RS112* (Kirpnick et al., 2005), nor mutation in most studies in *S. typhimurium* (Marshall et al., 1976; Wong et al., 1989; Kubo et al., 2002). Diazinon induced gene mutation in a single Ames assay in *S. typhimurium* in the presence (but not the absence) of metabolic activation (Wong et al., 1989). Moreover, diazinon did not induce DNA damage in the rec assay in *B. subtilis* without metabolic activation (Shirasu et al., 1976). A study in an acellular system with calf thymus DNA showed non-intercalative binding of diazinon with DNA (Kashanian et al., 2008).

4.2.2 Receptor-mediated mechanisms

(a) Neurotoxicity-pathway receptors

Diazinon is bioactivated to diazoxon in insects and mammals (Section 4.1.3; Casida & Quistad, 2004). Diazoxon can covalently modify the catalytic serine residue and inhibit the activity of several B-esterases, including the recognized target acetylcholinesterase, resulting in acute neurotoxicity in insects and mammals. 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 diazinon that can affect neurotoxicity include the cannabinoid receptor and butyrylcholinesterase (Quistad et al., 2002; Costa et al., 2011). The mechanistic relevance of these effects to carcinogenicity is unknown.

(b) Sex-hormone pathway disruption

(i) Humans

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

Diazinon showed weak estrogenic activity in vitro in the E-Calex assay, in ovarian carcinoma cells, BG1, that are stably transfected with an estrogen-responsive luciferase reporter gene plasmid; the concentration that produced 10% of the maximal estradiol activity was 460 µM (Kojima et al., 2005).

Diazinon (10^{-6} to 100 µM) gave negative results for estrogenicity in estrogen-receptor-positive breast cancer cells (MCF-7), and did not cause estrogen-receptor-negative cells (MDA MB 231) to proliferate (Oh et al., 2007).

In androgen-receptor and estrogen-receptor α and β reporter-gene assays in Chinese hamster ovary cells (CHO-K1), diazinon did not show agonist or antagonist activity (Kojima et al., 2004, 2010).

(ii) Non-human mammalian experimental systems

In male mice treated daily by gavage for 4 weeks, diazinon (4.1 or 8.2 mg/kg bw) substantially reduced levels of luteinizing hormone and follicle-stimulating hormone, while a lower dose (2 mg/kg bw) was without effect (ElMazoudy & Attia, 2012). At 4.1 mg/kg bw, plasma testosterone concentration was nearly double that of controls (5.9 versus 3.1 ng/mL), and at 8.2 mg/kg bw it was roughly one third of that of controls (1.1 versus 3.1 ng/mL). For prolactin, a similar pattern was seen of increase in concentration in the group at 4.1 mg/kg bw, and decrease in the group at the highest dose; for estradiol, only the group at 4.1 mg/kg bw showed significant increase in concentration. Jayachandra &

Table 4.5 Genetic and related effects of diazinon in non-mammalian systems in vitro

Phylogenetic class	Test system (species, strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Prokaryote (bacteria)	*Salmonella typhimurium*, TA1535, TA1536, TA1537, TA1538	Mutation	Reverse mutation	–	–	1000 µg/plate		Marshall et al. (1976)
	Salmonella typhimurium, TA98, TA100	Mutation	Reverse mutation	–	–	1 mM [304 µg/mL]		Kubo et al. (2002)
	Salmonella typhimurium, TA98	Mutation	Reverse mutation	–	+	NR	Concentration tested was between non-toxic and 50% toxic concentration: 20–80 ppm [20–80 µg/mL]	Wong et al. (1989)
	Salmonella typhimurium, TA102, TA1535, TA1537	Mutation	Reverse mutation	–	–	80 ppm [80 µg/mL]		Wong et al. (1989)
	Bacillus subtilis	DNA damage	Rec-assay, differential toxicity	–	NT	20 µg/disk		Shirasu et al. (1976)
Yeast	*Saccharomyces cerevisiae* strain RS112	Chromosomal damage	Deletion assay Intrachromosomal recombination	–	–	10 000 µg/mL		Kirpnick et al. (2005)
Acellular systems	Calf thymus DNA	DNA damage	DNA binding	+	NT	4.92 µM [1.5 µg/mL]	Formation of stable 1 : 2 complex of DNA–diazinon	Kashanian et al. (2008)

[a] +, positive; –, negative

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested

D'Souza (2014) found decreased concentrations of gonadotropins at puberty and in adulthood in male offspring of Sprague-Dawley rats exposed to diazinon during mating, pregnancy, and lactation. At puberty and in adulthood, male offspring of dams exposed to diazinon (30 mg/kg bw) had significantly reduced plasma concentrations of luteinizing hormone, follicle-stimulating hormone, and prolactin; prolactin was also reduced at 15 mg/kg bw. At puberty, offspring also had reduced concentrations of testosterone, compared with control levels. Several abnormalities were found in sperm and other reproductive parameters in adults and pubescent animals at each dose level.

Serum testosterone concentrations were significantly reduced in male Sprague-Dawley rats exposed for 8 weeks to diazinon (10, 15 or 30 mg/kg bw per day by gavage; $P < 0.05$) Leong et al. (2013). After 1 week, serum testosterone concentrations were significantly increased by diazinon (15 or 30 mg/kg bw per day). A single high dose of diazinon (75 mg/kg bw) administered orally to Wistar rats for 28 days also increased serum testosterone concentrations (Alahyary et al., 2008).

Marked and dose-dependent decreases in progesterone compared with controls were seen in female Wistar rats treated orally with diazinon (50, 100, or 150 mg/kg bw per day for 14 days) (Johari et al., 2010). There were no significant changes for estrogen, luteinizing hormone, or follicle-stimulating hormone.

In an in-vitro study, diazinon increased the proliferation of the 17-β estradiol-sensitive MtT/Se cell line derived from rat pituitary tumour cells in which estrogen receptor α is dominant (Manabe et al., 2006).

(iii) Non-mammalian experimental systems

In female bluegill fish (*Lepomis macrochirus*), continuous exposure to diazinon (60 µg/L in aquaria water) reduced blood estradiol measurements at all time-points (24, 48, 72, and 96 hours, 1 and 2 weeks), with significant reductions at all time-points except 96 hours. Estradiol was undetectable at 24 hours and 2 weeks. Alterations in estradiol concentration reflected the damage present within the ovarian structure (Maxwell & Dutta, 2005).

(c) Other pathways

(i) Humans

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

In an in-vitro human pregnane X receptor (PXR) reporter-gene assay in a CHO-K1 cell line, diazinon did not exhibit agonist activity (Kojima et al., 2010).

(ii) Non-human mammalian experimental systems

Thyroid hormone status was evaluated in healthy Swiss albino mice, and in mice treated with diazinon alone for 9 and 17 weeks or in combination with a drug, and with and without *Schistosoma masoni* (Hanna et al., 2003). There were non-significant increases in triiodothyronine (T3) (by 16.5% and 22.4% at 9 and 17 weeks, respectively) and thyroxine (by 2.8% and 5.3% at 9 and 17 weeks, respectively) compared with controls.

In livers from mice exposed in utero to a low dose of diazinon (0.18 mg/kg bw to dams during pregnancy), hepatic metabolism of corticosterone was impaired. Plasma concentrations of corticosterone were elevated in resting male and female mice, but normal under stress (Cranmer et al., 1978). High doses (9 mg/kg bw) were without effect.

In in-vitro studies, diazinon was not an agonist for mouse peroxisome proliferator-activated receptors α or γ (PARP α or γ) in reporter-gene assays in CV-1 monkey kidney cells (Takeuchi et al., 2006; Kojima et al., 2010). Diazinon was not an agonist for the aryl hydrocarbon receptor (AhR) in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing

copies of a dioxin-responsive element (Takeuchi et al., 2008; Kojima et al., 2010).

(iii) Non-mammalian experimental systems

Thyroid-stimulating hormone (TSH) and thyroxine (T4) were substantially reduced at 24, 48, 72, and 96 hours in all dose groups in Caspian roach (*Rutilus rutilus*) fingerling fish from the north-east of the Islamic Republic of Iran exposed in aquaria to a diazon-based formulation (purity, 60%; 0, 1, 2, and 3 mg/L in fresh water for 96 hours) (Katuli et al., 2014). Triiodothyronine (T3) was also reduced except at the highest dose at 24 hours after exposure. Whole-body cortisol levels were increased in diazinon-exposed fish, but decreased to the control levels by 96 hours after fish were transferred to diazinon-free brackish water.

In adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*), the effective dose of diazinon that inhibited by 50% (EC_{50}) the stimulated cortisol secretion in response to adrenocorticotropic hormone (ACTH) was similar to the doses that were lethal to cells ($LC50/EC_{50} = 1.3$) (Bisson & Hontela, 2002).

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

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

In human erythrocytes, diazinon (0.0033–33 mM; for 60 or 180 minutes) significantly increased malondialdehyde concentrations and the activity of superoxide dismutase and glutathione peroxidase at all dose levels in a concentration- and duration-dependent manner. Catalase activity remained unchanged. In haemolized erythrocytes, superoxide dismutase activity was significantly decreased at 33 mM (both time-points), and glutathione peroxidase activity was significantly increased at 0.3 and 33 mM (both time-points).

Diazinon and its photolysis product IMPY increased lipid peroxidation in human lymphocytes (freshly prepared from one donor) in vitro (Colović et al., 2010). On incubation for 72 hours, there were significant elevations in amounts of thiobarbituric acid-reactive substances with diazinon at concentrations of 2×10^{-5} M or higher, and with IMPY at 2×10^{-6} M or higher. The effect of IMPY was approximately 50–80% stronger (statistically significant) than that of diazinon at the same concentrations.

(ii) Non-human mammalian experimental systems

In vivo

Most of the experimental studies of oxidative stress and diazinon were conducted in rats and examined a range of end-points, exposure durations, doses, administration routes, and tissues. Specifically, it was found that diazinon induces the production of free radicals and oxidative stress in rat tissues through alteration of antioxidant-enzyme activity, depletion of glutathione, and increasing lipid peroxidation. Increases in oxidative-stress biomarkers upon exposure to diazinon in vivo have been observed in blood (Shadnia et al., 2007; Sutcu et al., 2007; Abdou & ElMazoudy, 2010; Messarah et al., 2013; El-Demerdash & Nasr, 2014; Moallem et al., 2014), liver (Teimouri et al., 2006; Amirkabirian et al., 2007; Lari et al., 2013; Lari et al., 2014), myocardium (Akturk et al., 2006; Jafari et al., 2012; Razavi et al., 2014a), testis (Leong et al., 2013; Oksay et al., 2013), kidney (Shah & Iqbal, 2010; Boroushaki et al., 2013), brain (Jafari et al., 2012; Yilmaz et al., 2012), blood vessels (Razavi et al., 2014b), adipose (Pakzad et al., 2013) and spleen (Jafari et al., 2012). Some studies used pre-treatments with various antioxidants and demonstrated that diazinon-related oxidative stress is mitigated by antioxidants (Shadnia et al., 2007; Sutcu et al., 2007; Messarah et al., 2013; El-Demerdash & Nasr, 2014). Jafari et al. (2012) performed a comparative analysis of tissue

susceptibility to diazinon-associated oxidative stress, and observed that induction of oxidative stress in diazinon-treated rats is in the rank order of brain > heart > spleen.

A study in mice given a single intraperitoneal injection of diazinon (22 or 43 mg/kg bw) showed an increase in superoxide dismutase activity in the testis (Sarabia et al., 2009b). Two studies examined oxidative stress end-points in rabbits exposed to diazinon. Tsitsimpikou et al. (2013) reported histopathological lesions and oxidative stress in liver and kidneys after long-term exposure of rabbits to diazinon. Zafiropoulos et al. (2014) observed diazinon-induced oxidative stress in the rabbit myocardium.

In vitro

Four reports presented the effects of diazinon on oxidative stress end-points in rat or mouse cells in vitro. Slotkin et al. (Slotkin et al., 2007; Slotkin & Seidler, 2009) used rat neuronotypic pheochromocytoma PC12 cells to explore whether diazinon affects the lipid peroxidation and transcriptional profiles of oxidative-stress response genes. Diazinon (30 µM) significantly increased levels of thiobarbituric acid-reactive substances in PC12 cells. In addition, the same concentration of diazinon (30 µm) had both positive and negative effects (all less than 1.5-fold) on several glutathione synthesis-related genes, catalase, and superoxide dismutase isoforms (Slotkin & Seidler, 2009). Pizzurro et al. (2014) showed that diazinon and its oxygen metabolite diazoxon cause oxidative stress in cultures of primary rat hippocampal neurons as a mechanism of inhibition of neurite outgrowth. Antioxidants prevented neurite outgrowth inhibition by diazinon. The concentrations of both compounds used in these studies were not cytotoxic, and caused limited inhibition of acetylcholinesterase activity in astrocytes. Finally, Giordano et al. (2007) explored the role of oxidative stress on the neurotoxicity of diazinon and diazoxon in neuronal cells from wildtype mice (*Gclm*$^{+/+}$) and mice lacking the modifier subunit of glutamate cysteine ligase (*Gclm*$^{-/-}$), the first and limiting enzyme in the synthesis of glutathione. Both diazinon and diazoxon increased intracellular levels of reactive oxygen species and lipid peroxidation, and in both cases the effects were greater in neurons from *Gclm* null mice. There was no change in intracellular concentrations of glutathione, but there was a significant increase in levels of oxidized glutathione.

(iii) Non-mammalian experimental systems

Positive associations between exposure to diazinon and oxidative stress were reported in various tissues in fish models in vivo (Oruç & Usta, 2007; Uner et al., 2007; Girón-Pérez et al., 2009; Oruç, 2011; Banaee et al., 2013).

(b) Inflammation and immunomodulation

(i) Humans

Three publications (Hoppin et al., 2007; Valcin et al., 2007; Slager et al., 2010) suggested that exposure to diazinon, among other pesticides, may be associated with an increased incidence of chronic inflammatory and allergic diseases of the respiratory system (bronchitis and rhinitis) in agricultural workers exposed to these agents. They used data from the AHS, a large study of pesticide applicators and their spouses enrolled in Iowa and North Carolina, USA, in 1993–1997. [The Working Group noted that these data should be interpreted with caution since the exposures were to mixtures of pesticides and dust.]

In in-vitro studies using human lymphoblastic T-cell lines (Jurkat), diazinon (> 125 µM) significantly decreased induction of interferon γ (IFNγ) and interleukin 4 (IL4) promoters in the presence of phytohaemagglutinin, or without any stimulus, but had no effect on viability (≥ 1 mM) (Oostingh et al., 2009). Diazinon had similar effects in human peripheral blood mononuclear cells, reducing the secretion of TH1-cytokine IFNγ, and TH2 cytokines IL-4 and IL-13 significantly at concentrations above

10 µM. Shao et al. (2013) demonstrated upregulation of several adaptive immune-response genes by diazinon in the transcriptome of the human Jurkat T-cell line in vitro.

(ii) Non-human mammalian experimental systems

Pro-inflammatory effects of diazinon have been observed in studies in experimental animals. Pakzad et al. (2013) treated rats with diazinon (70 mg/kg bw) by daily gavage for 4 weeks and evaluated molecular changes in the adipose tissue, finding that levels of tumour necrosis factor α (TNFα) doubled after exposure to diazinon. Moallem et al. (2014) evaluated levels of TNFα in rat serum after oral exposure to diazinon at 20 mg/kg bw per day for 4 weeks and also observed a significant induction of more than threefold. Studies in female rabbits given diazinon (5 mg/kg bw per day) orally every other day for up to 12 months reported focal inflammation and fibrosis in the liver and kidneys (Tsitsimpikou et al., 2013).

Pathological effects of diazinon on the immune system have been reported. Jeong et al. (1995) observed a significant decrease in thymus weight at the highest dose (20 mg/kg bw) in $B6C3F_1$ mice given diazinon by intraperitoneal injection for 7 days. Long-term oral exposure to diazinon (300 mg/kg food, by dry weight) for 45 days in CD-1 mice resulted in necrotic degeneration of trabeculae (spleen and thymus), hyperplasia of cortex and medulla (lymph nodes, thymus) and hyperplasia of the white and red pulp of the spleen (Handy et al., 2002). In C57BL/6 female mice given diazinon (0.2, 2, or 25 mg/kg bw; five intraperitoneal injections per week) for 28 days, there was a decrease in the ratio of thymus weight to body weight at doses > 2 mg/kg bw, and gross histopathological changes were observed in the thymus and spleen of mice at 25 mg/kg bw (Neishabouri et al., 2004). In a study in rats given diazinon at a dose of 20 mg/kg bw (administered orally every second day, for 35 days), there was a marked increase in the number of spleen lymphocytes, without a significant gain in relative spleen weight (Baconi et al., 2013). Diazinon also caused an increase in the number of mononuclear cells per spleen weight. However, splenic lymphocyte proliferation stimulated with concanavalin A ex vivo was not affected.

Suppression of the humoral immune response by diazinon has been reported in studies in mice. Suppression of humoral functional responses, such as haemagglutination titration and IgM plaque-forming colonies, was observed in female C57BL/6 mice treated with diazinon at 25 mg/kg bw for 28 days (five intraperitoneal injections per week) (Neishabouri et al., 2004). In mice given diazinon at 50 mg/kg bw for 30 days, there was a gradual significant decrease in the concenetrations of interleukins IL-2, IL-4, IL-10, and IL-12, and IFNγ (both protein and mRNA) in the splenocyte cultures that were stimulated with phytohaemagglutinin (Alluwaimi & Hussein, 2007). In pregnant mice fed diets containing diazinon (9 mg/kg) throughout gestation, there were significant effects on serum concentrations of IgG1 and IgG2a in male and female offspring at age 3 months (Barnett et al., 1980). No effects were observed on levels of IgG2b, IgA, or IgM at any time-point.

Cell-mediated effects of diazinon on the immune system were demonstrated in studies in mice. Suppression of the cellular functional responses, such as delayed-type hypersensitivity to sheep erythrocytes and T-cell subtyping (CD4/CD8) was observed in female C57BL/6 mice treated with diazinon at 25 mg/kg bw for 28 days (five intraperitoneal injections per week) (Neishabouri et al., 2004).

(iii) Non-mammalian experimental systems

Positive associations between exposure to diazinon and immunotoxicity in fish have been observed. There have been several reports on the effects of diazinon on immune system parameters in Nile tilapia (*Oreochromis*

niloticus) (Girón-Pérez et al., 2007, 2008, 2009). Splenocyte proliferation and phagocytic indices were significantly decreased after acute exposure to diazinon (Girón-Pérez et al., 2007). Diazinon (1.96 mg/L) significantly increased respiratory burst and IgM concentration in splenocytes (Girón-Pérez et al., 2009). In an ex-vivo study, acetylcholinesterase activity was lower, and acetylcholine concentration was higher, in spleen from Nile tilapia exposed to diazinon than in non-exposed controls. Pre-exposure to acetylcholine depleted the proliferative function of spleen cells, suggesting that the immunotoxic effects of diazinon in fish may be indirect and could involve the lymphocyte cholinergic system (Girón-Pérez et al., 2008). Also in Nile tilapia, diazinon decreased lymphocyte count and suppressed humoral immune responses in vaccinated fish, as shown by a decrease in primary antibody response and antibody plaque-forming cell number (Khalaf-Allah, 1999). In a study in iridescent shark (*Pangasius hypophthalmus*) exposed to diazinon (0.5 and 1 ppm for 7 days), leukocytosis, lymphopenia, and neutrophilia were observed (Hedayati & Tarkhani, 2014).

4.2.4 Cell proliferation and death

(a) Humans

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

In experiments in vitro, a human teratocarcinoma cell line (NTera2/D1) (NT2) that has properties of neuronal precursor cells was used to explore the role of acetylcholinesterase in the modulation of apoptosis by diazinon (Aluigi et al., 2010). Diazinon (1 µM; a concentration that did not result in significant inhibition of acetylcholinesterase activity) increased the number of viable cells (by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay). At 10 and 100 µM, acetylcholinesterase activity was inhibited, and cell viability was decreased, as dose and duration increased. At 10 µM, various measures of apoptosis were affected by diazinon, including activation of caspases and nuclear fragmentation (measured by a flow-cytometry procedure).

Diazinon (3.9–1000 µM) had no negative effect on cell viability, and in fact showed a significant increase above control levels at any concentration of co-administration in lung epithelial carcinoma (A549) cells transfected with an insert encoding different promoter regions, including that for TNFα, and treated with recombinant human TNFα (rhTNFα) (0, 1, 20, or 300 ng/mL) (Oostingh et al., 2009). Diazinon at the same concentrations had no significant positive or negative effect on cell viability in a human lymphoblastic T-cell line (Jurkat) incubated with phytohaemagglutinin at 0 or 10 µg/mL.

In colonic epithelial cell lines established from primary cultures of surgically resected tissue, diazinon (0.05–50 µM; in dimethyl sulfoxide, DMSO) caused an increase in cell growth as measured by the MTT assay after 1 day (Greenman et al., 1997). After 3 days, cell growth remained elevated at 1 and 50 µM, but did not significantly differ from control levels at 0.5 and 10 µM.

In a colorectal adenocarcinoma cell line (Caco-2 cells), cell growth (as measured by the MTT assay) was not elevated above control levels after exposure to diazinon (15, 45, or 135 µM for 5 days) (Habibollahi et al., 2011). Indeed, cell viability substantially decreased with increasing exposure, but descendants of cells that were treated for 4.5 months with gradually increasing concentrations of diazinon (from 0.02 µM to 20 µM) were more resistant to effects on cell viability than were the parent cells. [Data on cell growth after a shorter period of exposure were not provided.]

In a lymphocyte culture derived from blood drawn from a healthy male (age, 30 years), cell proliferation potential (evaluated by cytokinesis-block proliferation index) was inhibited by diazinon (0.02–20 µM) (Colović et al., 2010). This

was also the case for similarly exposed skin fibroblasts (source not specified).

(b) Non-human mammalian experimental systems

(i) In vivo

In bioassays in rats and mice carried out by the National Toxicology Program (NTP), diazinon caused an increase in the incidence of proliferative lesions of the uterus (NTP, 1979). In female rats, the incidence of proliferative lesions of the uterus in treated animals was roughly double that in controls (P = 0.05, Cochran-Armitage trend). In female mice, the incidence of uterine hyperplasia was significantly increased (P = 0.05, Cochran-Armitage trend).

Male Wistar rats receiving diazinon at a dose of 15 or 30 mg/kg bw per day in corn oil by gavage for 4 weeks showed no differences in markers of apoptotic effects in brain tissue (Marzieh et al., 2013). Western-blot analyses of caspases 3 and 9 and related active forms, or Bax/Bcl2, did not differ between treated and control rats.

In an experiment on liver foci, male F344 rats were injected intraperitoneally with diethylnitrosamine as an initiator, and then received diets containing diazinon (500 or 100 ppm) for 6 weeks; diazinon had no effect on the number of foci that were positive for glutathione *S*-transferase placental (GSTP) form (Kato et al., 1995).

In adult male Wistar rats receiving daily doses of diazinon (15 mg/kg bw) in corn oil for 4 weeks, liver caspases 3 and 9 were activated and the Bax/Bcl2 ratio was increased (Lari et al., 2013). The antioxidant crocin had a protective effect, as indicated by decreased levels of caspases 3 and 9 activation and Bax/Bcl2 ratio in rats receiving diazinon plus crocin. In a follow-up study in similarly treated rats, proteomic analysis showed that levels of liver proteins involved in apoptosis pathways were perturbed (Lari et al., 2014). For example, levels of glucose-regulated protein GRP78 (a member of the family of heat-shock proteins that functions as an endoplasmic reticulum chaperone with anti-apoptotic properties) and regucalcin (RGN, involved in cellular calcium homeostasis) were reduced.

(i) In vitro

In a rat intestinal cell line (IEC-6) incubated with diazinon in DMSO, cell growth (MTT assay) was elevated after 1 day with diazinon at 1, 10 and 50 μM, after 2 days at 1 μM, and after 3 days at 1 or 10 μM (Greenman et al., 1997).

Diazinon (0.01–10 μM) induced cell proliferation in rat pituitary tumour cells (MtT/Se), which are responsive to stimulation by 17β-estradiol (Manabe et al., 2006).

Diazinon was tested in Swiss Webster mice, on cultures of neuronal and mixed cortical cell lines derived from fetal mixed cortical cells, and glial cultures derived from mice aged 1 or 2 days (Rush et al., 2010). Diazinon at a concentration of 30 or 100 μM caused a high percentage of neuronal death, while diazoxon had no measurable effect. The toxicity of diazinon was mitigated by co-exposure to a caspase inhibitor. Diazinon induced chromatin condensation characteristic of apoptosis. Glutamate receptor antagonists, as well as atropine and mecamylamine, were not protective, and addition of acetylcholine and its non-hydrolysable analogue, carbachol, did not increase toxicity as would be expected if inhibition of acetylcholinesterase activity were playing a role.

In a study designed to test the neuroprotective effects of cannabinoids, diazinon (50–200 μM) induced apoptosis in a dose-dependent fashion, as measured by TUNEL (terminal uridine deoxynucleotidyl transferase dUTP nick end labelling) staining, in the rat PC12 neuronal cell line (Sadri et al., 2010). Apoptosis was mitigated when cells were pre-treated with the cannabinoid receptor agonist WIN-55, 212-2.

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 website of the ToxCast research programme (EPA, 2015a). Z-Tetrachlorvinphos (CAS No. 22248-79-9; 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 the 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 characteristicsof 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 comprise 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).

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" in order 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 Working Group's analyses, 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 *IARC Monographs*. ToxPi is a dimensionless index score that integrates of multiple different assay results and displays them visually. The overall score for a chemical takes into account 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.

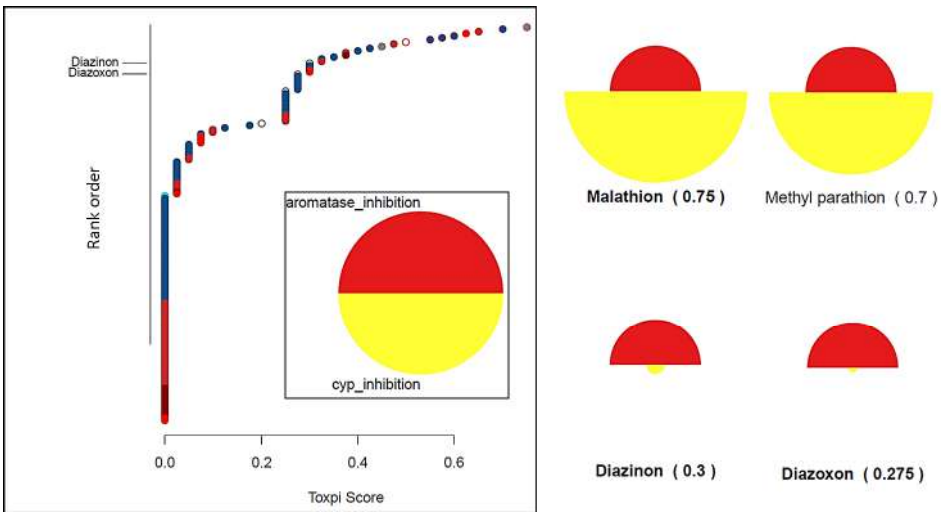
The list of ToxCast/Tox21 assay end-points included in the analysis by the Working Group, description of the target and/or model system for each end-point (e.g. cell type, species, detection technology, etc.), their mapping to 7 of the 10 "key characteristics" of known human carcinogens, and the decision 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 diazinon 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 diazinon and diazoxon in the ranked list is 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 diazinon and diazoxon are shown.

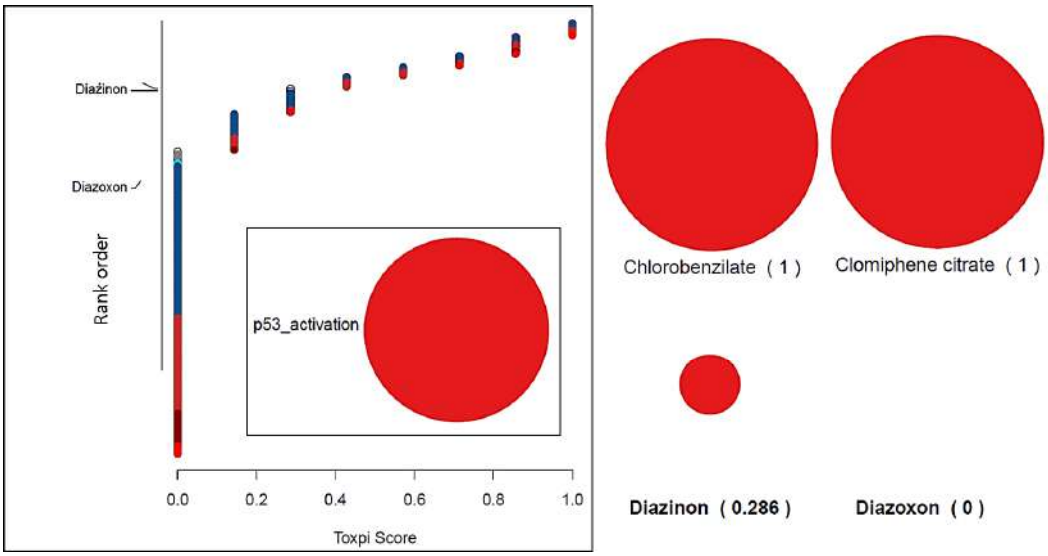
- Characteristic (1) *Is electrophilic or can undergo metabolic activation:* Diazinon and diazoxon were tested for 31 assay end-points and were found to be active for 3 and 2, respectively, of the assay end-points related to CYP inhibition. The highest ranked of the 178 chemicals included in the comparison was malathion, which was active for 20 out of 29 assay end-points. Diazinon and diazoxon were tested for two assays end-points related to aromatase inhibition, and were found to be active for one end-point each (Fig. 4.3).
- Characteristic (2) *Is genotoxic*: Diazinon and diazoxon were tested for nine assay end-points related to TP53 activity. Diazinon was found to be active for two assay end-points. The highest ranked chemicals tested were chlorobenzilate and clomiphene citrate, which were active for seven out of of nine assay end-points. Diazoxon was not active for any of these assay end-points (Fig. 4.4).
- Characteristic (4) *Induces epigenetic alterations:* Diazinon and diazoxon were found to be inactive for all 11 assay end-points for which they were tested (4 end-points related to DNA binding, and 7 end-points related to histone modification) (Fig. 4.5).
- Characteristic (5) *Induces oxidative stress*: Diazinon and diazoxon were tested for 18 assay end-points. Diazinon showed negligible

Fig. 4.3 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to enzyme inhibition



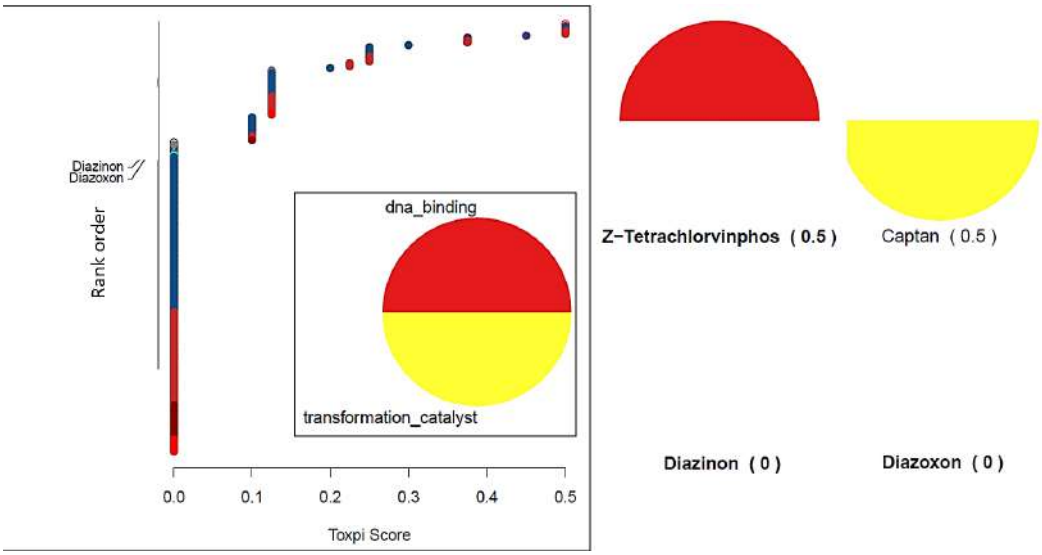
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 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 (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.4 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to genotoxicity



On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, 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 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 (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.5 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to epigenetic alterations



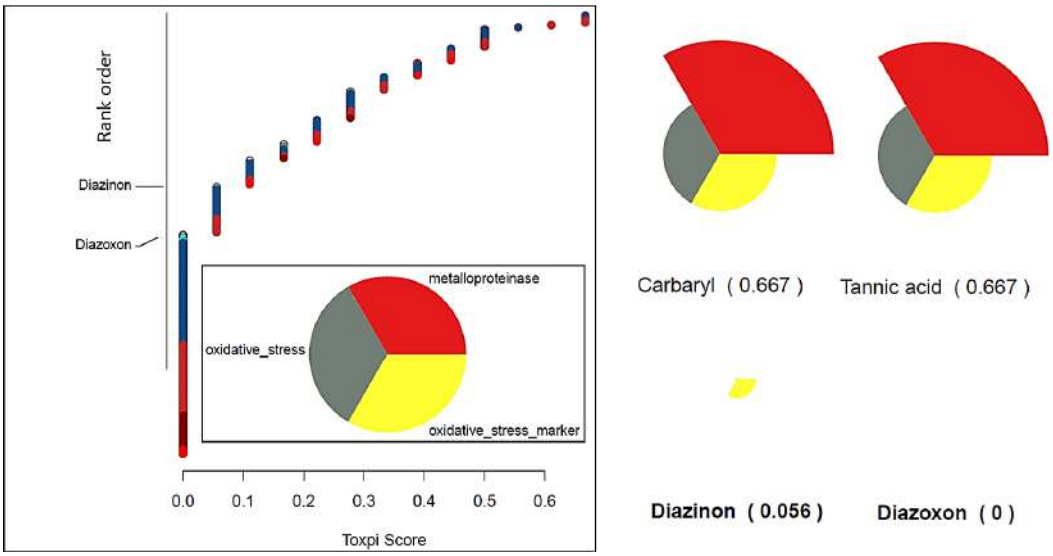
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 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 (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

activity. Diazoxon showed no activity (Fig. 4.6).

- Characteristic (6) *Induces chronic inflammation*: Diazinon and diazoxon were tested for 45 assay end-points; and no activity was observed for either chemical (Fig. 4.7).
- Characteristic (8) *Modulates receptor-mediated effects:* Diazinon and diazoxon were tested for 81 assay end-points. Diazinon was active for 16 of these end-points, including both end-points relating to AhR, a subset of end-points relating to estrogen receptor (both α and β), and other end-points relating to nuclear receptors. Diazoxon showed no activity for any of these assay end-points (Fig. 4.8).
- Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply:* Diazinon and diazoxon were both tested for 67 of the 68 assay end-points. Diazinon was found to be active for 3 assay end-points relating to cytotoxicity, while diazoxon was active for 1 end-point. In comparison to the highest ranked chemicals, ziram and clomiphene citrate, diazinon and diazoxon showed little cellular toxicity under the conditions of the assay (Fig. 4.9).

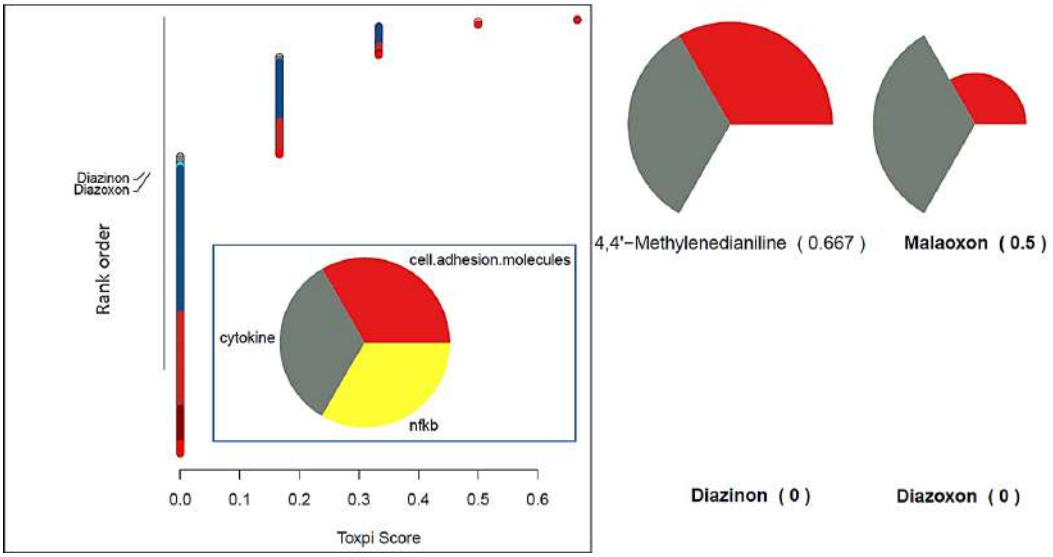
Overall, diazinon demonstrated activity in both AhR assays, and additional effects in a subset of assay end-points relating to estrogen receptor α and β. Diazoxon exhibited little activity across the 263 assay end-points, being found active for only 3 assay end-points. The limited activity of diazoxon may be attributed to the high reactivity and short half-life of this compound, which hinder interpretation of the results of the assay end-points.

Fig. 4.6 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to oxidative stress



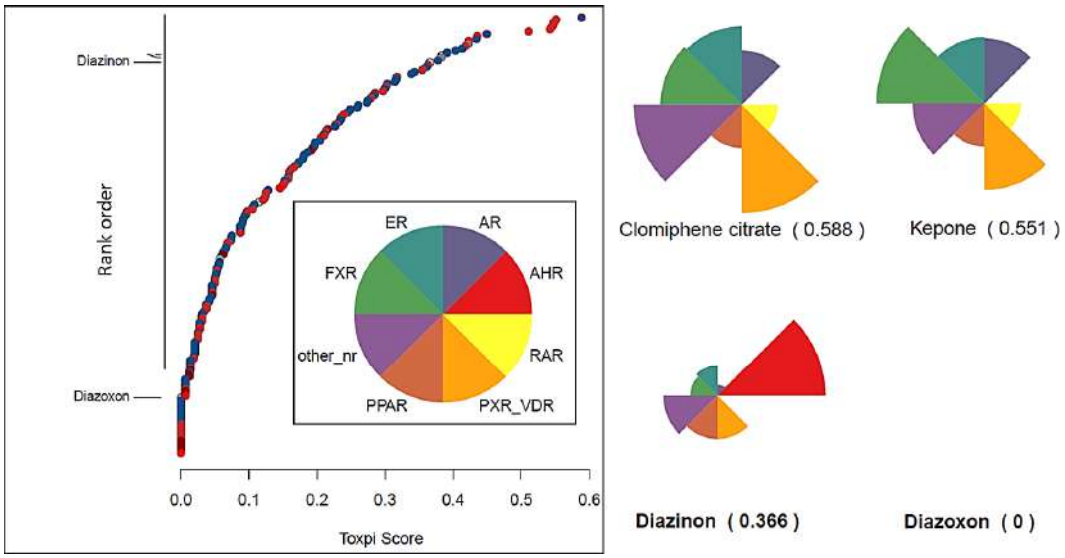
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the 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, carbaryl and tannic acid) and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.7 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to chronic inflammation



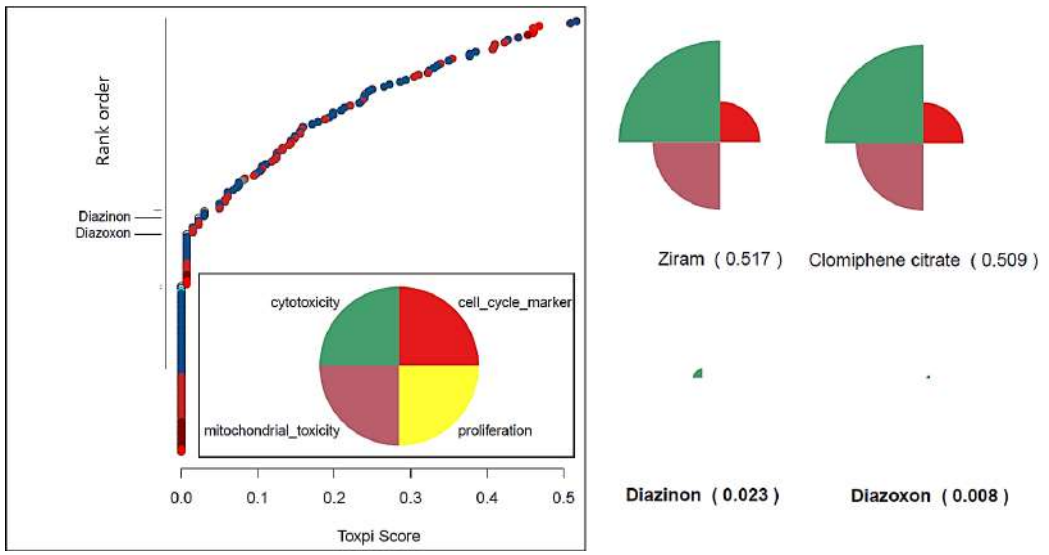
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the 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, 4,4′methylenedianiline and malaoxon) and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.8 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to receptor-mediated effects



On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, 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, clomiphene citrate and kepone) and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

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



On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, 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, clomiphene citrate and ziram) and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

4.4 Susceptibility

Indirect evidence for an association between risk of cancer and exposure to diazinon was reported from two studies in the same population. Searles Nielsen et al. (2005) explored the relationship between exposure to common residential insecticides (with chlorpyrifos and diazinon presumed to be most likely exposures, albeit not measured in this study), two common *PON1* polymorphisms, C-108T and Q192R, and occurrence of brain tumours in childhood. This population-based study with 66 cases and 236 controls found an inverse association between PON1 levels and occurrence of brain tumours in childhood; the risk of childhood brain tumour was non-significantly increased in relation to the inefficient *PON1* promoter allele [per $PON1_{-108T}$ allele, relative to $PON1_{-108CC}$: OR, 1.4; 95% CI, 1.0–2.2; *P* for trend, 0.07]. Notably, the association for childhood brain tumours was statistically significant among children whose mothers reported chemical treatment of the home for pests during pregnancy or childhood (per $PON1_{-108T}$ allele: among exposed, OR, 2.6; 95% CI, 1.2–5.5; among unexposed, OR, 0.9; 95% CI, 0.5–1.6) and for primitive neuroectodermal tumours (per $PON1_{-108T}$ allele: OR, 2.4; 95% CI, 1.1–5.4). The Q192R polymorphism was not associated with risk of childhood brain tumour, nor was the $PON1_{C-108T/Q192R}$ haplotype.

In a follow-up study, Searles Nielsen et al. (2010) examined the same single nucleotide polymorphisms (SNPs) for PON1 and six additional genetic polymorphisms that affect insecticide metabolism, using the same number of cases and controls born in Washington State, USA (66 cases, 236 controls) expanded with 26 cases and 50 controls from San Francisco, and 110 cases and 99 controls from Los Angeles. Of the six additional genetic polymorphisms studied, the $BCHE_{539T}$ allele, associated with reduced in-vivo activity of the butyrylcholinesterase enzyme, was associated with increased risk of childhood brain tumours only among insecticide-exposed individuals, but this association was not statistically significant.

4.5 Other adverse effects

4.5.1 Humans

In a nested case–control study, men with diazinon metabolites in urine samples were more likely to exhibit lower sperm concentration and motility (Swan, 2006). Sperm DNA damage was observed after incubation of spermatozoa from healthy volunteers with several organophosphate compounds and their oxons, including diazinon (concentration, 50–750 μM) (Salazar-Arredondo et al., 2008; see Section 4.2.1(a)(ii) in this *Monograph*).

4.5.2 Experimental systems

Diazinon was tested in thirteen regulatory toxicity submissions included in the Toxicity Reference Database (ToxRefDB) (EPA, 2015c). Specifically, study design, treatment group, and treatment-related effect information were captured for five long-term studies of toxicity or carcinogenicity, two short-term studies of toxicity, two studies of developmental toxicity, two multigenerational studies of reproductive toxicity, and two studies of developmental neurotoxicity. Diazinon was also tested in bioassays in both rats and mice by the United States National Cancer Institute (NTP, 1979). [The Working Group noted that although long-term studies with diazinon were available, the ability to determine a full range of adverse effect potential may be limited by sensitivity to the cholinergic effects of diazinon, which limits the available dosing range.]

Cholinergic effects were observed in numerous studies in which cholinesterase inhibition was evaluated, and included inhibition of plasma, erythrocyte, and brain cholinesterase

activity at doses as low as 0.1 mg/kg bw per day (NTP, 1979; EPA, 1988, 1991). 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. Mild hyperactivity was also noted in rats and mice in bioassays carried out by the National Cancer Institute (NTP, 1979).

Liver hypertrophy and increases in liver weight were observed in female rats at the highest dietary dose tested (212 mg/kg bw per day) (EPA, 1988).

Although not specifically attributed to the stomach, gastrointestinal-tract issues were observed in rabbits given diazinon at the highest dose (100 mg/kg bw per day) in a study of developmental toxicity. Congestion, erosion, and haemorrhage were observed in the gastrointestinal tract of rabbits that died (EPA, 1981).

Under various exposure conditions, including in utero and during lactation, diazinon has been shown to decrease testicular weight, decrease sperm count and quality, and alter levels of various endocrine hormones (Jayachandra & D'Souza, 2013, 2014; ElMazoudy & Attia, 2012).

In a long-term study in dogs, lung weights were decreased in females fed diets containing diazinon at all doses (range, 0.0037–9.1 mg/kg bw per day) (EPA, 1991). Weights of the mandibular salivary gland were decreased in female dogs exposed to diazinon at the two higher doses tested (4.5 and 9.1 mg/kg bw per day) (EPA, 1991). Reduced body weight was observed in males at the intermediate dose, and in males and females at the highest dose (EPA, 1991).

In rats given diazinon at a dose of 15 mg/kg bw per day by gavage for 4 weeks, mitochondrial-mediated apoptosis occurred in heart tissue, as measured by levels of apoptotic proteins (Bax, Bcl2, and caspase 3), and the effects were ameliorated by co-exposure to the antioxidant crocin at 50/kg bw per day (Razavi et al., 2013). Evidence for cardiotoxicity has also been demonstrated in the form of dose-dependent degeneration of cardiac and skeletal muscle fibres in female rats exposed to diazinon (Abdou & ElMazoudy, 2010). In female mice, uterine cystic hyperplasia was observed in 22 out of 46 mice receiving diazinon at the highest dose tested (200 ppm), compared with zero in the matched controls (NTP, 1979).

In a two-generation study of reproductive toxicity, reduced mating, litter size, and viability index were observed in rats at the highest dose of 35.15/41.43 mg/kg bw per day (males/females). Fertility and gestational interval were reduced in females at the highest dose (EPA, 1989b).

In a study of developmental toxicity in rats, diazinon (100 mg/kg bw per day) increased rudimentary T-14 ribs and decreased fetal weights (EPA, 1985). In a study of developmental neurotoxicity in rats, diazinon (24.2 mg/kg bw per day) decreased pup weight in males and females and delayed vaginal opening in females, and preputial separation in males (EPA, 2003).

In a study of developmental neurotoxicity in rats, diazinon (24.2 mg/kg bw per day) increased the number of errors and latent period in males assessed for learning and memory in a maze (EPA, 2003).

In a dose range-finding study for the study by EPA (2003), diazinon (38.06 mg/kg bw per day) decreased pup weight in males and females, and decreased surface righting reflex in females (EPA, 2002).

5. Summary of Data Reported

5.1 Exposure data

Diazinon is an organophosphate insecticide that was developed in the 1950s and acts on a wide range of insects on crops, gardens, livestock, and pets. Production volumes have been relatively low (about 5000 tonnes in the USA in 1990) and have decreased further since use of diazinon was restricted in the USA in 2004, and in the European Union in 2006. In the USA,

outdoor residential use accounted for most of the diazinon used. Exposures in agricultural workers vary considerably, with higher exposure related to higher volume of diazinon used, inappropriate application methods, inadequate worker protection, and poor hygienic practices. Diazinon has been found in soil and dust. Levels in water and food are reported to be low.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to diazinon, the Working Group identified 9 reports from 3 cohort studies, and 14 reports on 6 case–control studies, that reported on associations between cancer and exposure to diazinon specifically. Several large studies each provided multiple reports, notably the Agricultural Health Study cohort, case–control studies in the midwest USA, and the Cross-Canada Case–control Study of Pesticides and Health, which were considered to be key studies for the evaluation because of relatively large study size and because individual information was provided on specific pesticide exposures. Reports from more than two independent studies were available for non-Hodgkin lymphoma (NHL) and leukaemia. For cancers of the lung, breast, and prostate, results from two independent studies were available. For cancers of the colorectum, melanoma, bladder, kidney, multiple myeloma, Hodgkin lymphoma, soft tissue sarcoma, brain in childhood or in adults, stomach, and oesophagus, results from a single study for each cancer site were available for evaluation.

5.2.1 NHL

Two large case–control studies on NHL reported a positive association for diazinon: a pooled analysis from the USA (OR, 1.7; 95% CI, 1.2–2.5; including proxy respondents; OR, 1.3; 95% CI, 0.8–2.0; excluding proxy respondents), and a study from Canada (OR, 1.7; 95% CI, 0.9–3.2; including proxy respondents). The pooled analysis from the USA showed a positive exposure–response relationship with years of diazinon use when proxy respondents were excluded, and adjustment for other pesticides did not alter the results (OR, 1.9; 95% CI, 1.1–3.6; including proxy respondents). Subtype-specific analyses indicated a positive association for small lymphocytic lymphoma. The positive association for all NHL was not replicated in the Agricultural Health Study (OR, 1.0; 95% CI, 0.8–1.3), but analyses by subtype indicated an increased risk and positive exposure–response relationship with lifetime exposure days for follicular lymphoma (*P* for trend, 0.02) and suggestive evidence for a similar association for small B-cell lymphocytic lymphoma/chronic B-cell lymphocytic lymphoma/mantle cell lymphoma (*P* for trend, 0.06), as well as for all lympho-haematopoietic cancers combined (*P* for trend, 0.09). An association was absent for diffuse large B-cell lymphoma, the largest subtype within NHL, and there was some evidence of heterogeneity among subtypes. There was no evidence for major confounding by other pesticides.

The Working Group noted that: (i) positive associations for NHL or its subtypes were reported for both case–control studies and a large cohort study; (ii) both case–control studies and the cohort study suggest a positive exposure–response relationship; (iii) both case–control studies and the cohort study assessed exposure to multiple pesticides through self-reporting, which in the case of the cohort study was before diagnosis, thus excluding differential exposure misclassification as a likely explanation for the observed association in the cohort study; and (iv) there was no evidence that confounding by other pesticides could explain the observed associations.

5.2.2 Leukaemia

One case–control study on leukaemia in the USA (OR, 1.2; 95% CI, 0.6–2.1), and one case–control study nested in a cohort of farmworkers in California (OR, 1.32; 95% CI, 0.65–2.65) reported risk estimates for diazinon, neither reporting a consistently increased risk, although in one study elevated risks were reported for both chronic lymphocytic leukaemia (OR, 1.4; 95% CI, 0.5–4.4) and granulocytic leukaemia (OR, 1.9; 95% CI, 0.7–5.7). [The Working Group noted that in current classifications, chronic lymphocytic leukaemia would now be classified as NHL.] In the large Agricultural Health Study cohort, an exposure–response association (*P* for trend = 0.03) was observed for leukaemia with a rate ratio of > 3 for the highest exposure tertile. Adjustment for a list of other pesticides that were associated with increased risks within the Agricultural Health Study did not markedly alter the results.

The Working Group noted that: (i) the large Agricultural Health Study cohort provided evidence of a positive association between use of diazinon and leukaemia, which was strengthened by the presence of a monotonic increase in risk by cumulative exposure, and adjustment for other pesticides without changing the results; (ii) there was a suggestion of an increased risk for both lymphocytic and granulocytic leukaemia in a case–control study nested within a cohort from California (United Farm Workers of America).

5.2.3 Cancer of the lung

Within the large Agricultural Health Study cohort, risk estimates for cancer of the lung were reported multiple times for different updates for this prospective cohort, in 2004, 2005, and 2015. Results for cancer of the lung were very consistent over these three updates, consistently showing a positive exposure–response relationship (*P* for trend, 0.02). These risk estimates were fully adjusted for smoking; adjustment for other pesticides and other agricultural exposures did not markedly change the results. No case–control studies on cancer of the lung were identified that reported specifically on exposure to diazinon. However, one study nested in a cohort of pest-control workers from Florida, showed an increased risk of cancer of the lung associated with diazinon exposure that was not statistically significant (OR, 2.0; 95% CI, 0.7–5.5; compared with deceased controls; and OR, 1.3; 95% CI, 0.6–3.1; compared with living controls); limitations in the exposure assessment of this study were noted.

The Working Group noted that: (i) the cumulative exposure-dependent increased risk for cancer of the lung is a consistent and robust finding within the large Agricultural Health Study cohort, arguing against chance as an explanation; (ii) there was no evidence that confounding by other pesticides, smoking, or other established risk factors for cancer of the lung could explain the observed association. However, the Working Group also noted that no other cohort studies or good-quality case–control studies of cancer of the lung were identified that also reported on diazinon, thus meaning that this finding was not replicated in other study populations.

5.2.4 Cancer of the breast

Two studies were identified that reported on diazinon and cancer of the breast in women: a study nested in the United Farm Workers of America cohort and the Agricultural Health Study; neither provided consistent evidence of an increased risk.

5.2.5 Cancer of the prostate

One case–control study on cancer of the prostate was identified that reported on exposure to diazinon as assessed through a job-exposure matrix as one of 180 pesticides evaluated,

reporting an exposure–response relationship for diazinon. Limitations in the exposure assessment were noted, in particular the high correlation among pesticides assessed through the job-exposure matrix, and lack of adjustment for other pesticides. Within the large Agricultural Health Study cohort, three updates reported on cancer of the prostate in 2005, 2013, and 2015. Although based on large numbers, there was no evidence that risk of cancer of the prostate was elevated for those exposed to diazinon, and risk did not increase by cumulative exposure.

The Working Group noted that the increased risk of cancer of the prostate observed for the case–control study was not replicated in the Agricultural Health Study cohort.

5.2.6 Other cancer sites

For cancers of the bladder, colorectum, kidney, stomach, oesophagus, and tumours of the brain in childhood or in adults, and for melanoma, multiple myeloma, Hodgkin lymphoma, and soft tissue sarcoma, results from a single study for each site were available for evaluation.

For cancer of the kidney, there was some suggestion of an increased risk for the highest category of diazinon exposure (based on one report from the Agricultural Health Study).

For multiple myeloma and Hodgkin lymphoma, there was some suggestion of an elevated risk (based on the Cross-Canada Case–control Study). In the same study, an increased risk of soft tissue sarcoma was also observed, and the threefold increased risk observed did not change after adjusting for aldrin, which was the only other pesticide also associated with soft tissue sarcoma besides diazinon.

An increased risk of childhood tumours of the brain and garden use of diazinon was observed (based on a very small study), but other studies could not evaluate this association because of small numbers.

No increased risk was observed for cancers of the colorectum (based on the Agricultural Health Study), stomach and oesophagus (based on a case–control study), bladder (based on the Agricultural Health Study), melanoma (based on the Agricultural Health Study), or adult glioma (based on a case–control study).

The risk for all cancers combined was evaluated in the large Agricultural Health Study cohort, which showed an increased risk with an exposure–response relationship (P for trend = 0.009).

In conclusion, positive associations and exposure–response trends were noted for NHL, leukaemia, and cancer of the lung. The Working Group noted that the number of studies available was relatively small and confounding by other pesticides as an explanation for the increased risks could not be fully excluded.

5.3 Animal carcinogenicity data

Diazinon was tested for carcinogenicity in one 2-year feeding study in male and female mice, and two 2-year feeding studies in male and female rats.

Diazinon induced a significant increase in the incidence of hepatocellular carcinoma in male mice at the lowest dose. This increase could not be clearly related to the administration of diazinon because it was only observed in male mice at the lowest dose, at an incidence slightly above the upper limit of the range for historical controls for this tumour in this strain of mouse. There were no significant findings in males at the highest dose, or in female mice at any dose.

In the first study in rats, diazinon induced a significant increase in the incidence of leukaemia or lymphoma (combined) in male rats at the lowest dose. This could not clearly be related to the administration of diazinon because it was observed only in males at the lowest dose, at an incidence slightly above the upper limit of the range for historical controls for these tumours in this strain of rat. There were no significant

findings in males at the highest dose, or in female rats at any dose. There were no significant increases in tumour incidence in the second study.

5.4 Mechanistic and other relevant data

The majority of orally administered diazinon is absorbed, in humans, dogs, and rodents. Studies in human volunteers indicate that dermal absorption of diazinon is considerably slower than oral absorption. Few data on systemic tissue distribution in humans were available to the Working Group. Studies in experimental animals indicate that diazinon is widely distributed via blood. Overall, metabolism of diazinon involves cytochrome P450 (CYP450), paraoxonase 1 (PON1) and carboxylesterases. It is well established that diazinon metabolism is similar in humans and experimental species. Diazinon is rapidly metabolized to short-lived diazoxon or 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) by several cytochrome P450s. PON1 can metabolize diazoxon to IMPY and diethylphosphate (DEP). Carboxylesterase can degrade diazoxon to liberate IMPY. In humans and experimental animals, diazinon is excreted as IMPY, DEP, or other metabolites (e.g. diethylthiophosphate).

The evidence for the genotoxicity of diazinon is *strong* and appears to operate in humans. No studies in humans in vivo exposed to diazinon only were available. Studies in experimental animals in vivo showed either DNA damage (oxidative DNA damage, DNA strand breaks) or chromosomal damage (micronuclei). In vitro, human cell lines also showed DNA damage (DNA strand breaks) or chromosomal damage (micronucleus formation, sister-chromatid exchange). The results of studies in humans exposed to multiple compounds including diazinon are consistent with these findings. In studies in non-human species in vitro, results were mixed.

The evidence that diazinon can induce oxidative stress is *strong*. Diazinon induced oxidative stress in human and mammalian cells in vitro, and in a variety of tissues in numerous studies in rodents in vivo. Studies employing pre-exposures to various antioxidants mitigated the effects. Diazinon induces oxidative stress through alteration of antioxidant enzyme activity, depletion of glutathione, and increasing lipid peroxidation. Several studies in fish also report similar findings. Pro-inflammatory effects are also observed in vivo in studies in rodents.

The evidence for receptor-mediated mechanisms in the potential carcinogenicity of diazinon is *weak*. In vivo, diazinon modulated gonadotropin levels in several studies in rats. The diazinon metabolite diazoxon binds to acetylcholinesterase and other serine esterases such as butyrylcholinesterase. It is unclear what role, if any, the sequelae can play in carcinogenesis.

Overall, the effects on proliferation are *weak*, with a few studies showing apoptotic effects in some diazinon-exposed human and rodent cell lines, and in a few other studies showing no cell proliferation or apoptotic effect. Diazinon induced uterine cystic hyperplasia in mice.

Because of the limited available data, the evidence for immunosuppression as a mechanism of carcinogenicity for diazinon is *weak*. In human cell lines, diazinon decreased the induction of regulators of immune system function, while pathological effects on the immune system, suppression of humoral immune response, and cellular functional responses have been observed in rodents in vivo. Immunotoxicity was seen in model fish species.

There were few data on the other key characteristics of carcinogens.

In studies in humans and experimental animals, diazinon exhibited effects of sperm quality, count, and motility, with corresponding testicular pathology in animals. In addition to

cholinergic effects, non-neoplastic pathology was also observed in lung, stomach, heart, and liver tissues in studies in experimental animals.

Overall, the mechanistic data provide strong support for carcinogenicity findings of diazinon. This includes strong evidence for genotoxicity and oxidative stress. 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 diazinon. A positive association has been observed for non-Hodgkin lymphoma, leukaemia, and cancer of the lung.

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of diazinon.

6.3 Overall evaluation

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

6.4 Rationale

There is strong evidence that diazinon can operate through two key characteristics of known human carcinogens and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to diazinon is genotoxic, from studies in experimental animals in vivo, and in studies in animal cell lines. In addition, studies in human cell lines in vitro show effects on chromosomal damage; this demonstrates that this mechanism can operate in humans. Additional support for human relevance is provided by positive results in a study of a small number of volunteers exposed to diazinon.
- There is also strong evidence that diazinon can act to induce oxidative stress. This evidence is from studies in experimental animals in vivo, and studies in human and animal cell lines in vitro. This mechanism has been challenged experimentally by administering antioxidants, treatment that abrogated the effects of diazinon on oxidative stress.

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GLYPHOSATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 1071-83-6 (acid); also relevant:
38641-94-0 (glyphosate-isopropylamine salt)
40465-66-5 (monoammonium salt)
69254-40-6 (diammonium salt)
34494-03-6 (glyphosate-sodium)
81591-81-3 (glyphosate-trimesium)

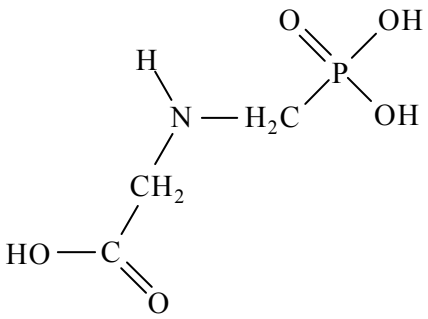
Chem. Abstr. Serv. Name: N-(phosphonomethyl)glycine

Preferred IUPAC Name: *N*-(phosphonomethyl)glycine

Synonyms: Gliphosate; glyphosate; glyphosate hydrochloride; glyphosate [calcium, copper (2+), dilithium, disodium, magnesium, monoammonium, monopotassium, monosodium, sodium, or zinc] salt

Trade names: Glyphosate products have been sold worldwide under numerous trade names, including: Abundit Extra; Credit; Xtreme; Glifonox; Glyphogan; Ground-Up; Rodeo; Roundup; Touchdown; Tragli; Wipe Out; Yerbimat (Farm Chemicals International, 2015).

1.1.2 Structural and molecular formulae and relative molecular mass



Molecular formula: $C_3H_8NO_5P$
Relative molecular mass: 169.07

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

1.1.3 Chemical and physical properties of the pure substance

Description: Glyphosate acid is a colourless, odourless, crystalline solid. It is formulated as a salt consisting of the deprotonated acid of glyphosate and a cation (isopropylamine, ammonium, or sodium), with more than one salt in some formulations.

Solubility: The acid is of medium solubility at 11.6 g/L in water (at 25 °C) and insoluble in common organic solvents such as acetone, ethanol, and xylene; the alkali-metal and

amine salts are readily soluble in water (Tomlin, 2000).

Volatility: Vapour pressure, 1.31×10^{-2} mPa at 25 °C (negligible) (Tomlin, 2000).

Stability: Glyphosate is stable to hydrolysis in the range of pH 3 to pH 9, and relatively stable to photodegradation (Tomlin, 2000). Glyphosate is not readily hydrolysed or oxidized in the field (Rueppel *et al.* 1977). It decomposes on heating, producing toxic fumes that include nitrogen oxides and phosphorus oxides (IPCS, 2005).

Reactivity: Attacks iron and galvanized steel (IPCS, 2005).

Octanol/water partition coefficient (P): log P, < –3.2 (pH 2–5, 20 °C) (OECD method 107) (Tomlin, 2000).

Henry's law: $< 2.1 \times 10^{-7}$ Pa m^3 mol^{-1} (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), $mg/m^3 = 6.92 \times$ ppm.

1.1.4 Technical products and impurities

Glyphosate is formulated as an isopropylamine, ammonium, or sodium salt in water-soluble concentrates and water-soluble granules. The relevant impurities in glyphosate technical concentrates are formaldehyde (maximum, 1.3 g/kg), *N*-nitrosoglyphosate (maximum, 1 mg/kg), and *N*-nitroso-*N*-phosphonomethylglycine (FAO, 2000). Surfactants and sulfuric and phosphoric acids may be added to formulations of glyphosate, with type and concentration differing by formulation (IPCS, 1994).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Glyphosate was first synthesized in 1950 as a potential pharmaceutical compound, but its herbicidal activity was not discovered until it was re-synthesized and tested in 1970 (Székács & Darvas, 2012). The isopropylamine, sodium, and ammonium salts were introduced in 1974, and the trimesium (trimethylsulfonium) salt was introduced in Spain in 1989. The original patent protection expired outside the USA in 1991, and within the USA in 2000. Thereafter, production expanded to other major agrochemical manufacturers in the USA, Europe, Australia, and elsewhere (including large-scale production in China), but the leading preparation producer remained in the USA (Székács & Darvas, 2012).

There are two dominant families of commercial production of glyphosate, the "alkyl ester" pathways, predominant in China, and the "iminodiacetic acid" pathways, with iminodiacetic acid produced from iminodiacetonitrile (produced from hydrogen cyanide), diethanol amine, or chloroacetic acid (Dill *et al.*, 2010; Tian *et al.*, 2012).

To increase the solubility of technical-grade glyphosate acid in water, it is formulated as its isopropylamine, monoammonium, potassium, sodium, or trimesium salts. Most common is the isopropylamine salt, which is formulated as a liquid concentrate (active ingredient, 5.0–62%), ready-to-use liquid (active ingredient, 0.5–20%), pressurized liquid (active ingredient, 0.75–0.96%), solid (active ingredient, 76–94%), or pellet/tablet (active ingredient, 60–83%) (EPA, 1993a).

There are reportedly more than 750 products containing glyphosate for sale in the USA alone (NPIC, 2010). Formulated products contain various non-ionic surfactants, most notably polyethyloxylated tallowamine (POEA), to

facilitate uptake by plants (Székács & Darvas, 2012). Formulations might contain other active ingredients, such as simasine, 2,4-dichlorophenoxyacetic acid (2,4-D), or 4-chloro-2-methylphenoxyacetic acid (IPCS, 1996), with herbicide resistance driving demand for new herbicide formulations containing multiple active ingredients (Freedonia, 2012).

(b) Production volume

Glyphosate is reported to be manufactured by at least 91 producers in 20 countries, including 53 in China, 9 in India, 5 in the USA, and others in Australia, Canada, Cyprus, Egypt, Germany, Guatemala, Hungary, Israel, Malaysia, Mexico, Singapore, Spain, Taiwan (China), Thailand, Turkey, the United Kingdom, and Venezuela (Farm Chemicals International, 2015). Glyphosate was registered in over 130 countries as of 2010 and is probably the most heavily used herbicide in the world, with an annual global production volume estimated at approximately 600 000 tonnes in 2008, rising to about 650 000 tonnes in 2011, and to 720 000 tonnes in 2012 (Dill *et al.*, 2010; CCM International, 2011; Hilton, 2012; Transparency Market Research, 2014).

Production and use of glyphosate have risen dramatically due to the expiry of patent protection (see above), with increased promotion of non-till agriculture, and with the introduction in 1996 of genetically modified glyphosate-tolerant crop varieties (Székács & Darvas, 2012). In the USA alone, more than 80 000 tonnes of glyphosate were used in 2007 (rising from less than 4000 tonnes in 1987) (EPA, 1997, 2011). This rapid growth rate was also observed in Asia, which accounted for 30% of world demand for glyphosate in 2012 (Transparency Market Research, 2014). In India, production increased from 308 tonnes in 2003–2004, to 2100 tonnes in 2007–2008 (Ministry of Chemicals & Fertilizers, 2008). China currently produces more than 40% of the global supply of glyphosate, exports almost 35% of the global supply (Hilton, 2012), and reportedly has sufficient production capacity to satisfy total global demand (Yin, 2011).

1.2.2 Uses

Glyphosate is a broad-spectrum, post-emergent, non-selective, systemic herbicide, which effectively kills or suppresses all plant types, including grasses, perennials, vines, shrubs, and trees. When applied at lower rates, glyphosate is a plant-growth regulator and desiccant. It has agricultural and non-agricultural uses throughout the world.

(a) Agriculture

Glyphosate is effective against more than 100 annual broadleaf weed and grass species, and more than 60 perennial weed species (Dill *et al.*, 2010). Application rates are about 1.5–2 kg/ha for pre-harvest, post-planting, and pre-emergence use; about 4.3 kg/ha as a directed spray in vines, orchards, pastures, forestry, and industrial weed control; and about 2 kg/ha as an aquatic herbicide (Tomlin, 2000). Common application methods include broadcast, aerial, spot, and directed spray applications (EPA, 1993a).

Due to its broad-spectrum activity, the use of glyphosate in agriculture was formerly limited to post-harvest treatments and weed control between established rows of tree, nut, and vine crops. Widespread adoption of no-till and conservation-till practices (which require chemical weed control while reducing soil erosion and labour and fuel costs) and the introduction of transgenic crop varieties engineered to be resistant to glyphosate have transformed glyphosate to a post-emergent, selective herbicide for use on annual crops (Duke & Powles, 2009; Dill *et al.* 2010). Glyphosate-resistant transgenic varieties have been widely adopted for the production of corn, cotton, canola, and soybean (Duke & Powles, 2009). Production of such crops accounted for 45% of worldwide demand for glyphosate in 2012 (Transparency Market Research, 2014). However, in Europe,

where the planting of genetically modified crops has been largely restricted, post-harvest treatment is still the most common application of glyphosate (Glyphosate Task Force, 2014). Intense and continuous use of glyphosate has led to the emergence of resistant weeds that may reduce its effectiveness (Duke & Powles, 2009).

(b) Residential use

Glyphosate is widely used for household weed control throughout the world. In the USA, glyphosate was consistently ranked as the second most commonly used pesticide (after 2,4-D) in the home and garden market sector between 2001 and 2007, with an annual use of 2000–4000 tonnes (EPA, 2011).

(c) Other uses

Glyphosate was initially used to control perennial weeds on ditch banks and roadsides and under power lines (Dill *et al.*, 2010). It is also used to control invasive species in aquatic or wetland systems (Tu *et al.*, 2001). Approximately 1–2% of total glyphosate use in the USA is in forest management (Mance, 2012).

Glyphosate has been used in a large-scale aerial herbicide-spraying programme begun in 2000 to reduce the production of cocaine in Colombia (Lubick, 2009), and of marijuana in Mexico and South America (Székács & Darvas, 2012).

(d) Regulation

Glyphosate has been registered for use in at least 130 countries (Dill *et al.*, 2010). In the USA, all uses are eligible for registration on the basis of a finding that glyphosate "does not pose unreasonable risks or adverse effects to humans or the environment" (EPA, 1993a). A review conducted in 2001 in connection with the registration process in the European Union reached similar conclusions regarding animal and human safety, although the protection of groundwater during non-crop use was identified as requiring particular attention in the short term (European Commission, 2002).

Nevertheless, as worldwide rates of adoption of herbicide-resistant crops and of glyphosate use have risen in recent years (Duke & Powles, 2009), restriction of glyphosate use has been enacted or proposed in several countries, although documented actions are few. In 2013, the Legislative Assembly of El Salvador voted a ban on the use of pesticides containing glyphosate (República de El Salvador, 2013). Sri Lanka is reported to have instituted a partial ban based on an increasing number of cases of chronic kidney disease among agricultural workers, but the ban was lifted after 2 months (ColomboPage, 2014). The reasons for such actions have included the development of resistance among weed species, as well as health concerns.

No limits for occupational exposure were identified by the Working Group.

1.3 Measurement and analysis

Several methods exist for the measurement of glyphosate and its major metabolite aminomethyl phosphonic acid (AMPA) in various media, including air, water, urine, and serum (Table 1.1). The methods largely involve derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) to reach sufficient retention in chromatographic columns (Kuang *et al.*, 2011; Botero-Coy *et al.*, 2013). Chromatographic techniques that do not require derivatization and enzyme-linked immunosorbent assays (ELISA) are under development (Sanchís *et al.*, 2012).

Table 1.1 Methods for the analysis of glyphosate

Sample matrix	Assay procedure	Limit of detection	Reference
Water	HPLC/MS (with online solid-phase extraction)	0.08 µg/L	Lee *et al.* (2001)
	ELISA	0.05 µg/L	Abraxis (2005)
	LC-LC-FD	0.02 µg/L	Hidalgo *et al.* (2004)
	Post HPLC column derivatization and FD	6.0 µg/L	EPA (1992)
	UV visible spectrophotometer (at 435 ng)	1.1 µg/L	Jan *et al.* (2009)
Soil	LC–MS/MS with triple quadrupole	0.02 mg/kg	Botero-Coy *et al.* (2013)
Dust	GC-MS-MID	0.0007 mg/kg	Curwin *et al.* (2005)
Air	HPLC/MS with online solid-phase extraction	0.01 ng/m^3	Chang *et al.* (2011)
Fruits and vegetables	HILIC/WAX with ESI-MS/MS	1.2 µg/kg	Chen *et al.* (2013)
Field crops (rice, maize and soybean)	LC–ESI-MS/MS	0.007–0.12 mg/kg	Botero-Coy *et al.* (2013b)
Plant vegetation	HPLC with single polymeric amino column	0.3 mg/kg	Nedelkoska & Low (2004)
Serum	LC–MS/MS	0.03 µg/mL 0.02 µg/mL (aminomethylphosphonic acid) 0.01 µg/mL (3-methylphosphinicopropionic acid)	Yoshioka *et al.* (2011)
Urine	HPLC with post-column reaction and FD	1 µg/L	Acquavella *et al.* (2004)
	ELISA	0.9 µg/L	Curwin *et al.* (2007)

ELISA, enzyme-linked immunosorbent assay; ESI-MS/MS, electrospray tandem mass spectrometry; FD, fluorescence detection; GC-MS-MID, gas chromatography-mass spectrometry in multiple ion detection mode; HILIC/WAX, hydrophilic interaction/weak anion-exchange liquid chromatography; HPLC/MS, high-performance liquid chromatography with mass spectrometry; HPLC, high-performance liquid chromatography; LC-ESI–MS/MS, liquid chromatography-electrospray–tandem mass spectrometry; LC–LC, coupled-column liquid chromatography; LC–MS/MS, liquid chromatography–tandem mass spectrometry

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Studies related to occupational exposure to glyphosate have included farmers and tree nursery workers in the USA, forestry workers in Canada and Finland, and municipal weed-control workers in the United Kingdom (Centre de Toxicologie du Québec, 1988; Jauhiainen *et al.*, 1991; Lavy *et al.*, 1992; Acquavella *et al.*, 2004; Johnson *et al.*, 2005). Para-occupational exposures to glyphosate have also been measured in farming families (Acquavella *et al.*, 2004; Curwin *et al.*, 2007). These studies are summarized in Table 1.2.

(b) Community exposure

Glyphosate can be found in soil, air, surface water, and groundwater (EPA, 1993a). Once in the environment, glyphosate is adsorbed to soil and is broken down by soil microbes to AMPA (Borggaard & Gimsing, 2008). In surface water, glyphosate is not readily broken down by water or sunlight (EPA, 1993a). Despite extensive worldwide use, there are relatively few studies

Table 1.2 Occupational and para-occupational exposure to glyphosate

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Forestry				
Canada, 1986		Arithmetic mean of air glyphosate concentrations:	Air concentrations of glyphosate were measured at the work sites of one crew (five workers) during ground spraying 268 urine samples were collected from 40 workers; glyphosate concentration was above the LOD (15 µg/L) in 14%	Centre de Toxicologie du Québec (1988)
	Signaller	Morning, 0.63 µg/m³ Afternoon, 2.25 µg/m³		
	Operator	Morning, 1.43 µg/m³ Afternoon, 6.49 µg/m³		
	Overseer	Morning, 0.84 µg/m³ Afternoon, 2.41 µg/m³		
	Mixer	Morning, 5.15 µg/m³ Afternoon, 5.48 µg/m³		
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, < 1.25–15.7 µg/m³ (mean, NR)	Clearing work was done with brush saws equipped with pressurized herbicide sprayers Air samples were taken from the workers' breathing zone (number of samples, NR) Urine samples were collected during the afternoons of the working week (number, NR) Glyphosate concentrations in urine were below the LOD (10 µg/L)	Jauhiainen *et al.* (1991)
USA, year NR	Workers in two tree nurseries (n = 14)	In dermal sampling, 1 of 78 dislodgeable residue samples were positive for glyphosate The body portions receiving the highest exposure were ankles and thighs	Dermal exposure was assessed with gauze patches attached to the clothing and hand rinsing Analysis of daily urine samples repeated over 12 weeks was negative for glyphosate	Lavy *et al.* (1992)
Weed control				
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m³ in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m³ in 33% of 12 personal air samples collected from workers with backpack with lance applications	[The Working Group noted that the reported air concentrations were substantially higher than in other studies, but was unable to confirm whether the data were for glyphosate or total spray fluid] Dermal exposure was also measured, but reported as total spray fluid, rather than glyphosate	Johnson *et al.* (2005)

Table 1.2 (continued)

Industry, country, year	Job/process	Results	Comments/additional data	Reference
Farming				
USA, 2001	Occupational and para-occupational exposure of 24 farm families (24 fathers, 24 mothers and 65 children). Comparison group: 25 non-farm families (23 fathers, 24 mothers and 51 children)	Geometric mean (range) of glyphosate concentrations in urine: Non-farm fathers, 1.4 µg/L (0.13–5.4) Farm fathers, 1.9 µg/L (0.02–18) Non-farm mothers, 1.2 µg/L (0.06–5.0) Farm mothers, 1.5 µg/L (0.10–11) Non-farm children, 2.7 µg/L (0.10–9.4) Farm children, 2.0 µg/L (0.02–18)	Frequency of glyphosate detection ranged from 66% to 88% of samples (observed concentrations below the LOD were not censored). Detection frequency and geometric mean concentration were not significantly different between farm and non-farm families (observed concentrations below the LOD were not censored)	Curwin *et al.* (2007)
USA, year NR	Occupational and para-occupational exposures of 48 farmers, their spouses, and 79 children	Geometric mean (range) of glyphosate concentration in urine on day of application: Farmers, 3.2 µg/L (< 1 to 233 µg/L) Spouses, NR (< 1 to 3 µg/L) Children, NR (< 1 to 29 µg/L)	24-hour composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Glyphosate was detected in 60% of farmers' samples, 4% of spouses' samples and 12% of children's samples the day of spraying and in 27% of farmers' samples, 2% of spouses' samples and 5% of children's samples 3 days after	Acquavella *et al.* (2004)

LOD, limit of detection; ND, not detected; NR, not reported

on the environmental occurrence of glyphosate (Kolpin *et al.*, 2006).

(i) Air

Very few studies of glyphosate in air were available to the Working Group. Air and rainwater samples were collected during two growing seasons in agricultural areas in Indiana, Mississippi, and Iowa, USA (Chang *et al.*, 2011). The frequency of glyphosate detection ranged from 60% to 100% in air and rain samples, and concentrations ranged from < 0.01 to 9.1 ng/m^3 in air samples and from < 0.1 to 2.5 µg/L in rainwater samples. Atmospheric deposition was measured at three sites in Alberta, Canada. Rainfall and particulate matter were collected as total deposition at 7-day intervals throughout the growing season. Glyphosate deposition rates ranged from < 0.01 to 1.51 µg/m^2 per day (Humphries *et al.*, 2005).

No data were available to the Working Group regarding glyphosate concentrations in indoor air.

(ii) Water

Glyphosate in the soil can leach into groundwater, although the rate of leaching is believed to be low (Borggaard & Gimsing, 2008; Simonsen *et al.*, 2008). It can also reach surface waters by direct emission, atmospheric deposition, and by adsorption to soil particles suspended in runoff water (EPA, 1993a; Humphries *et al.*, 2005). Table 1.3 summarizes data on concentrations of glyphosate or AMPA in surface water and groundwater.

(iii) Residues in food and dietary intake

Glyphosate residues have been measured in cereals, fruits, and vegetables (Table 1.4). Residues were detected in 0.04% of 74 305 samples of fruits, vegetables, and cereals tested from 27 member states of the European Union, and from Norway, and Iceland in 2007 (EFSA, 2009). In cereals, residues were detected in 50% of samples tested in Denmark in 1998–1999, and in 9.5% of samples tested from member states of the European Union, and from Norway and Iceland in 2007 (Granby & Vahl, 2001; EFSA, 2009). In the United Kingdom, food sampling for glyphosate residues has concentrated mainly on cereals, including bread and flour. Glyphosate has been detected regularly and usually below the reporting limit (Pesticide Residues Committee, 2007, 2008, 2009, 2010). Six out of eight samples of tofu made from Brazilian soy contained glyphosate, with the highest level registered being 1.1 mg/kg (Pesticide Residues Committee, 2007).

(iv) Household exposure

In a survey of 246 California households, 14% were found to possess at least one product containing glyphosate (Guha *et al.*, 2013).

(v) Biological markers

Glyphosate concentrations in urine were analysed in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Colombia (MLHB, 2013; Varona *et al.*, 2009). Glyphosate concentrations in Colombia were considerably higher than in Europe, with means of 7.6 µg/L and 0.02 µg/L, respectively (Table 1.5). In a study in Canada, glyphosate concentrations in serum ranged from undetectable to 93.6 µg/mL in non-pregnant women (n = 39), and were undetectable in serum of pregnant women (n = 30) and fetal cord serum (Aris & Leblanc, 2011).

1.4.2 Exposure assessment

Exposure assessment methods in epidemiological studies on glyphosate and cancer are discussed in Sections 1.4.2 and 2.1 of the *Monograph* on Malathion, in the present volume.

Table 1.3 Concentration of glyphosate and AMPA in water

Country, year of sampling	Number of samples/setting	Results	Comments/additional data	Reference
USA, 2002	51 streams/agricultural areas (154 samples)	Maximum glyphosate concentration, 5.1 µg/L Maximum AMPA concentration, 3.67 µg/L	The samples were taken following pre- and post-emergence application and during harvest season Glyphosate detected in 36% of samples; AMPA detected in 69% of samples	Battaglin *et al.*, (2005)
USA, 2002	10 wastewater treatment plants and two reference streams (40 samples)	Glyphosate, range ≤ 0.1–2 µg/L AMPA, range ≤ 0.1–4 µg/L	AMPA was detected more frequently (67.5%) than glyphosate (17.5%)	Kolpin *et al.* (2006)
Canada, 2002	3 wetlands and 10 agricultural streams (74 samples)	Range, < 0.02–6.08 µg/L	Glyphosate was detected in most of the wetlands and streams (22% of samples)	Humphries *et al.* (2005)
Colombia, year NR	5 areas near crops and coca eradication (24 samples)	Maximum concentration, 30.1 µg/L (minimum and mean, NR)	Glyphosate detected in 8% of samples (MDL, 25 µg/L)	Solomon *et al.*, (2007)
Denmark, 2010–2012	4 agricultural sites (450 samples)	Range, < 0.1–31.0 µg/L	Glyphosate detected in 23% of samples; AMPA detected in 25% of samples	Brüch *et al.* (2013)

AMPA, aminomethylphosphonic acid; MDL, method detection limit; NR, data not reported

Table 1.4 Concentrations of glyphosate in food

Country, year	Type of food	Results	Comments/additional data	Reference
Denmark, 1998, 1999	Cereals	> 50% of samples had detectable residues Means: 0.08 mg/kg in 1999 and 0.11 mg/kg in 1998	49 samples of the 1998 harvest 46 samples of the 1999 harvest	Granby & Vahl (2001)
27 European Union member states, Norway and Iceland, 2007	350 different food commodities	0.04% of 2302 fruit, vegetable and cereal samples 9.5% of 409 cereal samples	74 305 total samples	EFSA (2009)
Australia, 2006	Composite sample of foods consumed in 24 hours	75% of samples had detectable residues Mean, 0.08 mg/kg Range, < 0.005 to 0.5 mg/kg	20 total samples from 43 pregnant women	McQueen *et al.* (2012)

Table 1.5 Concentrations of glyphosate and AMPA in urine and serum in the general population

Country, period	Subjects	Results	Comments/additional data	Reference
Urine				
18 European countries, 2013	162 individuals	Arithmetic mean of glyphosate concentration: 0.21 µg/L (maximum, 1.56 µg/L) Arithmetic mean of AMPA concentration: 0.19 µg/L (maximum, 2.63 µg/L)	44% of samples had quantifiable levels of glyphosate and 36% had quantifiable levels of AMPA	MLHB (2013)
Colombia, 2005–2006	112 residents of areas sprayed for drug eradication	Arithmetic mean (range) of glyphosate concentration: 7.6 µg/L (ND–130 µg/L) Arithmetic mean (range) of AMPA concentration: 1.6 µg/L (ND–56 µg/L)	40% of samples had detectable levels of glyphosate and 4% had detectable levels of AMPA (LODs, 0.5 and 1.0 µg/L, respectively) Urinary glyphosate was associated with use in agriculture	Varona *et al.* (2009)
Serum				
Canada, NR	30 pregnant women and 39 non-pregnant women	ND in serum of pregnant women or cord serum; Arithmetic mean, 73.6 ng/L, (range, ND–93.6 ng/L) in non-pregnant women	No subject had worked or lived with a spouse working in contact with pesticides LOD, 15 µg/L	Aris & Leblanc (2011)

AMPA, aminomethylphosphonic acid; LOD, limit of detection; ND, not detected; NR, not reported

2. Cancer in Humans

2.1 General discussion of epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.2 of the *Monograph* on Malathion.

2.2 Cohort studies

See Table 2.1

The Agricultural Health Study (AHS), a large prospective cohort study conducted in Iowa and North Carolina in the USA, is the only cohort study to date to have published findings on exposure to glyphosate and the risk of cancer at many different sites (Alavanja *et al.*, 1996; NIH, 2015) (see Section 2.2 of the *Monograph* on Malathion, in the present volume, for a detailed description of this study).

The enrolment questionnaire from the AHS sought information on the use of 50 pesticides (ever or never exposure), crops grown and livestock raised, personal protective equipment used, pesticide application methods used, other agricultural activities and exposures, nonfarm occup ational exposures, and several lifestyle, medical, and dietary variables. The duration (years) and frequency (days per year) of use was investigated for 22 of the 50 pesticides in the enrolment questionnaire. [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 power.]

The first report of cancer incidence associated with pesticide use in the AHS cohort considered cancer of the prostate (Alavanja *et al.*, 2003). Risk estimates for exposure to glyphosate were not presented, but no significant exposure–response association with cancer of the prostate was found. In an updated analysis of the AHS (1993 to 2001), De Roos *et al.* (2005a) (see below) also found no association between exposure to glyphosate and cancer of the prostate (relative risk, RR, 1.1; 95% CI, 0.9–1.3) and no exposure–response trend (*P* value for trend = 0.69).

De Roos *et al.* (2005a) also evaluated associations between exposure to glyphosate and the incidence of cancer at several other sites. The prevalence of ever-use of glyphosate was 75.5% (> 97% of users were men). In this analysis, exposure to glyphosate was defined as: (a) ever personally mixed or applied products containing glyphosate; (b) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and (c) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between exposure to glyphosate and incidence of all cancers combined, and incidence of 12 cancer types: lung, melanoma, multiple myeloma, and non-Hodgkin lymphoma (see Table 2.1) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, and leukaemia (results not tabulated). Exposure to glyphosate was not associated with all cancers combined (RR, 1.0; 95% CI, 0.9–1.2; 2088 cases). For multiple myeloma, the relative risk was 1.1 (95% CI, 0.5–2.4; 32 cases) when adjusted for age, but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders (age, smoking, other pesticides, alcohol consumption, family history of cancer, and education); in analyses by cumulative exposure-days and intensity-weighted exposure-days, the relative risks were around 2.0 in the highest tertiles. Furthermore, the association between multiple myeloma and exposure to glyphosate only appeared within the subgroup for which complete data were available on all the covariates; even without any adjustment, the risk of multiple myeloma associated with glyphosate use was increased by twofold among the smaller subgroup with available covariate data

Table 2.1 Cohort studies of cancer and exposure to glyphosate

Reference, study 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
De Roos *et al.* (2005a) Iowa and North Carolina, USA 1993–2001	54 315 (after exclusions, from a total cohort of 57 311) licensed pesticide applicators Exposure assessment method: questionnaire; semi-quantitative assessment from self-administered questionnaire	Lung	Ever use	NR	0.9 (0.6–1.3)	Age, smoking, other pesticides, alcohol consumption, family history of cancer, education	AHS Cancer sites investigated: lung, melanoma, multiple myeloma and NHL (results tabulated) as well as oral cavity, colon, rectum, pancreas, kidney, bladder, prostate and leukaemia (results not tabulated) [Strengths: large cohort; specific assessment of glyphosate; semiquantitative exposure assessment. Limitations: risk estimates based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
			Cumulative exposure days:				
			1–20	40	1 (ref.)		
			21–56	26	0.9 (0.5–1.5)		
			57–2678	26	0.7 (0.4–1.2)		
			Trend-test *P* value: 0.21				
		Melanoma	Ever use	NR	1.6 (0.8–3)		
			1–20	23	1 (ref.)		
			21–56	20	1.2 (0.7–2.3)		
			57–2678	14	0.9 (0.5–1.8)		
			Trend-test *P* value: 0.77				
		Multiple myeloma	Ever use	NR	2.6 (0.7–9.4)		
			1–20	8	1 (ref.)		
			21–56	5	1.1 (0.4–3.5)		
			Trend-test *P* value: 0.27				
		NHL	Ever use	NR	1.1 (0.7–1.9)		
			1–20	29	1 (ref.)		
			21–56	15	0.7 (0.4–1.4)		
			57–2678	17	0.9 (0.5–1.6)		
			Trend-test *P* value: 0.73				

Table 2.1 (continued)

Reference, study 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
Flower *et al.* (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, 1975–1998	21 375; children (aged < 19 years) of licensed pesticide applicators in Iowa (n = 17 357) and North Carolina (n = 4018) Exposure assessment method: questionnaire	Childhood cancer	Maternal use of glyphosate (ever) Paternal use of glyphosate (prenatal)	13 6	0.61 (0.32–1.16) 0.84 (0.35–2.34)	Child's age at enrolment	AHS Glyphosate results relate to the Iowa participants only [Strengths: Large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; potential exposure to multiple pesticides; limited power for glyphosate exposure]
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 Exposure assessment method: questionnaire	Breast	Direct exposure to glyphosate Husband's use of glyphosate	82 109	0.9 (0.7–1.1) 1.3 (0.8–1.9)	Age, race, state	AHS [Strengths: large cohort; specific assessment of glyphosate. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]
Lee *et al.* (2007) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2002	56 813 licensed pesticide applicators Exposure assessment method: questionnaire	Colorectum Colon Rectum	Exposed to glyphosate Exposed to glyphosate Exposed to glyphosate	225 151 74	1.2 (0.9–1.6)	Age, smoking, state, total days of any pesticide application	AHS [Strengths: large cohort. Limitations: based on self-reported exposure, limited to licensed applicators, potential exposure to multiple pesticides]

Table 2.1 (continued)

Reference, study 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
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 years 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 intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides was assessed	Pancreas (C25.0–C25.9)	Ever exposure to glyphosate Low (< 185 days) High (≥ 185 days) Trend-test *P* value: 0.85	55 29 19	1.1 (0.6–1.7)	Age, smoking, diabetes	AHS [Strengths: large cohort. Limitations: based on self-reported exposure; limited to licensed applicators; potential exposure to multiple pesticides]

AHS, Agricultural Health Study; NHL, non-Hodgkin lymphoma; NR, not reported

(De Roos *et al.*, 2005b). [The study had limited power for the analysis of multiple myeloma; there were missing data on covariates when multiple adjustments were done, limiting the interpretation of the findings.] A re-analysis of these data conducted by Sorahan (2015) confirmed that the excess risk of multiple myeloma was present only in the subset with no missing information (of 22 cases in the restricted data set). In a subsequent cross-sectional analysis of 678 male participants from the same cohort, Landgren *et al.* (2009) did not find an association between exposure to glyphosate and risk of monoclonal gammopathy of undetermined significance (MGUS), a premalignant plasma disorder that often precedes multiple myeloma (odds ratio, OR, 0.5; 95% CI, 0.2–1.0; 27 exposed cases).

Flower *et al.* (2004) reported the results of the analyses of risk of childhood cancer associated with pesticide application by parents in the AHS. The analyses for glyphosate were conducted among 17 357 children of Iowa pesticide applicators from the AHS. Parents provided data via questionnaires (1993–1997) and the cancer follow-up (retrospectively and prospectively) was done through the state cancer registries. Fifty incident childhood cancers were identified (1975–1998; age, 0–19 years). For all the children of the pesticide applicators, risk was increased for all childhood cancers combined, for all lymphomas combined, and for Hodgkin lymphoma, compared with the general population. The odds ratio for use of glyphosate and risk of childhood cancer was 0.61 (95% CI, 0.32–1.16; 13 exposed cases) for maternal use and 0.84 (95% CI, 0.35–2.34; 6 exposed cases) for paternal use. [The Working Group noted that this analysis had limited power to study a rare disease such as childhood cancer.]

Engel *et al.* (2005) reported on incidence of cancer of the breast among farmers' wives in the AHS cohort, which included 30 454 women with no history of cancer of the breast before enrolment in 1993–1997. Information on pesticide use and other factors was obtained at enrolment by self-administered questionnaire from the women and their husbands. A total of 309 incident cases of cancer of the breast were identified until 2000. There was no difference in incidence of cancer of the breast for women who reported ever applying pesticides compared with the general population. The relative risk for cancer of the breast among women who had personally used glyphosate was 0.9 (95% CI, 0.7–1.1; 82 cases) and 1.3 (95% CI, 0.8–1.9; 109 cases) among women who never used pesticides but whose husband had used glyphosate. [No information on duration of glyphosate use by the husband was presented.] Results for glyphosate were not further stratified by menopausal status.

Lee *et al.* (2007) investigated the relationship between exposure to agricultural pesticides and incidence of cancer of the colorectum in the AHS. A total of 56 813 pesticide applicators with no prior history of cancer of the colorectum were included in this analysis, and 305 incident cancers of the colorectum (colon, 212; rectum, 93) were diagnosed during the study period, 1993–2002. Most of the 50 pesticides studied were not associated with risk of cancer of the colorectum, and the relative risks with exposure to glyphosate were 1.2 (95% CI, 0.9–1.6), 1.0 (95% CI, 0.7–1.5), and 1.6 (95% CI, 0.9–2.9) for cancers of the colorectum, colon, and rectum, respectively.

Andreotti *et al.* (2009) examined associations between the use of pesticides and cancer of the pancreas using a case–control analysis nested in the AHS. This analysis included 93 incident cases of cancer of the pancreas (64 applicators, 29 spouses) and 82 503 cancer-free controls who completed the enrolment questionnaire. Ever-use of 24 pesticides and intensity-weighted lifetime days [(lifetime exposure days) × (exposure intensity score)] of 13 pesticides were assessed. Risk estimates were calculated controlling for age, smoking, and diabetes. The odds ratio for ever- versus never-exposure to glyphosate was

1.1 (95% CI, 0.6–1.7; 55 exposed cases), while the odds ratio for the highest category of level of intensity-weighted lifetime days was 1.2 (95% CI, 0.6–2.6; 19 exposed cases).

Dennis *et al.* (2010) reported that exposure to glyphosate was not associated with cutaneous melanoma within the AHS. [The authors did not report a risk estimate.]

2.3 Case–control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

2.3.1 Non-Hodgkin lymphoma

See Table 2.2

(a) Case–control studies in the midwest USA

Cantor *et al.* (1992) conducted a case–control study of incident non-Hodgkin lymphoma (NHL) among males in Iowa and Minnesota, USA (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). A total of 622 white men and 1245 population-based controls were interviewed in person. The association with farming occupation and specific agricultural exposures were evaluated. When compared with non-farmers, the odds ratios for NHL were 1.2 (95% CI, 1.0–1.5) for men who had ever farmed, and 1.1 (95% CI, 0.7–1.9; 26 exposed cases; adjusted for vital status, age, state, cigarette smoking status, family history of lymphohaematopoietic cancer, high-risk occupations, and high-risk exposures) for ever handling glyphosate. [There was low power to assess the risk of NHL associated with exposure to glyphosate. There was no adjustment for other pesticides. These data were included in the pooled analysis by De Roos *et al.* (2003).]

Brown *et al.* (1993) reported the results of a study to evaluate the association between multiple myeloma and agricultural risk factors in the midwest USA (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). A population-based case–control study of 173 white men with multiple myeloma and 650 controls was conducted in Iowa, USA, an area with a large farming population. A non-significantly elevated risk of multiple myeloma was seen among farmers compared with never-farmers. The odds ratio related to exposure to glyphosate was 1.7 (95% CI, 0.8–3.6; 11 exposed cases). [This study had limited power to assess the association between multiple myeloma and exposure to glyphosate. Multiple myeloma is now considered to be a subtype of NHL.]

De Roos *et al.* (2003) used pooled data from three case–control studies of NHL conducted in the 1980s in Nebraska (Zahm *et al.*, 1990), Kansas (Hoar *et al.*, 1986), and in Iowa and Minnesota (Cantor *et al.*, 1992) (see the *Monograph* on Malathion, Section 2.2, for a detailed description of these studies) to examine pesticide exposures in farming as risk factors for NHL in men. The study population included 870 cases and 2569 controls; 650 cases and 1933 controls were included for the analysis of 47 pesticides controlling for potential confounding by other pesticides. Both logistic regression and hierarchical regression (adjusted estimates were based on prior distributions for the pesticide effects, which provides more conservative estimates than logistic regression) were used in data analysis, and all models were essentially adjusted for age, study site, and other pesticides. Reported use of glyphosate as well as several individual pesticides was associated with increased incidence of NHL. Based on 36 cases exposed, the odds ratios for the association between exposure to glyphosate and NHL were 2.1 (95% CI, 1.1–4.0) in the logistic regression analyses and 1.6 (95% CI, 0.9–2.8) in the hierarchical regression analysis. [The numbers of cases and controls were lower than those in the pooled analysis by Waddell *et al.* (2001) because only subjects with no missing data on pesticides were included. The strengths of this study when compared with other studies are that it was large,

Table 2.2 Case–control studies of leukaemia and lymphoma and exposure to glyphosate

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
USA							
Brown *et al.* (1990) Iowa and Minnesota, USA 1981–1983	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 years and Medicare for those aged ≥ 65 years Exposure assessment method: questionnaire	Leukaemia	Any glyphosate	15	0.9 (0.5–1.6)	Age, vital status, state, tobacco use, family history lymphopoietic cancer, high-risk occupations, high risk exposures	[Strengths: large population based study in a farming area. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]
Cantor *et al.* (1992) Iowa and Minnesota, USA 1980–1982	Cases: 622 (response rate, 89.0%); Iowa health registry records and Minnesota hospital and pathology records Controls: 1245 (response rate, 76–79%); population-based; no cancer of the lympho-haematopoietic system; frequency-matched to cases by age (5-year group), vital status, state. Random-digit dialling (aged < 65 years); Medicare records (aged ≥ 65 years); state death certificate files (deceased subjects) Exposure assessment method: questionnaire; in-person interview	NHL	Ever handled glyphosate	26	1.1 (0.7–1.9)	Age, vital status, state, smoking status, family history lymphopoietic cancer, high-risk occupations, high-risk exposures	Data subsequentially pooled in De Roos *et al.* (2003); white men only [Strengths: large population-based study in farming areas. Limitations: not controlled for exposure to other pesticides. Limited power for glyphosate exposure]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown *et al.* (1993) Iowa, USA 1981–1984	Cases: 173 (response rate, 84%); Iowa health registry Controls: 650 (response rate, 78%); Random-digit dialling (aged < 65 years) and Medicare (aged > 65 years) Exposure assessment method: questionnaire	Multiple myeloma	Any glyphosate	11	1.7 (0.8–3.6)	Age, vital status	[Strengths: population-based study. Areas with high prevalence of farming. Limitations: limited power for glyphosate exposure]
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	Any glyphosate exposure	36	2.1 (1.1–4)	Age, study area, other pesticides	Both logistic regression and hierarchical regression were used in data analysis, the latter providing more conservative estimates [Strengths: increased power when compared with other studies, population-based, and conducted in farming areas. Advanced analytical methods to account for multiple exposures] Included participants from Cantor *et al.* (1992), Zahm *et al.* (1990), Hoar *et al.* (1986), and Brown *et al.* (1990)

Table 2.2 (continued)

Reference, location, enrolment period	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.* (2004a) Iowa, Minnesota and Nebraska, USA 1980–1986	Cases: 872 (response rate, NR); diagnosed with NHL from 1980 to 1986 Controls: 2381 (response rate, NR); frequency-matched controls Exposure assessment method: questionnaire; information on use of pesticides and history of asthma was based on interviews	NHL	Exposed to glyphosate – non-asthmatics Exposed to glyphosate – asthmatics	53 6	1.4 (0.98–2.1) 1.2 (0.4–3.3)	Age, vital status, state	177 participants (45 NHL cases, 132 controls) reported having been told by their doctor that they had asthma
Canada							
McDuffie *et al.* (2001) Canada 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, some administered by telephone, some by post	NHL	Exposed to glyphosate Unexposed > 0 and ≤ 2 days > 2 days	51 464 28 23	1.2 (0.83–1.74) 1 1.0 (0.63–1.57) 2.12 (1.2–3.73)	Age, province of residence	Cross-Canada study [Strengths: large population based study. Limitations: no quantitative exposure data. Exposure assessment by questionnaire. Relatively low participation]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Karunanayake *et al.* (2012) Six provinces in Canada (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia) 1991–1994	Incident cases: 316 (response rate, 68.4%); men aged ≥ 19 years; ascertained from provincial cancer registries, except in Quebec (hospital ascertainment) Controls: 1506 (response rate, 48%); matched by age ± 2 years to be comparable with the age distribution of the entire case group (HL, NHL, MM, and STS) within each province of residence. Potential controls (men aged ≥ 19 years) selected at random within age constraints from the provincial health insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia) Exposure assessment method: questionnaire; stage 1 used a self-administered postal questionnaire; and in stage 2 detailed pesticide exposure information was collected by telephone interview	HL (ICDO2 included nodular sclerosis (M9656/3; M9663/3; M9664/3; M9665/3; M9666/3; M9667/3), lymphocytic predominance (M9651/3; M9657/3; M9658/3; M9659/3), mixed cellularity (M9652/3), lymphocytic depletion (M9653/3; M9654/3), miscellaneous (other M9650-M9669 codes for HL)	Glyphosate-based formulation Glyphosate-based formulation	38 38	1.14 (0.74–1.76) 0.99 (0.62–1.56)	Age group, province of residence Age group, province of residence, medical history	Cross Canada study Based on the statistical analysis of pilot study data, it was decided that the most efficient definition of pesticide exposure was a cumulative exposure ≥ 10 hours/year to any combination of pesticides. This discriminated (a) between incidental, bystander, and environmental exposure vs more intensive exposure, and (b) between cases and controls [Strengths: large study. Limitations: low response rates]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kachuri *et al.* (2013) Six Canadian provinces (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and Quebec) 1991–1994	Cases: 342 (response rate, 58%); men aged ≥ 19 years 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 years selected randomly using provincial health insurance records, random digit dialling, or voters' lists, frequency-matched to cases by age (±2 years) and province of residence Exposure assessment method: questionnaire	Multiple myeloma	Glyphosate use Use of glyphosate (> 0 and ≤ 2 days per year) Use of glyphosate (> 2 days per year)	32 15 12	1.19 (0.76–1.87) 0.72 (0.39–1.32) 2.04 (0.98–4.23)	Age, province of residence, use of a proxy respondent, smoking status, medical variables, family history of cancer	Cross-Canada study [Strengths: population-based case–control study. Limitations: relatively low response rates]
Sweden							
Nordström *et al.* (1998) Sweden 1987–1992	Cases: 111 (response rate, 91%); 121 HCL cases in men identified from Swedish cancer registry Controls: 400 (response rate, 83%); 484 (four controls/case) matched for age and county; national population registry Exposure assessment method: questionnaire; considered exposed if minimum exposure of 1 working day (8 h) and an induction period of at least 1 year	HCL	Exposed to glyphosate	4	3.1 (0.8–12)	Age	Overlaps with Hardell *et al.* (2002). HCL is a subtype of NHL [Strengths: population-based case–control study. Limitations: Limited power. There was no adjustment for other exposures]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hardell & Eriksson (1999) Northern and middle Sweden 1987–1990	Cases: 404 (192 deceased) (response rate, 91%); regional cancer registries Controls: 741 (response rate, 84%); live controls matched for age and county were recruited from the national population registry, and deceased cases matched for age and year of death were identified from the national registry for causes of death Exposure assessment method: questionnaire	NHL (ICD-9 200 and 202)	Ever glyphosate – univariate Ever glyphosate – multivariate	4 NR	2.3 (0.4–13) 5.8 (0.6–54)	Not specified in the multivariable analysis	Overlaps with Hardell *et al.* (2002) [Strengths: population-based study. Limitations: few subjects were exposed to glyphosate and the study had limited power. Analyses were "multivariate" but covariates were not specified]
Hardell *et al.* (2002) Sweden; four Northern counties and three counties in mid Sweden 1987–1992	Cases: 515 (response rate, 91% in both studies); Swedish cancer registry Controls: 1141 (response rates, 84% and 83%%); national population registry Exposure assessment method: questionnaire	NHL and HCL	Ever glyphosate exposure (univariate) Ever glyphosate exposure (multivariate)	8 8	3.04 (1.08–8.5) 1.85 (0.55–6.2)	Age, county, study site, vital status, other pesticides in the multivariate analysis	Overlaps with Nordström *et al.* (1998) and Hardell & Eriksson (1999), [Strengths: large population-based study. Limitations: limited power for glyphosate exposure]

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Eriksson *et al.* (2008) Sweden. Four health service areas (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	Any glyphosate	29	2.02 (1.1–3.71)	Age, sex, year of enrolment	[Strengths: population-based case-control. Limitations: limited power for glyphosate] * Exposure to other pesticides (e.g. MPCA) controlled in the analysis
			Any glyphosate*	29	1.51 (0.77–2.94)		
			≤ 10 days per year use	12	1.69 (0.7–4.07)		
			> 10 days per year use	17	2.36 (1.04–5.37)		
		NHL	1–10 yrs	NR	1.11 (0.24–5.08)		
			> 10 yrs	NR	2.26 (1.16–4.4)		
		B-cell lymphoma	Exposure to glyphosate	NR	1.87 (0.998–3.51)		
		Lymphocytic lymphoma/B-CLL	Exposure to glyphosate	NR	3.35 (1.42–7.89)		
		Diffuse large B-cell lymphoma	Exposure to glyphosate	NR	1.22 (0.44–3.35)		
		Follicular, grade I–III	Exposure to glyphosate	NR	1.89 (0.62–5.79)		
		Other specified B-cell lymphoma	Exposure to glyphosate	NR	1.63 (0.53–4.96)		
		Unspecified B-cell lymphoma	Exposure to glyphosate	NR	1.47 (0.33–6.61)		
		T-cell lymphoma	Exposure to glyphosate	NR	2.29 (0.51–10.4)		
		Unspecified NHL	Exposure to glyphosate	NR	5.63 (1.44–22)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Other studies in Europe							
Orsi *et al.* (2009) France 2000–2004	Cases: 491 (response rate, 95.7%); cases (244 NHL; 87 HL; 104 LPSs; 56 MM) were recruited from main hospitals of the French cities of Brest, Caen, Nantes, Lille, Toulouse and Bordeaux, aged 20–75 years; ALL cases excluded Controls: 456 (response rate, 91.2%); matched on age and sex, recruited in the same hospitals as the cases, mainly in orthopaedic and rheumatological departments and residing in the hospital's catchment area Exposure assessment method: questionnaire	NHL	Any glyphosate exposure	12	1.0 (0.5–2.2)	Age, centre, socioeconomic category (blue/ white collar)	[Limitations: limited power for glyphosate]
		HL	Any exposure to glyphosate	6	1.7 (0.6–5)		
		LPS	Any exposure to glyphosate	4	0.6 (0.2–2.1)		
		MM	Any exposure to glyphosate	5	2.4 (0.8–7.3)		
		All lymphoid neoplasms	Any exposure to glyphosate	27	1.2 (0.6–2.1)		
		NHL, diffuse large cell lymphoma	Occupational use of glyphosate	5	1.0 (0.3–2.7)		
		NHL, follicular lymphoma	Occupational exposure to glyphosate	3	1.4 (0.4–5.2)		
		LPS/CLL	Occupational exposure to glyphosate	2	0.4 (0.1–1.8)		
		LPS/HCL	Occupational exposure to glyphosate	2	1.8 (0.3–9.3)		

Table 2.2 (continued)

Reference, location, enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cocco *et al.* (2013) Czech Republic, France, Germany, Italy, Ireland and Spain 1998–2004	Cases: 2348 (response rate, 88%); cases were all consecutive adult patients first diagnosed with lymphoma during the study period, resident in the referral area of the participating centres Controls: 2462 (response rate, 81% hospital; 52% population); controls from Germany and Italy were randomly selected by sampling from the general population and matched to cases on sex, 5-year age-group, and residence area. The rest of the centres used matched hospital controls, excluding diagnoses of cancer, infectious diseases and immunodeficiency diseases Exposure assessment method: questionnaire; support of a crop-exposure matrix to supplement the available information, industrial hygienists and occupational experts in each participating centre reviewed the general questionnaires and job modules to assess exposure to pesticides	B-cell lymphoma	Occupational exposure to glyphosate	4	3.1 (0.6–17.1)	Age, sex, education, centre	EPILYMPH case-control study in six European countries

ALL, acute lymphocytic leukaemia; B-CLL, chronic lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; HCL, hairy cell leukaemia; HL, Hodgkin lymphoma; LPS, lymphoproliferative syndrome; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ref., reference; STS, soft tissue sarcoma

population-based, and conducted in farming areas. Potential confounding from multiple exposures was accounted for in the analysis.]

Using the data set of the pooled population-based case–control studies in Iowa, Minnesota, and Nebraska, USA, Lee *et al.* (2004a) investigated whether asthma acts as an effect modifier of the association between pesticide exposure and NHL. The study included 872 cases diagnosed with NHL from 1980 to 1986 and 2381 frequency-matched controls. Information on use of pesticides and history of asthma was based on interviews. A total of 177 subjects (45 cases, 132 controls) reported having been told by their doctor that they had asthma. Subjects with a history of asthma had a non-significantly lower risk of NHL than non-asthmatics, and there was no main effect of pesticide exposure. In general, asthmatics tended to have larger odds ratios associated with exposure to pesticides than non-asthmatics. There was no indication of effect modification: the odds ratio associated with glyphosate use was 1.4 (95% CI, 0.98–2.1; 53 exposed cases) among non-asthmatics and 1.2 (95% CI, 0.4–3.3; 6 exposed cases) for asthmatics, when compared with non-asthmatic non-exposed farmers). [This analysis overlapped with that of De Roos *et al.* (2003).]

(b) The cross-Canada case–control study

McDuffie *et al.* (2001) studied the associations between exposure to specific pesticides and NHL in a multicentre population-based study with 517 cases and 1506 controls among men of six Canadian provinces (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Odds ratios of 1.26 (95% CI, 0.87–1.80; 51 exposed cases; adjusted for age and province) and 1.20 (95% CI, 0.83–1.74, adjusted for age, province, high-risk exposures) were observed for exposure to glyphosate. In an analysis by frequency of exposure to glyphosate, participants with > 2 days of exposure per year had an odds ratio of 2.12 (95% CI, 1.20–3.73, 23 exposed cases) compared with those with some, but ≤ 2 days of exposure. [The study was large, but had relatively low participation rates.]

Kachuri *et al.* (2013) investigated the association between lifetime use of pesticides and multiple myeloma in a population-based case–control study among men in six Canadian provinces between 1991 and 1994 (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Data from 342 cases of multiple myeloma and 1357 controls were obtained for ever-use of pesticides, number of pesticides used, and days per year of pesticide use. The odds ratios were adjusted for age, province of residence, type of respondent, smoking and medical history. The odds ratio for ever-use of glyphosate was 1.19 (95% CI, 0.76–1.87; 32 cases). When the analysis was conducted by level of exposure, no association was found for light users (≤ 2 days per year) of glyphosate (OR, 0.72; 95% CI, 0.39–1.32; 15 exposed cases) while the odds ratio in heavier users (> 2 days per year) was 2.04 (95% CI, 0.98–4.23; 12 exposed cases). [The study had relatively low response rates. Multiple myeloma is now considered a subtype of NHL.]

(c) Case–control studies in Sweden

Nordström *et al.* (1998) conducted a population case–control study in Sweden on hairy cell leukaemia (considered to be a subgroup of NHL). The study included 121 cases in men and 484 controls matched for age and sex. An age-adjusted odds ratio of 3.1 (95% CI, 0.8–12; 4 exposed cases) was observed for exposure to glyphosate. [This study had limited power to detect an effect, and there was no adjustment for other exposures.]

Hardell & Eriksson (1999) reported the results of a population-based case–control study on the incidence of NHL in men associated with pesticide exposure in four northern counties in Sweden. Exposure data was collected by questionnaire (also supplemented by telephone interviews) from 404 cases (192 deceased) and 741

controls (matched by age, sex, county, and vital status). Increased risks of NHL were found for subjects exposed to herbicides and fungicides. The odds ratio for ever-use of glyphosate was 2.3 (95% CI, 0.4–13; 4 exposed cases) in a univariate analysis, and 5.8 (95% CI, 0.6–54) in a multivariable analysis. [The exposure frequency was low for glyphosate, and the study had limited power to detect an effect. The variables included in the multivariate analysis were not specified. This study may have overlapped partially with those of Hardell *et al.* (2002).]

Hardell *et al.* (2002) conducted a pooled analysis of two case–control studies, one on NHL (already reported in Hardell & Eriksson, 1999) and another on hairy cell leukaemia, a subtype of NHL (already reported by Nordström *et al.*, 1998). The pooled analysis of NHL and hairy cell leukaemia was based on 515 cases and 1141 controls. Increased risk was found for exposure to glyphosate (OR, 3.04; 95% CI, 1.08–8.52; 8 exposed cases) in the univariate analysis, but the odds ratio decreased to 1.85 (95% CI, 0.55–6.20) when study, study area, and vital status were considered in a multivariate analysis. [The exposure frequency was low for glyphosate and the study had limited power. This study partially overlapped with those of Hardell & Eriksson (1999) and Nordström *et al.* (1998).]

Eriksson *et al.* (2008) reported the results of a population based case–control study of exposure to pesticides as a risk factor for NHL. Men and women aged 18–74 years living in Sweden were included from 1 December 1999 to 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 (91%) cases and 1016 (92%) controls participated. Multivariable models included agents with 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, year of diagnosis or enrolment. The odds ratio for exposure to glyphosate was 2.02 (95% CI, 1.10–3.71) in a univariate analysis, and 1.51 (95% CI, 0.77–2.94) in a multivariable analysis. When exposure for more than 10 days per year was considered, the odds ratio was 2.36 (95% CI, 1.04–5.37). With a latency period of > 10 years, the odds ratio was 2.26 (95% CI, 1.16–4.40). The associations with exposure to glyphosate were reported also for lymphoma subtypes, and elevated odds ratios were reported for most of the cancer forms, including B-cell lymphoma (OR, 1.87; 95% CI, 0.998–3.51) and the subcategory of small lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89; [not adjusted for other pesticides]). [This was a large study; there was possible confounding from use of other pesticides including MCPA, but this was considered in the analysis.]

(d) Other case–control studies in Europe

Orsi *et al.* (2009) reported the results of a hospital-based case–control study conducted in six centres in France between 2000 and 2004. Incident cases with a diagnosis of lymphoid neoplasm aged 20–75 years and controls of the same age and sex as the cases were recruited in the same hospital, mainly in the orthopaedic and rheumatological departments during the same period. [The Working Group noted that the age of case eligibility was given in the publication as 20–75 years in the materials and methods section, but as 18–75 years in the abstract.] Exposures to pesticides were evaluated through specific interviews and case-by-case expert reviews. The analyses included 491 cases (244 cases of NHL, 87 cases of Hodgkin lymphoma), 104 of lymphoproliferative syndrome, and 56 cases of multiple myeloma), and 456 age- and sex-matched controls. Positive associations between some subtypes and occupational exposure to several pesticides

were noted. The odds ratios associated with any exposure to glyphosate were 1.2 (95% CI, 0.6–2.1; 27 exposed cases) for all lymphoid neoplasms combined, 1.0 (95% CI, 0.5–2.2; 12 exposed cases) for NHL, 0.6 (95% CI, 0.2–2.1; 4 exposed cases) for lymphoproliferative syndrome, 2.4 (95% CI, 0.8–7.3) for multiple myeloma, and 1.7 (95% CI, 0.6–5.0; 6 exposed cases) for Hodgkin lymphoma, after adjusting for age, centre, and socioeconomic category ("blue/white collar").

Cocco *et al.* (2013) reported the results of a pooled analysis of case–control studies conducted in six European countries in 1998–2004 (EPILYMPH, Czech Republic, France, Germany, Ireland, Italy, and Spain) to investigate the role of occupational exposure to specific groups of chemicals in the etiology of lymphoma overall, B-cell lymphoma, and its most prevalent subtypes. A total of 2348 incident cases of lymphoma and 2462 controls were recruited. Controls from Germany and Italy were randomly selected by sampling from the general population, while the rest of the centres used matched hospital controls. Overall, the participation rate was 88% for cases, 81% for hospital controls, and 52% for population controls. An occupational history was collected with farm work-specific questions on type of crop, farm size, pests being treated, type and schedule of pesticide use. In each study centre, industrial hygienists and occupational experts assessed exposure to specific groups of pesticides and individual compounds with the aid of agronomists. [Therefore any exposure misclassification would be non-differential.] Analyses were conducted for lymphoma and the most prevalent lymphoma subtypes adjusting for age, sex, education, and centre. Lymphoma overall, and B-cell lymphoma were not associated with any class of the investigated pesticides, while the risk of chronic lymphocytic leukaemia was elevated among those ever exposed to inorganic and organic pesticides. Only for a few individual agrochemicals was there a sizeable number of study subjects to conduct a meaningful analysis, and the odds ratio for exposure to glyphosate and B-cell lymphoma was 3.1 (95% CI, 0.6–17.1; 4 exposed cases and 2 exposed controls). [The study had a very limited power to assess the effects of glyphosate on risk of NHL.]

2.3.2 Other haematopoietic cancers

Orsi *et al.* (2009) also reported results for Hodgkin lymphoma (see Section 2.3.1).

Karunanayake *et al.* (2012) conducted a case–control study of Hodgkin lymphoma among white men, aged 19 years or older, in six regions of Canada (see the Malathion *Monograph*, Section 2.2, for a detailed description of this study). The analysis included 316 cases and 1506 age-matched (± 2 years) controls. Based on 38 cases exposed to glyphosate, the odds ratios were 1.14 (95% CI, 0.74–1.76) adjusted for age and province, and 0.99 (95% CI, 0.62–1.56) when additionally adjusted for medical history variables.

Brown *et al.* (1990) evaluated exposure to carcinogens in an agricultural setting and the relationship with leukaemia in a population-based case–control interview study in Iowa and Minnesota, USA, including 578 white men with leukaemia and 1245 controls. The exposure assessment was done with a personal interview of the living subjects or the next-of-kin. Farmers had a higher risk of all leukaemias compared with non-farmers, and associations were found for exposure to specific animal insecticides, including the organophosphates crotoxyphos, dichlorvos, famphur, pyrethrins, and methoxychlor. The odds ratio for glyphosate was 0.9 (95% CI, 0.5–1.6; 15 exposed cases; adjusted for vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures). [This was a large study in an agricultural setting, but had limited power for studying the effects of glyphosate use.]

2.4 Case–control studies on other cancer sites

2.4.1 Cancer of the oesophagus and stomach

Lee *et al.* (2004b) evaluated the risk of adenocarcinomas of the oesophagus and stomach associated with farming and agricultural pesticide use. The population-based case–control study was conducted in eastern Nebraska, USA. Subjects of both sexes 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. 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. For ever-use of glyphosate, the odds ratio was 0.8 (95% CI, 0.4–1.4; 12 exposed cases) for cancer of the stomach, and 0.7 (95% CI, 0.3–1.4; 12 exposed cases) for oesophageal cancer. [The study was conducted in a farming area, but the power to detect an effect of glyphosate use was limited.]

2.4.2 Cancer of the brain

Ruder *et al.* (2004) conducted a case–control study on glioma among nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. The study included 457 cases of glioma and 648 population-based controls, all adult men. Exposure assessment was done with interviews of the subject or the relatives. The response rates were 93% and 70% for cases and controls, respectively. No association were found with any of the pesticides assessed, including glyphosate. [Glyphosate use was assessed, but specific results were not presented.]

Carreón *et al.* (2005) evaluated the effects of rural exposures to pesticides on risk of glioma among women aged 18–80 years who were nonmetropolitan residents of Iowa, Michigan, Minnesota, and Wisconsin in the Upper Midwest Health Study, USA. A total of 341 cases of glioma and 528 controls were enrolled. A personal interview was carried out for exposure assessment. The response rates were 90% and 72%, respectively. After adjusting for age, age group, education, and farm residence, no association with glioma was observed for exposure to several pesticide classes or individual pesticides. There was a reduced risk for glyphosate (OR, 0.7; 95% CI, 0.4–1.3; 18 exposed cases). These results were not affected by the exclusion of proxy respondents (43% of cases, 2% of controls).

Lee *et al.* (2005) evaluated the association between farming and agricultural pesticide use and risk of adult glioma in a population-based case–control study in eastern Nebraska, USA. Cases of glioma were in men and women (n = 251) and were compared with population controls from a previous study (n = 498). A telephone interview was conducted for 89% of the cases and 83% of the controls. Adjusted odds ratios for farming and for use of individual and chemical classes of insecticides and herbicides were calculated using non-farmers as the reference category. Among men, ever living or working on a farm and duration of farming were associated with significantly increased risks of glioma, but the positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Some specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers, but most of the positive associations were limited to proxy respondents. There was a non-significant excess risk with glyphosate use for the overall group (OR, 1.5; 95% CI, 0.7–3.1; 17 exposed cases), but there was inconsistency between observations for self-respondents (OR,

0.4; 95% CI, 0.1–1.6) and observations for proxy respondents (OR, 3.1; 95% CI, 1.2–8.2). [The study had limited power to detect an effect of glyphosate use, and the inconsistencies for self and proxy respondents made the results difficult to interpret.]

2.4.3 Soft tissue sarcoma

Pahwa *et al.* (2011) reported the results of the soft tissue sarcoma component of the cross-Canada study in relation to specific pesticides, including 357 cases of soft tissue sarcoma and 1506 population controls from 1991–1994. The fully adjusted odds ratio for glyphosate use was 0.90 (95% CI, 0.58–1.40).

2.4.4 Cancer of the prostate

Band *et al.* (2011) report results of a case–control study including 1516 patients with cancer of the prostate (ascertained by the cancer registry of British Columbia, Canada, for 1983–90) and 4994 age-matched controls with cancers at all other cancer sites excluding lung and unknown primary site. Agricultural exposures were assessed by job-exposure matrix. A total of 60 cases were exposed to glyphosate (adjusted OR, 1.36; 95% CI, 0.83–2.25).

2.4.5 Childhood cancer

Parental exposure to pesticides, including glyphosate, was assessed in a population-based case–control study of childhood leukaemia in Costa Rica (Monge *et al.*, 2007). However, associations of childhood cancer with glyphosate were reported only for an "other pesticides" category that also included paraquat, chlorothalonil, and other chemicals. [Because glyphosate was not specifically assessed, this study was not evaluated by the Working Group.]

2.5 Meta-analyses

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including glyphosate. The meta-analysis for glyphosate included six studies (McDuffie *et al.*, 2001; Hardell *et al.*, 2002; De Roos *et al.*, 2003; 2005a; Eriksson *et al.*, 2008; Orsi *et al.*, 2009) and yielded a meta risk-ratio of 1.5 (95% CI, 1.1–2.0). [The Working Group noted that the most fully adjusted risk estimates from the articles by Hardell *et al.* (2002) and Eriksson *et al.* (2008) were not used in this analysis. After considering the adjusted estimates of the two Swedish studies in the meta-analysis, the Working Group estimated a meta risk-ratio of 1.3 (95% CI, 1.03–1.65), I^2 = 0%, *P* for heterogeneity 0.589.]

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

3.1.1 Dietary administration

Groups of 50 male and 50 female CD-1 mice [age not reported] were given diets containing glyphosate (purity, 99.7%) at a concentration of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 months. There was no treatment-related effect on body weight in male and female mice at the lowest or intermediate dose. There was a consistent decrease in body weight in the male and female mice at the highest dose compared with controls. Survival in all dose groups was similar to that of controls. There was a positive trend (*P* = 0.016, trend test; see EPA, 1985b) in the incidence of renal tubule adenoma in dosed male mice: 0/49, 0/49, 1/50 (2%), 3/50 (6%). [The Working Group noted that renal tubule adenoma is a rare tumour in CD-1 mice.] No data on tumours of the kidney

Table 3.1 Studies of carcinogenicity with glyphosate 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, CD-1 (M, F) 24 mo EPA (1985a, b, 1986, 1991a)	Diet containing glyphosate (technical grade; purity, 99.7%) at concentrations of 0, 1000, 5000, or 30 000 ppm, ad libitum, for 24 mo 50 M and 50 F/group [age, NR]	*Males* Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) *Females* No data provided on the kidney Report from the PWG of the EPA (1986): *Males* Renal tubule adenoma: 1/49 (2%), 0/49, 0/50, 1/50 (2%) Renal tubule carcinoma: 0/49, 0/49, 1/50 (2%), 2/50 (4%) Renal tubule adenoma or carcinoma (combined): 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%)	P for trend = 0.016; see Comments [NS] [P = 0.037; Cochran–Armitage trend test] [P = 0.034; Cochran–Armitage trend test]	No information was provided on renal tubule adenomas in female mice, or on statistical analyses of tumour data EPA recommended that additional renal sections be cut and evaluated from all control and treated male mice. The pathology report for these additional sections (EPA, 1985b) showed the same incidence of renal tubule adenomas as originally reported, with no significant difference in incidence when comparing control and treated groups; however, the test for linear trend in proportions resulted in P = 0.016 EPA (1986) convened a PWG and requested additional pathological and statistical information on kidney tumours observed in male mice treated with glyphosate
Mouse, CD-1 (M, F) 104 wk JMPR (2006)	Diet containing glyphosate (purity, 98.6%) at doses of 0, 100, 300, 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	*Males* Haemangiosarcoma: 0/50, 0/50, 0/50, 4/50 (8%) Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 2/50 (4%), 0/50, 2/50 (4%) *Females* Haemangiosarcoma: 0/50, 2/50 (4%), 0/50, 1/50 (2%) Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue: 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%)	[P < 0.001; Cochran–Armitage trend test] NS NS NS	

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, Swiss (M) 32 wk George *et al.* (2010)	Initiation–promotion study Skin application of glyphosate-based formulation (glyphosate, 41%; POEA, ~15%) (referred to as "glyphosate") dissolved in 50% ethanol; DMBA dissolved in 50% ethanol, and TPA dissolved in 50% acetone, used in the groups described below 20 M/group	Skin tumours [called "papillomas" by the authors, following gross examination only]		Short duration of treatment, no solvent controls, and lack of any histopathological evaluation Age at start, NR (mice weighed 12–15 g bw) [The Working Group concluded this was an inadequate study for the evaluation of glyphosate]
	Group I: untreated control (no treatment)	Group I: 0/20		
	Group II: glyphosate only: 25 mg/kg bw topically, 3 × /wk, for 32 wk	Group II: 0/20		
	Group III: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group III: 20/20*, 7.8 ± 1.1	*$P < 0.05$ vs groups VI and VII	
	Group IV: single topical application of glyphosate, 25 mg/kg bw, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group I: 0/20		
	Group V: 3 × /wk topical application of glyphosate, 25 mg/kg bw, for 3 wk, followed 1 wk later by TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group V: 0/20		
	Group VI: single topical application of DMBA, 52 µg/mouse	Group VI: 0/20		
	Group VII: topical application of TPA, 5 µg/mouse, 3 × /wk, for 32 wk	Group VII: 0/20		
	Group VIII: single topical application of DMBA, 52 µg/mouse, followed 1 wk later by topical treatment with glyphosate, 25 mg/kg bw, 3 × /wk, for 32 wk	Group VIII: 8/20*, 2.8 ± 0.9	*$P < 0.05$ vs group VI	

bw, body weight; DMBA, 7,12-dimethylbenz[*a*]anthracene; EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NR, not reported; NS, not significant; POEA, polyethoxylated tallowamine; PWG, pathology working group; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; vs, versus; wk, week; yr, year

were provided for female mice. No other tumour sites were identified (EPA, 1985a). Subsequent to its initial report (EPA, 1985a), the United States Environmental Protection Agency (EPA) recommended that additional renal sections be cut and evaluated from all male mice in the control and treated groups. The pathology report for these additional sections (EPA, 1985b) indicated the same incidence of renal tubule adenoma as originally reported, with no significant increase in incidence between the control group and treated groups by pairwise comparison. However, as already reported above, the test for linear trend in proportions resulted in a significance of $P = 0.016$. The EPA (1986) also requested that a pathology working group (PWG) be convened to evaluate the tumours of the kidney observed in male mice treated with glyphosate, including the additional renal sections. In this second evaluation, the PWG reported that the incidence of adenoma of the renal tubule was 1/49 (2%), 0/49, 0/50, 1/50 (2%) [not statistically significant]; the incidence of carcinoma of the renal tubule was 0/49, 0/49, 1/50 (2%), 2/50 (4%) [$P = 0.037$, trend test for carcinoma]; and the incidence of adenoma or carcinoma (combined) of the renal tubule was 1/49 (2%), 0/49, 1/50 (2%), 3/50 (6%) [$P = 0.034$, trend test for combined]. [The Working Group considered that this second evaluation indicated a significant increase in the incidence of rare tumours, with a dose-related trend, which could be attributed to glyphosate. Chandra & Frith (1994) reported that only 1 out of 725 [0.14%] CD-1 male mice in their historical database had developed renal cell tumours (one carcinoma).]

[The Working Group noted the differences in histopathological diagnosis between pathologists. Proliferative lesions of the renal tubules are typically categorized according to published criteria as hyperplasia, adenoma, or carcinoma. The difference is not trivial, because focal hyperplasia, a potentially preneoplastic lesion, should be carefully differentiated from the regenerative changes of the tubular epithelium. There is a morphological continuum in the development and progression of renal neoplasia. Thus larger masses may exhibit greater heterogeneity in histological growth pattern, and cytologically more pleomorphism and atypia than smaller lesions (Eustis *et al.*, 1994). Of note, a renal tumour confirmed by the PWG after re-evaluation of the original slides (EPA, 1986), had not been seen in the re-sectioned kidney slides (EPA, 1985b). This may be related to the growth of tumour that – in contrast to tumours in other organs – is not spherical but elliptical because of the potential expansion in tubules. In addition, the concept of tubular expansion without compression of adjacent parenchyma may be at the basis of the discrepancy between the first (EPA, 1985a, b) and second evaluation (EPA, 1986).]

In another study reported to the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), groups of 50 male and 50 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly for the first 13 weeks and every 4 weeks thereafter to give doses of 0, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was no treatment-related effect on body weight or survival in any of the dosed groups. There was an increase in the incidence of haemangiosarcoma in males – 0/50, 0/50, 0/50, 4/50 (8%) [$P < 0.001$, Cochran–Armitage trend test], and in females – 0/50, 2/50 (4%), 0/50, 1/50 (2%) [not statistically significant], and an increase in the incidence of histiocytic sarcoma in the lymphoreticular/haemopoietic tissue in males – 0/50, 2/50 (4%), 0/50, 2/50 (4%), and in females – 0/50, 3/50 (6%), 3/50 (6%), 1/50 (2%) [not statistically significant for males or females]. [The Working Group considered that this study was adequately reported.]

3.1.2 Initiation–promotion

Groups of 20 male Swiss mice [age at start not reported; body weight, 12–15 g] were given a glyphosate-based formulation (glyphosate, 41%; polyethoxylated tallowamine, ~15%) (referred to as glyphosate in the article) that was dissolved in 50% ethanol and applied onto the shaved back skin (George *et al.*, 2010). Treatment groups were identified as follows:

- Group I – untreated control;
- Group II – glyphosate only (25 mg/kg bw), applied topically three times per week for 32 weeks;
- Group III – single topical application of dimethylbenz[*a*]anthracene (DMBA; in ethanol; 52 µg/mouse), followed 1 week later by 12-*O*-tetradecanoylphorbol-13-acetate (TPA; in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group IV – single topical application of glyphosate (25 mg/kg bw) followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group V – glyphosate (25 mg/kg bw) applied topically three times per week for 3 weeks (total of nine applications), followed 1 week later by TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks;
- Group VI – single topical application of DMBA (in ethanol; 52 µg/mouse);
- Group VII –TPA (in acetone; 5 µg/mouse), applied topically three times per week for 32 weeks; and
- Group VIII –single topical application of DMBA (in ethanol; 52 µg/mouse), followed 1 week later by glyphosate (25 mg/kg bw), applied topically three times per week for 32 weeks.

All mice were killed at 32 weeks. Skin tumours were observed only in group III (positive control, DMBA + TPA, 20/20) and group VIII (DMBA + glyphosate, 8/20; $P < 0.05$ versus group VI [DMBA only, 0/20]). No microscopic examination was conducted and tumours were observed "as a minute wart like growth [that the authors called squamous cell papillomas], which progressed during the course of experiment." [The glyphosate formulation tested appeared to be a tumour promoter in this study. The design of the study was poor, with short duration of treatment, no solvent controls, small number of animals, and lack of histopathological examination. The Working Group concluded that this was an inadequate study for the evaluation of glyphosate.]

3.1.3 Review articles

Greim *et al.* (2015) have published a review article containing information on five long-term bioassay feeding studies in mice. Of these studies, one had been submitted for review to the EPA (EPA, 1985a, b, 1986, 1991a), and one to the JMPR (JMPR, 2006); these studies are discussed in Section 3.1.1. The review article reported on an additional three long-term bioassay studies in mice that had not been previously available in the open literature, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The three additional long-term bioassay studies in mice are summarized below. [The Working Group was unable to evaluate these studies, which are not included in Table 3.1 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information was lacking on statistical methods, choice of doses, body-weight gain, survival data, details of histopathological examination, and/or stability of dosed feed mixture).]

In the first study (identified as Study 12, 1997a), groups of 50 male and 50 female CD-1

mice [age at start not reported] were given diets containing glyphosate (purity, 94–96%) at a concentration of 0, 1600, 8000, or 40 000 ppm for 18 months. The increase in the incidence of bronchiolo-alveolar adenoma and carcinoma, and of lymphoma, was reported to be not statistically significant in males and females receiving glyphosate. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the second study (identified as Study 13, 2001), groups of 50 male and 50 female Swiss albino mice [age at start not reported] were given diets containing glyphosate (purity, > 95%) at a concentration of 0 (control), 100, 1000, or 10 000 ppm for 18 months. The authors reported a statistically significant increase in the incidence of malignant lymphoma (not otherwise specified, NOS) in males at the highest dose: 10/50 (20%), 15/50 (30%), 16/50 (32%), 19/50 (38%; $P < 0.05$; pairwise test); and in females at the highest dose: 18/50 (36%), 20/50 (40%), 19/50 (38%), 25/50 (50%; $P < 0.05$; pairwise test). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In the third study (identified as Study 14, 2009a), groups of 51 male and 51 female CD-1 mice [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 500, 1500, or 5000 ppm for 18 months. Incidences for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS), and hepatocellular adenoma and carcinoma in males, and for bronchiolo-alveolar adenoma and carcinoma, malignant lymphoma (NOS) and pituitary adenoma in females, were included in the article. In males, the authors reported that there was a significant positive trend [statistical test not specified] in the incidence of bronchiolo-alveolar carcinoma (5/51, 5/51, 7/51, 11/51) and of malignant lymphoma (0/51, 1/51, 2/51, 5/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

3.2 Rat

See Table 3.2

3.2.1 Drinking-water

Groups of 10 male and 10 female Sprague-Dawley rats (age, 5 weeks) were given drinking-water containing a glyphosate-based formulation at a dose of 0 (control), 1.1×10^{-8} % (5.0×10^{-5} mg/L), 0.09% (400 mg/L) or 0.5% (2.25×10^{3} mg/L), ad libitum, for 24 months (Séralini *et al.*, 2014). [The study reported is a life-long toxicology study on a glyphosate-based formulation and on genetically modified NK603 maize, which the authors stated was designed as a full study of long-term toxicity and not a study of carcinogenicity. No information was provided on the identity or concentration of other chemicals contained in this formulation.] Survival was similar in treated and control rats. [No data on body weight were provided.] In female rats, there was an almost twofold increase in the incidence of tumours of the mammary gland (mainly fibroadenoma and adenocarcinoma) in animals exposed to the glyphosate-based formulation only versus control animals: control, 5/10 (50%); lowest dose, 9/10 (90%); intermediate dose, 10/10 (100%) [$P < 0.05$; Fisher exact test]; highest dose, 9/10 (90%). [The Working Group concluded that this study conducted on a glyphosate-based formulation was inadequate for evaluation because the number of animals per group was small, the histopathological description of tumours was poor, and incidences of tumours for individual animals were not provided.]

In another study with drinking-water, Chruscielska *et al.* (2000) gave groups of 55 male and 55 female Wistar rats (age, 6–7 weeks) drinking-water containing an ammonium salt

of glyphosate as a 13.85% solution [purity of glyphosate, not reported] that was used to make aqueous solutions of 0 (control), 300, 900, and 2700 mg/L, for 24 months [details on the dosing regimen were not reported]. The authors reported that survival and body-weight gain were similar in treated and control animals. No significant increase in tumour incidence was reported in any of the treated groups. [The Working Group noted the limited information provided on dosing regimen, histopathological examination method, and tumour incidences.]

3.2.2 Dietary administration

The JMPR report included information on a 1-year feeding study in which groups of 24 male and 24 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 year (JMPR, 2006). There was a treatment-related decrease in body-weight gain at the two highest doses (significant at 20 000 ppm for both sexes, and at 8000 ppm only in females). There was no treatment-related decrease in survival. No significant increase in tumour incidence was observed in any of the treated groups. [The Working Group noted the short duration of exposure.]

The JMPR report also included information on a 104-week feeding study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7–98.9%) at a concentration that was adjusted to provide doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 weeks (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose. There was no significant treatment-related decrease in survival or increase in tumour incidence in any of the treated groups.

Information was also included in the JMPR report on a 24-month feeding study in which groups of 52 male and 52 female Wistar-Alpk:APfSD rats [age at start not reported] were given diets containing glyphosate (purity, 97.6%) at a concentration of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 24 months (JMPR, 2006). There was a treatment-related decrease in body-weight gain in males and females at the highest dose, and a corresponding significant increase in survival in males. No significant increase in tumour incidence was observed in any of the treated groups.

The EPA (1991a, b, c, d) provided information on a long-term study in which groups of 60 male and 60 female Sprague-Dawley rats (age, 8 weeks) were given diets containing glyphosate (technical grade; purity, 96.5%) at a concentration of 0 ppm, 2000 ppm, 8000 ppm, or 20 000 ppm, ad libitum, for 24 months. Ten animals per group were killed after 12 months. There was no compound-related effect on survival, and no statistically significant decreases in body-weight gain in male rats. In females at the highest dose, body-weight gain was significantly decreased, starting on day 51. In males at the lowest dose, there was a statistically significant increase in the incidence of pancreatic islet cell adenoma compared with controls: 8/57 (14%) versus 1/58 (2%), $P \leq 0.05$ (Fisher exact test). Additional analyses by the EPA (1991a) (using the Cochran–Armitage trend test and Fisher exact test, and excluding rats that died or were killed before week 55) revealed a statistically significant higher incidence of pancreatic islet cell adenoma in males at the lowest and highest doses compared with controls: lowest dose, 8/45 (18%; $P = 0.018$; pairwise test); intermediate dose, 5/49 (10%); highest dose, 7/48 (15%; $P = 0.042$; pairwise test) versus controls, 1/43 (2%). The range for historical controls for pancreatic islet cell adenoma reported in males at this laboratory was 1.8–8.5%. [The Working Group noted that there was no statistically significant positive trend in the incidence of these tumours, and no apparent progression to carcinoma.] There was also a statistically significant positive trend in the incidence of hepatocellular adenoma in

Table 3.2 Studies of carcinogenicity with glyphosate in rats

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 24 mo Séralini *et al.* (2014)	Drinking-water containing a glyphosate-based formulation at a concentration of 0 (control), 1.1×10^{-8}% (glyphosate, 5.0×10^{-5} mg/L), 0.09% (glyphosate, 400 mg/L) or 0.5% (glyphosate, 2.25×10^{3} mg/L), ad libitum, for 24 mo 10 M and 10 F/group (age, 5 wk)	*Males* No significant increase in tumour incidence observed in any of the treated groups *Females* Mammary tumours (mainly fibroadenomas and adenocarcinomas): 5/10 (50%), 9/10 (90%), 10/10 (100%)*, 9/10 (90%) Pituitary lesions (hypertrophy, hyperplasia, and adenoma): 6/10 (60%), 8/10 (80%), 7/10 (70%), 7/10 (70%)	NS *[$P < 0.05$] [NS]	Data are from an in-depth life-long toxicology study on a glyphosate-based formulation and NK603 genetically modified maize; authors stated that the study was designed as a full chronic toxicity and not a carcinogenicity study. No information provided on the identity or concentration of other chemicals contained in this formulation Histopathology poorly described and tumour incidences for individual animals not discussed in detail. Small number of animals per group [The Working Group concluded this was an inadequate study for the evaluation of glyphosate carcinogenicity]
Rat, Wistar (M, F) 24 mo Chruscielska *et al.* (2000)	Drinking-water containing ammonium salt of glyphosate (13.85% solution) [purity of glyphosate, NR] was used to make aqueous solutions of 0, 300, 900, and 2700 mg/L [Details on dosing regimen, NR] 55 M and 55 F/group (age, 6–7 wk)	No significant increase in tumour incidence observed in any of the treated groups	NS	Limited information on dosing regimen, histopathological examination methods, and tumour incidences
Rat, Wistar-Alpk:APfSD (M, F) 1 yr JMPR (2006)	Diet containing glyphosate (purity, 95.6%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 1 yr 24 M and 24 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	Short duration of exposure
Rat, Sprague-Dawley (M, F) 104 wk JMPR (2006)	Diet containing glyphosate (purity, 98.7–98.9%) at doses of 0, 10, 100, 300, or 1000 mg/kg bw, ad libitum, for 104 wk 50 M and 50 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	
Rat, Wistar-Alpk:APfSD (M, F) 24 mo JMPR (2006)	Diet containing glyphosate (purity, 97.6%) at concentrations of 0, 2000, 6000, or 20 000 ppm, ad libitum, for 2 yr 52 M and 52 F/group [age, NR]	No significant increase in tumour incidence observed in any groups of treated animals	NS	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) 24 mo EPA (1991a, b, c, d)	Diet containing glyphosate (technical grade; purity, 96.5%) at concentrations of 0, 2000, 8000, or 20 000 ppm, ad libitum, for 24 mo 60 M and 60 F/group (age, 8 wk) 10 rats/group killed after 12 mo	*Males* *Pancreas (islet cell):* Adenoma: 1/58 (2%), 8/57 (14%)*, 5/60 (8%), 7/59 (12%) Carcinoma: 1/58 (2%), 0/57, 0/60, 0/59 Adenoma or carcinoma (combined): 2/58 (3%), 8/57 (14%), 5/60 (8%), 7/59 (12%) *Liver:* Hepatocellular adenoma: 2/60 (3%), 2/60 (3%), 3/60 (6%), 7/60 (12%) Hepatocellular carcinoma: 3/60 (5%), 2/60 (3%), 1/60 (2%), 2/60 (3%) *Females* *Pancreas (islet cell):* Adenoma: 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 Carcinoma: 0/60, 0/60, 0/60, 0/59 Adenoma or carcinoma (combined): 5/60 (8%), 1/60 (2%), 4/60 (7%), 0/59 *Thyroid:* C-cell adenoma: 2/60 (3%), 2/60 (3%), 6/60 (10%), 6/60 (10%) C-cell carcinoma: 0/60, 0/60, 1/60, 0/60	Adenoma, * $P \leq 0.05$ (Fisher exact test with Bonferroni inequality); see comments Adenoma, P for trend = 0.016; see comments NS Adenoma, P for trend = 0.031; see comments	Historical control range for pancreatic islet cell adenoma reported in males at this laboratory, 1.8–8.5% EPA (1991a) performed additional analyses using the Cochran–Armitage trend test and Fisher exact test, and excluding animals that died or were killed before wk 54–55: *Males* *Pancreas (islet cell):* Adenoma: 1/43 (2%), 8/45 (18%; P = 0.018), 5/49 (10%), 7/48 (15%; P = 0.042) Carcinoma: 1/43 (2%), 0/45 (0%), 0/49 (0%), 0/48 (0%) Adenoma or carcinoma (combined): 2/43 (5%), 8/45 (18%), 5/49 (10%), 7/48 (15%) [There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma] *Liver:* Hepatocellular adenoma: 2/44 (5%; P for trend = 0.016), 2/45 (4%), 3/49 (6%), 7/48 (15%) Hepatocellular carcinoma: 3/44 (7%); 2/45 (4%), 1/49 (2%), 2/48 (4%) Hepatocellular adenoma or carcinoma (combined): 5/44 (11%), 4/45 (9%), 4/49 (8%), 9/48 (19%) [There was no apparent progression to carcinoma] *Females* *Thyroid:* C-cell adenoma: 2/57 (4%; P for trend = 0.031), 2/60 (3%), 6/59 (10%), 6/55 (11%) C-cell carcinoma: 0/57, 0/60, 1/59 (2%), 0/55 C-cell adenoma or carcinoma (combined): 2/57 (4%), 2/60 (3%), 7/59 (12%), 6/55 (11%) [There was no apparent progression to carcinoma]

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Rat Sprague-Dawley (M, F) Lifetime (up to 26 mo) EPA (1991a, b, c, d)	Diet containing glyphosate (purity, 98.7%) at concentrations of 0 ppm, 30 ppm (3 mg/kg bw per day), 100 ppm (10 mg/kg bw per day), 300 ppm (31 mg/kg bw per day), ad libitum, up to 26 mo 50 M and 50 F/group [age, NR]	*Males* *Pancreas (islet cell):* Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%) Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%) Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%) *Females* *Pancreas (islet cell):* Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%) Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%) Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)	Adenoma, *[$P < 0.05$; Fisher exact test] NS	[There was no statistically significant positive trend in the incidence of pancreatic tumours, and no apparent progression to carcinoma]

bw, body weight; d, day; F, female; M, male; mo, month; NR, not reported; NS, not significant; wk, week; yr, year

males (P = 0.016) and of thyroid follicular cell adenoma in females (P = 0.031). [The Working Group noted that there was no apparent progression to carcinoma for either tumour type.]

The EPA (1991a, b, c, d) provided information on another long-term study in which groups of 50 male and 50 female Sprague-Dawley rats [age at start not reported] were given diets containing glyphosate (purity, 98.7%) at a concentration of 0, 30 (3 mg/kg bw per day), 100 (10 mg/kg bw per day), or 300 ppm (31 mg/kg bw per day), ad libitum, for life (up to 26 months). No information was provided on body weight or survival of the study animals. An increase in the incidence of pancreatic islet cell adenoma was reported in males at the lowest dose: controls, 0/50 (0%); lowest dose, 5/49 (10%) [$P < 0.05$; Fisher exact test]; intermediate dose, 2/50 (4%); highest dose, 2/50 (4%). [The Working Group noted that there was no statistically significant positive dose-related trend in the incidence of these tumours, and no apparent progression to carcinoma.]

3.2.3 Review articles

Greim *et al.* (2015) have published a review article containing information on nine long-term bioassay feeding studies in rats. Of these studies, two had been submitted for review to the EPA (1991a, b, c, d), two to the JMPR (JMPR, 2006), and one had been published in the openly available scientific literature (Chruscielska *et al.*, 2000); these studies are discussed earlier in Section 3.2. The review article reported on an additional four long-term bioassay studies in rats that had not been previously published, but had been submitted to various organizations for registration purposes. The review article provided a brief summary of each study and referred to an online data supplement containing the original data on tumour incidence from study reports. The four additional long-term bioassay studies in rats are summarized below. [The Working Group did not evaluate these studies, which are not included in Table 3.2 and Section 5.3, because the information provided in the review article and its supplement was insufficient (e.g. information lacking on statistical methods, choice of doses, body-weight gain, survival data, details on histopathological examination and/or stability of dosed feed mixture).]

In one study (identified as Study 4, 1996), groups of 50 male and 50 female Wistar rats [age at start not reported] were given diets containing glyphosate (purity, 96%) at a concentration of 0, 100, 1000, or 10 000 ppm, ad libitum, for 24 months. It was reported that hepatocellular adenomas and hepatocellular carcinomas were found at non-statistically significant incidences in both males and females. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In one study in Sprague-Dawley rats (identified as Study 5, 1997), groups of 50 male and 50 female rats [age at start not reported] were given diets containing glyphosate technical acid [purity not reported] at a concentration of 0, 3000, 15 000, or 25 000 ppm, ad libitum, for 24 months. There was no significant increase in tumour incidence in the treated groups. [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information.]

In a second study in Sprague Dawley rats (identified as Study 6, 1997b), groups of 50 males and 50 females [age at start not reported] were given diets containing glyphosate (purity, 94.6–97.6%) at a concentration of 0, 3000, 10 000, or 30 000 ppm, ad libitum, for 24 months. Non-significant increases in tumour incidences compared with controls were noted for skin keratoacanthoma in males at the highest dose, and for fibroadenoma of the mammary gland in females at the lowest and intermediate doses. [The Working Group was unable to evaluate this

study because of the limited experimental data provided in the review article and supplemental information.]

In another study in male and female Wistar rats (identified as Study 8, 2009b), groups of 51 male and 51 female rats [age at start not reported] were fed diets containing glyphosate (purity, 95.7%) at a concentration of 0, 1500, 5000, or 15 000 ppm, ad libitum, for 24 months. The highest dose was progressively increased to reach 24 000 ppm by week 40. A non-significant increase in tumour incidence was noted for adenocarcinoma of the mammary gland in females at the highest dose (6/51) compared with controls (2/51). [The Working Group was unable to evaluate this study because of the limited experimental data provided in the review article and supplemental information. The Working Group noted that tumours of the mammary gland had been observed in other studies in rats reviewed for the present *Monograph*.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Introduction

The herbicidal activity of glyphosate is attributed to interference with the production of essential aromatic amino acids (EPA, 1993b). In plants, glyphosate competitively inhibits the activity of enolpyruvylshikimate phosphate synthase, an enzyme that is not present in mammalian cells. Glyphosate is degraded by soil microbes to aminomethylphosphonic acid (AMPA) (see Fig. 4.1), a metabolite that can accumulate in the environment. In mammals, glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine; however, it has been suggested that glyphosate can undergo gut microbial metabolism in humans (Motojyuku *et al.*, 2008) and rodents (Brewster *et al.*, 1991).

4.1.2 Absorption

(a) Humans

Data on the absorption of glyphosate via intake of food and water in humans were not available to the Working Group. Inhalation of glyphosate is considered to be a minor route of exposure in humans, because glyphosate is usually formulated as an isopropylamine salt with a very low vapour pressure (Tomlin, 2000).

In the Farm Family Exposure Study, 60% of farmers had detectable levels of glyphosate in 24-hour composite urine samples taken on the day they had applied a glyphosate-based formulation (Acquavella *et al.*, 2004). Farmers who did not use rubber gloves had higher urinary concentrations of glyphosate than those who did use gloves [indicating that dermal absorption is a relevant route of exposure]. In a separate study, detectable levels of glyphosate were found in urine samples from farm families and non-farm families (Curwin *et al.*, 2007).

In accidental and deliberate intoxication cases involving ingestion of glyphosate-based formulations, glyphosate was readily detectable in the blood (Zouaoui *et al.*, 2013). After deliberate or accidental ingestion, one glyphosate-based formulation was found to be more lethal to humans than another (Sørensen & Gregersen, 1999). [Greater lethality was attributed to the presence of trimethylsulfonium counterion, which might facilitate greater absorption after oral exposure.]

Small amounts of glyphosate can be absorbed after dermal exposures in humans in vitro. For example, when an aqueous solution of 1% glyphosate was applied in an in-vitro human skin model, only 1.4% of the applied dose was absorbed through the skin. Glyphosate is typically formulated as an isopropylamine salt, and is dissolved in a water-based vehicle, while the

stratum corneum is a lipid-rich tissue (Wester *et al.*, 1991). In-vitro studies using human skin showed that percutaneous absorption of a glyphosate-based formulation was no more than 2% of the administered dose over a concentration range of 0.5–154 μg/cm^2 and a topical volume range of 0.014–0.14 mL/cm^2. In addition, very little glyphosate (≤ 0.05% of the administered dose) was sequestered in the stratum corneum after dermal application (Wester *et al.*, 1991).

In the human Caco-2 cell line, an in-vitro model of intestinal enterocytes, glyphosate (> 10 mg/mL) was shown to significantly disrupt barrier properties, leading to an increase in paracellular permeability (transport of substances that pass through the intercellular space between the cells) (Vasiluk *et al.*, 2005).

(b) Experimental systems

Three studies have been conducted to investigate the absorption of a single oral dose of glyphosate in rats (Brewster *et al.*, 1991; Chan & Mahler, 1992; EPA, 1993b).

In male Sprague-Dawley rats given [^{14}C]-labelled glyphosate (10 mg/kg bw), the majority of the radiolabel was associated with the gastrointestinal contents and small intestinal tissue 2 hours after administration (Brewster *et al.*, 1991). Approximately 35–40% of the administered dose was found to be absorbed from the gastrointestinal tract. Urinary and faecal routes of elimination were equally important. [The Working Group concluded that glyphosate is incompletely absorbed from the gastrointestinal tract after oral exposure in rats.]

In a study by the United States National Toxicology Programme (NTP) in Fisher 344 rats, 30% of the administered oral dose (5.6 mg/kg bw) was absorbed, as determined by urinary excretion data (Chan & Mahler, 1992). This finding was in accordance with the previously described study of oral exposure in rats (Brewster *et al.*, 1991).

In a study reviewed by the EPA, Sprague-Dawley rats were given an oral dose of glyphosate (10 mg/kg bw); 30% and 36% of the administered dose was absorbed in males and females, respectively (EPA, 1993b). At a dose that was ~10-fold higher (1000 mg/kg bw), oral absorption of glyphosate by the rats was slightly reduced.

In a 14-day feeding study in Wistar rats given glyphosate at dietary concentrations of up to 100 ppm, only ~15% of the administered dose was found to be absorbed (JMPR, 2006). In New Zealand White rabbits or lactating goats given glyphosate as single oral doses (6–9 mg/kg bw), a large percentage of the administered dose was recovered in the faeces [suggesting very poor gastrointestinal absorption of glyphosate in these animal models] (JMPR, 2006).

In monkeys given glyphosate by dermal application, percutaneous absorption was estimated to be between 1% and 2% of the administered dose (Wester *et al.*, 1991). Most of the administered dose was removed by surface washes of the exposed skin.

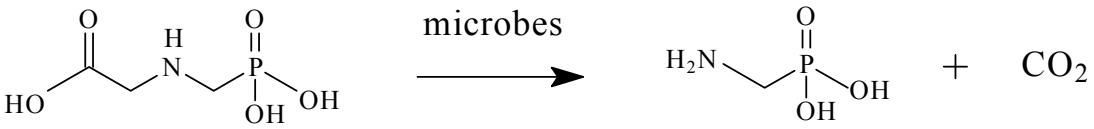
4.1.3 Distribution

(a) Humans

No data in humans on the distribution of glyphosate in systemic tissues other than blood were available to the Working Group. In cases of accidental or deliberate intoxication involving ingestion of glyphosate-based formulations, glyphosate was measured in blood. Mean blood concentrations of glyphosate were 61 mg/L and 4146 mg/L in mild-to-moderate cases of intoxication and in fatal cases, respectively (Zouaoui *et al.*, 2013).

One report, using optical spectroscopy and molecular modelling, indicated that glyphosate could bind to human serum albumin, mainly by hydrogen bonding; however, the fraction of glyphosate that might bind to serum proteins in blood was not actually measured (Yue *et al.*, 2008).

Fig. 4.1 Microbial metabolism of glyphosate to AMPA



Glyphosate is degraded to AMPA by microbial metabolism
Compiled by the Working Group

(b) Experimental systems

In Sprague-Dawley rats given a single oral dose of glyphosate (100 mg/kg bw), glyphosate concentrations in plasma reached peak levels, then declined slowly from day 1 to day 5 (Bernal *et al.*, 2010). The plasma data appeared to fit a one-compartment model with an elimination rate constant of k_{el} = 0.021 hour^{-1}. [The Working Group estimated the elimination half-life of glyphosate to be 33 hours.] Tissue levels of glyphosate were not determined in this study. In a study by Brewster *et al.* (1991), the tissue levels of glyphosate at 2, 6.3, 28, 96, and 168 hours in Sprague-Dawley rats given a single oral dose (10 mg/kg bw) declined rapidly. Tissues with the greatest amounts of detectable radiolabel (> 1% of the administered dose) were the small intestine, colon, kidney, and bone. Peak levels were reached in small intestine tissue and blood by 2 hours, while peak levels in other tissues occurred at 6.3 hours after dosing. After 7 days, the total body burden of [^{14}C]-labelled residues was ~1% of the administered dose, and was primarily associated with the bone (~1 ppm). In every tissue examined after administration of [^{14}C]-labelled glyphosate, essentially 100% of the radiolabel that was present in the tissue was unmetabolized parent glyphosate. Thus, essentially 100% of the body burden was parent compound, with no significant persistence of glyphosate after 7 days (Brewster *et al.*, 1991). In a 14-day feeding study in Wistar rats given diets containing glyphosate at 100 ppm, glyphosate reached steady-state levels in the blood by day 6 (JMPR, 2006). The tissue concentrations of glyphosate had the following rank order: kidneys > spleen > fat > liver. Tissue levels declined rapidly after cessation of exposure to glyphosate. A second study in rats given glyphosate (10 mg/kg bw per day, 14 days) followed by a single oral dose of [^{14}C]-glyphosate (at 10 mg/kg bw) showed that repeated dosing did not alter the tissue distribution of glyphosate (JMPR, 2006).

In rhesus monkeys, tissues harvested 7 days after dermal exposures to [^{14}C]-labelled glyphosate did not contain radiolabel at detectable levels (Wester *et al.*, 1991).

4.1.4 Metabolism and modulation of metabolic enzymes

(a) Metabolism

Glyphosate is degraded in the environment by soil microbes, primarily to AMPA and carbon dioxide (Fig. 4.1; Jacob *et al.*, 1988). A minor pathway for the degradation of glyphosate in bacteria (*Pseudomonas sp.* strain LBr) is via conversion to glycine (Jacob *et al.*, 1988). In a case of deliberate poisoning with a glyphosate-based formulation, small amounts of AMPA (15.1 µg/mL) were detectable in the blood (Motojyuku *et al.*, 2008) [suggesting that this pathway might also operate in humans]. In rats given a single high oral dose of glyphosate (100 mg/kg bw), small amounts of AMPA were detected in the plasma (Bernal *et al.*, 2010). In

male Sprague-Dawley rats given an oral dose of glyphosate (10 mg/kg bw), a very small amount of AMPA (< 0.04% of the administered dose) was detected in the colon 2 hours after dosing; this was attributed to intestinal microbial metabolism (Brewster *et al.*, 1991).

(b) Modulation of metabolic enzymes

(i) Humans

In human hepatic cell lines, treatment with one of four glyphosate-based formulations produced by the same company was shown to enhance CYP3A4 and CYP1A2 levels, while glutathione transferase levels were reduced (Gasnier *et al.*, 2010). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by the adjuvants contained in the formulation.]

(ii) Experimental systems

Exposure of Wistar rats to a glyphosate-based formulation significantly altered some hepatic xenobiotic enzyme activities (Larsen *et al.*, 2014). Liver microsomes obtained from male and female rats treated with the formulation exhibited ~50% reductions in cytochrome P450 (CYP450) content compared with control (untreated) rats. However, opposing effects were observed when assessing 7-ethoxycoumarin *O*-deethylase activity (7-ECOD, a non-specific CYP450 substrate). Female rats treated with the glyphosate-based formulation exhibited a 57% increase in hepatic microsomal 7-ECOD activity compared with controls, while male rats treated with the formulation exhibited a 58% decrease in this activity (Larsen *et al.*, 2014). [The Working Group noted that it was not clear whether the effects were caused by glyphosate alone or by adjuvants contained in the formulation.]

4.1.5 Excretion

(a) Humans

Excretion of glyphosate in humans was documented in several biomonitoring studies. For example, as part of the Farm Family Exposure Study, urinary concentrations of glyphosate were evaluated immediately before, during, and after glyphosate application in 48 farmers and their spouses and children (Acquavella *et al.*, 2004). Dermal contact with glyphosate during mixing, loading, and application was considered to be the main route of exposure in the study. On the day the herbicide was applied, 60% of the farmers had detectable levels of glyphosate in 24-hour composite urine samples, as did 4% of their spouses and 12% of children. For farmers, the geometric mean concentration was 3 µg/L, the maximum value was 233 µg/L, and the highest estimated systemic dose was 0.004 mg/kg bw (Acquavella *et al.*, 2004). In a separate study, detectable levels of glyphosate were excreted in the urine of members of farm families and of non-farm families, with geometric means ranging from 1.2 to 2.7 µg/L (Curwin *et al.*, 2007).

In a study of a rural population living near areas sprayed for drug eradication in Colombia (see Section 1.4.1, Table 1.5), mean urinary glyphosate concentrations were 7.6 µg/L (range, undetectable to 130 µg/L) (Varona *et al.*, 2009). AMPA was detected in 4% of urine samples (arithmetic mean, 1.6 µg/L; range, undetectable to 56 µg/L).

(b) Experimental systems

In an NTP study in Fisher 344 rats given a single oral dose of [^{14}C]-labelled glyphosate (5.6 or 56 mg/kg bw), it was shown that > 90% of the radiolabel was eliminated in the urine and faeces within 72 hours (Chan & Mahler, 1992). In Sprague-Dawley rats given [^{14}C]-labelled glyphosate at an oral dose of 10 or 1000 mg/kg bw, ~60–70% of the administered dose was excreted in the faeces, and the remainder in the urine (EPA,

1993b). By either route, most (98%) of the administered dose was excreted as unchanged parent compound. AMPA was the only metabolite found in the urine (0.2–0.3% of the administered dose) and faeces (0.2–0.4% of the administered dose). [The large amount of glyphosate excreted in the faeces is consistent with its poor oral absorption.] Less than 0.3% of the administered dose was expired as carbon dioxide.

In rhesus monkeys given glyphosate as an intravenous dose (9 or 93 μg), > 95% of the administered dose was excreted in the urine (Wester *et al.*, 1991). Nearly all the administered dose was eliminated within 24 hours. In contrast, in rhesus monkeys given glyphosate by dermal application (5400 μg/20 cm^2), only 2.2% of the administered dose was excreted in the urine within 7 days (Wester *et al.*, 1991).

Overall, systemically absorbed glyphosate is not metabolized efficiently, and is mainly excreted unchanged into the urine.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Glyphosate has been studied for genotoxic potential in a wide variety of assays. Studies carried out in exposed humans, in human cells in vitro, in other mammals in vivo and in vitro, and in non-mammalian systems in vivo and in vitro, respectively, are summarized in Table 4.1, Table 4.2, Table 4.3, Table 4.4, and Table 4.5. [A review article by Kier & Kirkland (2013) summarized the results of published articles and unpublished reports of studies pertaining to the genotoxicity of glyphosate and glyphosate formulations. A supplement to this report contained information on 66 unpublished regulatory studies. The conclusions and data tables for each individual study were included in the supplement; however, the primary study reports from which these data were extracted were not available to the Working Group. The information provided in the supplement was insufficient regarding topics such as details of statistical methods, choice of the highest dose tested, and verification of the target tissue exposure. The Working Group determined that the information in the supplement to Kier & Kirkland (2013) did not meet the criteria for data inclusion as laid out in the Preamble to the *IARC Monographs*, being neither "reports that have been published or accepted for publication in the openly available scientific literature" nor "data from governmental reports that are publicly available" (IARC, 2006). The review article and supplement were not considered further in the evaluation.]

(a) Humans

(i) Studies in exposed humans

See Table 4.1

In exposed individuals (n = 24) living in northern Ecuador in areas sprayed with a glyphosate-based formulation, a statistically significant increase in DNA damage (DNA strand breaks) was observed in blood cells collected 2 weeks to 2 months after spraying (Paz-y-Miño *et al.*, 2007). The same authors studied blood cells from individuals (n = 92) in 10 communities in Ecuador's northern border, who were sampled 2 years after the last aerial spraying with a herbicide mix containing glyphosate, and showed that their karyotypes were normal compared with those of a control group (Paz-y-Miño *et al.*, 2011).

Bolognesi *et al.* (2009) studied community residents (137 women of reproductive age and their 137 spouses) from five regions in Colombia. In three regions with exposures to glyphosate-based formulations from aerial spraying, blood samples were taken from the same individuals at three time-points (before spraying (baseline), 5 days after spraying and 4 months after spraying) to determine the frequency of micronucleus formation in lymphocytes. The baseline frequency of binucleated cells with micronuclei was significantly higher in subjects

from the three regions where there had been aerial spraying with glyphosate-formulations and in a fourth region with pesticide exposure (but not through aerial spraying), compared with a reference region (without use of pesticide). The frequency of micronucleus formation in peripheral blood lymphocytes was significantly increased, compared with baseline levels in the same individuals, after aerial spraying with glyphosate-based formulations in each of the three regions (see Table 4.1; Bolognesi *et al.*, 2009). Immediately after spraying, subjects who reported direct contact with the glyphosate-based spray showed a higher frequency of binucleated cells with micronuclei. However, the increase in frequency of micronucleus formation observed immediately after spraying was not consistent with the rates of application used in the regions, and there was no association between self-reported direct contact with pesticide sprays and frequency of binucleated cells with micronuclei. In subjects from one but not other regions, the frequency of binucleated cells with micronuclei was significantly decreased 4 months after spraying, compared with immediately after spraying.

(ii) Human cells in vitro

See Table 4.2

Glyphosate induced DNA strand breaks (as measured by the comet assay) in liver Hep-2 cells (Mañas *et al.*, 2009a), lymphocytes (Mladinic *et al.*, 2009b; Alvarez-Moya *et al.*, 2014), GM38 fibroblasts, the HT1080 fibrosarcoma cell line (Monroy *et al.*, 2005), and the TR146 buccal carcinoma line (Koller *et al.*, 2012). DNA strand breaks were induced by AMPA in Hep-2 cells (Mañas *et al.*, 2009b), and by a glyphosate-based formulation in the TR146 buccal carcinoma cell line (Koller *et al.*, 2012).

In human lymphocytes, AMPA (Mañas *et al.*, 2009b), but not glyphosate (Mañas *et al.*, 2009a), produced chromosomal aberrations. Glyphosate did not induce a concentration-related increase in micronucleus formation in human lymphocytes at levels estimated to correspond to occupational and residential exposure (Mladinic *et al.*, 2009a). Sister-chromatid exchange was induced by glyphosate (Bolognesi *et al.*, 1997), and by a glyphosate-based formulation (Vigfusson & Vyse, 1980; Bolognesi *et al.*, 1997) in human lymphocytes exposed in vitro.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

The ability of glyphosate or a glyphosate-based formulation to induce DNA adducts was studied in mice given a single intraperitoneal dose. Glyphosate induced DNA adducts (8-hydroxy deoxyguanosine) in the liver, but not in the kidney, while a glyphosate-based formulation caused a slight increase in DNA adducts in the kidney, but not in the liver (Bolognesi *et al.*, 1997). Peluso *et al.* (1998) showed that a glyphosate-based formulation (glyphosate, 30.4%), but not glyphosate alone, caused DNA adducts (as detected by ^{32}P-DNA post-labelling) in mouse liver and kidney. Glyphosate and a glyphosate-based formulation produced DNA strand breaks in the liver and kidney after a single intraperitoneal dose (Bolognesi *et al.*, 1997).

In mice given a single dose of glyphosate by gavage, no genotoxic effect was observed by the dominant lethal test (EPA, 1980a).

After a single intraperitoneal dose, no chromosomal aberrations were observed in the bone marrow of rats treated with glyphosate (Li & Long 1988), while chromosomal aberrations were increased in the bone marrow of mice given a glyphosate-based formulation (glyphosate isopropylamine salt, ~41%) (Prasad *et al.*, 2009). A single oral dose of a glyphosate-based formulation did not cause chromosomal aberrations in mice (Dimitrov *et al.*, 2006).

In mice treated by intraperitoneal injection, a single dose of glyphosate did not cause

Table 4.1 Genetic and related effects of glyphosate in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Blood	NR	DNA damage	DNA strand breaks, comet assay	24 exposed individuals in northern Ecuador; areas sprayed with glyphosate-based formulation (sampling 2 weeks to 2 months after spraying); control group was 21 non-exposed individuals	+ $P < 0.001$		Paz-y-Miño *et al.* (2007)
Blood	NR	Chromosomal damage	Chromosomal aberrations	92 individuals in 10 communities, northern border of Ecuador; sampling 2 years after last aerial spraying with herbicide mix containing glyphosate); control group was 90 healthy individuals from several provinces without background of smoking or exposure to genotoxic substances (hydrocarbons, X-rays, or pesticides)	–	182 karyotypes were considered normal [Smoking status, NR]	Paz-y-Miño *et al.* (2011)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	55 community residents, Nariño, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P < 0.001$]	*P* values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	53 community residents, Putumayo, Colombia; area with aerial glyphosate-based formulation spraying for coca and poppy eradication (glyphosate was tank-mixed with an adjuvant)	+ [$P = 0.01$]	*P* values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	27 community residents, Valle del Cauca, Colombia; area where glyphosate-based formulation was applied through aerial spraying for sugar-cane maturation (glyphosate was applied without adjuvant)	+ [$P < 0.001$]	*P* values for after spraying vs before spraying in the same individuals	Bolognesi *et al.* (2009)

[a] +, positive; –, negative
NR, not reported; vs, versus

micronucleus formation in the bone marrow (Rank *et al.*, 1993), although two daily doses did (Bolognesi *et al.*, 1997; Mañas *et al.*, 2009a). AMPA, the main metabolite of glyphosate, also produced micronucleus formation after two daily intraperitoneal doses (Mañas *et al.*, 2009b). Conflicting results for micronucleus induction were obtained in mice exposed intraperitoneally to a glyphosate-based formulation. A single dose of the formulation at up to 200 mg/kg bw did not induce micronucleus formation in the bone marrow in one study (Rank *et al.* 1993), while it did increase micronucleus formation at 25 mg/kg bw in another study (Prasad *et al.*, 2009). After two daily intraperitoneal doses, a glyphosate-based formulation did not induce micronucleus formation at up to 200 mg/kg bw according to Grisolia (2002), while Bolognesi *et al.* (1997) showed that the formulation did induce micronucleus formation at 450 mg/kg bw. In mice given a single oral dose of a glyphosate-based formulation at 1080 mg/kg bw, no induction of micronuclei was observed (Dimitrov *et al.*, 2006).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Glyphosate did not induce unscheduled DNA synthesis in rat primary hepatocytes, or *Hprt* mutation (with or without metabolic activation) in Chinese hamster ovary cells (Li & Long, 1988).

In bovine lymphocytes, chromosomal aberrations were induced by glyphosate in one study (Lioi *et al.*, 1998), but not by a glyphosate formulation in another study (Siviková & Dianovský, 2006). Roustan *et al.* (2014) demonstrated, in the CHO-K1 ovary cell line, that glyphosate induced micronucleus formation only in the presence of metabolic activation, while AMPA induced micronucleus formation both with and without metabolic activation. Sister-chromatid exchange was observed in bovine lymphocytes exposed to glyphosate (Lioi *et al.*, 1998) or a glyphosate formulation (in the absence but not the presence of metabolic activation) (Siviková & Dianovský, 2006).

(iii) Non-mammalian systems in vivo

See Table 4.5

Fish and other species

In fish, glyphosate produced DNA strand breaks in the comet assay in sábalo (Moreno *et al.*, 2014), European eel (Guilherme *et al.*, 2012b), zebrafish (Lopes *et al.*, 2014), and Nile tilapia (Alvarez-Moya *et al.*, 2014). AMPA also induced DNA strand breaks in the comet assay in European eel (Guilherme *et al.*, 2014b). A glyphosate-based formulation produced DNA strand breaks in numerous fish species, such as European eel (Guilherme *et al.*, 2010, 2012b, 2014a; Marques *et al.*, 2014, 2015), sábalo (Cavalcante *et al.*, 2008; Moreno *et al.*, 2014), guppy (De Souza Filho *et al.*, 2013), bloch (Nwani *et al.*, 2013), neotropical fish *Corydoras paleatus* (de Castilhos Ghisi & Cestari, 2013), carp (Gholami-Seyedkolaei *et al.*, 2013), and goldfish (Cavaş & Könen, 2007).

AMPA, the main metabolite of glyphosate, induced erythrocytic nuclear abnormalities (kidney-shaped and lobed nuclei, binucleate or segmented nuclei and micronuclei) in European eel (Guilherme *et al.*, 2014b). Micronucleus formation was induced by different glyphosate-based formulations in various fish (Grisolia, 2002; Cavaş & Könen, 2007; De Souza Filho *et al.*, 2013; Vera-Candioti *et al.*, 2013).

Glyphosate-based formulations induced DNA strand breaks in other species, including caiman (Poletta *et al.*, 2009), frog (Meza-Joya *et al.*, 2013), tadpoles (Clements *et al.*, 1997), and snail (Mohamed, 2011), but not in oyster (Akcha *et al.*, 2012), clam (dos Santos & Martinez, 2014), and mussel glochidia (Conners & Black, 2004). In earthworms, one glyphosate-based formulation induced DNA strand breaks while two others did not (Piola *et al.*, 2013; Muangphra *et al.*, 2014), highlighting the potential importance of components other than the active ingredient in the formulation.

Table 4.2 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in human cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Glyphosate							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	3 mM [507.2 µg/mL]	$P < 0.01$; dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas et al. (2009a)
Lymphocytes	DNA damage	DNA strand breaks, standard and hOGG1 modified comet assay	+	+	3.5 µg/mL	With the hOGG1 modified comet assay, + S9, the increase was significant ($P < 0.01$) only at the highest dose tested (580 µg/mL)	Mladinic *et al.* (2009b)
Lymphocytes	DNA damage	DNA strand breaks, comet assay	+	NT	0.0007 mM [0.12 µg/mL]	$P \leq 0.01$	Alvarez-Moya *et al.* (2014)
Fibroblast GM 38	DNA damage	DNA strand breaks, comet assay	+	NT	4 mM [676 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Fibroblast GM 5757	DNA damage	DNA strand breaks, comet assay	(+)	NT	75 mM [12 680 µg/mL]	Glyphosate (ineffective alone, data NR) increased strand breaks induced by H_2O_2 (40 or 50 µM) ($P < 0.004$ vs H_2O_2 alone)	Lueken *et al.* (2004)
Fibrosarcoma HT1080	DNA damage	DNA strand breaks, comet assay	+	NT	4.75 mM [803 µg/mL]	$P < 0.001$	Monroy *et al.* (2005)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	6 mM [1015 µg/mL]		Mañas *et al.* (2009a)
Lymphocytes	Chromosomal damage	Micronucleus formation	–	(+)	580 µg/mL	$P < 0.01$ at the highest exposure + S9 No concentration-related increase in micronuclei containing the centromere signal (C+)	Mladinic *et al.* (2009a)

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results[a]		Dose (LED or HID)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	1000 µg/mL	$P < 0.05$	Bolognesi *et al.* (1997)
AMPA							
Liver Hep-2	DNA damage	DNA strand breaks, comet assay	+	NT	4.5 mM [500 µg/mL]	$P < 0.05$ at 4.5 mM; $P < 0.01$ at up to 7.5 mM Dose–response relationship ($r \geq 0.90$; $P < 0.05$)	Mañas *et al.* (2009b)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	1.8 mM [200 µg/mL]	$P < 0.05$	Mañas *et al.* (2009b)
Glyphosate-based formulations							
Liver HepG2	DNA damage	DNA strand breaks, comet assay	(+)	NT	5 ppm	Glyphosate, 400 g/L Dose-dependent increase; greatest increase at 10 ppm Statistical analysis, NR	Gasnier *et al.* (2009)
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Glyphosate acid, 450g/L Dose-dependent increase ($P \leq 0.05$)	Koller *et al.* (2012)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	250 µg/mL	$P < 0.001$ No growth at 25 mg/mL	Vigfusson & Vyse (1980)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	100 µg/mL	Glyphosate, 30.4% $P < 0.05$	Bolognesi *et al.* (1997)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

AMPA, aminomethyl phosphonic acid; HID, highest ineffective dose; hOGG1, human 8-hydroxyguanosine DNA-glycosylase; LED, lowest effective dose; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant; SCGE, single cell gel electrophoresis; vs, versus

Micronucleus formation was induced by a glyphosate-based formulation (glyphosate, 36%) in earthworms (Muangphra *et al.*, 2014), and by a different glyphosate-based formulation in caiman (Poletta *et al.*, 2009, 2011), and frog (Yadav *et al.*, 2013).

Insects

In standard *Drosophila melanogaster*, glyphosate induced mutation in the test for somatic mutation and recombination, but not in a cross of flies characterized by an increased capacity for CYP450-dependent bioactivation (Kaya *et al.*, 2000). A glyphosate-based formulation also caused sex-linked recessive lethal mutations in *Drosophila* (Kale *et al.*, 1995).

Plants

In plants, glyphosate produced DNA damage in *Tradescantia* in the comet assay (Alvarez-Moya *et al.*, 2011). Chromosomal aberration was induced after exposure to glyphosate in fenugreek (Siddiqui *et al.*, 2012), and in onion in one study (Frescura *et al.*, 2013), but not in another (Rank *et al.*, 1993). A glyphosate-based formulation also induced chromosomal aberration in barley roots (Truta *et al.*, 2011) and onion (Rank *et al.*, 1993), but not in *Crepis capillaris* (hawksbeard) (Dimitrov *et al.*, 2006). Micronucleus formation was not induced by glyphosate in *Vicia faba* bean (De Marco *et al.*, 1992) or by a glyphosate-based formulation in *Crepis capillaris* (Dimitrov *et al.*, 2006).

(iv) Non-mammalian systems in vitro

See Table 4.6

Glyphosate induced DNA strand breaks in erythrocytes of tilapia fish, as demonstrated by comet assay (Alvarez-Moya *et al.*, 2014).

Glyphosate did not induce mutation in *Bacillus subtillis, Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100, or in *Escherichia coli* WP2, with or without metabolic activation (Li & Long, 1988). However, Rank *et al.* (1993) demonstrated that a glyphosate-based formulation was mutagenic in *S. typhimurium* TA98 in the absence of metabolic activation, and in *S. typhimurium* TA100 in the presence of metabolic activation.

4.2.2 Receptor-mediated mechanisms

(a) Sex-hormone pathway disruption

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

In hormone-dependent T47D breast cancer cells, the proliferative effects of glyphosate (10^{-6} to 1 μM) (see Section 4.2.4) and those of 17β-estradiol (the positive control) were mitigated by the estrogen receptor antagonist, ICI 182780; the proliferative effect of glyphosate was completely abrogated by the antagonist at a concentration of 10 nM (Thongprakaisang *et al.*, 2013). Glyphosate also induced activation of the estrogen response element (ERE) in T47D breast cancer cells that were stably transfected with a triplet ERE-promoter-luciferase reporter gene construct. Incubation with ICI 182780 at 10 nM eliminated the response. When the transfected cells were incubated with both 17β-estradiol and glyphosate, the effect of 17β-estradiol was reduced and glyphosate behaved as an estrogen antagonist. After 6 hours of incubation, glyphosate increased levels of estrogen receptors ERα and ERβ in a dose-dependent manner in T47D cells; after 24 hours, only ERβ levels were increased and only at the highest dose of glyphosate. [These findings suggested that the proliferative effects of glyphosate on T47D cells are mediated by ER.]

In human hepatocarcinoma HepG2 cells, four glyphosate-based formulations produced by the same company had a marked effect on the activity and transcription of aromatase, while glyphosate alone differed from controls, but not significantly so (Gasnier *et al.*, 2009).

Table 4.3 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammals in vivo

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Glyphosate								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	300 mg/kg bw	i.p.; 1×; sampled after 8 and 24 h	Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	–	270 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Single dose tested only $P < 0.05$ after 4 h	Bolognesi *et al.* (1997)
Mouse, CD-1 (M)	Uterus after mating	Mutation	Dominant lethal test	–	2000 mg/kg bw	Oral gavage; 1 ×	Proportion of early resorptions evaluated after mating of non-treated females with glyphosate-treated male mice	EPA (1980)
Rat, Sprague-Dawley (M, F)	Bone marrow	Chromosomal damage	Chromosomal aberrations	–	1000 mg/kg bw	i.p.; 1 ×; sampled after 6, 12 and 24 h	Single dose tested only	Li & Long (1988)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 and 48 h	Glyphosate isopropylamine salt	Rank *et al.* (1993)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	300 mg/kg bw	i.p.; 2 × 150 mg/kg bw with 24 h interval; sampled 6 or 24 h after the last injection	Single dose tested only $P < 0.05$ after 24 h	Bolognesi *et al.* (1997)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	400 mg/kg bw	i.p.; one injection per 24 h, 2 × 200, sampled 24 h after the last injection	$P < 0.01$ at the highest dose (400 mg/kg bw)	Mañas *et al.* (2009a)
AMPA								
Mouse, Balb C (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	200 mg/kg bw	i.p.; one injection per 24 h, 2 × 100, sampled 24 h after the last injection	$P < 0.01$ at the lowest dose (200 mg/kg bw)	Mañas *et al.* (2009b)
Glyphosate-based formulations								
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA adducts, 8-OHdG by LC/UV	–	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA adducts, 8-OHdG by LC/UV	+	~300 mg/kg bw	i.p.; 1 ×, sampled after 8 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M, F)	Kidney	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M, F)	Liver	DNA damage	DNA adducts, ^{32}P-DNA post labelling	+	400 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 30.4%	Peluso *et al.* (1998)
Mouse, Swiss CD1 (M)	Liver	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, Swiss CD1 (M)	Kidney	DNA damage	DNA strand breaks, alkaline elution assay	+	~300 mg/kg bw	i.p.; 1 ×; sampled after 4 and 24 h	Glyphosate, 30.4% Single dose tested only $P < 0.05$ only after 4 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow (PCE)	Chromosomal damage	Chromosomal aberrations	–	1080 mg/kg bw	p.o. in distilled water; 1 ×; sampled after 6, 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

Table 4.3 (continued)

Species, strain (sex)	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss albino (M)	Bone marrow	Chromosomal damage	Chromosomal aberrations	+	25 mg/kg bw	i.p.; 1 ×; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% The percentage of aberrant cells was increased vs control in a dose- and time-dependent manner ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, NMRI-bom (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 1 ×; sampled after 24 h	Glyphosate isopropylammonium salt, 480 g/L The percentage of PCE decreased	Rank *et al.* (1993)
Mouse, Swiss (M, F)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	–	200 mg/kg bw	i.p.; 2 × within 24 h interval and sampled 24 h after the last injection	Glyphosate isopropylammonium salt, 480 g/L	Grisolia (2002)
Mouse, Swiss albino (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	25 mg/kg bw	i.p.; 1 × ; sampled after 24, 48 and 72 h	Glyphosate isopropylamine salt, > 41% Significant induction of micronuclei vs control at both doses and all times ($P < 0.05$)	Prasad *et al.* (2009)
Mouse, Swiss CD1 (M)	Bone marrow (PCE)	Chromosomal damage	Micronucleus formation	+	450 mg/kg bw	i.p.; 2 × 225 mg/kg with 24 h interval; sampled 6 or 24 h after the last injection	Glyphosate, 30.4% Single dose tested only $P < 0.05$ after 6 h and 24 h	Bolognesi *et al.* (1997)
Mouse, C57BL (M)	Bone marrow	Chromosomal damage	Micronucleus formation	–	1080 mg/kg bw	p.o. in distilled water; 1 × ; sampled after 24, 48, 72, 96 and 120 h	Single dose tested only	Dimitrov *et al.* (2006)

[a] +, positive; -, negative; (+) or (-) positive/negative in a study with limited quality

bw, body weight; F, female; h, hour; HID, highest effective dose; i.p., intraperitoneal; LC, liquid chromatography; LED, lowest effective dose; M, male; PCE, polychromatic erythrocytes; p.o., oral; 8-OHdG, 8-hydroxydeoxyguanosine; UV, ultraviolet

Table 4.4 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results[a]		Dose (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Rat, Fisher F334	Hepatocytes	DNA damage	Unscheduled DNA synthesis	–	NT	125 µg/mL		Li & Long (1988)
Hamster, Chinese	CHO-K_1BH_4 ovary, cell line	Mutation	*Hprt* mutation	–	–	22 500 µg/mL		Li & Long (1988)
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	–	+	10 µg/mL	$P \leq 0.001$, in the dark +S9 Negative –S9 in the dark or with light irradiation	Roustan *et al.* (2014)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	17 µM [3 µg/mL]	$P < 0.05$	Lioi *et al.* (1998)
AMPA								
Hamster, Chinese	CHO-K1 ovary cell line	Chromosomal damage	Micronucleus formation	+	+	0.01 µg/mL	$P \leq 0.05$, in the dark –S9 Highest increase was observed at very low dose (0.0005 µg/mL) –S9 but with light-irradiation ($P < 0.01$)	Roustan *et al.* (2014)
Glyphosate-based formulations								
Bovine	Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	1120 µM [190 µg/mL]	Glyphosate, 62%	Siviková & Dianovský (2006)
Bovine	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	–	56 µM [9.5 µg/mL]	Glyphosate, 62% Time of exposure, 24 h $P < 0.01$, –S9, at ≥ 56 µM	Siviková & Dianovský (2006)

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

AMPA, aminomethyl phosphonic acid; HIC, highest ineffective concentration; *Hprt*, hypoxanthine guanine phosphoribosyl transferase gene; LEC, lowest effective concentration; NT, not tested

Table 4.5 Genetic and related effects of glyphosate, AMPA, and glyphosate-based formulations in non-mammalian systems in vivo

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Glyphosate							
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	0.48 mg/L	Time of exposure 6, 24, and 96 h For erythrocytes, $P = 0.01$ after 6 h, and $P = 0.014$ after 96 h; no significant increase after 24 h For gill cells, $P = 0.02$ only after 6 h at 2.4 mg/L	Moreno *et al.* (2014)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.0179 mg/L	Time of exposure 1 and 3 days $P < 0.05$	Guilherme *et al.* (2012b)
Fish	*Danio rerio* (zebrafish), sperm	DNA damage	DNA strand breaks, acridine orange method	+	10 mg/L	After 96 h, DNA integrity was 78.3 ± 3.5%, significantly reduced from control (94.7 ± 0.9%) and 5 mg/L (92.6 ± 1.9%), ($P < 0.05$)	Lopes *et al.* (2014)
Fish	*Oreochromis niloticus* (Nile tilapia) branchial erythrocytes	DNA damage	DNA strand breaks, comet assay	+	7 µM [1.2 mg/L]	Time of exposure, 10 days $P < 0.001$ with concentrations ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Oyster	Oyster spermatozoa	DNA damage	DNA strand breaks, comet assay	–	0.005 mg/L	Time of exposure, 1 h	Akcha *et al.* (2012)
Insect	*Drosophila* standard cross	Mutation	SMART	+	1 mM [0.169 mg/L]	Purity, 96% Increased frequency of small single spots (≥ 1 mM) and total spots (≥ 2 mM) $P = 0.05$	Kaya *et al.* (2000)
Insect	*Drosophila melanogaster*, high bioactivation cross	Mutation	SMART	–	10 mM [1.69 mg/L]	Purity, 96%	Kaya *et al.* (2000)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Plant systems	*Tradescantia* clone 4430 (spiderworts), staminal hair nuclei	DNA damage	DNA strand breaks, comet assay	+	0.0007 mM [0.12 µg/mL]	Glyphosate isopropylamine salt $P < 0.01$ for directly exposed nuclei (dose-dependent increase) and plants	Alvarez-Moya *et al.* (2011)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	3%	Single dose tested only Partial but significant reversal with distilled water	Frescura *et al.* (2013)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	–	2.88 µg/mL	Glyphosate isopropylamine	Rank *et al.* (1993)
Plant systems	*Trigonella foenum-graecum* L. (fenugreek)	Chromosomal damage	Chromosomal aberrations	+	0.2%	$P < 0.001$; positive dose–response relationship	Siddiqui *et al.* (2012)
Plant systems	*Vicia faba* (bean)	Chromosomal damage	Micronucleus formation	–	1400 ppm (1400 µg/g of soil)	Tested with two types of soil, but not without soil	De Marco *et al.* (1992)
AMPA							
Fish	*Anguilla anguilla* L. (European eel)	DNA damage	DNA strand breaks, comet assay	+	0.0118 mg/L	Time of exposure, 1 and 3 days $P < 0.05$ after 1 day of exposure	Guilherme *et al.* (2014b)
Fish	*Anguilla anguilla* L. (European eel)	Chromosomal damage	Other (ENA)	+	0.0236 mg/L	$P < 0.05$ only at highest dose after 3 day exposure (not after 1 day)	Guilherme *et al.* (2014b)
Glyphosate-based formulations							
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay	+	0.058 mg/L	$P < 0.05$ Positive dose–response relationship	Guilherme *et al.* (2010)
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 30.8% Time of exposure, 1 and 3 days With FPG, $P < 0.05$; with comet assay alone, $P < 0.05$ at 116 µg/L	Guilherme *et al.* (2012b)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Anguilla anguilla* L. (European eel), blood cells	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.116 mg/L	Single dose tested only Time of exposure, 3 days; recovery from non-specific DNA damage, but not oxidative DNA damage, 14 days after exposure $P < 0.05$	Guilherme *et al.* (2014a)
Fish	*Anguilla anguilla* L. (European eel), liver	DNA damage	DNA strand breaks, comet assay improved with the DNA-lesion-specific FPG and Endo III	+	0.058 mg/L	Glyphosate-based formulation, 485 g/L Time of exposure, 3 days $P < 0.05$	Marques *et al.* (2014, 2015)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and bronchial cells	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Single dose tested only, for 6, 24, and 96 h $P < 0.05$ for both erythrocytes and bronchial cells	Cavalcante *et al.* (2008)
Fish	*Prochilodus lineatus* (sábalo), erythrocytes and gill cells	DNA damage	DNA strand breaks, comet assay	+	1 mg/L	Glyphosate-based formulation, 480 g/L Time of exposure, 6, 24 and 96 h $P < 0.001$ after 24 and 96 h in erythrocytes and 24 h in gill cells	Moreno *et al.* (2014)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2.83 µL/L [1.833 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) $P < 0.05$	De Souza Filho *et al.* (2013)
Fish	*Channa punctatus* (bloch), blood and gill cells	DNA damage	DNA strand breaks, comet assay	+	3.25 mg/L	Exposure continued for 35 days; blood and gill cells collected on day 1, 7, 14, 21, 28 and 35 $P < 0.01$, for blood and gill cells; DNA damage increased with time and concentration	Nwani *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	DNA damage	DNA strand breaks, comet assay	+	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6, and 9 days $P < 0.01$, in blood and in liver cells	de Castilhos Ghisi & Cestari (2013)
Fish	*Cyprinus carpio* Linnaeus (carp), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	2 mg/L (10% LC_{50}, 96 h)	Glyphosate, equivalent to 360 g/L Single dose tested only, for 16 days $P < 0.01$	Gholami-Seyedkolaei *et al.* (2013)
Fish	*Carassius auratus* (goldfish), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days After 48 h: $P < 0.05$ (5 mg/L) and $P < 0.001$ (10 and 15 mg/L)	Cavaş & Könen (2007)
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Micronucleus formation	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Corydoras paleatus* (blue leopard corydoras, mottled corydoras and peppered catfish), blood and hepatic cells	Chromosomal damage	Micronucleus formation	–	0.0067 mg/L	Glyphosate, 48% (corresponding to 3.20 µg/L) Single dose tested only, for 3, 6 and 9 days	de Castilhos Ghisi & Cestari (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Tilapia rendalli* (redbreast tilapia) blood erythrocytes	Chromosomal damage	Micronucleus formation	+	42 mg/kg bw	Glyphosate, 480 g/L Increased frequency of micronucleus formation vs control ($P < 0.05$) in blood samples collected 4 days after a single intra-abdominal injection of 42, 85, or 170 mg/kg bw	Grisolia (2002)
Fish	*Carassius auratus* (goldfish), erythrocytes	Chromosomal damage	Micronucleus formation	+	5 ppm	Glyphosate equivalent to 360 g/L Time of exposure, 2, 4 and 6 days Statistically significant differences: 96 h ($P < 0.05$); 144 h ($P < 0.01$)	Cavaş & Könen (2007)
Fish	*Poecilia reticulata* (guppy) gill erythrocytes	Chromosomal damage	Micronucleus formation, ENA	+	1.41 µL/L [0.914 mg/L]	Glyphosate, 64.8%, m/v (648 g/L) Micronucleus formation, $P < 0.01$ Other nuclear abnormalities, $P < 0.05$ at 1.41 to 5.65 µL/L; concentration-dependent ($r^2 = 0.99$)	De Souza Filho *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	3.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.05$, with 3.9 and 7.8 mg/L for 48 and 96 h	Vera-Candioti *et al.* (2013)
Fish	*Cnesterodon decemmaculatus* (Jenyns, 1842) peripheral blood erythrocytes	Chromosomal damage	Micronucleus formation	+	22.9 mg/L	Glyphosate, 48% Time of exposure, 48 and 96 h $P < 0.01$, with 22.9 and 45.9 mg/L, and $P < 0.05$ at 68.8 mg/L, for 96 h	Vera-Candioti *et al.* (2013)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Fish	*Prochilodus lineatus* (sábalo) erythrocytes	Chromosomal damage	Chromosomal aberrations	–	10 mg/L	Single dose tested only, for 6, 24, and 96 h Nuclear abnormalities (lobed nuclei, segmented nuclei and kidney-shaped nuclei)	Cavalcante *et al.* (2008)
Fish	*Anguilla anguilla* L. (European eel), peripheral mature erythrocytes	Chromosomal damage	Other (ENA)	+	0.058 mg/L	Time of exposure, 1 and 3 days Chromosomal breakage and/or chromosomal segregational abnormalities after 3 days of exposure, $P < 0.05$	Guilherme *et al.* (2010)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	DNA damage	DNA strand breaks, comet assay	–	19 800 mg/L	Glyphosate, 66.2% Single dose tested only; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching	Poletta *et al.* (2011)
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	0.500 mg/egg	Glyphosate, 66.2% In-ovo exposure; blood sampling at the time of hatching $P < 0.05$ in both experiments (50–1000 µg/egg in experiment 1; 500–1750 µg/egg in experiment 2)	Poletta *et al.* (2009)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Caiman	*Caiman latirostris* (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus fomation	+	19.8 g/L	Glyphosate, 66.2% One dose tested; in-ovo exposure First spraying exposure at the beginning of incubation period, a second exposure on day 35, then incubation until hatching. Micronucleus formation, $P < 0.001$ Damage index, $P < 0.001$	Poletta *et al.* (2011)
Frog tadpole	*Rana catesbeiana* (ouaouaron), blood	DNA damage	DNA strand breaks, comet assay	+	1.687 mg/L, p.o.	Time of exposure, 24 h $P < 0.05$, with 6.75 mg/L; and $P < 0.001$ with 27 mg/L (with 108 mg/L, all died within 24 h)	Clements *et al.* (1997)
Frog	*Eleutherodactylus johnstonei* (Antilles coqui), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	0.5 µg a.e./cm^2	Glyphosate-based formulation, 480 g/L Exposure to an homogenate mist in a 300 cm^2 glass terrarium Time of exposure: 0.5, 1, 2, 4, 8 and 24 h $P < 0.05$	Meza-Joya *et al.* (2013)
Frog	*Euflictis cyanophlyctis* (Indian skittering frog), erythrocytes	Chromosomal damage	Micronucleus formation	+	1 mg a.e./L	Glyphosate isopropylamine salt, 41% Time of exposure: 24, 48, 72, and 96 h $P < 0.001$ at 24, 48, 72 and 96 h	Yadav *et al.* (2013)
Snail	*Biomphalaria alexandrina*, haemolymph	DNA damage	DNA strand breaks, comet assay	+	10 mg/L	Glyphosate, 48% Single dose tested only, for 24 h. The percentage of damaged DNA was 21% vs 4% (control) No statistical analysis	Mohamed (2011)
Oyster	Oysters, spermatozoa	DNA damage	DNA strand breaks, comet assay	–	5 µg/L	Glyphosate, 200 µg equivalent/L Time of exposure, 1 h	Akcha *et al.* (2012)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Clam	*Corbicula fluminea* (Asian clam) haemocytes	DNA damage	DNA strand breaks, comet assay	–	10 mg/L	Time of exposure, 96 h Significant increase when atrazine (2 or 10 mg/L) was added to glyphosate ($P < 0.05$) No increase after exposure to atrazine or glyphosate separately	dos Santos & Martinez (2014)
Mussels	*Utterbackia imbecillis* (Bivalvia: Unionidae) glochidia mussels (larvae)	DNA damage	DNA strand breaks, comet assay	–	5 mg/L	Glyphosate, 18% Doses tested: 2.5 and 5 mg/L for 24 h NOEC, 10.04 mg/L	Conners & Black (2004)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	240 µg a.e./cm^2	Monoammonium salt, 85.4%, a.e. Epidermic exposure during 72 h (on filter paper)	Piola *et al.* (2013)
Worm	Earthworm, *Eisenia andrei*, coelomocytes	DNA damage	DNA strand breaks, comet assay	+	15 µg a.e./cm^2	Monoammonium salt, 72%, a.e. Epidermic exposure during 72 h (on filter paper) $P < 0.001$	Piola *et al.* (2013)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	DNA damage	DNA strand breaks, comet assay	–	251.50 µg/cm^2	Active ingredient, 36% (w/v) Epidermic exposure 48 h on filter paper; LC_{50}, 251.50 µg/cm^2	Muangphra *et al.* (2014)
Worm	Earthworm, *Pheretima peguana*, coelomocytes	Chromosomal damage	Micronucleus formation	+	251.50 µg/cm^2	Active ingredient, 36% (w/v) Exposure, 48 h on filter paper; LC_{50}, 251.50 µg/cm^2 filter paper $P < 0.05$, for total micro-, bi-, and trinuclei frequencies at 0.25 µg/cm^2; when analysed separately, micro- and trinuclei frequencies significantly differed from controls only at the LC_{50}	Muangphra *et al.* (2014)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results[a]	Dose (LED or HID)	Comments	Reference
Insect	*Drosophila melanogaster*	Mutation	Sex-linked recessive lethal mutations	+	1 ppm	Single dose tested only $P < 0.001$	Kale *et al.* (1995)
Plant systems	*Allium cepa* (onion)	Chromosomal damage	Chromosomal aberrations	+	1.44 µg/mL	Glyphosate-based formulation, 480 g/L The doses of formulation were calculated as glyphosate isopropylamine $P < 0.005$	Rank *et al.* (1993)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Chromosomal aberrations	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)
Plant systems	*Hordeum vulgare* L. cv. Madalin (barley roots)	Chromosomal damage	Chromosomal aberrations	(+)	360 µg/mL (0.1%)	Reported as "significant"	Truta *et al.* (2011)
Plant systems	*Crepis capillaris* (hawksbeard)	Chromosomal damage	Micronucleus formation	–	0.5%	The highest dose tested (1%) was toxic	Dimitrov *et al.* (2006)

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

a.e., acid equivalent; AMPA, aminomethyl phosphonic acid; bw, body weight; ENA, erythrocytic nuclear abnormalities; Endo III, endonuclease III; FPG, formamidopyrimidine glycosylase; h, hour; HID, highest ineffective dose; LC_{50}, median lethal dose; LED, lowest effective dose; NOEC, no-observed effect concentration; p.o., oral; SMART, somatic mutation and recombination test

Table 4.6 Genetic and related effects of glyphosate and glyphosate-based formulations on non-mammalian systems in vitro

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Glyphosate								
Eukaryote Fish	*Oreochromis niloticus* (Nile tilapia), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	NT	7 µM [1.2 µg/mL]	Glyphosate isopropylamine, 96% $P \leq 0.001$; positive dose–response relationship for doses ≥ 7 µM	Alvarez-Moya *et al.* (2014)
Prokaryote (bacteria)	*Scytonema javanicum* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Wang *et al.* (2012)
Prokaryote (bacteria)	*Anabaena spherica* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Microcystis viridis* (cyanobacteria)	DNA damage	DNA strand breaks, FADU assay	(+)	NT	10 µM [1.7 µg/mL] (in combination with UVB)	Co-exposure to glyphosate (not tested alone; single dose tested only) enhanced UVB-induced increases	Chen *et al.* (2012)
Prokaryote (bacteria)	*Bacillus B. subtilis*	Differential toxicity	Rec assay	–	NT	2000 µg/disk		Li & Long (1988)
Prokaryote (bacteria)	*Salmonella typhimurium* TA1535, TA1537, TA1538, TA98 and TA100	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)
Prokaryote (bacteria)	*Escherichia coli* WP2	Mutation	Reverse mutation	–	–	5000 µg/plate		Li & Long (1988)

Table 4.6 (continued)

Phylogenetic class	Test system (species; strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Acellular systems	Prophage superhelical PM2 DNA	DNA damage	DNA strand breaks	(–)	NT	75 mM [12.7 mg/mL] (in combination with H_2O_2 (100 µM)	Glyphosate inhibited H_2O_2-induced damage of PM2 DNA at concentrations where synergism was observed in cellular DNA damage (data NR)	Lueken *et al.* (2004)
Glyphosate-based formulations								
Prokaryote (bacteria)	*Salmonella typhimurium* TA98	Mutation	Reverse mutation	+	–	360 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)
Prokaryote (bacteria)	*Salmonella typhimirium* TA100	Mutation	Reverse mutation	–	+	720 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank *et al.* (1993)

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

FADU, fluorometric analysis of DNA unwinding; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; UVB, ultraviolet B

Additionally, although all four glyphosate-based formulations dramatically reduced the transcription of ERα and ERβ in ERE-transfected HepG2 cells, glyphosate alone had no significant effect. Glyphosate and all four formulations reduced androgen-receptor transcription in the breast cancer cell line MDA-MB453-kb2, which has a high level of androgen receptor, with the formulations showing greater activity than glyphosate alone.

In a human placental cell line derived from choriocarcinoma (JEG3 cells), 18 hours of exposure to a glyphosate-based formulation (IC_{50} = 0.04%) decreased aromatase activity (Richard *et al.*, 2005). Glyphosate alone was without effect. The concentrations used did not affect cell viability.

Glyphosate, at non-overtly toxic concentrations, decreased aromatase activity in fresh human placental microsomes and transformed human embryonic kidney cells (293) transfected with human aromatase cDNA (Benachour *et al.*, 2007). A glyphosate-based formulation, at non-overtly toxic concentrations, had the same effect. The formulation was more active at equivalent doses than glyphosate alone.

In human androgen receptor and ERα and ERβ reporter gene assays using the Chinese hamster ovary cell line (CHO-K1), glyphosate had neither agonist nor antagonist activity (Kojima *et al.*, 2004, 2010).

(ii) Non-human mammalian experimental systems

In vivo

No data were available to the Working Group.

In vitro

Benachour *et al.* (2007) and Richard *et al.* (2005) reported that glyphosate and a glyphosate-based formulation inhibited aromatase activity in microsomes derived from equine testis. Richard *et al.* (2005) reported an absorbance spectrum consistent with an interaction between a nitrogen atom of glyphosate and the active site of the purified equine aromatase enzyme.

In the mouse MA-10 Leydig cell tumour cell line, a glyphosate-based formulation (glyphosate, 180 mg/L) markedly reduced [$(Bu)_2$] cAMP-stimulated progesterone production (Walsh *et al.*, 2000). The inhibition was dose-dependent, and occurred in the absence of toxicity or parallel reductions in total protein synthesis. In companion studies, the formulation also disrupted steroidogenic acute regulatory protein expression, which is critical for steroid hormone synthesis. Glyphosate alone did not affect steroidogenesis at any dose tested up to 100 µg/L. Forgacs *et al.* (2012) found that glyphosate (300 µM) had no effect on testosterone production in a novel murine Leydig cell line (BLTK1). Glyphosate did not modulate the effect of recombinant human chorionic gonadotropin, which served as the positive control for testosterone production.

(iii) Non-mammalian experimental systems

Gonadal tissue levels of testosterone, 17β-estradiol and total microsomal protein were significantly reduced in adult snails (*Biomphalaria alexandrina*) exposed for 3 weeks to a glyphosate-based formulation (glyphosate, 48%) at the LC_{10} (10% lethal concentration) (Omran & Salama, 2013). These effects persisted after a 2-week recovery period, although the impact on 17β-estradiol was reduced in the recovery animals. The formulation also induced marked degenerative changes in the ovotestis, including absence of almost all the gametogenesis stages. CYP450 1B1, measured by enzyme-linked immunosorbent assay (ELISA), was substantially increased in the treated snails, including after the recovery period.

Glyphosate (0.11 mg/L for 7 days) did not increase plasma vittelogenin levels in juvenile rainbow trout (Xie *et al.*, 2005).

(b) Other pathways

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Glyphosate did not exhibit agonist activity in an assay for a human pregnane X receptor (PXR) reporter gene in a CHO-K1 cell line (Kojima *et al.*, 2010).

(ii) Non-human mammalian experimental systems

In vivo

In rats, glyphosate (300 mg/kg bw, 5 days per week, for 2 weeks) had no effect on the formation of peroxisomes, or the activity of hepatic carnitine acetyltransferase and catalase, and did not cause hypolipidaemia, suggesting that glyphosate does not have peroxisome proliferator-activated receptor activity (Vainio *et al.*, 1983).

In vitro

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

(iii) Non-mammalian experimental systems

As a follow-up to experiments in which injection of glyphosate, or incubation with a glyphosate-based formulation (glyphosate, 48%), caused chick and frog (*Xenopus laevis*) cephalic and neural crest terata characteristic of retinoic acid signalling dysfunction, Paganelli *et al.*, (2010) measured retinoic acid activity in tadpoles exposed to a glyphosate-based formulation. Retinoic activity measured by a reporter gene assay was increased by the formulation, and a retinoic acid antagonist blocked the effect. This indicated a possible significant modulation of retinoic acid activity by glyphosate.

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Several studies examined the effects of glyphosate on oxidative stress parameters in the human keratinocyte cell line HaCaT. Gehin *et al.* (2005) found that a glyphosate-based formulation was cytotoxic to HaCaT cells, but that addition of antioxidants reduced cytotoxicity. Elie-Caille *et al.* (2010) showed that incubation of HaCaT cells with glyphosate at 21 mM (the half maximal inhibitory concentration for cytotoxicity, IC_{50}) for 18 hours increased production of hydrogen peroxide (H_2O_2) as shown by dichlorodihydrofluorescein diacetate assay. Similarly, George & Shukla (2013) exposed HaCaT cells to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) and evaluated oxidative stress using the dichlorodihydrofluorescein diacetate assay. The formulation (0.1 mM) increased maximum oxidant levels by approximately 90% compared with vehicle, an effect similar to that of H_2O_2 (100 mM). Pre-treatment of the cells with the antioxidant *N*-acetylcysteine abrogated generation of oxidants by both the formulation and by H_2O_2. *N*-Acetylcysteine also inhibited cell proliferation induced by the glyphosate-based formulation (0.1 mM). [The Working Group noted the recognized limitations of using dichlorodihydrofluorescein diacetate 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.]

Chaufan *et al.* (2014) evaluated the effects of glyphosate, AMPA (the main metabolite of glyphosate), and a glyphosate-based formulation on oxidative stress in HepG2 cells. The formulation, but not glyphosate or AMPA, had adverse effects. Specifically, the formulation increased levels of reactive oxygen species, nitrotyrosine formation, superoxide dismutase activity, and glutathione, but did not have an effect on catalase or glutathione-*S*-transferase activities. Coalova *et al.* (2014) exposed Hep2 cells to a glyphosate-based formulation (glyphosate as isopropylamine salt, 48%) at the LC_{20} (concentration not otherwise specified) and evaluated various parameters of oxidative stress. Exposure to the formulation for 24 hours increased catalase activity and glutathione levels, but did not have an effect on superoxide dismutase or glutathione-*S*-transferase activity.

Using blood samples from non-smoking male donors, Mladinic *et al.* (2009b) examined the effects of in-vitro exposure to glyphosate on oxidative DNA damage in primary lymphocyte cultures and on lipid peroxidation in plasma. Both parameters were significantly elevated at glyphosate concentrations of 580 µg/mL (~3.4 mM), but not at lower concentrations. Kwiatkowska *et al.* (2014) examined the effects of glyphosate, its metabolite AMPA, and *N*-methylglyphosate (among other related compounds) in human erythrocytes isolated from healthy donors. The erythrocytes were exposed at concentrations of 0.01–5 mM for 1, 4, or 24 hours before flow cytometric measurement of the production of reactive oxygen species with dihydrorhodamine 123. Production of reactive oxygen species was increased by glyphosate (≥ 0.25 mM), AMPA (≥ 0.25 mM), and *N*-methylglyphosate (≥ 0.5 mM).

(ii) Non-human mammalian experimental systems

Most of the studies of oxidative stress and glyphosate were conducted in rats and mice, and examined a range of exposure durations, doses, preparations (glyphosate and glyphosate-based formulations), administration routes and tissues. In addition, various end-points were evaluated to determine whether oxidative stress is induced by exposure to glyphosate. Specifically, it was found that glyphosate induces production of free radicals and oxidative stress in mouse and rat tissues through alteration of antioxidant enzyme activity, depletion of glutathione, and increases in lipid peroxidation. Increases in biomarkers of oxidative stress upon exposure to glyphosate in vivo have been observed in blood plasma (Astiz *et al.*, 2009b), liver (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), skin (George *et al.*, 2010), kidney (Bolognesi *et al.*, 1997; Astiz *et al.*, 2009b), and brain (Astiz *et al.*, 2009b). Several studies demonstrated similar effects with a glyphosate-based formulation in the liver (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011; Jasper *et al.*, 2012), kidney (Bolognesi *et al.*, 1997; Cavuşoğlu *et al.*, 2011) and brain (Cattani *et al.*, 2014), or with a pesticide mixture containing glyphosate in the testes (Astiz *et al.*, 2013). Pre-treatment with antioxidants has been shown to mitigate the induction of oxidative stress by a glyphosate-based formulation (Cavuşoğlu *et al.*, 2011) and by a pesticide mixture containing glyphosate (Astiz *et al.*, 2013).

DNA damage associated with oxidative stress after exposure to glyphosate (e.g. as reported in Bolognesi *et al.*, 1997) is reviewed in Section 4.2.1.

(iii) Non-mammalian experimental systems

Positive associations between exposure to glyphosate and oxidative stress were reported in various tissues in aquatic organisms (reviewed in Slaninova *et al.*, 2009). Glyphosate and various glyphosate-based formulations have been tested in various fish species for effects on a plethora of end-points (e.g. lipid peroxidation, DNA

damage, expression of antioxidant enzymes, levels of glutathione), consistently presenting evidence that glyphosate can cause oxidative stress in fish (Lushchak *et al.*, 2009; Ferreira *et al.*, 2010; Guilherme *et al.*, 2010, 2012a, b, 2014a, b; Modesto & Martinez, 2010a, b; Cattaneo *et al.*, 2011; Glusczak *et al.*, 2011; de Menezes *et al.*, 2011; Ortiz-Ordoñez *et al.*, 2011; Nwani *et al.*, 2013; Marques *et al.*, 2014, 2015; Sinhorin *et al.*, 2014; Uren Webster *et al.*, 2014). Similar effects were observed in bullfrog tadpoles exposed to a glyphosate-based formulation (Costa *et al.*, 2008), and in the Pacific oyster exposed to a pesticide mixture containing glyphosate (Geret *et al.*, 2013).

(b) Inflammation and immunomodulation

(i) Humans

Studies in exposed humans

No data were available to the Working Group.

Human cells in vitro

Nakashima *et al.* (2002) investigated the effects of glyphosate on cytokine production in human peripheral blood mononuclear cells. Glyphosate (1 mM) had a slight inhibitory effect on cell proliferation, and modestly inhibited the production of IFN-gamma and IL-2. The production of TNF-α and IL-1 β was not affected by glyphosate at concentrations that significantly inhibited proliferative activity and T-cell-derived cytokine production.

(ii) Non-human mammalian experimental systems

Kumar *et al.* (2014) studied the pro-inflammatory effects of glyphosate and farm air samples in wildtype C57BL/6 and TLR4$^{-/-}$ mice, evaluating cellular response, humoral response, and lung function. In the bronchoalveolar lavage fluid and lung digests, airway exposure to glyphosate (1 or 100 µg) significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and IgG2a levels. Airway exposure to glyphosate (100 ng, 1 µg, or 100 µg per day for 7 days) also produced substantial pulmonary inflammation, confirmed by histological examination. In addition, glyphosate-rich farm-air samples significantly increased circulating levels of IL-5, IL-10, IL-13 and IL-4 in wildtype and in TLR4$^{-/-}$ mice. Glyphosate was also tested in wildtype mice and significantly increased levels of IL-5, IL-10, IL-13, and IFN-γ (but not IL-4). The glyphosate-induced pro-inflammatory effects were similar to those induced by ovalbumin, and there were no additional or synergistic effects when ovalbumin was co-administered with glyphosate.

Pathological effects of glyphosate on the immune system have been reported in 13-week rat and mouse feeding studies by the NTP (Chan & Mahler, 1992). Relative thymus weight was decreased in male rats exposed for 13 weeks, but increased in male mice. Treatment-related changes in haematological parameters were observed in male rats at 13 weeks and included mild increases in haematocrit [erythrocyte volume fraction] and erythrocytes at 12 500, 25 000, and 50 000 ppm, haemoglobin at 25 000 and 50 000 ppm, and platelets at 50 000 ppm. In female rats, small but significant increases occurred in lymphocyte and platelet counts, leukocytes, mean corpuscular haemoglobin, and mean corpuscular volume at 13 weeks.

Blakley (1997) studied the humoral immune response in female CD-1 mice given drinking-water containing a glyphosate-based formulation at concentrations up to 1.05% for 26 days. The mice were inoculated with sheep erythrocytes to produce a T-lymphocyte, macrophage-dependent antibody response on day 21 of exposure. Antibody production was not affected by the formulation.

(iii) Non-mammalian experimental systems

A positive association between exposure to glyphosate and immunotoxicity in fish has been reported. Kreutz *et al.* (2011) reported alterations

in haematological and immune-system parameters in silver catfish (*Rhamdia quelen*) exposed to sublethal concentrations (10% of the median lethal dose, LC_{50}, at 96 hours) of a glyphosate-based herbicide. Numbers of blood erythrocytes, thrombocytes, lymphocytes, and total leukocytes were significantly reduced after 96 hours of exposure, while the number of immature circulating cells was increased. The phagocytic index, serum bacteria agglutination, and total peroxidase activity were significantly reduced after 24 hours of exposure. Significant decreases in serum bacteria agglutination and lysozyme activity were found after 10 days of exposure. No effect on serum bactericidal and complement natural haemolytic activity was seen after 24 hours or 10 days of exposure to glyphosate.

el-Gendy *et al.* (1998) demonstrated effects of a glyphosate-based formulation (glyphosate, 48%) at 1/1000 of the concentration recommended for field application on humoral and cellular immune response in bolti fish (*Tilapia nilotica*). The mitogenic responses of splenocytes to phytohaemagglutinin, concanavalin A, and lipopolysaccharide in fish exposed to glyphosate for 96 hours were gradually decreased and reached maximum depression after 4 weeks. Glyphosate also produced a concentration-dependent suppression of in-vitro plaque-forming cells in response to sheep erythrocytes.

4.2.4 Cell proliferation and death

(a) Humans

(i) Studies in exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

Cell proliferation potential was explored in HaCaT keratinocytes exposed to a glyphosate-based formulation (glyphosate, 41%; concentration, up to 0.1 mM) (George & Shukla, 2013). The formulation increased the number of viable cells, as assessed by the MTT assay (based on reduction of the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) at concentrations up to 0.1 mM, while concentration- and incubation-time-dependent reductions were seen at higher concentrations (up to 1 mM). The formulation (0.01 or 0.1 mM for 72 hours) significantly enhanced cell proliferation (measured by staining for either proliferating cell nuclear antigen or 5-bromo-2′-deoxyuridine); at 0.1 mM, the increases exceeded levels for the positive control, tetradecanoyl-phorbol-13-acetate. The proportion of S-phase cells (assessed using flow cytometry) and the expression of G1/S cell-cycle regulatory proteins (cyclins D1 and E, CDK2, CDK4, and CDK6) increased after exposure to the formulation or the positive control.

Li *et al.* (2013) reported that glyphosate and AMPA inhibited cell growth in eight human cancer cell lines, but not in two immortalized normal prostate cell lines. An ovarian (OVCAR-3) and a prostate (C4–2B) cell line showed the greatest loss in viability, with glyphosate or AMPA at 15–50 mM. Further assays were conducted on AMPA, but not glyphosate, in two prostate cancer cell lines (C4–2B and PC-3), and found cell-cycle arrest (decreased entry of cells into S-phase) and increased apoptosis. [The Working Group noted that the findings from these assays with AMPA are of unclear relevance to the effects of glyphosate.]

Glyphosate (10^{-6} to 1 μM) increased growth by 15–30% relative to controls in hormone-dependent T47D breast cancer cells, but only when endogenous estrogen was minimized in the culture medium (by substitution with 10% dextran-charcoal treated fetal bovine serum). Glyphosate did not affect the growth of hormone-independent MDA-MB231 breast cancer cells cultured in either medium (Thongprakaisang *et al.*, 2013).

Glyphosate (up to 30 μM) did not show cell proliferation potential (5-bromo-2′-deoxyuridine) and did not activate caspase 3 or TP53 in human neuroprogenitor ReN CX cells (Culbreth *et al.*, 2012).

Several studies evaluated the impact of glyphosate or glyphosate-based formulations on apoptotic cell death in the HepG2 human hepatoma cell line. Glyphosate-based formulations induced apoptosis in HepG2 cells, while glyphosate alone was generally without effect or showed effects at considerably higher concentrations (Gasnier *et al.*, 2009, 2010; Mesnage *et al.*, 2013; Chaufan *et al.*, 2014; Coalova *et al.*, 2014). For example, 23.5% of the nuclei of HepG2 cells exposed to a glyphosate-based formulation showed condensed and fragmented chromatin ($P < 0.01$), and caspases 3 and 7 were significantly activated, both effects being indicative of apoptosis (Chaufan *et al.*, 2014). Caspases were unaffected by glyphosate or AMPA alone. Glyphosate and AMPA did not affect cell viability at concentrations up to 1000 mg/L, a concentration that increased rather than decreased cell viability after 48 and 72 hours of incubation. In contrast, cells exposed to glyphosate-based formulation at lower concentrations were not viable. Similarly, Coalova *et al.* (2014) reported that a glyphosate-based formulation (glyphosate, 48%) induced apoptotic cell death in HepG2 cells. Apoptosis was indicated by activation of caspases 3 and 7, and the significant fraction (17.7%) of nuclei with condensed and fragmented chromatin ($P < 0.001$).

In studies with glyphosate and nine different glyphosate-based formulations in three cell lines, glyphosate alone did not increase the activity of adenylate kinase (Mesnage *et al.*, 2013). The activity of caspases 3 and 7 was significantly increased by glyphosate in HepG2 and embryonic kidney HEK293 cells, and elevated (although not significantly) about 1.8 times above control levels in placental choriocarcinoma JEG-3 cells. Two formulations containing an ethoxylated adjuvant induced adenylate kinase activity to a greater extent than caspase activity. All formulations were reported to be more cytotoxic than glyphosate. [In concentration–response curves, glyphosate showed an effect on mitochondrial succinate dehydrogenase activity, a measure of cell viability, that was similar to that shown by one formulation. The calculated 50% lethal concentration in JEG3 cells for mitochondrial succinate dehydrogenase activity was greater for three formulations, although the values appeared inconsistent with the concentration–response curves.]

In HUVEC primary neonate umbilical cord vein cells, and 293 embryonic kidney and JEG3 placental cell lines, Benachour & Séralini (2009) found that glyphosate at relatively high concentrations induced apoptosis, as indicated by induction of caspases 3 and 7, and DNA staining and microscopy. At comparable or lower concentrations, four glyphosate-based formulations all caused primarily necrotic cell death. The umbilical cord HUVEC cells were the most sensitive (by about 100-fold) to the apoptotic effects of glyphosate.

Heu *et al.* (2012) evaluated apoptosis in immortalized human keratinocytes (HaCaT) exposed to glyphosate (5–70 mM). Based on annexin V, propidium iodide and mitochondrial staining, exposures leading to 15% cytotoxicity gave evidence of early apoptosis, while increases in late apoptosis and necrosis were observed at higher levels of cytotoxicity.

(b) Non-human mammalian experimental systems

(i) In vivo

In male Wistar rats, glyphosate (10 mg/kg bw, injected intraperitoneally three times per week for 5 weeks) reduced, but not significantly, the inner mitochondrial membrane integrity of the substantia nigra and cerebral cortex (Astiz *et al.* 2009a). Caspase 3 activity was unaltered in these tissues. Mitochondrial cardiolipin content was significantly reduced, particularly in the substantia nigra, where calpain activity was substantially higher. Glyphosate induced DNA fragmentation in the brain and liver.

(ii) In vitro

In adult Sprague Dawley rat testicular cells exposed in vitro, glyphosate (up to 1%; for 24 or 48 hours) did not provoke cell-membrane alterations (Clair *et al.*, 2012). However, caspase 3 and 7 activity increased with exposure in Sertoli cells alone, and in Sertoli and germ cell mixtures. On the other hand, a glyphosate-based formulation (a 0.1% solution, containing 0.36 g/L of glyphosate) induced membrane alterations and decreased the activity of caspase 3 and 7 in Leydig cells, and in Sertoli and germ cell mixtures. In a separate study, glyphosate increased apoptosis in primary Sertoli cell cultures from mice (Zhao *et al.*, 2013).

Glyphosate (5–40 mM, for 12, 24, 48, or 72 hours) significantly increased cell death in a time- and concentration-dependent manner in differentiated rat pheochromocytoma PC12 (neuronal) cells Gui *et al.* (2012). Apoptotic changes included cell shrinkage, DNA fragmentation, decreased Bcl2 expression, and increased Bax expression. Both autophagy and apoptosis were implicated, as pre-treatment with the pan-caspase inhibitor Z-VAD or the autophagy inhibitor 3-MA inhibited cell loss.

Induction of apoptosis by glyphosate or glyphosate-based formulations was also studied in other cell lines. Glyphosate (10 μM) induced apoptosis in rat heart H9c2 cells, the effect being enhanced when glyphosate was given in combination with the adjuvant TN-20 (5 μM), (Kim *et al.*, 2013). A glyphosate-based formulation induced apoptosis in mouse 3T3-L1 fibroblasts, and inhibited their transformation to adipocytes (Martini *et al.*, 2012). A glyphosate-based formulation (10 mM) did not increase rat hepatoma HTC cell death, but did affect mitochondrial membrane potential (Malatesta *et al.*, 2008).

Glyphosate (up to 30 μM) did not activate caspase 3 or show cell proliferation potential (5-bromo-2′-deoxyuridine) in a mouse neuroprogenitor cell line, but did activate Tp53 at the highest concentration tested (Culbreth *et al.*, 2012).

4.2.5 Other mechanisms

No data on immortalization, epigenetic alterations, altered DNA repair, or genomic instability after exposure to glyphosate were available to the Working Group.

4.3 Data relevant to comparisons across agents and end-points

No data on high-throughput screening or other relevant data were available to the Working Group. Glyphosate was not tested by the Tox21 and ToxCast research programmes of the government of the USA (Kavlock *et al.* 2012; Tice *et al.*, 2013).

4.4 Cancer susceptibility data

No studies that examined genetic, life-stage, or other susceptibility factors with respect to adverse health outcomes that could be associated with exposure to glyphosate were identified by the Working Group.

4.5 Other adverse effects

4.5.1 Humans

In the USA in the past decade, poison-control centres have reported more than 4000 exposures to glyphosate-containing herbicides, of which several hundred were evaluated in a health-care facility, and fatalities were rare (Rumack, 2015). In a pesticide surveillance study carried out by the National Poisons Information Service of the United Kingdom, glyphosate was among the most common pesticide exposure implicated in severe or fatal poisoning cases between 2004 and 2013 (Perry *et al.*, 2014). Deliberate poisonings with glyphosate resulting in toxicity and fatality

have been reported in many countries, including Australia (Stella & Ryan, 2004), Denmark (Mortensen *et al.*, 2000), India (Mahendrakar *et al.*, 2014), Japan (Motojyuku *et al.*, 2008), Republic of Korea (Park *et al.*, 2013), New Zealand (Temple & Smith, 1992), Sri Lanka (Roberts *et al.*, 2010), Taiwan, China (Chen *et al.*, 2009), and Thailand (Sribanditmongkol *et al.*, 2012).

Glyphosate demonstrated no potential for photo-irritation or photo-sensitization in 346 volunteers exposed dermally on normal or abraded skin (Hayes & Laws, 1991). On the other hand, Mariager *et al.* (2013) reported severe burns after prolonged accidental dermal exposure to a glyphosate-based formulation.

4.5.2 Experimental systems

Glyphosate was tested in nine regulatory submissions included in the Toxicity Reference Database (ToxRefDB) and reviewed by the EPA (EPA, 2015). Specifically, study design, treatment group, and treatment-related effect information were captured for four long-term studies and/or carcinogenicity studies, one short-term study, two multigeneration studies of reproductivity, and two studies of developmental toxicity. The NTP also tested glyphosate in a 13-week study in rats and mice (Chan & Mahler, 1992).

In a long-term combined study of toxicity and carcinogenicity in rats given glyphosate at nominal doses of 100, 400, and 1000 mg/kg bw per day, inflammation was observed in the stomach mucosa of females at the intermediate and highest doses (EPA, 1990, 1991b). In males at the highest dose, liver weight, cataracts and lens degeneration in the eyes, and urine specific gravity were increased, while body weight, body-weight gain, and urinary pH were decreased. Pancreatic acinar cell atrophy was observed in males at the highest dose. Pancreatic inflammation was also observed in male rats at the highest dose in a short-term study (nominal doses of 50, 250, and 1000 mg/kg bw per day) (EPA, 1987). In the study by the NTP, cytoplasmic alteration was observed in the parotid and submandibular salivary glands of rats (Chan & Mahler, 1992).

In a study of carcinogenicity in mice given glyphosate at doses of 150, 1500, or 4500 mg/kg bw per day, liver hypertrophy and necrosis were observed in males at the highest dose (EPA, 1983). Other effects in males at the highest dose included increased testes weight, interstitial nephritis, and decreased body weight. In females at the highest dose, ovary weights were increased, proximal tubule epithelial basophilia and hypertrophy was observed, and body weights were decreased. In the study by the NTP, cytoplasmic alteration was observed in the parotid salivary glands in mice (Chan & Mahler, 1992).

Developmental and reproductive toxicity

In a study of developmental toxicity in rats given glyphosate at a dose of 300, 1000, or 3500 mg/kg bw per day, reduced implantation rates and fewer live fetuses were observed in dams at the highest dose (EPA, 1980b). In fetuses at the highest dose, unossified sternebra were observed and fetal weight was reduced.

5. Summary of Data Reported

5.1 Exposure data

Glyphosate is a broad-spectrum herbicide that is effective at killing or suppressing all plant types, including grasses, perennials, and woody plants. The herbicidal activity of glyphosate was discovered in 1970 and since then its use has increased to a point where it is now the most heavily used herbicide in the world, with an annual global production volume in 2012 of more than 700 000 tonnes used in more than 750 different products. Changes in farming practice and the development of genetically modified crops that are resistant to glyphosate have contributed to the increase in use.

There is little information available on occupational or community exposure to glyphosate. Glyphosate can be found in soil, air, surface water and groundwater, as well as in food. It has been detected in air during agricultural herbicide-spraying operations. Glyphosate was detected in urine in two studies of farmers in the USA, in urban populations in Europe, and in a rural population living near areas sprayed for drug eradication in Columbia. However, urinary concentrations were mostly below the limit of detection in several earlier studies of forestry workers who sprayed glyphosate. Exposure of the general population occurs mainly through diet.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to glyphosate, the Working Group identified seven reports from the Agricultural Health Study (AHS) cohort and several reports from case–control studies. The AHS cohort, the pooled analyses of the case–control studies in the midwest USA, and the cross-Canada study were considered key investigations because of their relatively large size. Reports from two or more independent studies were available for non-Hodgkin lymphoma (NHL), multiple myeloma, Hodgkin lymphoma, glioma, and prostate. For the other cancer sites, results from only one study were available for evaluation.

5.2.1 NHL and other haematopoietic cancers

Two large case–control studies of NHL from Canada and the USA, and two case–control studies from Sweden reported statistically significant increased risks of NHL in association with exposure to glyphosate. For the study in Canada, the association was seen among those with more than 2 days/year of exposure, but no adjustment for other pesticides was done. The other three studies reported excesses for NHL associated with exposure to glyphosate, after adjustment for other pesticides (reported odds ratio were 2.1 (95% CI, 1.1–4.0); 1.85 (95% CI, 0.55–6.2); and 1.51 (95% CI, 0.77–2.94). Subtype-specific analyses in a Swedish case–control study indicated positive associations for total NHL, as well as all subtypes, but this association was statistically significant only for the subgroup of lymphocytic lymphoma/chronic lymphocytic leukaemia (OR, 3.35; 95% CI, 1.42–7.89). An elevated risk (OR, 3.1; 95% CI, 0.6–17.1) was also found for B-cell lymphoma in an European study based on few cases. One hospital-based case–control study from France did not find an association between exposure to glyphosate and NHL (OR, 1.0; 95% CI, 0.5–2.2) based on few exposed cases.

A roughly twofold excess of multiple myeloma, a subtype of NHL, was reported in three studies: only among the highest category of glyphosate use (> 2 days/year) in the large Canadian case–control study, in a case–control study from Iowa, USA, and in a French case–control study (all not statistically significant). These three studies did not adjust for the effect of other pesticides. In the AHS, there was no association with NHL (OR, 1.1; 0.7–1.9). For multiple myeloma, relative risk was 1.1 (95% CI, 0.5–2.4) when adjusted for age only; but was 2.6 (95% CI, 0.7–9.4) when adjusted for multiple confounders. No excess in leukaemia was observed in a case–control study in Iowa and Minnesota, USA, or in the AHS.

In summary, case–control studies in the USA, Canada, and Sweden reported increased risks for NHL associated with exposure to glyphosate. The increased risk persisted in the studies that adjusted for exposure to other pesticides. The AHS cohort did not show an excess of NHL. The Working Group noted that there were excesses reported for multiple myeloma in three studies; however, they did not weight this evidence as strongly as that of NHL because of the possibility that chance could not be excluded; none of the

risk estimates were statistically significant nor were they adjusted for other pesticide exposures.

5.2.2 Other cancer sites

No association of glyphosate with cancer of the brain in adults was found in the Upper Midwest Health case–control study. No associations in single case–control studies were found for cancers of the oesophagus and stomach, prostate, and soft-tissue sarcoma. For all other cancer sites (lung, oral cavity, colorectal, pancreas, kidney, bladder, breast, prostate, melanoma) investigated in the large AHS, no association with exposure to glyphosate was found.

5.3 Animal carcinogenicity data

Glyphosate was tested for carcinogenicity in male and female mice by dietary administration in two studies, and in male and female rats by dietary administration in five studies and in drinking-water in one study. A glyphosate-based formulation was also tested in drinking-water in one study in male and female rats, and by skin application in one initiation–promotion study in male mice.

There was a positive trend in the incidence of renal tubule carcinoma and of renal tubule adenoma or carcinoma (combined) in males in one feeding study in CD-1 mice. Renal tubule carcinoma is a rare tumour in this strain of mice. No significant increase in tumour incidence was seen in female mice in this study. In the second feeding study, there was a significant positive trend in the incidence of haemangiosarcoma in male CD-1 mice. No significant increase in tumour incidence was seen in female mice in this study.

For the five feeding studies in rats, two studies in the Sprague-Dawley strain showed a significant increase in the incidence of pancreatic islet cell adenoma in males – one of these two studies also showed a significant positive trend in the incidences of hepatocellular adenoma in males and of thyroid C-cell adenoma in females. Two studies (one in Sprague-Dawley rats, one in Wistar rats) found no significant increase in tumour incidence at any site. One study in Wistar rats was inadequate for the evaluation because of the short duration of exposure.

In the study in Wistar rats given drinking-water containing glyphosate, there was no significant increase in tumour incidence.

A glyphosate-based formulation was found to be a skin-tumour promoter in the initiation–promotion study in male Swiss mice. The study of a glyphosate-based formulation in drinking-water in Sprague-Dawley rats was inadequate for the evaluation because of the small number of animals per group, and the limited information provided on tumour histopathology and incidence in individual animals. These studies of a chemical mixture containing glyphosate were considered inadequate to evaluate the carcinogenicity of glyphosate alone.

5.4 Mechanistic and other relevant data

Direct data on absorption of glyphosate in humans were not available to the Working Group. Glyphosate was detected in the urine of agricultural workers in several studies, and in the blood of poisoning cases, indicative of absorption. Some evidence for absorption through human skin (~2%) was reported in studies in vitro. The minor role of dermal absorption was also shown in a study in non-human primate model in vivo. However, no study examined the rates of absorption in humans. In rodents, several studies showed up to 40% absorption after oral administration of a single or repeated dose.

Glyphosate was measured in human blood. No data on parenchymal tissue distribution for glyphosate in humans were available to the Working Group. In rats given glyphosate by oral administration, concentrations in tissues had

the following rank order: kidneys > spleen > fat > liver. Repeated administration had no effect on the distribution of glyphosate. In a study in rats, the half-life of glyphosate in plasma was estimated to be more than 1 day, indicating that glyphosate is not rapidly eliminated.

In the environment, glyphosate is degraded by soil microbes, primarily to aminomethylphosphonic acid (AMPA) and carbon dioxide. Glyphosate is not efficiently metabolized in humans or other mammals. In rats, small amounts of AMPA were detected in the plasma and in the colon, with the latter being attributed to intestinal microbial metabolism. In humans, small amounts of AMPA are detectable in blood in cases of deliberate glyphosate poisoning. Few studies examined the possible effects of glyphosate-based formulations on metabolizing enzymes, but no firm conclusions could be drawn from these studies.

Studies in rodents showed that systemically absorbed glyphosate is excreted unchanged into the urine, and that the greatest amount is excreted in the faeces, indicating poor absorption. Glyphosate was detected in the urine of humans who were exposed occupationally to glyphosate. AMPA has also been detected in human urine.

Glyphosate is not electrophilic.

A large number of studies examined a wide range of end-points relevant to genotoxicity with glyphosate alone, glyphosate-based formulations, and AMPA.

There is strong evidence that glyphosate causes genotoxicity. The evidence base includes studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. In-vivo studies in mammals gave generally positive results in the liver, with mixed results for the kidney and bone marrow. The end-points that have been evaluated in these studies comprise biomarkers of DNA adducts and various types of chromosomal damage. Tests in bacterial assays gave consistently negative results.

The evidence for genotoxicity caused by glyphosate-based formulations is strong. There were three studies of genotoxicity end-points in community residents exposed to glyphosate-based formulations, two of which reported positive associations. One of these studies examined chromosomal damage (micronucleus formation) in circulating blood cells before and after aerial spraying with glyphosate-based formulations and found a significant increase in micronucleus formation after exposure in three out of four different geographical areas. Additional evidence came from studies that gave largely positive results in human cells in vitro, in mammalian model systems in vivo and in vitro, and studies in other non-mammalian organisms. The end-points that were evaluated in these studies comprised biomarkers of DNA adducts and various types of chromosomal damage. The pattern of tissue specificity of genotoxicity end-points observed with glyphosate-based formulations is similar to that observed with glyphosate alone. Tests in bacterial assays gave generally negative results.

For AMPA, the evidence for genotoxicity is moderate. While the number of studies that examined the effects of AMPA was not large, all of the studies gave positive results. Specifically, genotoxicity was reported in a study in humans in vitro, a study in mammals in vivo, a study in mammals in vitro, and one study in eels in vivo.

Strong evidence exists that glyphosate, AMPA, and glyphosate-based formulations can induce oxidative stress. Evidence came from studies in many rodent tissues in vivo, and human cells in vitro. In some of these studies, the mechanism was challenged by co-administration of antioxidants and observed amelioration of the effects. Similar findings have been reported in fish and other aquatic species. Various end-points (e.g. lipid peroxidation markers, oxidative DNA adducts, dysregulation of antioxidant enzymes) have been evaluated in numerous studies. This

increased the confidence of the Working Group in the overall database.

There is weak evidence that glyphosate or glyphosate-based formulations induce receptor-mediated effects. In multiple experiments, glyphosate-based formulations affected aromatase activity; glyphosate was active in a few of these studies. Some activity in other nuclear receptor-mediated pathways has been observed for glyphosate or glyphosate-based formulations. In one series of experiments, glyphosate was not found to be a ligand to several receptors and related proteins (aryl hydrocarbon receptor, peroxisome proliferator-activated receptors, pregnane X receptor).

There is weak evidence that glyphosate may affect cell proliferation or death. Several studies in human and rodent cell lines have reported cytotoxicity and cell death, the latter attributed to the apoptosis pathway. Studies that examined the effects of glyphosate alone or a glyphosate-based formulation found that glyphosate alone had no effect, or a weaker effect than the formulation.

There is weak evidence that glyphosate may affect the immune system, both the humoral and cellular response, upon long-term treatment in rodents. Several studies in fish, with glyphosate or its formulations, also reported immunosuppressive effects.

With regard to the other key characteristics of human carcinogens (IARC, 2014), the Working Group considered that the data were too few for an evaluation to be made.

Severe or fatal human poisoning cases have been documented worldwide. In rodents, organ and systemic toxicity from exposures to glyphosate are demonstrated by liver-weight effects and necrosis in animals at high doses. Additionally, effects on the pancreas, testes, kidney and ovaries, as well as reduced implantations and unossified sternebra were seen at similar doses.

No data on cancer-related susceptibility after exposure to glyphosate were available to the Working Group.

Overall, the mechanistic data provide strong evidence for genotoxicity and oxidative stress. 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 glyphosate. A positive association has been observed for non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Glyphosate 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 glyphosate in Group 2A.

In addition to limited evidence for the carcinogenicity of glyphosate in humans and sufficient evidence for the carcinogenicity of glyphosate in experimental animals, there is strong evidence that glyphosate can operate through two key characteristics of known human carcinogens, and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to glyphosate or glyphosate-based formulations is genotoxic based on studies in humans in vitro and studies in experimental animals.

One study in several communities in individuals exposed to glyphosate-based formulations also found chromosomal damage in blood cells; in this study, markers of chromosomal damage (micronucleus formation) were significantly greater after exposure than before exposure in the same individuals.

- There is strong evidence that glyphosate, glyphosate-based formulations, and aminomethylphosphonic acid can act to induce oxidative stress based on studies in experimental animals, and in studies in humans in vitro. This mechanism has been challenged experimentally by administering antioxidants, which abrogated the effects of glyphosate on oxidative stress. Studies in aquatic species provide additional evidence for glyphosate-induced oxidative stress.

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TETRACHLORVINPHOS

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 22248-79-9 [also 22350-76-1 for the analogous (*E*)- isomer; and 961-11-5 for the mixed (*Z*)- + (*E*)- isomers]

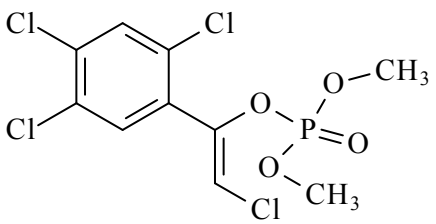
Chem. Abstr. Serv. Name: Phosphoric acid, (1*Z*)-2-chloro-1-(2,4,5-trichlorophenyl) ethenyl dimethyl ester

Preferred IUPAC Name: (1*Z*)-2-chloro-1-(2,4,5-trichlorophenyl)ethenyl dimethyl phosphate

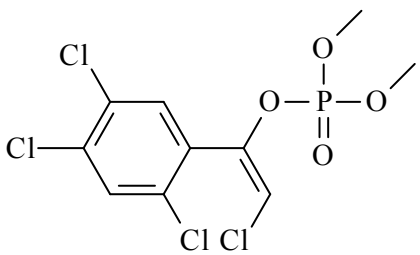
Synonyms: CVMP; stirofos; stirophos; TCVP; tetrachlorvinfos, vinfos

Trade Names: Tetrachlorvinphos products are sold worldwide under several trade names, including Appex, Dust M, Gardcide, Gardona, Rabon, Rabon Oral Larvicide (ROL), and Rabond Ravap (IARC, 1983; ChemIDplus, 2015).

1.1.2 Structural and molecular formulae, and relative molecular mass



(1*Z*)-2-chloro-1-(2,4,5-trichlorophenyl) ethenyl dimethyl phosphate (CAS No., 22248-79-9)



(1*E*)-2-chloro-1-(2,4,5-trichlorophenyl) ethenyl dimethyl ester phosphoric acid (CAS No., 22350-76-1)

Molecular formula: $C_{10}H_9Cl_4O_4P$

Relative molecular mass: 365.96

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: Off-white, tan to brown crystalline solid with a mild chemical odour (EPA, 1995a; NCBI, 2015)

Solubility: Very slightly soluble at 11 mg/L in water at 20 °C, soluble at < 200 g/kg at 20 °C in acetone, < 150 g/kg at 20 °C in xylene, 400 g/kg in chloroform and dichloromethane (IARC, 1983)

Volatility: Vapour pressure (*Z*-isomer), 4.2×10^{-8} mm Hg (20 °C) (IARC, 1983), not expected to volatilize from dry soil surfaces

Stability: Stable below 100 °C; slowly hydrolysed by water, neutral, and acid environments, and more quickly in an alkaline environment (IARC, 1983; NCBI, 2015)

Octanol/water partition coefficient (P): log P, 3.53 (Hansch et al., 1995)

Henry's law: 1.8×10^{-9} atm m^3 mol^{-1} at 25 °C (NIH, 2015), not expected to volatilize from water

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 15.0 ppm.

1.1.4 Technical products and impurities

The technical product typically contains 98% *Z*-stereoisomer and 2% *E*-stereoisomer (Worthing, 1979).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Tetrachlorvinphos, a phenyl organophosphate insecticide, was introduced and first used commercially in 1966 in the USA (EPA, 2006).

Tetrachlorvinphos is produced by the reaction of trimethyl phosphate with 2,2,2′,4′,5′-pentachloroacetophenone (IARC, 1983; Tomlin, 2000).

Tetrachlorvinphos is formulated as wettable powder (active ingredient, a.i., 50%), dust (a.i., 1–3%), granular (a.i., typically 0.2–7.8%), and emulsifiable concentrate (a.i., 3–24%), impregnated material (a.i., 13–14.5%, as pet collars and cattle ear tags), ready-to-use liquid (a.i., 1–2%, as spray-on/wipe-on/backrub materials for pets, horses, and cattle), and pressurized liquid (a.i., 1%, as flea and tick spray for cats). Tetrachlorvinphos is also available in pelleted/tableted form and as mineral blocks for livestock (EPA, 2006). It is sometimes formulated in conjunction with the insect growth regulator *S*-methoprene (EPA, 2006).

(b) Production volume

In 1978, the total world production volume of tetrachlorvinphos was reported to be 450 tonnes (IARC, 1983). As of 2002, approximately 400 tonnes of tetrachlorvinphos active ingredient were used annually in the USA, of which about 200 tonnes were used for poultry (EPA, 2002a). Tetrachlorvinphos was not listed among the top 25 agricultural pesticides (by mass of a.i. per year) in the USA between 1987 and 1997 (EPA, 1999); however, 47 unique tetrachlorvinphos products were reported to be available in the USA from 12 primary registrant companies (NPIRS, 2015).

No production, import, or use quantities were available for countries other than the USA. Tetrachlorvinphos was not listed on the 2007 list of high production chemicals published by the Organisation for Economic Co-operation and Development (OECD), suggesting that tetrachlorvinphos was not produced or imported at levels greater than 1000 tonnes per year in any member country or region, although products containing tetrachlorvinphos were reportedly available for sale to consumers in several countries (OECD, 2009).

1.2.2 Uses

Tetrachlorvinphos is a selective insecticide and miticide with contact and stomach action (NIH, 2015). It can be used against ectoparasites on poultry, against flies in dairies and livestock barns, as a larvicide in livestock, and to control fleas on pets (EPA, 2002a). It has also been used on crops, where it is effective against various pests of fruits, vegetables, cereals, and cash crops, including fruit flies and moths in cotton, maize, rice, tobacco, vegetables, and fruit (NIH, 2015). Target pests also include fleas, ticks, lice, flies (adults and larvae), chiggers, mites, spiders, wasps, and cattle grubs. In addition, tetrachlorvinphos has been used on agricultural premises, agricultural equipment, and recreational areas (NTP, 1978).

(a) Agriculture

Tetrachlorvinphos can be applied dermally to livestock to control flies and mites; it can be used as an oral larvicide in cattle, pigs, goats, and horses; in cattle ear tags and as a feed additive to control flies; in poultry dust boxes to control poultry mites; and as paint on and sprays in poultry houses (EPA, 2002a).

(b) Residential use

Tetrachlorvinphos is used in pet flea and tick collars, shampoos, and as a dust or powder, aerosol, and pump spray for direct treatment of pets and in pet sleeping areas. In the USA, tetrachlorvinphos is used in an estimated 10% of households with dogs or cats (EPA, 2002a).

(c) Public health

Tetrachlorvinphos has been used as a spray to control nuisance and public health pests in and around refuse sites, recreational areas, and for outdoor use as sprays for fleas, ticks, and mites, around kennels, yards, camping grounds, parks, foot paths, and roadways (EPA, 2002a).

(d) Regulation

No maximum residue limit (MRL) for tetrachlorvinphos was listed in the Codex Alimentarius (Codex Alimentarius, 2015).

Tetrachlorvinphos was revoked for use in the European Union as of 2003 under Directive 91/414/EEC (European Commission, 1991). It was used in some member states for slightly different periods, e.g. France, 1972–1998; and the Netherlands, 1973–1999 (CTGB, 2015; Ministère de l'Agriculture et de la Forêt, 2015).

Use of tetrachlorvinphos remains allowable for pets, livestock and poultry in the USA, but no tetrachlorvinphos products were currently registered for use on any plant commodity in the USA, as crop uses were voluntarily cancelled in 1987 (EPA, 2006). In 2006, the United States Environmental Protection Agency (EPA) modified the allowable use of tetrachlorvinphos to reduce risks (EPA, 2006). The EPA has established maximum tolerances for tetrachlorvinphos in eggs, milk, and other animal products (NIH, 2015).

Tetrachlorvinphos is reportedly registered for use in Canada, South Africa, and Australia, as well as in the USA (Paranjape et al., 2015).

No occupational exposure limits for tetrachlorvinphos were available to the Working Group.

1.3 Measurement and analysis

Tetrachlorvinphos can be measured in air, water, soil, dust, fruits and vegetables, and urine and faeces (Table 1.1). The metabolites found in urine include 2,4,5-trichlorophenylethanediol glucuronide and dimethylphosphate, a nonspecific metabolite of several organophosphate pesticides (Beynon et al., 1973; Bravo et al., 2004).

Tetrachlorvinphos is not persistent in the environment. It is broken down in air within 24 hours, and in soil over a few weeks (NIH, 2015).

Table 1.1 Methods of analysis for tetrachlorvinphos

Sample matrix	Assay procedure	Limit of detection	Reference
Air	GC/ECD	10 µg/m³	OSHA (2015)
Aqueous	GC/FPD or GC/NPD	NR	EPA (2007)
Water	GC/MS	11 ng/L	Beceiro-González et al. (2007)
Solids (soils, sediments, sludges)	GC/FPD or GC/NPD	NR	EPA (2007)
Dust	GC/MS-EI-MID	50 ng/g	Quirós-Alcalá et al. (2011)
Fruits and vegetables	GC/MS	70 µg/kg	Fillion et al. (2000)
Urine	Isotope dilution GC-MS/MS	0.6 µg/L (dimethyl phosphate)	Bravo et al. (2004)

ECD, electron-capture detection; EI, electron impact; FPD, flame-photometric detector; GC, gas chromatography; MID, multiple ion detection mode; MS, mass spectrometry; NPD, nitrogen-phosphorous detector; NR, not reported

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Workers including farmers, ranchers and pesticide applicators may be exposed to tetrachlorvinphos during mixing, loading, application and entering treated areas (EPA, 2006). No data on occupational exposure levels were available to the Working Group.

(b) Community exposure

Adults and children in the general population can be exposed to tetrachlorvinphos when treating pets, or through dermal contact with pets treated with pet collars, powders, or aerosol sprays (EPA, 2006). Several studies have shown transferable residues from fur of pets treated with veterinary products containing tetrachlorvinphos (Davis et al., 2008; Rotkin-Ellman & Solomon, 2009). In one study, five dogs and five cats wearing flea collars containing tetrachlorvinphos were followed for 14 days. After 3 days with the flea collar, average residue levels were 57.98 µg/wipe in dogs and 43.40 µg/wipe in cats). After 14 days, the average residues were 5.67 µg/wipe in dogs and 8.19 µg/wipe in cats (Rotkin-Ellman & Solomon, 2009).

In a second study, the transfer of tetrachlorvinphos to humans from dogs treated with flea collars was estimated for a sample of 55 dogs. Researchers used cotton gloves to pet the dogs: the average amounts of tetrachlorvinphos transferred from the fur of the neck and the back to gloves were 22 400 ± 2900 and 80 ± 20 µg/glove, respectively, at 5 days after the collar application. The amounts transferred declined notably with time after application. T-shirts worn by children living with the treated dogs 7–11 days after treatment contained tetrachlorvinphos at 1.8 ± 0.8 µg/g shirt. 2,4,5-Trichloromandelic acid, a biomarker of exposure to tetrachlorvinphos, was detected in the urine of adults and children exposed to treated dogs (range, 1.4–582 ng/mL in adults and 2.1–1558 ng/mL in children) (Davis et al., 2008).

Other pathways are dietary exposure due to the use of tetrachlorvinphos on livestock and crops, and inhalation after outdoor application; exposure through drinking-water is expected to be minimal due to the localized nature of most applications (EPA, 2006). Very little information on environmental exposure to tetrachlorvinphos was available to the Working Group.

In Venezuela, tetrachlorvinphos was detected in 19% of red peppers and 25% of lettuces sampled (Quintero et al., 2008).

In 2006 in the USA, dust samples for 13 urban homes in Oakland, California, and 15 farmworker homes in Salinas, an agricultural community in California were collected. Detection frequencies

of tetrachlorvinphos were 4% in Oakland and 10% in Salinas. The concentration ranged from less than the limit of detection (LOD) to 15.8 ng/g in Oakland and from < LOD to 271 ng/g in Salinas (Quirós-Alcalá et al., 2011).

1.4.2 Exposure assessment and biological markers

(a) Exposure assessment

Exposure assessment methods in epidemiological studies on tetrachlorvinphos and cancer are discussed in Section 1.4.2 and Section 2.1.2 of the *Monograph* on Malathion, in the present volume.

(b) Biological markers

Urinary dimethyl phosphate reflects recent exposure to organophosphate insecticides. (EPA, 2006), but interpretation of such data is always difficult because the results cannot be attributed to any specific organophosphate.

Cholinesterase inhibition is often used as a marker of exposure to organophosphate insecticides; however, no changes in plasma or erythrocyte cholinesterase activity were observed during 4 weeks in a study in five subjects treated with a tetrachlorvinphos-based formulation (Rider & Puletti, 1969).

2. Cancer in Humans

Tetrachlorvinphos was previously evaluated by the Working Group as *Group 3*, *not classifiable as to its carcinogenicity to humans*, based on limited evidence in experimental animals (IARC, 1983, 1987). No data in humans were available at that time.

2.1 Summary of frequently cited epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.2 of the *Monograph* on Malathion in the present volume. The scope of the available epidemiological studies is discussed in Section 2.1 of the *Monograph* on Malathion, and includes a consideration of chance, bias and confounding, and exposure assessment.

2.2 Cohort studies

There were no cohort studies available that provided a specific assessment of exposure to tetrachlorvinphos.

Settimi et al. (1999) reported results from a cohort of workers in a cigarette factory in Bologna, Italy, where tobacco was treated with tetrachlorvinphos, γ-hexane, and methyl bromide. The cohort of 1733 (972 women and 761 men) included workers who had been employed for at least 6 months in cigarette manufacturing and related jobs between 1 January 1962 and 1 January 1990. The cohort was traced for vital status until 1 July 1996 using municipal offices, finding 1250 living (715 women, 535 men), 467 deceased (247 women, 220 men), and 16 (10 women, 6 men) lost to follow-up (0.9%). Standardized mortality ratios (SMRs) were calculated using sex-specific mortality rates for the population of the Emilia Romagna region, adjusted for age and calendar period. Standardized mortality ratios for total mortality and total cancer ranged from 0.8 to 1.1. Mortality for cancer of the stomach was significantly lower among men (SMR, 0.5; 95% CI, 0.2–1.0; $P < 0.05$). Mortality for non-Hodgkin lymphoma (NHL) was significantly elevated among women (SMR, 2.7; 95% CI, 1–5.6; $P > 0.05$), especially among those employed for ≥ 15 years (SMR, 8.3; 95% CI, 2.3–21.4). No deaths from NHL occurred

among men. Mortality from cancer of the brain was elevated among men (SMR, 2.0; 95% CI, 0.5–5.0; 3 deaths) and women (SMR, 1.7; 95% CI, 0.5–4.3; 3 deaths). [This study was limited by the lack of specific information about individual exposure to tetrachlorvinphos, and by the small numbers of specific cancers. Lack of information on personal use of tobacco was not considered to be a significant limitation because the observed rate of cancer of the lung and respiratory disease was about that expected.]

There were other studies of workers in the cigarette/tobacco industry, but no use of tetrachlorvinphos was reported.

2.3 Case–control studies on lympho-haematopoietic cancers

See Table 2.1

Brown et al. (1990) evaluated the relationship between tetrachlorvinphos and leukaemia for a case–control study of white men in the USA (Iowa and Minnesota) (see the *Monograph* on Malathion, Section 2.2.2, for a detailed description of this study). The odds ratio for leukaemia among farmers reporting use of tetrachlorvinphos was 2.9 (95% CI, 0.8–10.6; 5 exposed cases and 5 exposed controls), compared with non-farmers, adjusted for age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures [This study overlapped with Waddell et al., 2001 and De Roos et al., 2003. The strengths of this study were that it was population-based and enrolled incident cases, and there was detailed exposure assessment of exposure to pesticides from farmers who could provide such information. A limitation was that it was not possible to evaluate by level of exposure given the small number of exposed cases.]

Waddell et al. (2001) pooled data from three population-based case–control studies of NHL (748 cases, 2236 controls) among men in the midwestern USA (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) to evaluate several pesticides, including tetrachlorvinphos (see the *Monograph* on Malathion, Section 2.2.2, for a detailed description of this study). Comparing farmers using tetrachlorvinphos to non-farmers yielded an odds ratio of 1.8 (95% CI, 0.7–4.7; 9 exposed cases and 17 exposed controls) after adjusting for age, state, and respondent type. Adjustment for other potential confounders did not affect the odds ratios. The odds ratio for tetrachlorvinphos was not adjusted for reported use of other pesticides. [The strengths of this study were that it was population-based and enrolled incident cases, and there was detailed exposure assessment of pesticides from farmers who could provide such information. A limitation was that it was not possible to evaluate by level of exposure given the small number of exposed cases.]

De Roos et al. (2003) re-analysed data from the pooled studies of NHL in four midwestern states (Iowa, Kansas, Minnesota, and Nebraska) in the USA using logistic regression and hierarchical regression to distinguish between individual pesticides and scenarios of farmers' exposure to multiple pesticides (see the *Monograph* on Malathion, Section 2.2.2, for a detailed description of this study). This analysis included 650 cases of NHL and 1933 controls. Based on three exposed cases and 11 exposed controls, the odds ratio for tetrachlorvinphos (adjusted for age, state and other pesticides) was 0.4 (95% CI, 0.1–1.8) for logistic regression, and 0.8 (95% CI, 0.3–1.9) for hierarchical regression. [The results from De Roos et al. (2003) may have differed from analyses by Waddell et al. (2001): there were fewer cases (650 versus 748) and controls (19 033 versus 2236) in these analyses because of exclusion of individuals with missing information on other pesticides included in the hierarchical model, and because the hierarchical model adjusted for effects of other pesticides.]

Table 2.1 Case-control studies on cancer and exposure to tetrachlorvinphos

Reference, location enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled
Brown et al. (1990) Iowa and Minnesota, USA 1981–1984	Cases: 578 (response rate, 86%); white men, newly diagnosed, age ≥ 30 yr; cancer registry or hospital records Controls: 1245 (response rate, 77–79%); population-based; frequency matched on 5-yr age group, vital status, state of residence; random-digit dialling for those aged < 65 yr and Medicare for those aged ≥ 65 yr Exposure assessment method: questionnaire; in-person interview with subject or proxy; farming and pesticide use history for subjects who worked on farm, listing 23 animal insecticides, 34 crop insecticides, 38 herbicides, 16 fungicides. Exposure defined as ever personally handled, mixed or applied; ORs for diazinon refer to use on crops	Total leukaemia	Ever used TCVP	5	2.9 (0.8–10.6)	Age, vital status, state, tobacco use, family history of lympho-haematopoietic cancer, high-risk occupations, high-risk exposures
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 748 (response rate, NR); white men, newly diagnosed, age ≥ 21 yr (Iowa & Minnesota: 462; Kansas: 150; Nebraska: 136) Controls: 2236 (response rate, NR); white men, population-based, frequency matched on: 5-yr age group, vital status, state of residence (Iowa & Minnesota: 927; Kansas: 823; Nebraska: 486) Exposure assessment method: questionnaire; use of pesticides obtained by questionnaire from subjects, or surrogates if subjects deceased. In-person interviews in Minnesota and Iowa, and telephone interviews in Kansas and Nebraska. Sought information on specific pesticides, when and frequency of use, type of application, and use of protective equipment	NHL	Ever used	9	1.8 (0.7–4.7)	Age, state of residence, respondent type (proxy/ direct)

Table 2.1 (continued)

Reference, location enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled
De Roos et al. (2003) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records; white men Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files; white men Exposure assessment method: see Waddell et al. (2001); questionnaire and interview (direct or next-of-kin); analyses focused on 47 pesticides to which ≥ 20 persons were exposed; any subject with a missing or "don't know" response for any of the 47 pesticides was excluded from all analyses	NHL	Ever (logistic regression) Ever (hierarchical regression)	3 3	0.4 (0.1–1.8) 0.8 (0.3–1.9)	Age, study site, all the other 46 pesticides to which 20 or more persons were exposed

CI, confidence interval; TCVP, tetrachlorvinphos; yr, year

3. Cancer in Experimental Animals

Studies of carcinogenicity previously assessed by and leading to the previous evaluation of *limited evidence* in experimental animals for the carcinogenicity of tetrachlorvinphos are also included in the present monograph (IARC, 1983, 1987).

3.1 Mouse

See Table 3.1

Oral administration

In a study by the National Cancer Institute (NCI), groups of 50 male and 50 female B6C3F$_1$ mice (age, 35 days) were given diets containing tetrachlorvinphos (purity, 98%) at a dose of 8000 or 16 000 ppm ad libitum 7 days per week for 80 weeks, and then held for an additional 12 weeks (NTP, 1978). Groups of 10 male and 10 female mice held untreated for 90–92 weeks served as matched untreated controls. Since the numbers of mice in the matched-control groups were small, pooled-control groups were also used for statistical comparisons. Matched controls from the study on tetrachlorvinphos were combined with matched controls from long-term studies performed on malathion, toxaphene, endrin, and lindane that were conducted at the same time in the same laboratory. The pooled untreated controls for tetrachlorvinphos consisted of a total of 50 male and 50 female mice. For this bioassay, mice receiving tetrachlorvinphos were maintained in a room housing mice treated with dieldrin or malathion, together with their respective matched controls. There was a dose-related decrease in mean body weights in treated male and female mice compared with the matched controls throughout the exposure period. Survival in all dose groups was similar to that of controls.

There was a significant positive trend in the incidence of hepatocellular carcinoma in treated males compared with either matched or pooled controls: matched controls, 0/9; pooled controls, 5/49 (10%); lower dose, 36/50 (72%); higher dose, 40/50 (80%); $P < 0.001$. Pairwise comparison of lower- and higher-dose groups of males with matched- or pooled-control groups showed significant increases in the incidences of hepatocellular carcinoma in the treated groups in every case. The incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) was also increased in treated males. In treated females, the incidence of hepatocellular carcinoma by itself was not significantly increased. However, the incidence of liver neoplastic nodules [hepatocellular adenoma] alone – pooled controls, 1/48 (2%); lower dose, 14/49 (29%), $P < 0.001$; higher dose, 9/47 (19%), $P = 0.007$) – and the incidence of liver neoplastic nodules [hepatocellular adenoma] or hepatocellular carcinoma (combined) – pooled controls, 3/48 (6%); lower dose, 19/49 (39%), $P < 0.001$; higher dose, 11/47 (23%), $P = 0.019$ – showed significant dose-related positive trends, and also significantly increased incidences in the groups at the lower and higher dose compared with pooled controls. The incidence of liver neoplastic nodules [hepatocellular adenoma] or hepatocellular carcinoma (combined) in females was also significantly increased in the group at the lower dose (19/49, $P = 0.020$) compared with matched controls (0/9). There were no significant increases in tumour incidence at any other site in treated mice (NTP, 1978). [The Working Group noted the small number of matched controls, and the exposure in a room where other chemicals were also being studied.]

Parker et al. (1985) treated groups of 80 male and 80 female B6C3F$_1$ mice (age, 7–8 weeks) with diets containing tetrachlorvinphos (purity, 98%) at a dose of 0, 17.5, 64, 320, 1600, 8000, or 16 000 ppm (in these groups, the test chemical was reported by the authors to be representative

Table 3.1 Studies of carcinogenicity with tetrachlorvinphos in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) ≤ 92 wk NTP (1978)	Diet containing TCVP (purity, 98%) at a concentration of 0 ppm (matched control), 0 ppm (pooled control), 8000 ppm, or 16 000 ppm, ad libitum, 7 days/wk for 80 wk, and then held untreated for an additional 10–12 wk 50 M and 50 F (age, 35 days)/treated group; 10 M and 10 F/matched untreated control group Due to the small number of mice in the matched-control group, pooled controls (50 M and 50 F) were also used for statistical comparisons; matched controls from this study were combined with matched controls from long-term studies with other chemicals that were conducted at the same time in the same laboratory	*Males* Liver: Neoplastic nodule [hepatocellular adenoma]: 0/9, 3/49 (6%), 11/50 (22%)*, 2/50 (4%) Hepatocellular carcinoma: 0/9‡, 5/49 (10%)‡, 36/50 (72%)**, 40/50 (80%)** [Hepatocellular adenoma] or carcinoma (combined): 0/9‡, 8/49 (16%)‡, 47/50 (94%)**, 42/50 (84%)** Kidney: Renal tubule carcinoma: 0/9, NR, 0/50, 1/50 *Females* Liver: Neoplastic nodule [hepatocellular adenoma]: 0/9, 1/48‡‡ (2%), 14/49 (29%)***, 9/47 (19%)† Hepatocellular carcinoma: 0/9, 2/48 (4%), 5/49 (10%), 2/47 (4%) [Hepatocellular adenoma] or carcinoma (combined): 0/9, 3/48 (6%)‡‡‡, 19/49 (39%)††, 11/47 (23%)††† Kidney: Renal tubule adenoma: 0/9, NR, 1/49, 0/46	Fisher exact test and Cochran-Armitage trend test *$P = 0.024$ (vs pooled controls) **$P < 0.001$ (vs matched and pooled controls) ***$P < 0.001$ (vs pooled controls) †$P = 0.007$ (vs pooled controls) ††$P < 0.001$ (vs pooled controls), $P = 0.020$ (vs matched controls) †††$P = 0.019$ (vs pooled controls) ‡ $P < 0.001$ for trend ‡‡ $P = 0.018$ for trend ‡‡‡ $P = 0.030$ for trend	Mice receiving TCVP were maintained in a room housing mice given dieldrin or malathion, together with their respective matched controls There was a small number of matched controls, and the study was conducted in same room as studies with other chemicals (malathion, toxaphene, endrin, and lindane) Dose-related decrease in mean body weights in treated males and females were observed throughout exposure period

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) 103 wk Parker et al. (1985)	Diet containing TCVP (purity, 98%) at a concentration of 0, 17.5, 64, 320, 1600, 8000, 16 000 ppm (all groups receiving TCVP of current production), or 16 000 ppm (NCI test material) ad libitum, 7 days/wk for 103 wk 80 M and 80 F (age, 7–8 wk)/treated group; 160 M and 160 F mice/double control group; 10 treated and 20 control mice of each sex per group were killed and examined at 6, 12, and 18 mo Two samples of TCVP were used; mice at 16 000 ppm were fed test material from the previous NCI bioassay (NTP, 1978); mice in all other treatment groups received TCVP that was reported to be "more representative of current production" [of technical TCVP]	*Males* Liver: Hepatocellular adenoma: 2/99 (2%), 0/50, 0/49, 0/50, 1/50 (2%), 5/50 (10%), 3/50 (6%), 4/46 (9%) Hepatocellular carcinoma: 24/99 (24%), 14/50 (28%), 14/49 (29%), 9/50 (18%), 11/50 (22%), 13/50 (26%), 20/50 (40%), 31/46 (67%)* Hepatocellular adenoma or carcinoma (combined): 26/99 (26%), 14/50 (28%), 14/49 (29%), 9/50 (18%), 12/50 (24%), 18/50 (36%), 23/50 (46%), 35/46 (76%)*	*$P \leq 0.05$ (Cox test with Bonferroni correction)	Additional pathology review was conducted by a "consultant pathologist," and the results were as follows: *Males* Liver: Adenomatous nodules: 2/99 (2%), 3/50 (6%), 2/49 (4%), 1/50 (2%), 2/50 (4%), 3/50 (6%), 9/50 (18%), 9/46 (20%) Hepatocellular carcinoma: 14/99 (14%), 7/50 (14%), 6/49 (12%), 4/50 (8%), 4/50 (8%), 1/50 (2%), 2/50 (4%), 6/46 (13%) Adenoma or carcinoma (combined): 16/99 (16%), 10/50 (20%), 8/49 (16%), 5/50 (10%), 6/50 (12%), 4/50 (8%), 11/50 (22%), 15/46 (33%)

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) 103 wk Parker et al. (1985) (cont.)		Kidney: Renal tubule adenoma: 1/99 (1%), 0/50, 0/49, 0/50, 1/50 (2%), 0/50, 2/50 (4%), 2/46 (4%) Renal tubule carcinoma: 0/99, 0/50, 0/49, 0/50, 1/50 (2%), 1/50 (2%), 9/50 (18%)*, 10/46 (22%)* Renal tubule adenoma or carcinoma (combined): 1/99 (1%), 0/50, 0/49, 0/50, 2/50 (4%), 1/50 (2%), 11/50 (22%)*, 12/46 (26%)* *Females* Liver: Hepatocellular adenoma: 0/99, 1/48 (2%), 0/49, 0/50, 1/49 (2%), 2/49 (4%), 3/50 (6%), 1/47 (2%) Hepatocellular carcinoma: 0/99, 0/48, 0/49, 0/50, 3/49 (6%), 5/49 (10%), 4/50 (8%), 5/47 (11%)* Hepatocellular adenoma or carcinoma (combined): 0/99, 1/48 (2%), 0/49, 0/50, 4/49 (8%), 7/49 (14%)*, 7/50 (14%)*, 6/47 (13%)* Kidney: Renal tubule adenoma or carcinoma (combined): 0/99, 0/48, 0/49, 0/50, 0/49, 0/49, 0/50, 2/47 (4%)		Kidney: Renal tubule adenoma: 0/99, 0/50, 0/49, 0/50, 0/50, 0/50, 10/50 (20%)*, 10/46 (22%)* Renal tubule carcinoma: 0/99, 0/50, 0/49, 0/50, 0/50, 0/50, 1/50 (2%), 2/46 (4%) Renal tubule adenoma or carcinoma (combined): 0/99, 0/50, 0/49, 0/50, 0/50, 0/50, 11/50 (22%)*, 12/46 (26%)* *Females* Liver: Adenomatous nodules: 0/99, 0/48, 0/49, 0/50, 0/49, 0/49, 1/50 (2%), 1/47 (2%) Hepatocellular carcinoma: 0/99, 0/48, 0/49, 0/50, 1/49 (2%), 0/49, 0/50, 1/47 (2%) Adenoma or carcinoma (combined): 0/99, 0/48, 0/49, 0/50, 1/49 (2%), 0/49, 1/50 (2%), 2/47 (4%) The total numbers of liver nodular lesions identified by the study and consultant pathologists were similar, but the subsequent histological diagnoses of these nodules depended on differences in classification used by each pathologist. Similarly, the study pathologist considered the majority of renal tumours to be carcinomas, while the consultant pathologist diagnosed most of these as adenoma

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) 103 wk EPA (1988)	Diet containing TCVP at a concentration of 0, 17.5, 64, 320, 1600, 8000, or 16 000 ppm, ad libitum, 7 days/wk for 103 wk 80 M and 80 F (age, 7–8 wk)/treated group; 160 M and 160 F/double control group; 10 treated and 20 control mice of each sex per group were killed at 6, 12, and 18 mo	*Males* Liver: Hepatocellular adenoma: 2/80 (2%), 1/37 (3%), 0/42, 0/35, 1/39 (3%), 5/47 (11%), 3/47 (6%) Hepatocellular carcinoma: 26/113 (23%), 17/58 (29%), 16/58 (28%), 10/51 (20%), 14/55 (25%), 13/60 (22%), 22/59 (37%) Hepatocellular adenoma or carcinoma (combined): 28/113 (25%)*, 18/58 (31%), 16/58 (28%), 10/51 (20%), 15/55 (27%), 18/60 (30%), 25/59 (42%)* Kidney: Renal tubule adenoma: 0/113**, 0/58, 0/68, 0/62, 1/65 (2%), 0/70, 4/69 (6%)* Renal tubule carcinoma: 0/71**, 0/37, 0/39, 0/31, 1/36 (3%), 1/47 (2%), 9/46 (20%)** Renal tubule adenoma or carcinoma (combined): 0/113**, 0/58, 0/68, 0/62, 2/65 (3%), 1/70 (1%), 13/69 (19%)** *Females* Liver: Hepatocellular adenoma: 0/113**, 1/57 (2%), 1/57 (2%), 0/59, 1/57 (2%), 2/56 (4%), 3/58 (5%)* Hepatocellular carcinoma: 1/119 (1%)**, 0/58, 0/69, 0/70, 4/69 (6%), 5/66 (8%)*, 5/68 (7%)* Hepatocellular adenoma or carcinoma (combined): 1/119 (1%)**, 1/58 (2%), 1/69, 0/70, 5/69 (7%)*, 7/66 (11%)**, 8/68 (12%)**	*$P \leq 0.05$ (pairwise comparison by Fisher exact test, or trend test by Cochran-Armitage test) **$P \leq 0.01$ (pairwise comparison by Fisher exact test, or trend test by Cochran-Armitage test)	Evaluation of the study published by Parker et al. (1985). The study reviewed by the EPA reported results on a single sample of TCVP ("current production" technical TCVP). Tumour incidences in the EPA (1988) evaluation were different to those reported by Parker et al. (1985), because the EPA used the number of tumour-bearing animals/number of animals at risk, excluding animals that died before appearance of first tumour, as the criteria for tumour incidence

EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NCI, National Cancer Institute; NR, not reported; TCVP, tetrachlorvinphos; wk, week

of "current production of technical tetrachlorvinphos"), and 16 000 ppm (the test chemical in this group was the same as in the NCI bioassay reported above; NTP, 1978). A group of 160 male and 160 female mice served as a double control. In each group, 10 treated and 20 control mice of each sex were killed and examined at 6, 12, and 18 months. Survival of males and females in the groups receiving the two highest doses was significantly greater than in the control group, while survival in all other dose groups was comparable to that of controls. Statistical evaluation of data on body weight revealed significantly lower mean values for all treatment groups compared with the control group at week 50. Exposure to tetrachlorvinphos caused a significant increase ($P \leq 0.05$) in the incidence of hepatocellular carcinoma – 31 out of 46 (67%) versus 24 out of 99 (24%) in controls – and hepatocellular adenoma or carcinoma (combined) – 35 out of 46 (76%) versus 26 out of 99 (26%) in controls) in males receiving the NCI study material at 16 000 ppm. In female mice exposed to the NCI study material at 16 000 ppm there was a significant increase ($P \leq 0.05$) in the incidence of hepatocellular carcinoma – 5 out of 47 (11%) versus 0 out of 99 in controls– and in the incidence of hepatocellular adenoma or carcinoma (combined)– 6 out of 47 (13%) versus 0 out of 99 in controls. Additionally, in the females exposed to "current production" technical tetrachlorvinphos at 8000 ppm – 7 out of 49 (14%) versus 0/99 in controls – or 16 000 ppm – 7 out of 50 (14%) versus 0 out of 99. Also, in both high-dose groups of male mice, there was a significant increase ($P \leq 0.05$) in the incidence of renal tubule carcinoma of the kidney –"current production" technical tetrachlorvinphos, 9 out of 50 (18%); and NCI study material, 10 out of 46 (22%) versus 0 out of 99 in controls – and the incidence of renal tubule adenoma or carcinoma (combined) of the kidney –"current production" technical tetrachlorvinphos, 11 out of 50 (22%); and NCI study material, 12 out of 46 (26%) versus 1 out of 99 (1%) in controls.

Parker et al. (1985) also reported an additional pathology review conducted by a "consultant" pathologist. The only statistically significant finding reported by the consultant pathologist was an increase in the incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) of the kidney in both high-dose groups of males fed tetrachlorvinphos at 16 000 ppm; renal tubule adenoma or carcinoma (combined): "current production" technical tetrachlorvinphos, 11 out of 50 (22%); and NCI study material, 12 out of 46 (26%) versus 0/99 in controls. The study pathologist considered the majority of renal tumours to be carcinomas, whereas the consultant pathologist diagnosed most of these tumours as adenomas.

The EPA (1988) evaluated the study described above (Parker et al., 1985). This evaluation reported results on only one sample of tetrachlorvinphos ("current production" technical tetrachlorvinphos). The tumour incidences reported in the EPA (1988) evaluation differed with those reported by Parker et al. (1985), because the EPA used the number of tumour-bearing animals per number of animals at risk, excluding animals that died before appearance of first tumour, as the criteria for tumour incidence. Using this criterion, the EPA found that exposure to tetrachlorvinphos caused a significant increase ($P \leq 0.05$) in the incidence of hepatocellular adenoma or carcinoma (combined): 25 out of 59 (42%) in male mice at 16 000 ppm versus 28 out of 113 (25%) in controls. In female mice, there was a significant increase ($P \leq 0.05$) in the incidence of hepatocellular carcinoma in groups at 8000 ppm (5 out of 66 (8%) versus 1 out of 119 (1%) in controls) and 16 000 ppm (5 out of 68 (8%) versus 1 out of 119 (1%) in controls), and a significant increase ($P \leq 0.01$) in the incidence of hepatocellular adenoma or carcinoma (combined) in the groups at 8000 ppm (7 out of 66 (11%) versus 1 out of 119 (1%) in controls) and 16 000 ppm (8 out of 68 (12%) versus 1 out of 119 (1%) in controls). Also, in male mice at the highest dose there was

a significant increase ($P \leq 0.01$) in the incidences of renal tubule carcinoma of the kidney (9 out of 46 (20%) versus 0 out of 71 in controls) and renal tubule adenoma or carcinoma (combined) (13 out of 69 (19%) versus 0 out of 113 in controls). All the above significant increases in tumour incidence by pairwise comparison were associated with a significant positive trend in the incidence of the related tumour.

[The Working Group noted that while the tumour incidences reported by EPA (1988) and Parker et al. (1985) differed because of the different criteria used, the two reported significance mainly at the same tumour sites.]

3.2 Rat

See Table 3.2

Oral administration

In a study by the NCI, groups of 50 male and 50 female Osborne-Mendel rats (age, 35 days) were given feed containing tetrachlorvinphos (purity, 98%) at a time-weighted average dose of 4250 ppm (8000 ppm for 5 weeks, then lowered to 4000 ppm for 75 weeks), or 8500 ppm (16 000 ppm for 5 weeks, then lowered to 8000 ppm for 75 weeks) ad libitum 7 days per week for 80 weeks, and then held untreated for an additional 31 weeks (NTP, 1978). Initial doses were lowered by 50% at 5 weeks on study because toxicity was observed that indicated excessive mortality might occur before the end of the study. Groups of 10 male and 10 female rats held untreated for 111 weeks served as matched untreated controls. Since the numbers of rats in the matched-control groups were small, pooled-control groups were also used for statistical comparisons. Matched controls from the study on tetrachlorvinphos were combined with matched controls from long-term studies on malathion, toxaphene, endrin, and lindane that were conducted at the same time in the same laboratory. The pooled controls for statistical tests consisted of 55 males and 55 females. There was a dose-related decrease in mean body weights in treated male and female rats compared with the matched controls throughout the exposure period. Survival of males at the higher dose was only 48% at the end of the study. Survival of males and females in all other dosed groups was similar to or higher than that of controls (NTP, 1978).

In female rats at the higher dose, there was a significant increase in the incidence of thyroid C-cell adenoma (7 out of 46 versus 1 out of 46 in pooled controls; $P = 0.027$,) and in the incidence of cortical adenoma of the adrenal gland (5 out of 50 versus 0 out of 50 in pooled controls; $P = 0.022$). There was also a significant positive trend ($P < 0.02$) in the incidence of both types of neoplasm. Haemangioma of the spleen was also reported at a significantly higher incidence in males at the lower dose compared with the corresponding pooled controls (4 out of 48 (8%) versus 0 out of 47; $P = 0.049$) [the Working Group considered that these tumours may not have been associated with treatment, since there were only four tumours in the group at the lower dose, none at the higher dose, and the statistical test result for a positive dose-related trend was not significant]. There were no significant increases in tumour incidence at any other site in treated rats (NTP, 1978). [The Working Group noted the small number of matched controls, the early toxicity causing halving of doses at week 5 of the study, and that the rats were dosed for only 80 weeks and held for 31 weeks before termination.]

The EPA (1988) provided information on a long-term study in which groups of Porton Wistar rats [age not reported] were given diets containing tetrachlorvinphos [purity not reported] at a dose of 0 (60 males and 60 females; controls), 5 (40 males and 40 females), 25 (40 males and 40 females), 125 (40 males and 40 females), or 2000 (20 males and 20 females) ppm, ad libitum, 7 days per week for 2 years. In groups of treated male and female rats, final

Table 3.2 Studies of carcinogenicity with tetrachlorvinphos in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Osborne-Mendel (M, F) 111 wk NTP (1978)	Diet containing TCVP (purity, 98%) at a concentration of 0 ppm (matched control), 0 ppm (pooled control), 4250 ppm TWA (8000 ppm for 5 wk, then lowered to 4000 ppm for 75 wk), or 8500 ppm TWA (16 000 ppm for 5 wk, then lowered to 8000 ppm for 75 wk), ad libitum, 7 days/wk for 80 wk, and then held untreated for an additional 31 wk 50 M and 50 F (age, 35 days)/treated groups; 10 M and 10 F/matched untreated control group Since the numbers of rats in the matched-control groups was small, pooled-control groups also were used for statistical comparisons; matched controls from this study were combined with those from long-term studies with malathion, toxaphene, endrin, and lindane that were conducted at the same time in the same laboratory; the pooled controls for statistical tests consisted of 55 M and 55 F	*Males* Spleen: Haemangioma: 0/10, 0/52, 4/48 (8%)*, 0/47 *Females* Thyroid: C-cell adenoma: 1/9 (11%), 1/46 (2%)†, 2/50 (4%), 7/46 (15%)** Adrenal: Cortical adenoma: 0/9, 0/50†, 2/49 (4%), 5/50 (10%)**	*$P = 0.049$ (Fisher exact test, vs pooled controls) **$P < 0.03$ (Fisher exact test, vs pooled controls) † $P < 0.02$ for trend (Cochran-Armitage test)	Initial doses were lowered by 50% at wk 5 because observed toxicity indicated that excessive mortality might occur before the end of the study Study had a small number of matched controls and early toxicity led to halving of doses at experimental wk 5 of study. The animals were dosed for only 80 wk and then held for 31 wk Dose-related decrease in mean body weights in treated males and females were observed throughout exposure period; survival of males at the highest dose was only 48% at the end of the study
Porton Wistar (M, F) 2 yr EPA (1988)	Diet containing TCVP at a concentration of 0, 5, 25, 125, or 2000 ppm, ad libitum, 7 days/wk for 2 yr 60 M and 60 F/control group; 20 M and 20 F/highest-dose group; 40 M and 40 F/all other dose groups	No TCVP-related tumours reported	NS	Purity of study material and age of animals at study start, NR Final body weights significantly lower than those of controls for both sexes Small number of animals at the highest dose

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Sprague-Dawley (M, F) 2 yr EPA (1995b)	Diet containing TCVP at a concentration of 0, 100, 1000, or 2000 ppm, ad libitum, 7 days/wk for 2 yr 50 M and 50 F/group	*Males* Thyroid: C-cell adenoma: 8/47 (17%), 10/47 (21%), 7/48 (15%), 13/45 (29%) C-cell carcinoma: 0/47, 1/47 (2%), 1/48 (2%), 0/45 Adrenal: Pheochromocytoma, benign: 4/49 (8%)*, 2/49 (4%), 6/49 (12%), 9/50 (18%) Pheochromocytoma, malignant: 0/49, 0/49, 1/49 (2%), 0/50 Pheochromocytoma, benign or malignant (combined): 4/49 (8%)*, 2/49 (4%), 6/49 (12%), 9/50 (18%) *Females* Thyroid: C-cell adenoma: 6/64 (9%), 4/50 (8%), 5/49 (10%), 4/65 (6%) C-cell carcinoma: 0/64, 0/50, 1/49 (2%), 1/65 (2%) Adrenal: Pheochromocytoma, benign: 0/48, 1/50 (2%), 2/50 (4%), 0/49 Pheochromocytoma, malignant: 1/48 (2%), 0/50, 0/50, 0/49	*$P = 0.018$, trend test	Purity of study material and age of animals at study start, NR

F, female; M, male; mo, month; NR, not reported; NS, not significant; TCVP, tetrachlorvinphos; TWA, time-weighted average; vs, versus; wk, week; yr, year

body weights were significantly lower than those of the controls. There were no compound-related lesions reported. [The Working Group noted the small number of rats at the highest dose.]

The EPA (1995b) also provided information on a long-term study in which groups of 50 male and 50 female Sprague-Dawley rats [age not reported] were given diets containing tetrachlorvinphos [purity not reported] at a dose of 0, 100, 1000, or 2000 ppm ad libitum 7 days per week for 2 years. [No information on survival or body weight was provided.] In male rats, there was a significant positive trend ($P = 0.018$) in the incidence of adrenal pheochromocytoma (benign or malignant, combined). The incidences were: controls, 4 out of 49; lowest dose, 2 out of 49; intermediate dose, 6 out of 49; highest dose, 9 out of 50. In male rats at the highest dose, there was also a non-significant increase in the incidence of thyroid C-cell adenoma (13 out of 45 (29%) versus 8 out of 47 (17%) in controls).

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Very little literature on absorption of tetrachlorvinphos was available to the Working Group. No studies on oral absorption in humans were identified by the Working Group. Because of the lipophilicity of tetrachlorvinphos, it is expected that oral or dermal absorption occurs via passive diffusion. One study showed that tetrachlorvinphos used in dog flea collars could be transferred to the pet owner's clothing (Davis et al., 2008). Urinary concentrations of 2,4,5-trichloromandelic acid (a tetrachlorvinphos metabolite) excreted by the dog owners were significantly higher on days immediately after placement of the flea collar on dogs than before treatment. [This result, although it summarizes excretion data, suggested that dermal absorption of tetrachlorvinphos might occur in humans.]

(b) Experimental systems

A study in rats (Porton strain) given a single oral dose of [^{14}C]-tetrachlorvinphos (16.5–22 mg/kg bw; radiolabelled at both vinyl carbon atoms) indicated efficient absorption from the gastrointestinal tract; 78% of the administered dose was eliminated in the urine within 4 days (Akintonwa & Hutson, 1967). In dogs given [^{14}C]-tetrachlorvinphos orally (0.24–0.47 mg/kg bw), 92% of the radiolabel was excreted in the urine and faeces within 4 days (Akintonwa & Hutson, 1967), again indicating effective absorption of tetrachlorvinphos.

In dairy cows given diet containing [^{14}C]-tetrachlorvinphos at a concentration of 50 ppm for five consecutive days resulted in absorption and subsequent rapid metabolism of tetrachlorvinphos to several polar metabolites, as assessed by thin-layer chromatography (Akhtar & Foster, 1980b). Nearly all the metabolites, and only trace amounts of parent compound, were excreted in the urine (Akhtar & Foster, 1980b).

Following dermal application to male CD rats of radiolabelled tetrachlorvinphos (0.1 mg/cm^2 for 10 hours), 9.57% of the administered dose was recovered in the skin, urine, faeces, and carcass, while 84% was recovered unabsorbed (EPA, 2000).

4.1.2 Distribution

(a) Humans

No data on the distribution of tetrachlorvinphos in human tissue were available to the Working Group.

(b) Experimental systems

Administration of tetrachlorvinphos to dairy cows resulted in its delivery to the liver and kidney (Akhtar & Foster, 1980b). In addition, studies of toxicokinetics in rodents indicated that tetrachlorvinphos was available systemically after oral dosing. After 4 days, 0.5% of the radiolabel was present in the skin and hair of rats (Akintonwa & Hutson, 1967).

4.1.3 Metabolism and modulation of metabolic enzymes

(a) Metabolism

(i) Overview

Tetrachlorvinphos is somewhat unique among organophosphate pesticides in that it does not require a bioactivation step in vivo to elicit its toxicological effects (see Fig. 4.1). Tetrachlorvinphos, a phosphoric acid triester, is essentially a "preformed" oxon that can directly inhibit serine hydrolases, such as acetylcholinesterase and other B-esterases. Cytochrome P450 (CYP)-catalysed demethylation and esterase-catalysed hydrolysis are both important routes of tetrachlorvinphos detoxification (Fig. 4.1). It was reported that horse plasma butyrylcholinesterase activity was significantly inhibited by tetrachlorvinphos, indicating that esterases can interact with tetrachlorvinphos (Karanth et al., 2008). For example, a single oral dose of tetrachlorvinphos (500 mg/kg) significantly inhibited liver carboxylesterase activity in rats (Moroi & Kuga, 1982). [The Working Group could not identify any evidence that paraoxonase 1 (PON-1) hydrolysed tetrachlorvinphos, but again, based on structural precedent, and the fact that 2,4,5-trichlorphenacyl chloride and downstream metabolites are formed in vitro and in vivo (Akhtar & Foster, 1980a, b), it seems reasonable and likely that PON-1 will hydrolyse tetrachlorvinphos.]

(ii) Humans

No data on metabolism in humans were available to the Working Group.

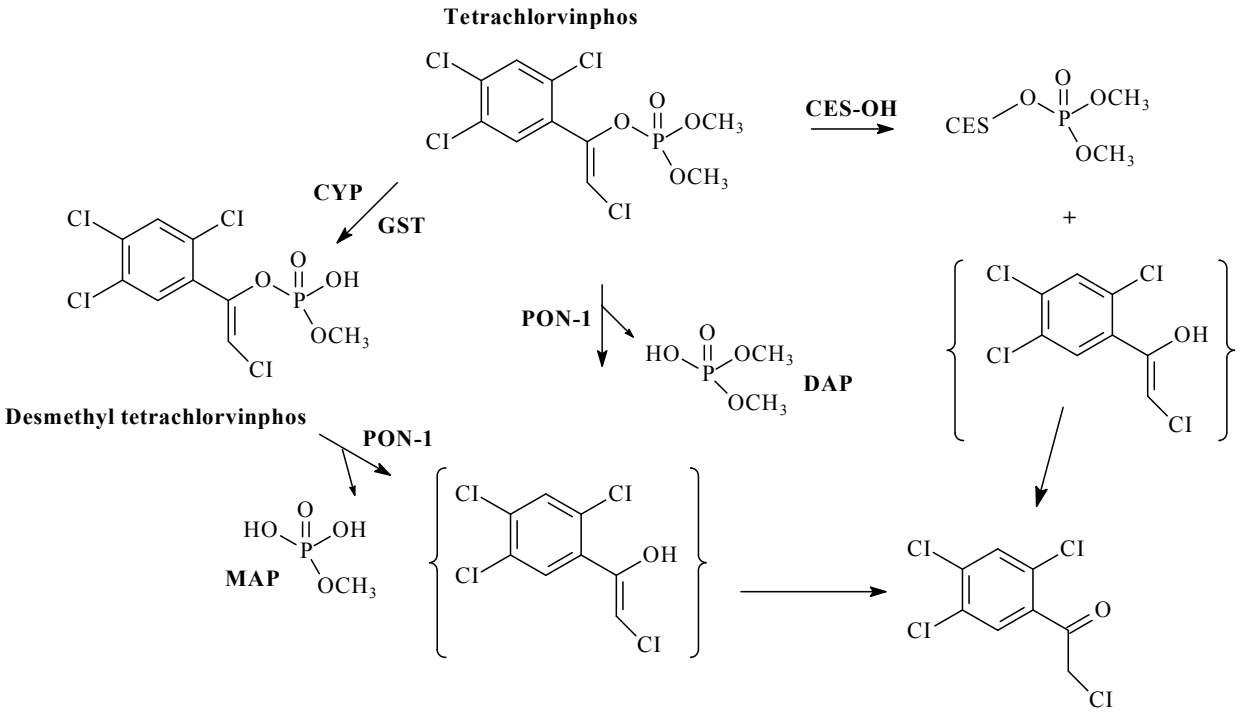
(iii) Experimental systems

Dogs were shown to metabolize tetrachlorvinphos more rapidly than rats; this was attributed to the higher activities of CYP [isoform not specified] and glutathione transferases that metabolize tetrachlorvinphos in dog liver compared with rat liver (Crawford et al., 1976). Addition of glutathione to the soluble fraction of liver from mouse, rat, rabbit, and pig caused the demethylation of tetrachlorvinphos, thus forming desmethyl tetrachlorvinphos (Fig. 4.1; Hutson et al., 1972). Glutathione acts as the acceptor of the transferred methyl group yielding *S*-methyl glutathione. In addition, demethylation of tetrachlorvinphos can also be catalysed by the hepatic microsomal fraction in an NADPH-dependent reaction, thus implicating CYP (Fig. 4.1; Crawford et al., 1976).

In lactating cows, oral administration of food containing [^{14}C]-tetrachlorvinphos at a concentration of 50 ppm for five consecutive days resulted in rapid metabolism to several polar metabolites, as assessed by thin-layer chromatography (Akhtar & Foster, 1980b). Extensive demethylation of tetrachlorvinphos was also noted in this study. Similar biotransformation pathways of tetrachlorvinphos were observed using the soluble fraction of goose and turkey liver homogenates (Akhtar & Foster, 1980a).

Hydrolytic degradation of tetrachlorvinphos and desmethyl tetrachlorvinphos are also likely important routes of degradation (Akhtar & Foster, 1980b). The resulting metabolites are mono- and di-alkyl phosphates and 2,4,5-trichlorophenacyl chloride (Fig. 4.1). 2,4,5-Trichlorophenacyl chloride can be further metabolized to 2,4,5-trichloroacetophenone via the spontaneous formation of *S*-(2,4-dichlorophenacyl) glutathione, which is converted to the ketone by an enzyme-catalysed glutathione-dependent reaction. In dairy cows, it

Fig. 4.1 Biotransformation of tetrachlorvinphos



Cytochrome P450 (CYP)-catalysed reactions produce desmethyl tetrachlorvinphos. On the basis of structural similarity with other oxons, it is likely that PON-1 catalyses the hydrolysis of tetrachlorvinphos and that carboxylesterases will react directly with tetrachlorvinphos. GST, glutathione transferase. CES-OH represents carboxylesterase; the OH functionality represents the catalytic (nucleophilic) serine residue in the active site that reacts with the electrophilic phosphoric acid triester. Metabolites in brackets have not been isolated, but are likely intermediates in the formation of 2,4,5-trichlorophenacyl chloride. MAP, monoalkyl phosphate; DAP, dialkyl phosphate.
Compiled by the Working Group

was suggested that 2,4,5-trichloroacetophenone could be converted to 1-(2,4,5-trichlorophenyl) ethanol by a keto reductase activity (Akhtar & Foster, 1980b). Alternatively, 2,4,5-trichlorphenacyl chloride can be converted to 2,4,5-trichloromandelic acid, as shown in (Akhtar & Foster, 1980b). These metabolites are readily excreted in the urine as glucuronide conjugates.

(b) Modulation of metabolic enzymes

Tetrachlorvinphos did not inhibit CYP19 aromatase activity in human placental microsomes in vitro (Vinggaard et al., 2000). In hepatocytes harvested from human liver biopsies, and in rat primary hepatocytes, tetrachlorvinphos induced CYP1A1, as measured by 7-ethoxyresorufin-*O*-deethylase (EROD) activity (Delescluse et al., 1998). No similar activity was seen in a human carcinoma cell line (HepG2) or an immortalized human keratinocyte cell line (HaCaT).

In rats, two hepatic monooxygenase enzyme activities were induced in a dose-related manner after oral administration of tetrachlorvinphos (60 and 250 mg/kg) for 10 days (Moroi et al., 1976). The increases were observed in aminopyrine demethylase *o*-ethyl *O*-*p*-nitrophenylphenylphosphonothioate detoxification.

4.1.4 Excretion

(a) Humans

No data on excretion of tetrachlorvinphos in humans were available to the Working Group.

(b) Experimental systems

In rats (Porton strain) given a single oral dose of [^{14}C]-tetrachlorvinphos (16.5–22 mg/kg bw; radiolabelled at both vinyl carbon atoms), on average 78% of the administered dose was excreted in the urine, 16.5% in the faeces, and 0.5% in expired gases over 4 days (Akintonwa & Hutson, 1967). A significant fraction of the faecal radiolabel was identified as [^{14}C]-tetrachlorvinphos, indicating incomplete absorption. Similarly, in dairy cows given diets containing [^{14}C]-tetrachlorvinphos (5 or 50 ppm), ~76–82% of the administered dose was also eliminated in the urine, as metabolites (Gutenmann et al., 1971; Akhtar & Foster, 1980b). Only trace amounts of parent compound were detectable (Akhtar & Foster, 1980b). After hydrolysis of glucuronide and sulfate conjugates in the urine, the metabolites identified were (percentage of administered dose indicated): desmethyl tetrachlorvinphos (13.2%), 1-(2,4,5-trichlorophenyl) ethanol (34.8%), (2,4,5-trichlorophenyl)ethane-1,2-diol (28.1%), and 2,4,5-trichloromandelic acid (6.1%) (Gutenmann et al., 1971; Akhtar & Foster, 1980b).

4.2 Mechanisms of carcinogenesis

This section summarizes evidence for the key characteristics of carcinogens (IARC, 2014) for which there were adequate data for evaluation, concerning whether tetrachlorvinphos is genotoxic, modulates receptor-mediated events, and alters cell proliferation, cell death or nutrient supply.

4.2.1 Genetic and related effects

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the results of studies carried out in human cells in vitro, in experimental animals in vivo, in non-human mammalian cells in vitro, and in non-mammalian systems in vitro, respectively.

(a) Humans

See Table 4.1

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

A significant increase in the frequency of chromosomal aberrations was observed in human cultured lymphocytes exposed to tetrachlorvinphos in the absence of metabolic activation (Kurinnyĭ & Pilinskaia, 1977).

(b) Experimental systems

See Table 4.2, Table 4.3, Table 4.4

In one study, tetrachlorvinphos significantly increased the frequency of micronucleus formation in the bone marrow of Swiss mice after repeated doses administered intraperitoneally (100 mg/kg bw) or orally (3000 ppm, in the diet) (Amer & Fahmy, 1983). In the same study, no increase in the frequency of micronucleus formation was seen after dermal exposure (1350 mg/kg bw, twice per week, for 2 weeks).

In primary cultures of mouse spleen cells, tetrachlorvinphos significantly increased the frequency of chromosomal aberrations and sister-chromatid exchanges in the absence of metabolic activation (Amer & Aly, 1992). In Chinese hamster ovary cells, tetrachlorvinphos induced chromosomal aberrations in the absence but not in the presence of metabolic activation with S9 (EPA, 2002b).

In bacterial studies, tetrachlorvinphos did not induce primary DNA damage in *Escherichia coli PQ37* (Ruiz & Marzin, 1997), or mutations in *Salmonella typhimurium* (Dean, 1972; Bartsch et al., 1980; Brooks et al., 1982; Moriya et al.,

Table 4.1 Genetic and related effects of tetrachlorvinphos in humans cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Cultured lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	2 µg/mL	$P < 0.05$	Kurinnyĭ & Pilinskaia (1977)

[a] +, positive
HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

Table 4.2 Genetic and related effects of tetrachlorvinphos in non-human mammals in vivo

Species, strain	Tissue	End-point	Test	Results[a]	Doses (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss	Bone marrow	Chromosomal damage	Micronucleus formation	+ [a]	50 and 100 (LED) mg/kg bw	Intraperitoneal, 1–4×		Amer & Fahmy (1983)
Mouse, Swiss	Bone marrow	Chromosomal damage	Micronucleus formation	+	3000 (LED) and 6000 ppm, in diet	Oral, ≤ 10 wk		Amer & Fahmy (1983)
Mouse, Swiss	Bone marrow	Chromosomal damage	Micronucleus formation	(–)	1350 mg/kg bw	Dermal, 2× per wk, for 2 wk	Only one dose tested	Amer & Fahmy (1983)

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality
bw, body weight; HID, highest ineffective dose; LED, lowest effective dose (units as reported); NT, not tested; vs, versus; wk, week

Table 4.3 Genetic and related effects of tetrachlorvinphos in non-human mammalian cells in vitro

Species, strain	Tissue, cell line	End-point	Test	Results		Concentration (LEC or HIC)	Reference
				Without metabolic activation	With metabolic activation		
Mouse, Swiss	Spleen cell primary cultures	Chromosomal damage	Chromosomal aberrations	+ [a]	NT	0.50 µg/mL	Amer & Aly (1992)
Mouse, Swiss	Spleen cell primary cultures	Chromosomal damage	Sister-chromatid exchange	+	NT	0.50 µg/mL	Amer & Aly (1992)
Chinese hamster	Ovary	Chromosomal damage	Chromosomal aberrations	NT	+	75.1 µg/mL	EPA (2002b)
Chinese hamster	Ovary	Chromosomal damage	Chromosomal aberrations	–	NT	59.9 µg/mL	EPA (2002b)

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality
HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested

Table 4.4 Genetic and related effects of tetrachlorvinphos in non-mammalian systems in vitro

Phylogenetic class	Test system (species, strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Reference
				Without metabolic activation	With metabolic activation		
Prokaryote (bacteria)	*Escherichia coli* PQ37	DNA damage	SOS chromotest	–	–	NR	Ruiz & Marzin (1997)
	Salmonella typhimurium TA98, TA100, TA102, TA1535, and TA1537	Mutation	Reverse mutation	–	–	500 µg/plate	Ruiz & Marzin (1997)
	Salmonella typhimurium TA98, TA100, TA1535, and TA1538	Mutation	Reverse mutation	–	–	2000 µg/plate	Brooks et al. (1982)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Mutation	Reverse mutation	–	–	5000 µg/plate	Moriya et al. (1983)
	Salmonella typhimurium TA98, TA100	Mutation	Reverse mutation	–	–	3 µmol/plate	Bartsch et al. (1980)
	Escherichia coli WP2 *hcr*	Mutation	Reverse mutation	–	–	5000 µg/plate	Moriya et al. (1983)
	Escherichia coli WP2 and WP2 *uvrA*	Mutation	Reverse mutation	–	–	2000 µg/plate	Brooks et al. (1982)
	Escherichia coli WP2	Mutation	Reverse mutation	–	NT	Tested dose, NR; semiquantitative paper disc method	Dean (1972)
Lower eukaryote (yeast)	*Saccharomyces cerevisiae* D4	Mutation	Gene conversion	–	NT	400 µg/mL	Brooks et al. (1982)
Plant systems	*Vicia faba*	Chromosomal damage	Chromosomal aberrations	+	NT	Saturated and 0.5-saturated solutions of TCVP tested;LEC, 0.5 saturated solution	Amer & Mikhael (1983)

[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; TCVP, tetrachlorvinphos

1983; Ruiz & Marzin, 1997) or *E. coli* (Brooks et al., 1982; Moriya et al., 1983). Moreover, tetrachlorvinphos failed to cause gene conversion in yeast *Saccharomyces cerevisiae* D4 (Brooks et al., 1982). On the other hand, tetrachlorvinphos did increase the frequency of chromosomal aberration in root-tip meristems of *Vicia faba* (Amer & Mikhael, 1983).

4.2.2 Receptor-mediated mechanisms

(a) Neurotoxicity-pathway receptors

Tetrachlorvinphos is a reactive oxon. It can covalently modify the catalytic serine residue of several B-esterases and inhibit their catalytic activity, including the canonical target acetylcholinesterase (Akintonwa & Hutson, 1967; Moroi et al., 1976), resulting in the acute neurotoxicity elicited in insects and mammalian species (Ogawa et al., 1990; see Section 4.5). Acetylcholinesterase is responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems. The inhibition of acetylcholinesterase results in acetylcholine overload and the overstimulation of nicotinic and muscarinic acetylcholine receptors. The relevance of these effects of tetrachlorvinphos to mechanisms of carcinogenesis is unknown.

(b) Humans

No data from exposed humans were available to the Working Group.

In an in-vitro assay for competitive binding, tetrachlorvinphos did not displace 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (40 nM) from the human aryl hydrocarbon receptor (AhR) when administered at a ~2000-fold molar excess (Delescluse et al., 1998).

In human androgen and estrogen receptor reporter-gene assays in the Chinese hamster ovary cell line (CHO-K1), tetrachlorvinphos was not an antagonist or agonist of human androgen receptor, nor an antagonist or agonist of estrogen receptors α and β (Kojima et al., 2004, 2010). In the same studies, tetrachlorvinphos was found to be an agonist for human pregnane X receptor in transfected CHO-K1 cells.

(c) Experimental systems

(i) In vivo

The effect of tetrachlorvinphos on thyroid function was studied in two experiments in animals. In the first study, thyroid uptake of iodine was significantly reduced 2, 6, and 24 hours after exposure to tetrachlorvinphos (a single intraperitoneal dose at 500 mg/kg) in male albino rats (Bojadziev & Manolov, 1975). No effect was seen on triiodothyronine (T3) or thyroxine (T4). A second study examined T3 or T4 in 10 horses of various breeds and ages (Berger et al., 2008). Six horses received a dietary supplement containing tetrachlorvinphos for 30 days and four horses served as controls. Tetrachlorvinphos significantly decreased serum cholinesterase activity (to < 50%) during and for 13 days after exposure, and induced behavioural changes. Thyroid hormone levels were highly variable and no significant changes were observed; the authors noted that, "detection of possible effects of thyroid hormones associated with tetrachlorvinphos exposure may require a larger number of horses and/or a longer treatment period."

Technical-grade tetrachlorvinphos substantially reduced oocyte maturation in freshwater catfish native to southern India (Haider & Upadhyaya, 1986). A subsequent study of in-vitro exposures reported significant inhibition of luteinizing hormone-induced germinal vesicle breakdown in isolated fish oocytes at all three concentrations used (1, 10, and 100 ppb) (Haider & Upadhyaya, 1986).

Other effects of tetrachlorvinphos on the thyroid, testis, ovary, and adrenal glands of rodents are discussed in Sections 4.2.3 and 4.5.

(ii) In vitro

Tetrachlorvinphos was not an agonist for the 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). Tetrachlorvinphos was also not an agonist for mouse peroxisome proliferator-activated receptors α or γ in reporter-gene assays in CV-1 monkey kidney cells (Takeuchi et al., 2006; Kojima et al., 2010).

4.2.3 Cell proliferation and death

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Lesions indicative of altered cell proliferation and death were observed in studies of carcinogenicity in mice and rats (NTP, 1978; Parker et al., 1985). Other adverse effects reported in studies of carcinogenicity in rodents are discussed in Section 4.5.

In a study of carcinogenicity in B6C3F$_1$ mice, necropsy at study week 26 revealed hyperplasia of the renal inner cortical tubular epithelium in males and females fed diets containing tetrachlorvinphos at 8000 or 16 000 ppm (NTP, 1978; see Section 3.1). At 78 weeks, intraluminal necrotic debris was present at high doses in both sexes, and in males the parietal epithelium of the Bowman's capsule was devoid of cuboid cells.

In the mouse liver at 26 weeks, lesions (e.g. hepatocyte enlargement) but not hyperplasia, were reported in groups at 8000 and 16 000 ppm. At a necropsy at week 53, "scattered necrotic hepatocytes" and bile duct hyperplasia were reported for these groups (NTP, 1978). Liver-weight increases were reported in 28-day and 13-week studies in rats (Ogawa et al., 1990; EPA, 2002c).

In female mice, corpora lutea were not observed in the ovaries at any necropsy of mice in the group at the highest dose (Parker et al., 1985). In male mice, hyperchromatic degenerate cells were observed in the seminiferous tubules after week 26 in the groups at 8000 and 16 000 ppm. The size and secretory activity of seminal vesicles was decreased in males at the highest dose. In males and females, adrenal hypertrophy was observed in the group at 16 000 ppm.

In a separate study of ovarian follicles explanted from C57Bl/6J female mice after treatment of the colony with tetrachlorvinphos for skin parasites, Nayudu et al. (1994) reported premature termination of follicular growth and release of oocytes with immature nuclei and without cumulus cells. The duration and pattern of in-vitro growth was markedly altered in follicles isolated from exposed mice. In follicles isolated from the offspring (age, 21 days) of exposed parents (C57BI/6J females and CBA/J males), in-vitro growth was improved, but did not follow the linear growth pattern seen in follicles of unexposed mice.

In a study of carcinogenicity, parafollicular cell (C-cell) and follicular cell hyperplasia was observed in male and female rats (NTP, 1978). The C-cell hyperplasia was described as mostly unilateral, and microscopically as having a fairly uniform and diffuse increase of C-cells scattered between the thyroid follicles. The follicular cell hyperplasia was sometimes bilateral, appearing as nodular alterations on the surface of the thyroid. Microscopically they were described as variable, "multifocal and cystic or having inward papillary projections of variable thickness," lined by regular appearing follicular cells.

4.2.4 Other mechanisms

In six horses exposed to tetrachlorvinphos for 30 days (see also Section 4.2.2), there was no effect on the expression of cytokines (interferon-γ, INF-γ) and interleukin (IL-12p40), or cyclooxygenase-2 in concanavalin A-stimulated peripheral blood mononuclear cells (Berger et al.,

2008). However, when assessed in non-stimulated cells of treated animals, INF-γ was decreased (≈-20-fold transcription compared with reference value) non-significantly towards the end of treatment and even more so (≈70-fold) after treatment ($P = 0.064$) (Berger et al., 2008).

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 ToxCast/Tox21 assay battery 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. 22248–79–9; 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 the ToxCast/Tox21 assays.

Detailed information about the chemicals, assays and associated data analysis procedures 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 comprise 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)*: 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 was 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 in the present volume (see Annex 1). 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).

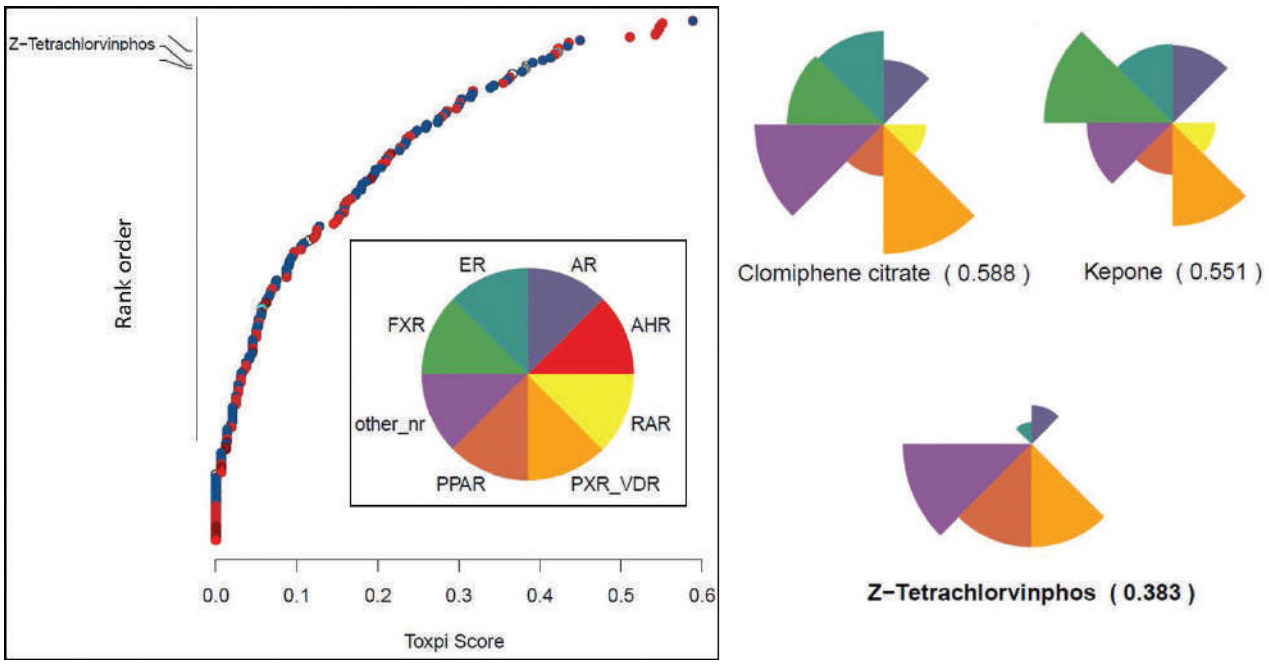
4.3.3 Specific effects across 7 of the 10 "key characteristics" based on in-vitro screening data

The relative effects of tetrachlorvinphos 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 (see Fig. 4.2). 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 position of *Z*-tetrachlorvinphos in the ranked list is 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 right-hand side, the two highest-ranked chemicals in each analysis are shown to represent the maximum ToxPi scores (with the scores in parentheses). Because *Z*-tetrachlorvinphos was not tested against many of the assay end-points for most characteristics discussed below, the ToxPi chart of *Z*-tetrachlorvinphos is shown only for the "modulates receptor-mediated effects" key characteristic.

Characteristic (1). *Is electrophilic or can undergo metabolic activation: Z*-tetrachlorvinphos was tested only for the two assay end-points relating to aromatase inhibition, demonstrating activity for a one cell-based end-point, but not for an end-point in a cell-free inhibition assay. *Z*-tetrachlorvinphos was not tested for any of the other 29 end-points in cell-free CYP-inhibition assays.

Characteristic (2) *Is genotoxic*: *Z*-tetrachlorvinphos was tested for 6 of the 9 assay end-points related to TP53 activity, showing activity for 2 end-points. In comparison, the most active chemical in the data set, chlorobenzilate, showed activity for 7 out of the 9 assay end-points for which it was tested. One of the active assay end-points, from a multiplexed transcription factor assay platform, has been demonstrated to be

Fig. 4.2 ToxPi ranking for *Z*-tetrachlorvinphos using ToxCast assay end-points mapped to receptor-mediated effects



On the left-hand side, the relative rank of Z-tetrachlorvinphos is shown (*y*-axis) with respect to its 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 Toxicity ForeCaster (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 citrate and kepone) and the target chemical (*Z*-tetrachlorvinphos) are shown with their respective ToxPi score in parentheses.
Compiled by the Working Group

confounded by oxidative stress (Martin et al., 2010).

Characteristic (4) *Induces epigenetic alterations: Z*-tetrachlorvinphos was active for all 4 of the DNA-binding assay end-points, but was not tested for any of the 7 cell-free enzymatic assay end-points assigned to the transformation assay grouping. [The Working Group noted that the positive response in the multiplexed transcription factor profiling assay platform was most likely due to the activation of oxidative stress.]

Characteristic (5) *Induces oxidative stress*: *Z*-tetrachlorvinphos was tested for all 6 assay end-points related to oxidative stress markers and exhibited intermediate activity, being active for 3 out of 6 assay end-points. Comparison with the most active chemicals, carbaryl and tannic acid, is limited due to the incomplete testing of *Z*-tetrachlorvinphos. *Z*-tetrachlorvinphos did activate NRF2, metal-response element and antioxidant-response element transcription.

Characteristic (6) *Induces chronic inflammation*: *Z*-tetrachlorvinphos was active for one out of two NF-kB assay end-points for which only 7 of the 185 chemicals in the analysis were active. *Z*-tetrachlorvinphos was not tested in the panel of 43 assay end-points that comprise the cytokine and cell-adhesion molecule assay groupings.

Characteristic (8) *Modulates receptor-mediated effects: Z*-tetrachlorvinphos was tested for 81 of the assay end-points mapped to this

characteristic. When compared with other chemicals evaluated by the *IARC Monographs*, *Z*-tetrachlorvinphos demonstrated appreciable capacity to interact with nuclear and other receptors similar to the two highest-ranking chemicals (clomiphene citrate and kepone). *Z*-tetrachlorvinphos showed consistent PXR activation and activity in assay end-points representative of antagonists of PPAR and other nuclear receptors. *Z*-tetrachlorvinphos activated 2 of the 18 estrogen-receptor assay end-points, both cell-based transcriptional assays. Of the 11 androgen receptor assay end-points, *Z*-tetrachlorvinphos was active in both assays run in an antagonist mode and in 1 out of 2 protein complementation assay end-points that test for agonist and antagonist activity (Fig. 4.2).

Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply: Z*-tetrachlorvinphos was tested for 27 out of the 68 assay end-points. *Z*-tetrachlorvinphos showed moderate impact on the assay end-points in this group when compared with the two highest-ranking chemicals, clomiphene citrate and ziram. *Z*-tetrachlorvinphos was active in the only assay for mitochondrial toxicity in which it was tested.

Overall, *Z*-tetrachlorvinphos was active for 36 of the 137 assay end-points for which it was tested. The results of ToxPi analysis of the ToxCast/Tox21 data for *Z*-tetrachlorvinphos supported findings in other model systems, as described in Section 4.2. These include aromatase inhibition, multiple nuclear receptor activities, oxidative stress, and some cytotoxic effects.

4.4 Susceptibility

No relevant studies of susceptibility to tetrachlorvinphos in humans or rodents were available to the Working Group.

4.5 Other adverse effects

4.5.1 Human

Few data on toxicity in humans were available to the Working Group.

4.5.2 Experimental systems

Regulatory submissions and published studies in rodents and dogs provide evidence for adverse effects including in the cholinergic system, liver, kidney, adrenals, and thyroid.

Effects on cholinesterase activity have been observed in different species, including rodents (Ogawa et al., 1990; EPA, 2002d), dogs (EPA, 1994) and horses (Berger et al., 2008). In a 28-day study in Slc:Wistar rats, serum and erythrocyte cholinesterase was inhibited in a dose-dependent manner from a dose of 10 mg/kg bw per day administered by intragastric gavage (Ogawa et al., 1990). Similarly, dose-dependent inhibition of plasma cholinesterase activity was observed after single oral doses of tetrachlorvinphos in rats, beginning at 8 mg/kg in males (19% inhibition) and at 20 mg/kg in females (35.5% inhibition), while inhibition of brain cholinesterase activity occurred at higher doses (EPA, 2002d). In a study of six exposed horses (Berger et al., 2008), tetrachlorvinphos significantly decreased serum cholinesterase activity (to < 50%) during and for 13 days after exposure, and induced behavioural changes.

Liver granuloma was observed in male and female $B6C3F_1$ mice at both dietary doses in a study of carcinogenicity (1200 and 2400 mg/kg per day) (NTP, 1978). Liver granuloma was also observed in a study of carcinogenicity in Osborne-Mendel rats, in females (at 212.5 and 425 mg/kg per day) and in males at the highest dose (NTP, 1978). Histological changes in the liver were seen in a 2-year study in male and female Sprague-Dawley rats given tetrachlorvinphos at a dose of 43 mg/kg per day (EPA, 1995c). In Slc:Wistar rats, liver weights increased and there was

accompanying vacuolization and necrosis at the highest dose (10, 100, and 1000 mg/kg per day) in a 28-day study (Ogawa et al., 1990). Centrolobular hepatocellular hypertrophy was seen in female Sprague Dawley rats, and in males at the intermediate dose, in a 13-week dietary study with tetrachlorvinphos (0, 100, 2000, or 5000 ppm; 0, 6.7, 142 or 375 mg/kg per day in males; 0, 10, 197 or 467 mg/kg per day in females) (EPA, 2002c). Liver weights increased, while body weights and body weight gains were reduced in males and females at the two highest doses.

Increased incidence and severity of bilateral basophilic tubules of the kidneys were also reported in male Sprague Dawley rats fed diets containing tetrachlorvinphos at 2000 or 5000 ppm in the 13-week study. In females, adrenal gland weights increased, as did fat deposition in the adrenal cortex. In the 28-day study in rats given tetrachlorvinphos by oral gavage, adrenal gland and kidney weight increases were observed at the highest dose, with accompanying pathology (Ogawa et al., 1990).

Effects on the thyroid gland reported in the study of carcinogenicity in Osborne-Mendel rats included C-cell and follicular cell hypertrophy in males and females at both doses (NTP, 1978). In the 28-day study in rats, thyroid gland weights were increased at 1000 mg/kg per day (Ogawa et al., 1990). In the 13-week study in rats, thyroid follicular cell hypertrophy was seen in males and females fed diets containing tetrachlorvinphos at 2000 or 5000 ppm (EPA, 2002c).

Decreased body weights were observed in rats and mice at both doses in studies of carcinogenicity, and there was increased mortality in male rats at the highest dose (NTP, 1978). In a study of developmental neurotoxicity in rats treated with tetrachlorvinphos (10, 50, or 200 mg/kg per day), pup weight decreased at the highest dose (EPA, 2005). Decreased thickness of the striatum, corpus callosum, and hippocampus were observed in males and females at the highest dose, and decreased thickness of the cerebellum was observed in males at the highest dose.

5. Summary of data reported

5.1 Exposure data

Tetrachlorvinphos is an organophosphate insecticide with anticholinesterase activity, which was first used commercially in 1966. It is effective against a wide range of flies, moths, fleas, ticks, and other insects; it can be sprayed on surfaces or applied to animals dermally, orally, or on treated collars and ear tags. Tetrachlorvinphos is banned for all uses in the European Union, and is not permitted on crops in the USA. It is still used in flea collars for dogs and cats in the USA, and this is one of the main sources of exposure for the general population. No data were available on occupational exposure to tetrachlorvinphos.

5.2 Human carcinogenicity data

Very few studies were available on the carcinogenicity of tetrachlorvinphos in humans. Excesses in the incidence of cancer of the brain among men and women, and of non-Hodgkin lymphoma (NHL) among women were reported in a cohort study of workers in a cigarette factory where tobacco was treated with tetrachlorvinphos; however, numbers were small, and individual exposure to the pesticide was not characterized. Analyses of data pooled from three case–control studies found an excess of NHL, but the excess was attenuated in further analyses that adjusted for exposure from other pesticides. An excess incidence of leukaemia (not otherwise specified) reported in a case–control study was based on only five exposed cases. Although excesses of cancer of the brain, NHL, and leukaemia (not otherwise specified) were observed, there were few studies for each cancer

site, small numbers, and lack of information on exposure specifically to tetrachlorvinphos, and therefore these data were considered inadequate to make an evaluation regarding carcinogenicity.

5.3 Animal carcinogenicity data

Tetrachlorvinphos was tested for carcinogenicity in male and female mice in two feeding studies, and in male and female rats in three feeding studies.

In the first study in mice, tetrachlorvinphos significantly increased the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in treated males; there was also a significant positive trend in the incidence of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). In treated females, there was a significant increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined).

In the second study in mice, tetrachlorvinphos significantly increased the incidence of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) in treated males and females. There was also a significant increase in the incidence of renal tubule carcinoma, and of renal tubule adenoma or carcinoma (combined), in treated males.

In one study in rats, tetrachlorvinphos caused a significant increase in the incidence of thyroid C-cell adenoma and adrenal cortical adenoma in females at the highest dose (with a significant positive trend for both types of tumours), and of haemangioma of the spleen in males at the lowest dose. In another study in rats, there was a significant positive trend in the incidence of adrenal pheochromocytoma (benign or malignant, combined) in treated males. There were no significant increases in the incidence of any tumours in the third study in rats.

5.4 Mechanistic and other relevant data

Tetrachlorvinphos is efficiently absorbed in rats, dogs, and cattle after oral administration; however, other routes have not been explored. In humans, one study suggested that tetrachlorvinphos can be absorbed through dermal exposure. Wide systemic distribution into parenchymal tissues and in blood was demonstrated in studies of tetrachlorvinphos in cattle and rats. Tetrachlorvinphos itself is a reactive oxon moiety that is able to react with proteins, with greatest affinity for esterases. No data on metabolism in humans were available. Data were available for rats and dogs and showed relatively complete metabolism of tetrachlorvinphos through cytochrome P450, demethylation, and hydrolytic degradation. Urine is the primary route of elimination for tetrachlorvinphos, as established from studies in rats and cattle. Primary excreted metabolites are desmethyl tetrachlorvinphos, 1-(2,4,5-trichlorophenyl)ethanol, 1-(2,4,5-trichlorophenyl)ethane-1,2-diol,and2,4,5-trichloromandelic acid.

With respect to the key characteristics of human carcinogens, adequate data were available to evaluate whether tetrachlorvinphos is genotoxic, modulates receptor-mediated events, and alters cell proliferation, cell death or nutrient supply.

The evidence is *moderate* that tetrachlorvinphos is genotoxic. The overall database is sparse but consistent. The evidence includes chromosomal damage in one in-vivo study in mice treated by intraperitoneal and oral, but not dermal, routes of exposure, two in-vitro studies in rodents, and one in-vitro study in human lymphocytes. Studies of gene mutation in bacteria gave clearly negative results in the presence or absence of metabolic activation.

The evidence is *weak* that tetrachlorvinphos modulates receptor-mediated effects. A well-established mechanism for the neurotoxic effects

of tetrachlorvinphos is inhibitory binding to acetylcholinesterase. The relevance of these effects to carcinogenesis is not clear. In animals in vivo, no effect was seen on thyroid hormones, although tetrachlorvinphos reduced iodine uptake in rats. In vitro, tetrachlorvinphos was not an agonist of the aryl hydrocarbon receptor, or mouse peroxisome proliferator activated receptors α and gamma. In human cells in vitro, tetrachlorvinphos interacted with multiple nuclear and other receptors, with mixed effects.

The evidence is *moderate* that tetrachlorvinphos alters cell proliferation in the mouse kidney and biliary tract, and rat thyroid gland, as demonstrated by hyperplasia.

No data were available to the Working Group concerning susceptibility to cancer after exposure to tetrachlorvinphos.

Overall, the mechanistic data are uninformative for carcinogenicity related to tetrachlorvinphos.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of tetrachlorvinphos.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Tetrachlorvinphos is *possibly carcinogenic to humans (Group 2B).*

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LIST OF ABBREVIATIONS

2,4-D	4-dichlorophenoxyacetic acid
7-ECOD	7-ethoxycoumarin *O*-deethylase
8-OH-dG	8-oxo-2'-deoxyguanosine
AB	alveolar bud
ACTH	adrenocorticotropic hormone
AhR	aryl hydrocarbon receptor
AHS	Agricultural Health Study
AMPA	aminomethylphosphonic acid
bw	body weight
CLL	chronic B-cell lymphocytic lymphoma
DAP	dialkylphosphate
DEP	diethylphosphate
DETP	diethylthiophosphate
DMBA	dimethylbenz[*a*]anthracene
DMP	dimethylphosphate
DMSO	dimethyl sulfoxide
DMTP	dimethylthiophosphate
ELISA	enzyme-linked immunosorbent assay
EPA	United States Environmental Protection Agency
ER	estrogen receptor
FAO	Food and Agriculture Organization of the United Nations
FMOC-Cl	9-fluorenylmethyl chloroformate
GC-MS	gas chromatography-mass spectrometry
GST	glutathione transferase
IDA	iminodiacetic acid
IFNγ	interferon gamma
IMPY	2-isopropyl-4-methyl-6-hydroxypyrimidine
IW-LED	intensity-weighted life-time exposure days
JEM	job-exposure matrix
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LED	life-time exposure days
MCL	mantle cell lymphoma
MDA	malathion dicarboxylic acid

MMA	malathion monocarboxylic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
NCI	National Cancer Institute
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NTP	United States National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PON	paraoxonase
PPAR	peroxisome proliferator-activated receptor
ppm	parts per million
POEA	polyethyloxylated tallow amine
PWG	pathology working group
PXR	pregnane X receptor
SD	standard deviation
SEER	Surveillance, Epidemiology, and End Results Program
SLL	small B-cell lymphocytic lymphoma
SNP	single-nucleotide polymorphism
T3	triiodothyronine
T4	thyroxine
TEB	terminal end bud
THPI	*cis*-1,2,3,6-tetrahydrophthalimide
TNFα	tumour necrosis factor alpha
ToxCast™	Toxicity Forecaster
ToxPi	Toxicological Prioritization Index
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TSH	thyroid-stimulating hormone
TUNEL	terminal uridine deoxynucleotidyl transferase dUTP nick end labelling
TWA	time-weighted average
UMHS	Upper Midwest Health Study

ANNEX 1. SUPPLEMENTAL MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online from http://publications.iarc.fr/549), contains a spreadsheet (.xlsx) and a zip folder containing several ToxPi software output files (.csv) analysed by the Working Group for Volume 112 of the *IARC Monographs*. The spreadsheet lists the ToxCast/Tox21 assay end-points, the associated target and/or model system (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" (EPA, 2015). The ToxPi files integrate the results by "key characteristic" and can be accessed using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

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M IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of some organophosphate insecticides and herbicides, including diazinon, glyphosate, malathion, parathion, and tetrachlorvinphos.

Diazinon acts on a wide range of insects on crops, gardens, livestock, and pets, but most uses have been restricted in the USA, Canada, and the European Union since the 1980s. Glyphosate is the most heavily used agricultural and residential herbicide in the world, and has been detected in soil, air, surface water, and groundwater, as well as in food. Malathion is one of the oldest and most widely used organophosphate insecticides, and has a broad spectrum of applications in agriculture and public health, notably mosquito control. The insecticide parathion has been largely banned or restricted throughout the world due to toxicity to wildlife and humans. Tetrachlorvinphos is banned in the European Union, but continues to be used in the USA and elsewhere as an insecticide on animals, including in pet flea collars.

The *IARC Monographs* Working Group reviewed epidemiological evidence, animal bioassays, and mechanistic and other relevant data to reach conclusions as to the carcinogenic hazard to humans of these agents.