

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER



IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

P R E A M B L E

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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

1 established as being effective during previous *Monograph* meetings but remain,
2 predominantly, the prerogative of each individual Working Group.

3 2. Objective and scope

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

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

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

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

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

33 Some epidemiological and experimental studies indicate that different agents may act at
34 different stages in the carcinogenic process, and several different mechanisms may be
35 involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of
36 carcinogenicity at any stage in the carcinogenesis process, independently of the underlying
37 mechanisms. Information on mechanisms may, however, be used in making the overall
38 evaluation (IARC, 1991; Vainio *et al.*, 1992; IARC, 2005, 2006; see also Part B, Sections 4
39 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international
40 scientific conferences to determine whether a broad-based consensus has emerged on how
41 specific mechanistic data can be used in an evaluation of human carcinogenicity. The results
42 of such conferences are reported in IARC Scientific Publications, which, as long as they still
43 reflect the current state of scientific knowledge, may guide subsequent Working Groups.

44 Although the *Monographs* have emphasized hazard identification, important issues may
45 also involve dose-response assessment. In many cases, the same epidemiological and
46 experimental studies used to evaluate a cancer hazard can also be used to estimate a dose-

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1 response relationship. A *Monograph* may undertake to estimate dose-response relationships
2 within the range of the available epidemiological data, or it may compare the dose-response
3 information from experimental and epidemiological studies. In some cases, a subsequent
4 publication may be prepared by a separate Working Group with expertise in quantitative
5 dose-response assessment.

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

16 3. Selection of agents for review

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

24 The scientific literature is surveyed for published data relevant to an assessment of
25 carcinogenicity. Ad-hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993,
26 1998 and 2003 made recommendations as to which agents should be evaluated in the
27 *Monographs* series. Recent recommendations are available on the *Monographs* programme
28 website (<http://monographs.iarc.fr>). IARC may schedule other agents for review as it
29 becomes aware of new scientific information or as national health agencies identify an urgent
30 public health need related to cancer.

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

39 4. Data for the *Monographs*

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

43 Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily
44 cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section

1 4). Only those data considered by the Working Group to be relevant to making the evaluation
2 are included.

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

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

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

17 5. Meeting participants

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

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

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

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

44 (d) Observers with relevant scientific credentials may be admitted to a meeting by IARC
45 in limited numbers. Attention will be given to achieving a balance of Observers from
46 constituencies with differing perspectives. They are invited to observe the meeting and

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1 should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair,
2 draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting
3 chair and subgroup chairs may grant Observers an opportunity to speak, generally after they
4 have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC*
5 *Monographs* meetings (available at <http://monographs.iarc.fr>).

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

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

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

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

25 6. Working procedures

26 A separate Working Group is responsible for developing each volume of *Monographs*. A
27 volume contains one or more *Monographs*, which can cover either a single agent or several
28 related agents. Approximately one year in advance of the meeting of a Working Group, the
29 agents to be reviewed are announced on the *Monographs* programme website
30 (<http://monographs.iarc.fr>) and participants are selected by IARC staff in consultation with
31 other experts. Subsequently, relevant biological and epidemiological data are collected by
32 IARC from recognized sources of information on carcinogenesis, including data storage and
33 retrieval systems such as PubMed. Meeting participants who are asked to prepare preliminary
34 working papers for specific sections are expected to supplement the IARC literature searches
35 with their own searches.

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

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

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

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

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

21 B. SCIENTIFIC REVIEW AND EVALUATION

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

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

- 34 1. Exposure data
- 35 2. Studies of cancer in humans
- 36 3. Studies of cancer in experimental animals
- 37 4. Mechanistic and other relevant data
- 38 5. Summary
- 39 6. Evaluation and rationale

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

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

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1 **1. Exposure data**

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

7 **(a) General information on the agent**

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

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

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

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

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

26 **(b) Analysis and detection**

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

31 **(c) Production and use**

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

39 The countries where companies report production of the agent, and the number of
40 companies in each country, are identified. Available data on production, international trade
41 and uses are obtained for representative regions. It should not, however, be inferred that those
42 areas or nations are necessarily the sole or major sources or users of the agent. Some
43 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

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1 to confounding. In some circumstances, however, correlation studies may be more
2 informative than analytical study designs (see, for example, the *Monograph* on arsenic in
3 drinking-water; IARC, 2004).

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

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

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

19 **(b) Quality of studies considered**

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

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

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

40 Secondly, the authors should have taken into account — in the study design and analysis
41 — other variables that can influence the risk of disease and may have been related to the
42 exposure of interest. Potential confounding by such variables should have been dealt with
43 either in the design of the study, such as by matching, or in the analysis, by statistical
44 adjustment. In cohort studies, comparisons with local rates of disease may or may not be
45 more appropriate than those with national rates. Internal comparisons of frequency of disease
46 among individuals at different levels of exposure are also desirable in cohort studies, since

1 they minimize the potential for confounding related to the difference in risk factors between
2 an external reference group and the study population.

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

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

15 (c) Meta-analyses and pooled analyses

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

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

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

40 (d) Temporal effects

41 Detailed analyses of both relative and absolute risks in relation to temporal variables,
42 such as age at first exposure, time since first exposure, duration of exposure, cumulative
43 exposure, peak exposure (when appropriate) and time since cessation of exposure, are
44 reviewed and summarized when available. Analyses of temporal relationships may be useful
45 in making causal inferences. In addition, such analyses may suggest whether a carcinogen

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1 acts early or late in the process of carcinogenesis, although, at best, they allow only indirect
2 inferences about mechanisms of carcinogenesis.

3 **(e) Use of biomarkers in epidemiological studies**

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

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

24 **(f) Criteria for causality**

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

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

43 A number of scenarios may increase confidence in a causal relationship. On the one hand,
44 an agent may be specific in causing tumours at one site or of one morphological type. On the
45 other, carcinogenicity may be evident through the causation of multiple tumour types.
46 Temporality, precision of estimates of effect, biological plausibility and coherence of the

1 overall database are considered. Data on biomarkers may be employed in an assessment of
2 the biological plausibility of epidemiological observations.

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

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

24 3. Studies of cancer in experimental animals

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

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

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

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

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

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

22 **(a) Qualitative aspects**

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

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

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

1 **(b) Quantitative aspects**

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

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

18 **(c) Statistical analyses**

19 Factors considered include the adequacy of the information given for each treatment
20 group: (i) number of animals studied and number examined histologically, (ii) number of
21 animals with a given tumour type and (iii) length of survival. The statistical methods used
22 should be clearly stated and should be the generally accepted techniques refined for this
23 purpose (Peto *et al.*, 1980; Gart *et al.*, 1986; Portier & Bailer, 1989; Bieler & Williams,
24 1993). The choice of the most appropriate statistical method requires consideration of
25 whether or not there are differences in survival among the treatment groups; for example,
26 reduced survival because of non-tumour-related mortality can preclude the occurrence of
27 tumours later in life. When detailed information on survival is not available, comparisons of
28 the proportions of tumour-bearing animals among the effective number of animals (alive at
29 the time the first tumour was discovered) can be useful when significant differences in
30 survival occur before tumours appear. The lethality of the tumour also requires consideration:
31 for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset
32 and can be assessed using life-table methods; non-fatal or incidental tumours that do not
33 affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in
34 tumour prevalence. Because tumour lethality is often difficult to determine, methods such as
35 the Poly-K test that do not require such information can also be used. When results are
36 available on the number and size of tumours seen in experimental animals (e.g. papillomas on
37 mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other
38 more complicated statistical procedures may be needed (Sherman *et al.*, 1994; Dunson *et al.*,
39 2003).

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

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

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

11 **4. Mechanistic and other relevant data**

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

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

25 **(a) Toxicokinetic data**

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

35 **(b) Data on mechanisms of carcinogenesis**

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

1 (i) Changes in physiology

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

8 (ii) Functional changes at the cellular level

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

18 (iii) Changes at the molecular level

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

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

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

29 Tests for genetic and related effects are described in view of the relevance of gene
30 mutation and chromosomal aberration/aneuploidy to carcinogenesis (Vainio *et al.*,
31 1992; McGregor *et al.*, 1999). The adequacy of the reporting of sample
32 characterization is considered and, when necessary, commented upon; with regard to
33 complex mixtures, such comments are similar to those described for animal
34 carcinogenicity tests. The available data are interpreted critically according to the end-
35 points detected, which may include DNA damage, gene mutation, sister chromatid
36 exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The
37 concentrations employed are given, and mention is made of whether the use of an
38 exogenous metabolic system *in vitro* affected the test result. These data are listed in
39 tabular form by phylogenetic classification.

40 Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and
41 cultured mammalian cells suggest that genetic and related effects could occur in
42 mammals. Results from such tests may also give information on the types of genetic
43 effect produced and on the involvement of metabolic activation. Some end-points
44 described are clearly genetic in nature (e.g. gene mutations), while others are
45 associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for

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1 tumour promotion, cell transformation and gap-junction intercellular communication
2 may be sensitive to changes that are not necessarily the result of genetic alterations
3 but that may have specific relevance to the process of carcinogenesis. Critical
4 appraisals of these tests have been published (Montesano *et al.*, 1986; McGregor *et*
5 *al.*, 1999).

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

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

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

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

37 **(c) Other data relevant to mechanisms**

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

42 High-output data, such as those derived from gene expression microarrays, and high-
43 throughput data, such as those that result from testing hundreds of agents for a single end-
44 point, pose a unique problem for the use of mechanistic data in the evaluation of a
45 carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret
46 changes in individual end-points (e.g. changes in expression in one gene) without considering
47 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 website (<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.

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1 (c) Cancer in experimental animals

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

9 (d) Mechanistic and other relevant data

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

15 6. Evaluation and rationale

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

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

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

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

30 (a) Carcinogenicity in humans

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

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

41 **Limited evidence of carcinogenicity:** A positive association has been observed between
42 exposure to the agent and cancer for which a causal interpretation is considered by the

1 Working Group to be credible, but chance, bias or confounding could not be ruled out
2 with reasonable confidence.

3 ***Inadequate evidence of carcinogenicity:*** The available studies are of insufficient quality,
4 consistency or statistical power to permit a conclusion regarding the presence or absence
5 of a causal association between exposure and cancer, or no data on cancer in humans are
6 available.

7 ***Evidence suggesting lack of carcinogenicity:*** There are several adequate studies covering the
8 full range of levels of exposure that humans are known to encounter, which are mutually
9 consistent in not showing a positive association between exposure to the agent and any
10 studied cancer at any observed level of exposure. The results from these studies alone or
11 combined should have narrow confidence intervals with an upper limit close to the null
12 value (e.g. a relative risk of 1.0). Bias and confounding should be ruled out with
13 reasonable confidence, and the studies should have an adequate length of follow-up. A
14 conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the
15 cancer sites, conditions and levels of exposure, and length of observation covered by the
16 available studies. In addition, the possibility of a very small risk at the levels of exposure
17 studied can never be excluded.

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

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

24 (b) Carcinogenicity in experimental animals

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

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

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

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

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

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

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

16 **(c) Mechanistic and other relevant data**

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

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

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

45 For complex exposures, including occupational and industrial exposures, the chemical
46 composition and the potential contribution of carcinogens known to be present are considered
47 by the Working Group in its overall evaluation of human carcinogenicity. The Working

1 Group also determines the extent to which the materials tested in experimental systems are
2 related to those to which humans are exposed.

3 **(d) Overall evaluation**

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

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

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

16 **Group 1: The agent is *carcinogenic to humans*.**

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

22 **Group 2.**

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

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

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

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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.

References

- Bieler, G.S. & Williams, R.L. (1993) Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics*, 49(3), 793-801
- Breslow, N.E. & Day, N.E. (1980) *Statistical Methods in Cancer Research, Vol. 1, The Analysis of Case-Control Studies* (IARC Scientific Publications No. 32), Lyon, IARC

- 1 Breslow, N.E. & Day, N.E. (1987) *Statistical Methods in Cancer Research, Vol. 2, The Design and Analysis of*
2 *Cohort Studies* (IARC Scientific Publications No. 82), Lyon, IARC
- 3 Buffler, P., Rice, J., Baan, R., Bird, M. & Boffetta, P., eds (2004) *Mechanisms of Carcinogenesis* (IARC
4 Scientific Publications No. 157), Lyon, IARC
- 5 Capen, C.C., Dybing, E., Rice, J.M. & Wilbourn, J.D. (1999) *Species Differences in Thyroid, Kidney and*
6 *Urinary Bladder Carcinogenesis* (IARC Scientific Publications No. 147), Lyon, IARC
- 7 Coglianò, V.J., Baan, R.A., Straif, K., Grosse, Y., Secretan, M.B., El Ghissassi, F. & Kleihues, P. (2004) The
8 science and practice of carcinogen identification and evaluation. *Environmental Health Perspect.*, 112(13),
9 1269–1274
- 10 Coglianò, V., Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F. & Boyle, P. (2005) Transparency in
11 IARC Monographs. *Lancet Oncol.*, 6(10), 747
- 12 Dunson, D.B., Chen, Z. & Harry, J. (2003) A Bayesian approach for joint modeling of cluster size and subunit-
13 specific outcomes. *Biometrics*, 59(3), 521–30
- 14 Fung, K.Y., Krewski, D. & Smythe, R.T. (1996) A comparison of tests for trend with historical controls in
15 carcinogen bioassay. *Can. J. Statist.*, 24, 431–454
- 16 Gart, J.J., Krewski, D., Lee, P.N., Tarone, R.E. & Wahrendorf, J. (1986) *Statistical Methods in Cancer*
17 *Research, Vol. 3, The Design and Analysis of Long-term Animal Experiments* (IARC Scientific Publications
18 No. 79), Lyon, IARC
- 19 Greenland, S. (1998) Meta-analysis. In: Rothman, K.J. & Greenland, S., eds, *Modern Epidemiology*,
20 Philadelphia, Lippincott Williams & Wilkins, pp. 643–673
- 21 Greim, H., Gelbke, H.-P., Reuter, U., Thielmann, H.W. & Edler, L. (2003) Evaluation of historical control data
22 in carcinogenicity studies. *Hum. exp. Toxicol.*, 22, 541–549
- 23 Haseman, J.K., Huff, J. & Boorman, G.A. (1984) Use of historical control data in carcinogenicity studies in
24 rodents. *Toxicol. Pathol.*, 12(2), 126–135
- 25 Hill, A.B. (1965) The environment and disease: Association or causation? *Proc. R. Soc. Med.*, 58, 295–300
- 26 Hoel, D.G., Kaplan, N.L. & Anderson, M.W. (1983) Implication of nonlinear kinetics on risk estimation in
27 carcinogenesis. *Science*, 219, 1032–1037
- 28 Huff, J.E., Eustis, S.L. & Haseman, J.K. (1989) Occurrence and relevance of chemically induced benign
29 neoplasms in long-term carcinogenicity studies. *Cancer Metastasis Rev.*, 8, 1–21
- 30 IARC (1977) *IARC Monographs Programme on the Evaluation of the Carcinogenic Risk of Chemicals to*
31 *Humans*. Preamble (IARC intern. tech. Rep. No. 77/002)
- 32 IARC (1978) *Chemicals with Sufficient Evidence of Carcinogenicity in Experimental Animals – IARC*
33 *Monographs Volumes 1-17* (IARC intern. tech. Rep. No. 78/003)
- 34 IARC (1979) *Criteria to Select Chemicals for IARC Monographs* (IARC intern. tech. Rep. No. 79/003)
- 35 IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Suppl. 4,*
36 *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans* (IARC Monographs,
37 Volumes 1 to 29), Lyon, IARC
- 38 IARC (1983) *Approaches to Classifying Chemical Carcinogens According to Mechanism of Action* (IARC
39 intern. tech. Rep. No. 83/001)
- 40 IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7, Overall*
41 *Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*, Lyon, IARC
- 42 IARC (1988) *Report of an IARC Working Group to Review the Approaches and Processes Used to Evaluate the*
43 *Carcinogenicity of Mixtures and Groups of Chemicals* (IARC intern. tech. Rep. No. 88/002)
- 44 IARC (1991) *A Consensus Report of an IARC Monographs Working Group on the Use of Mechanisms of*
45 *Carcinogenesis in Risk Identification* (IARC intern. tech. Rep. No. 91/002)
- 46 IARC (2004) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 84, Some Drinking-*
47 *water Disinfectants and Contaminants, including Arsenic*, Lyon, IARC, pp. 39–267

PREAMBLE

25

- 1 IARC (2005) *Report of the Advisory Group to Recommend Updates to the Preamble to the IARC Monographs*
2 (IARC Int. Rep. No. 05/001)
- 3 IARC (2006) *Report of the Advisory Group to Review the Amended Preamble to the IARC Monographs* (IARC
4 Int. Rep. No. 06/001)
- 5 McGregor, D.B., Rice, J.M. & Venitt, S., eds (1999) *The Use of Short- and Medium-term Tests for Carcinogens*
6 *and Data on Genetic Effects in Carcinogenic Hazard Evaluation* (IARC Scientific Publications No. 146),
7 Lyon, IARC
- 8 Montesano, R., Bartsch, H., Vainio, H., Wilbourn, J. & Yamasaki, H., eds (1986) *Long-term and Short-term*
9 *Assays for Carcinogenesis—A Critical Appraisal* (IARC Scientific Publications No. 83), Lyon, IARC
- 10 OECD (2002) *Guidance Notes for Analysis and Evaluation of Chronic Toxicity and Carcinogenicity Studies*
11 (Series on Testing and Assessment No. 35), Paris, OECD
- 12 Peto, R., Pike, M.C., Day, N.E., Gray, R.G., Lee, P.N., Parish, S., Peto, J., Richards, S. & Wahrendorf, J. (1980)
13 Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments.
14 In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Suppl. 2, *Long-*
15 *term and Short-term Screening Assays for Carcinogens: A Critical Appraisal*, Lyon, IARC, pp. 311–426
- 16 Portier, C.J. & Bailer, A.J. (1989) Testing for increased carcinogenicity using a survival-adjusted quantal
17 response test. *Fundam. appl. Toxicol.*, 12(4), 731–737
- 18 Sherman, C.D., Portier, C.J. & Kopp-Schneider, A. (1994) Multistage models of carcinogenesis: an
19 approximation for the size and number distribution of late-stage clones. *Risk Anal.*, 14(6), 1039–1048
- 20 Stewart, B.W. & Kleihues, P., eds (2003) *World Cancer Report*, Lyon, IARC
- 21 Tomatis, L., Aitio, A., Wilbourn, J. & Shuker, L. (1989) Human carcinogens so far identified. *Jpn. J. Cancer*
22 *Res.*, 80, 795–807
- 23 Toniolo, P., Boffetta, P., Shuker, D.E.G., Rothman, N., Hulka, B. & Pearce, N., eds (1997) *Application of*
24 *Biomarkers in Cancer Epidemiology* (IARC Scientific Publications No. 142), Lyon, IARC
- 25 Vainio, H., Magee, P., McGregor, D. & McMichael, A., eds (1992) *Mechanisms of Carcinogenesis in Risk*
26 *Identification* (IARC Scientific Publications No. 116), Lyon, IARC
- 27 Vainio, H., Wilbourn, J.D., Sasco, A.J., Partensky, C., Gaudin, N., Heseltine, E. & Eragne, I. (1995)
28 [Identification of human carcinogenic risk in IARC Monographs.] *Bull. Cancer*, 82, 339–348 (in French)
- 29 Vineis, P., Malats, N., Lang, M., d'Errico, A., Caporaso, N., Cuzick, J. & Boffetta, P., eds (1999) *Metabolic*
30 *Polymorphisms and Susceptibility to Cancer* (IARC Scientific Publications No. 148), Lyon, IARC
- 31 Wilbourn, J., Haroun, L., Heseltine, E., Kaldor, J., Partensky, C. & Vainio, H. (1986) Response of experimental
32 animals to human carcinogens: an analysis based upon the IARC Monographs Programme. *Carcinogenesis*,
33 7, 1853–1863

34

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VOLUME 112: SOME ORGANOPHOSPHATE INSECTICIDES AND HERBICIDES:
DIAZINON, GLYPHOSATE, MALATHION, PARATHION, AND TETRACHLORVINPHOS
Lyon, France: 3-10 March 2015

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NOTE REGARDING CONFLICTS OF INTERESTS: Each participant submitted WHO's Declaration of Interests, which covers employment and consulting activities, individual and institutional research support, and other financial interests. Participants identified as Invited Specialists did 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. The Declarations were updated and reviewed again at the opening of the meeting.

NOTE REGARDING OBSERVERS: 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.

Posted on 26 January 2015, updated 30 March 2015



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)

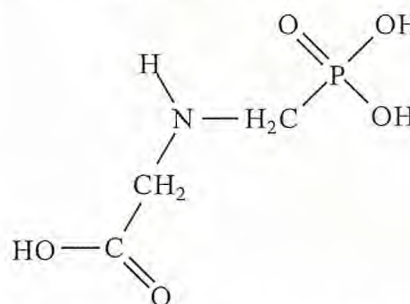
Chem. Abstr. Serv. Name: N-(phosphonomethyl)glycine

Preferred IUPAC Name: N-(phosphonomethyl)glycine

Synonyms: Glyphosate; 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 m³ mol⁻¹ (Tomlin, 2000).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), mg/m³ = 6.92 × 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), diethanolamine, 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 (IPC5, 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,

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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).

Glyphosate

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	Yoshioka et al. (2011)
		0.02 µg/mL	
		(aminomethylphosphonic acid)	
		0.01 µg/mL	
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](#); [Jauhainen 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
<i>Forestry</i>				
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/m ³ Afternoon, 2.25 µg/m ³		
	Operator	Morning, 1.43 µg/m ³ Afternoon, 6.49 µg/m ³		
	Overseer	Morning, 0.84 µg/m ³ Afternoon, 2.41 µg/m ³		
	Mixer	Morning, 5.15 µg/m ³ Afternoon, 5.48 µg/m ³		
Finland, year NR	Workers performing silvicultural clearing (n = 5)	Range of air glyphosate concentrations, < 1.25–15.7 µg/m ³ (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)	Jauhainen <i>et al.</i> (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 <i>et al.</i> (1992)
<i>Weed control</i>				
United Kingdom, year NR	Municipal weed control workers (n = 18)	Median, 16 mg/m ³ in 85% of 21 personal air samples for workers spraying with mechanized all-terrain vehicle Median, 0.12 mg/m ³ 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 <i>et al.</i> (2005)

Table 1.2 (continued)

Industry, country, year	Job/process	Results	Comments/additional data	Reference
<i>Farming</i>				
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)	<i>Curwin et al. (2007)</i>
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	<i>Acquavella et al. (2004)</i>

LOD, limit of detection; ND, not detected; NR, not reported

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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/m³ 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/m² 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 (MLHE, 2013; Varona *et al.*, 2009). Glyphosate concentrations in Colombia were considerably higher than in Europe, with means of 7.6 ng/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 ng/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 Section 2.0 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
<i>Urine</i>				
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)
<i>Serum</i>				
Canada, NR	30 pregnant women and 39 non-pregnant women	ND in serum of pregnant women or cord serum; Arithmetic mean, 73.6 µg/L, (range, ND–93.6 µg/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

2.0 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 2.0 of the *Monograph* on Malathion.

2.1 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 2.0 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 occupational 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 \times days/year); and (c) intensity-weighted cumulative exposure days (years of use \times days/year \times 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
<u>De Roos <i>et al.</i> (2005a)</u> 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	147	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 <i>P</i> value: 0.21				
		Melanoma	Ever use	75	1.6 (0.8–3)	Age only (results in this row only)	
			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 <i>P</i> value: 0.77				
			Multiple myeloma	Ever use	32		
		Ever use		32	2.6 (0.7–9.4)		
		1–20		8	1 (ref.)		
		21–56		5	1.1 (0.4–3.5)		
		Trend-test <i>P</i> value: 0.27					
NHL	Ever use	92		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 <i>P</i> 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
<u>Flower et al. (2004)</u> Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, 1975–1998	21 375; children (aged < 19 years) of licensed pesticide applicators in Iowa (<i>n</i> = 17 357) and North Carolina (<i>n</i> = 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]
<u>Engel et al. (2005)</u> 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]
<u>Lee et al. (2007)</u> 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

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 <i>et al.</i> (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 <i>P</i> 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.]

2.2 Case-control studies on non-Hodgkin lymphoma, multiple myeloma, and leukaemia

2.2.1 Non-Hodgkin lymphoma

See Table 2.2

(a) Case-control studies in the midwest USA

Canlor 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 2.0, 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 2.0, 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 (Canlor et al., 1992) (see the *Monograph* on Malathion, Section 2.0, 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 Wadell 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							
<u>Brown et al. (1990)</u> 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]
<u>Cantor et al. (1992)</u> 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 subsequently pooled in <u>De Roos et al. (2003)</u> ; 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
<u>Brown et al. (1993)</u> 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]
<u>De Roos et al. (2003)</u> 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 <u>Cantor et al. (1992)</u> , <u>Zahm et al. (1990)</u> , <u>Hoar et al. (1986)</u> , and <u>Brown et al. (1990)</u>

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
<u>Lee et al. (2004a)</u> 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
<u>McDuffie et al. (2001)</u> 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
<u>Karunanayake et al. (2012)</u> 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
<u>Kachuri et al. (2013)</u> 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]
<u>Nordström et al. (1998)</u> 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 <u>Hardell et al. (2002)</u> . HCL is a subtype of NHL [Strengths: population-based case-control study. Limitations: Limited power. There was no adjustment for other exposures]

Glyphosate

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
<u>Hardell & Eriksson (1999)</u> 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 <u>Hardell et al. (2002)</u> [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]
<u>Hardell et al. (2002)</u> 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 <u>Nordström et al. (1998)</u> and <u>Hardell & Eriksson (1999)</u> , [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
<u>Eriksson <i>et al.</i> (2008)</u> Sweden. Four health service areas (Lund, Linköping, Örebro and Umeå) 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
<i>Other studies in Europe</i>							
<u>Orsi <i>et al.</i> (2009)</u> 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
<u>Cocco et al. (2013)</u> 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

Glyphosate

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 2.0, 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 2.0, 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.]

2.2.2 Other haematopoietic cancers

Orsi et al. (2009) also reported results for Hodgkin lymphoma (see Section 2.2.1).

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 2.0, 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.]

2.3 Case-control studies on other cancer sites

2.3.1 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.]

2.3.2 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.]

2.3.3 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).

2.3.4 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).

2.3.5 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.]

2.4. 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]	<i>Males</i> Renal tubule adenoma: 0/49, 0/49, 1/50 (2%), 3/50 (6%) <i>Females</i> No data provided on the kidney Report from the PWG of the EPA (1986) : <i>Males</i> 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%)	<i>P</i> for trend = 0.016; see Comments [NS] [<i>P</i> = 0.037; Cochran-Armitage trend test] [<i>P</i> = 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 <i>P</i> = 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 IMPR (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]	<i>Males</i> 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%) <i>Females</i> 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%)	[<i>P</i> < 0.001; Cochran-Armitage trend test] NS NS NS	

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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 & Prith (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), $1.1 \times 10^{-8}\%$ (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, [Chruscicka et al. \(2000\)](#) gave groups of 55 male and 55 female Wistar rats (age, 6–7 weeks) drinking-water containing an ammonium salt

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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), $1.1 \times 10^{-6}\%$ (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)	<i>Males</i> No significant increase in tumour incidence observed in any of the treated groups <i>Females</i> 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	

Glyphosate

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	<p><i>Males</i></p> <p><i>Pancreas (islet cell):</i> 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%)</p> <p><i>Liver:</i> 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%)</p> <p><i>Females</i></p> <p><i>Pancreas (islet cell):</i> 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</p> <p><i>Thyroid:</i> 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</p>	<p>Adenoma, * $P \leq 0.05$ (Fisher exact test with Bonferroni inequality); see comments</p> <p>Adenoma, P for trend = 0.016; see comments</p> <p>NS</p> <p>Adenoma, P for trend = 0.031; see comments</p>	<p>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:</p> <p><i>Males</i> <i>Pancreas (islet cell):</i> 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]</p> <p><i>Liver:</i> 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]</p> <p><i>Females</i> <i>Thyroid:</i> 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]</p>

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]	<p><i>Males</i></p> <p><i>Pancreas (islet cell):</i></p> <p>Adenoma: 0/50 (0%), 5/49* (10%), 2/50 (4%), 2/50 (4%)</p> <p>Carcinoma: 0/50 (0%), 0/49 (0%), 0/50 (0%), 1/50 (2%)</p> <p>Adenoma or carcinoma (combined): 0/50 (0%), 5/49 (10%), 2/50 (4%), 3/50 (6%)</p> <p><i>Females</i></p> <p><i>Pancreas (islet cell):</i></p> <p>Adenoma: 2/50 (4%), 1/50 (2%), 1/50 (2%), 0/50 (0%)</p> <p>Carcinoma: 0/50 (0%), 1/50 (2%), 1/50 (2%), 1/50 (2%)</p> <p>Adenoma or carcinoma (combined): 2/50 (10%), 2/50 (2%), 2/50 (74%), 1/50 (2%)</p>	<p>Adenoma, *$P < 0.05$; Fisher exact test]</p> <p>NS</p>	[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 (Motoyuku *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

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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 $\mu\text{g}/\text{cm}^2$ and a topical volume range of 0.014–0.14 mL/cm^2 . In addition, very little glyphosate ($\leq 0.05\%$ 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 ($> 10 \text{ mg}/\text{mL}$) 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 (IMPR, 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] (IMPR, 2006).

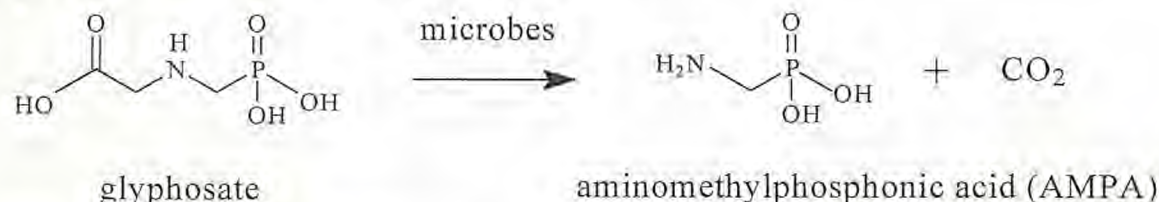
In monkeys given glyphosate by dermal application, percutaneous absorption was estimated to be between 1% and 2% of the administered dose (Wester et al., 1991). Most of the administered dose was removed by surface washes of the exposed skin.

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 $k_{el} = 0.021 \text{ hour}^{-1}$. [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 (IMPR, 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 (IMPR, 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 $\mu\text{g/mL}$) were detectable in the blood (Motoyuku *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 [¹⁴C]-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 [¹⁴C]-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 cm²), 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 (*Monrov 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 ³²P-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/ 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	+ $P < 0.001$		<i>Paz-y-Miño et al. (2007)</i>
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]	<i>Paz-y-Miño et al. (2011)</i>
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	<i>Bolognesi et al. (2009)</i>
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	<i>Bolognesi et al. (2009)</i>
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	<i>Bolognesi et al. (2009)</i>

* +, positive; –, negative
NR, not reported; vs, versus

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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 (Cavas & 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; Cavas & 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-Jaya *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 (Connors & 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 ₂ O ₂ (40 or 50 µM) ($P < 0.004$ vs H ₂ O 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)

Glyphosate

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	$P < 0.05$	<u>Bolognesi et al. (1997)</u>
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 ($r \geq 0.90$; $P < 0.05$)	<u>Mañas et al. (2009b)</u>
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	1.8 mM [200 µg/mL]	$P < 0.05$	<u>Mañas et al. (2009b)</u>
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	<u>Gasnier et al. (2009)</u>
Buccal carcinoma TR146	DNA damage	DNA strand breaks, SCGE assay	+	NT	20 µg/mL	Glyphosate acid, 450g/L Dose-dependent increase ($P \leq 0.05$)	<u>Koller et al. (2012)</u>
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	250 µg/mL	$P < 0.001$ No growth at 25 mg/ mL	<u>Vigfusson & Vyse (1980)</u>
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	100 µg/mL	Glyphosate, 30.4% $P < 0.05$	<u>Bolognesi et al. (1997)</u>

^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 subtilis*, *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 $1 \mu\text{M}$) (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 (Thongprakaitsang 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 ER α and ER β in a dose-dependent manner in T47D cells; after 24 hours, only ER β 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
<i>Glyphosate</i>								
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	<i>Bolognesi et al. (1997)</i>
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	<i>Bolognesi et al. (1997)</i>
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	<i>Peluso et al. (1998)</i>
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	<i>Peluso et al. (1998)</i>
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	<i>Bolognesi et al. (1997)</i>
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	<i>Bolognesi et al. (1997)</i>
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	<i>EPA (1980)</i>
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	<i>Li & Long (1988)</i>
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	<i>Rank et al. (1993)</i>
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	<i>Bolognesi et al. (1997)</i>

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)	<i>Mañas et al.</i> (2009a)
<i>AMPA</i>								
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)	<i>Mañas et al.</i> (2009b)
<i>Glyphosate-based formulations</i>								
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	<i>Bolognesi et al.</i> (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 $P < 0.05$	<i>Bolognesi et al.</i> (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%	<i>Peluso et al.</i> (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%	<i>Peluso et al.</i> (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	<i>Bolognesi et al.</i> (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	<i>Bolognesi et al.</i> (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	<i>Dimitrov et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (2006)

* +, 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 ₁ BH 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 ≤ 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 ≤ 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 positiveAMPA, 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
<i>Glyphosate</i>							
Fish	<i>Prochilodus lineatus</i> (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	<i>Anguilla anguilla</i> 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	<i>Danio rerio</i> (zebrafish), sperm	DNA damage	DNA strand breaks, acridine orange method	+	10 mg/L	After 96 h, DNA integrity was $78.3 \pm 3.5\%$, significantly reduced from control ($94.7 \pm 0.9\%$) and 5 mg/L ($92.6 \pm 1.9\%$), ($P < 0.05$)	Lopes et al. (2014)
Fish	<i>Oreochromis niloticus</i> (Nile tilapia) branchial erythrocytes	DNA damage	DNA strand breaks, comet assay	+	7 μ M [1.2 mg/L]	Time of exposure, 10 days $P < 0.001$ with concentrations $\geq 7 \mu$ M	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	<i>Drosophila</i> 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	<i>Drosophila melanogaster</i> , 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	<i>Tradescantia</i> 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	<i>Allium cepa</i> (onion)	Chromosomal damage	Chromosomal aberrations	+	3%	Single dose tested only Partial but significant reversal with distilled water	Frescura et al. (2013)
Plant systems	<i>Allium cepa</i> (onion)	Chromosomal damage	Chromosomal aberrations	–	2.88 µg/mL	Glyphosate isopropylamine	Rank et al. (1993)
Plant systems	<i>Trigonella foenum-graecum</i> L. (fenugreek)	Chromosomal damage	Chromosomal aberrations	+	0.2%	$P < 0.001$; positive dose-response relationship	Siddiqui et al. (2012)
Plant systems	<i>Vicia faba</i> (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	<i>Anguilla anguilla</i> 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	<i>Anguilla anguilla</i> 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	<i>Anguilla anguilla</i> 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	<i>Anguilla anguilla</i> 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	<i>Anguilla anguilla</i> 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	<i>Anguilla anguilla</i> 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	<i>Prochilodus lineatus</i> (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	<i>Prochilodus lineatus</i> (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	<i>Poecilia reticulata</i> (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	<i>Channa punctatus</i> (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	<i>Corydoras paleatus</i> (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	<i>Cyprinus carpio</i> 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	<i>Carassius auratus</i> (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	<i>Prochilodus lineatus</i> (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	<i>Corydoras paleatus</i> (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	<i>Tilapia rendalli</i> (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	<u>Grisolia (2002)</u>
Fish	<i>Carassius auratus</i> (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 ($P < 0.01$)	<u>Cavas & Könen (2007)</u>
Fish	<i>Poecilia reticulata</i> (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 ($r^2 = 0.99$)	<u>De Souza Filho et al. (2013)</u>
Fish	<i>Cnesterodon decemmaculatus</i> (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	<u>Vera-Candioti et al. (2013)</u>
Fish	<i>Cnesterodon decemmaculatus</i> (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	<u>Vera-Candioti et al. (2013)</u>

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results ^a	Dose (LED or HID)	Comments	Reference
Fish	<i>Prochilodus lineatus</i> (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	<i>Anguilla anguilla</i> 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	<i>Caiman latirostris</i> (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	<i>Caiman latirostris</i> (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	<i>Caiman latirostris</i> (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus formation	+	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)

Glyphosate

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results ^a	Dose (LED or HID)	Comments	Reference
Caiman	<i>Caiman latirostris</i> (broad-snouted caiman), erythrocytes	Chromosomal damage	Micronucleus formation	+	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	<i>Rana catesbeiana</i> (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	<i>Eleutherodactylus johnstonei</i> (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	<i>Euflectis cyanophlyctis</i> (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	<i>Biomphalaria alexandrina</i> , 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	<i>Corbicula fluminea</i> (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	<i>Utterbackia imbecillis</i> (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	Connors & Black (2004)
Worm	Earthworm, <i>Eisenia andrei</i> , 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, <i>Eisenia andrei</i> , 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, <i>Pheretima peguana</i> , 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, <i>Pheretima peguana</i> , 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)

Glyphosate

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results ^a	Dose (LED or HID)	Comments	Reference
Insect	<i>Drosophila melanogaster</i>	Mutation	Sex-linked recessive lethal mutations	+	1 ppm	Single dose tested only $P < 0.001$	<u>Kale et al. (1995)</u>
Plant systems	<i>Allium cepa</i> (onion)	Chromosomal damage	Chromosomal aberrations	+	1.44 µg/mL	Glyphosate-based formulation, 480 g/L The doses of formulation were calculated as glyphosate isopropylamine $P < 0.005$	<u>Rank et al. (1993)</u>
Plant systems	<i>Crepis capillaris</i> (hawksbeard)	Chromosomal damage	Chromosomal aberrations	-	0.5%	The highest dose tested (1%) was toxic	<u>Dimitrov et al. (2006)</u>
Plant systems	<i>Hordeum vulgare</i> L. cv. Madalin (barley roots)	Chromosomal damage	Chromosomal aberrations	(+)	360 µg/mL (0.1%)	Reported as "significant"	<u>Truta et al. (2011)</u>
Plant systems	<i>Crepis capillaris</i> (hawksbeard)	Chromosomal damage	Micronucleus formation	-	0.5%	The highest dose tested (1%) was toxic	<u>Dimitrov et al. (2006)</u>

^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; LC₅₀, 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	<i>Oreochromis niloticus</i> (Nile tilapia), erythrocytes	DNA damage	DNA strand breaks, comet assay	+	NT	7 μM [1.2 μg/mL]	Glyphosate isopropylamine, 96% <i>P</i> ≤ 0.001; positive dose–response relationship for doses ≥ 7 μM	Alvarez-Moya et al. (2014)
Prokaryote (bacteria)	<i>Scytonema javanicum</i> (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)	<i>Anabaena spherica</i> (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)	<i>Microcystis viridis</i> (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)	<i>Bacillus B. subtilis</i>	Differential toxicity	Rec assay	–	NT	2000 μg/disk		Li & Long (1988)
Prokaryote (bacteria)	<i>Salmonella typhimurium</i> TA1535, TA1537, TA1538, TA98 and TA100	Mutation	Reverse mutation	–	–	5000 μg/plate		Li & Long (1988)
Prokaryote (bacteria)	<i>Escherichia coli</i> WP2	Mutation	Reverse mutation	–	–	5000 μg/plate		Li & Long (1988)

Glyphosate

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 ₂ O ₂ (100 µM)	Glyphosate inhibited H ₂ O ₂ -induced damage of PM2 DNA at concentrations where synergism was observed in cellular DNA damage (data NR)	Lueken et al. (2004)
<i>Glyphosate-based formulations</i>								
Prokaryote (bacteria)	<i>Salmonella typhimurium</i> TA98	Mutation	Reverse mutation	+	-	360 µg/plate	Glyphosate isopropylammonium salt, 480 g/L	Rank et al. (1993)
Prokaryote (bacteria)	<i>Salmonella typhimurium</i> 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 ER α and ER β 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 (IC₅₀ = 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 ER α and ER β 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 μ g/L. Forgacs *et al.* (2012) found that glyphosate (300 μ M) 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, 17 β -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 LC₁₀ (10% lethal concentration) (Omran & Salama, 2013). These effects persisted after a 2-week recovery period, although the impact on 17 β -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 vitellogenin levels in juvenile rainbow trout (Xie *et al.*, 2005).

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(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 PPAR α or PPAR γ 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, IC₅₀) for 18 hours increased production of hydrogen peroxide (H₂O₂) 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 H₂O₂ (100 mM). Pre-treatment of the cells with the antioxidant N-acetylcysteine abrogated generation of oxidants by both the formulation and by H₂O₂. 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 LC₂₀ (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

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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- γ 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 μ g) significantly increased the total cell count, eosinophils, neutrophils, and IgG1 and

IgG2a levels. Airway exposure to glyphosate (100 ng, 1 μ g, or 100 μ g 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 boliti 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 μ M) 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 μ M) 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 & Seralini (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 μ M) induced apoptosis in rat heart H9c2 cells, the effect being enhanced when glyphosate was given in combination with the adjuvant TN-20 (5 μ M), (*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 μ M) 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 (Motoyuku *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. 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.

References

- Abraxis (2005). Glyphosate Plate Kit Part No. 500086. Warminster (PA): Abraxis, LLC. Available from: http://www.abraxiskits.com/uploads/products/docfiles/184_PN500086L/SER.pdf, accessed 28 July 2015.
- Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P *et al.* (2004). Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environ Health Perspect*, 112(3):321–6. doi:10.1289/ehp.6667 PMID:14998747
- Akcha F, Spagnol C, Rouxel J (2012). Genotoxicity of diuron and glyphosate in oyster spermatozoa and embryos. *Aquat Toxicol*, 106–107:104–13. doi:10.1016/j.aquatox.2011.10.018 PMID:22115909
- Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF *et al.* (2003). Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol*, 157(9):800–14. doi:10.1093/aje/kwg040 PMID:12727674
- Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF *et al.* (1996). The Agricultural Health Study. *Environ Health Perspect*, 104(4):362–9. doi:10.1289/ehp.96104362 PMID:8732939
- Alvarez-Moya C, Silva MR, Arámbula AR, Sandoval AI, Vasquez HC, González Montes RM (2011). Evaluation of genetic damage induced by glyphosate isopropylamine salt using *Tradescantia* bioassays. *Genet Mol Biol*, 34(1):127–30. doi:10.1590/S1415-47572010005000108 PMID:21637555
- Alvarez-Moya C, Silva MR, Ramírez CV, Gallardo DG, Sánchez RL, Aguirre AC *et al.* (2014). Comparison of the *in vivo* and *in vitro* genotoxicity of glyphosate isopropylamine salt in three different organisms. *Genet Mol Biol*, 37(1):105–10. doi:10.1590/S1415-47572014000100016 PMID:24688297
- Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA *et al.* (2009). Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. *Int J Cancer*, 124(10):2495–500. doi:10.1002/ijc.24185 PMID:19142867
- Aris A, Leblanc S (2011). Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol*, 31(4):528–33. doi:10.1016/j.reprotox.2011.02.004 PMID:21338670
- Astiz M, de Alaniz MJ, Marra CA (2009a). Effect of pesticides on cell survival in liver and brain rat tissues. *Ecotoxicol Environ Saf*, 72(7):2025–32. doi:10.1016/j.ecoenv.2009.05.001 PMID:19493570
- Astiz M, de Alaniz MJ, Marra CA (2009b). Antioxidant defense system in rats simultaneously intoxicated with agrochemicals. *Environ Toxicol Pharmacol*, 28(3):465–73. doi:10.1016/j.etap.2009.07.009 PMID:21784044
- Astiz M, Hurtado de Catalfo GE, García MN, Galletti SM, Errecalde AL, de Alaniz MJ *et al.* (2013). Pesticide-induced decrease in rat testicular steroidogenesis is differentially prevented by lipoate and tocopherol. *Ecotoxicol Environ Saf*, 91:129–38. doi:10.1016/j.ecoenv.2013.01.022 PMID:23465731
- Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP *et al.* (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. *Prostate*, 71(2):168–83. doi:10.1002/pros.21232 PMID:20799287
- Battaglin WA, Kolpin DW, Scribner EA, Kuivila KM, Sandstrom MW (2005). Glyphosate, other herbicides, and transformation products in midwestern streams, 20021. *J Am Water Resour Assoc*, 41(2):323–32. doi:10.1111/j.1752-1688.2005.tb03738.x
- Benachour N, Séralini GE (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem Res Toxicol*, 22(1):97–105. doi:10.1021/tx800218n PMID:19105591
- Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE (2007). Time- and dose-dependent effects of Roundup on human embryonic and placental cells. *Arch Environ Contam Toxicol*, 53(1):126–33. doi:10.1007/s00244-006-0154-8 PMID:17486286
- Bernal J, Bernal JL, Martin MT, Nozal MJ, Anadón A, Martínez-Larrañaga MR *et al.* (2010). Development and validation of a liquid chromatography-fluorescence-mass spectrometry method to measure glyphosate and aminomethylphosphonic acid in rat plasma. *J Chromatogr B Analyt Technol Biomed Life*

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- Sci*, 878(31):3290–6. doi:10.1016/j.jchromb.2010.10.013 PMID:21106459
- Blair A, Thomas K, Coble J, Sandler DP, Hines CJ, Lynch CF *et al.* (2011). Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. *Occup Environ Med*, 68(7):537–41. doi:10.1136/oem.2010.059469 PMID:21257984
- Blakley BR (1997). Effect of Roundup and Tordon 202C herbicides on antibody production in mice. *Vet Hum Toxicol*, 39(4):204–6. PMID:9251167
- Bolognesi C, Bonatti S, Degan P, Gallerani E, Peluso M, Rabboni R *et al.* (1997). Genotoxic activity of glyphosate and its technical formulation Roundup. *J Agric Food Chem*, 45(5):1957–62. doi:10.1021/jf9606518
- Bolognesi C, Carrasquilla G, Volpi S, Solomon KR, Marshall EJ (2009). Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *J Toxicol Environ Health A*, 72(15–16):986–97. doi:10.1080/15287390902929741 PMID:19672767
- Bonini MG, Rota C, Tomasi A, Mason RP (2006). The oxidation of 2',7'-dichlorofluorescein to reactive oxygen species: a self-fulfilling prophesy? *Free Radic Biol Med*, 40(6):968–75. doi:10.1016/j.freeradbiomed.2005.10.042 PMID:16540392
- Borggaard OK, Gimsing AL (2008). Fate of glyphosate in soil and the possibility of leaching to ground and surface waters: a review. *Pest Manag Sci*, 64(4):441–56. doi:10.1002/ps.1512 PMID:18161065
- Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013). Improvements in the analytical methodology for the residue determination of the herbicide glyphosate in soils by liquid chromatography coupled to mass spectrometry. *J Chromatogr A*, 1292:132–41. doi:10.1016/j.chroma.2012.12.007 PMID:23332301
- Botero-Coy AM, Ibáñez M, Sancho JV, Hernández F (2013b). Direct liquid chromatography-tandem mass spectrometry determination of underivatized glyphosate in rice, maize and soybean. *J Chromatogr A*, 1313:157–65. doi:10.1016/j.chroma.2013.07.037 PMID:23891211
- Brewster DW, Warren J, Hopkins WE 2nd (1991). Metabolism of glyphosate in Sprague-Dawley rats: tissue distribution, identification, and quantitation of glyphosate-derived materials following a single oral dose. *Fundam Appl Toxicol*, 17(1):43–51. doi:10.1016/0272-0590(91)90237-X PMID:1916078
- Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM *et al.* (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res*, 50(20):6585–91. PMID:2208120
- Brown LM, Burmeister LF, Everett GD, Blair A (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control*, 4(2):153–6. doi:10.1007/BF00053156 PMID:8481493
- Brüch W, Rosenborg AE, Jöhler RK, Gudmunsson L, Nielsen CB, Plauborg F *et al.* (2013). Monitoring results 1999–2012. The Danish Pesticide Leaching Assessment Programme. Available from: http://pesticidvarsling.dk/publ_result/index.html, accessed 1 December 2014.
- Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM *et al.* (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res*, 52(9):2447–55. PMID:1568215
- Carreón T, Butler MA, Ruder AM, Waters MA, Davis-King KE, Calvert GM *et al.*; Brain Cancer Collaborative Study Group (2005). Gliomas and farm pesticide exposure in women: the Upper Midwest Health Study. *Environ Health Perspect*, 113(5):546–51. doi:10.1289/ehp.7456 PMID:15866761
- Cattaneo R, Clasen B, Loro VL, de Menezes CC, Pretto A, Baldisserotto B *et al.* (2011). Toxicological responses of *Cyprinus carpio* exposed to a commercial formulation containing glyphosate. *Bull Environ Contam Toxicol*, 87(6):597–602. doi:10.1007/s00128-011-0396-7 PMID:21931962
- Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, Tasca CI *et al.* (2014). Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. *Toxicology*, 320:34–45. doi:10.1016/j.tox.2014.03.001 PMID:24636977
- Cavalcante DG, Martínez CB, Sofia SH (2008). Genotoxic effects of Roundup on the fish *Prochilodus lineatus*. *Mutat Res*, 655(1–2):41–6. doi:10.1016/j.mrgeniox.2008.06.010 PMID:18638566
- Cavaş T, Könen S (2007). Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (*Carassius auratus*) exposed to a glyphosate formulation using the micronucleus test and the comet assay. *Mutagenesis*, 22(4):263–8. doi:10.1093/mutage/gem012 PMID:17426049
- Cavuşoğlu K, Yapar K, Oruç E, Yalçın E (2011). Protective effect of *Ginkgo biloba* L. leaf extract against glyphosate toxicity in Swiss albino mice. *J Med Food*, 14(10):1263–72. doi:10.1089/jmf.2010.0202 PMID:21859351
- CCM International (2011). Outlook for China glyphosate industry 2012–2016. Available from: http://www.researchandmarkets.com/reports/2101356/outlook_for_china_glyphosate_industry_20122016, accessed 28 July 2015.
- Centre de Toxicologie du Québec (1988). Etude de l'exposition professionnelle des travailleurs forestiers exposés au glyphosate. Québec: Le Centre Hospitalier de l'Université Laval. Available from: <http://www.santequebec.ca/Bibliothèquevirtuelle/santequebec/35567000039898.pdf>, accessed 28 July 2015. [French]
- Chan R, Mahler J (1992). NTP technical report on the toxicity studies of glyphosate (CAS No. 1071–83–6)

- administered in dosed feed to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 16:1–58. PMID:[12409170](#)
- Chandra M, Frith CH (1994). Spontaneous renal lesions in CD-1 and B6C3F1 mice. *Exp Toxicol Pathol*, 46(3):189–98. doi:[10.1016/S0940-2993\(11\)80080-1](#) PMID:[8000238](#)
- Chang PC, Simcik MF, Capel PD (2011). Occurrence and fate of the herbicide glyphosate and its degradate aminomethylphosphonic acid in the atmosphere. *Environ Toxicol Chem*, 30(3):548–55. doi:[10.1002/etc.431](#) PMID:[21128261](#)
- Chaufan G, Coalova I, Ríos de Molina MC (2014). Glyphosate commercial formulation causes cytotoxicity, oxidative effects, and apoptosis on human cells: differences with its active ingredient. *Int J Toxicol*, 33(1):29–38. doi:[10.1177/1091581813517906](#) PMID:[24434723](#)
- Chen L, Xie M, Bi Y, Wang G, Deng S, Liu Y (2012). The combined effects of UV-B radiation and herbicides on photosynthesis, antioxidant enzymes and DNA damage in two bloom-forming cyanobacteria. *Ecotoxicol Environ Saf*, 80:224–30. doi:[10.1016/j.ecoenv.2012.03.007](#) PMID:[22464588](#)
- Chen M-X, Cao Z-Y, Jiang Y, Zhu Z-W (2013). Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry. *J Chromatogr A*, 1272:90–9. doi:[10.1016/j.chroma.2012.11.069](#) PMID:[23261284](#)
- Chen YJ, Wu ML, Deng JF, Yang CC (2009). The epidemiology of glyphosate-surfactant herbicide poisoning in Taiwan, 1986–2007: a poison center study. *Clin Toxicol (Phila)*, 47(7):670–7. doi:[10.1080/15563650903140399](#) PMID:[19640238](#)
- Chruscielska K, Brzezinski J, Kita K, Kalhorn D, Kita I, Graffstein B *et al.* (2000). Glyphosate - Evaluation of chronic activity and possible far-reaching effects. Part 1. Studies on chronic toxicity. *Pestycydy (Warsaw)*, 3–4:11–20.
- Clair E, Mesnage R, Travert C, Séralini GÉ (2012). Aglyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. *Toxicol In Vitro*, 26(2):269–79. doi:[10.1016/j.tiv.2011.12.009](#) PMID:[22200534](#)
- Clements C, Ralph S, Petras M (1997). Genotoxicity of select herbicides in *Rana catesbeiana* tadpoles using the alkaline single-cell gel DNA electrophoresis (comet) assay. *Environ Mol Mutagen*, 29(3):277–88. doi:[10.1002/\(SICI\)1098-2280\(199712\)29:3<277::AID-EM8>3.0.CO;2-9](#) PMID:[9142171](#)
- Coalova I, Ríos de Molina MC, Chaufan G (2014). Influence of the spray adjuvant on the toxicity effects of a glyphosate formulation. *Toxicol In Vitro*, 28(7):1306–11. doi:[10.1016/j.tiv.2014.06.014](#) PMID:[24999230](#)
- Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M *et al.* (2013). Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. *Occup Environ Med*, 70(2):91–8. doi:[10.1136/oemed-2012-100845](#) PMID:[23117219](#)
- ColomboPage News Desk (2014). Sri Lanka lifts ban on sale of glyphosate. ColomboPage, Sri Lanka Internet Newspaper [online newspaper]. 13 May, 12:13 am Sri Lanka time. Available from: http://www.colomboPage.com/archive/14A/May13_1399920230CH.php, accessed June 2015.
- Connors DE, Black MC (2004). Evaluation of lethality and genotoxicity in the freshwater mussel *Urtterbackia imbecillis* (Bivalvia: Unionidae) exposed singly and in combination to chemicals used in lawn care. *Arch Environ Contam Toxicol*, 46(3):362–71. doi:[10.1007/s00244-003-3003-z](#) PMID:[15195808](#)
- Costa MJ, Monteiro DA, Oliveira-Neto AL, Rantin FT, Kalinin AL (2008). Oxidative stress biomarkers and heart function in bullfrog tadpoles exposed to Roundup Original. *Ecotoxicology*, 17(3):153–63. doi:[10.1007/s10646-007-0178-5](#) PMID:[17987383](#)
- Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ (2012). Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. *Neurotoxicology*, 33(6):1499–510. doi:[10.1016/j.neuro.2012.05.012](#) PMID:[22634143](#)
- Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Ward EM *et al.* (2005). Pesticide contamination inside farm and nonfarm homes. *J Occup Environ Hyg*, 2(7):357–67. doi:[10.1080/154596205910016006](#) PMID:[16020099](#)
- Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H *et al.* (2007). Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in Iowa. *Ann Occup Hyg*, 51(1):53–65. doi:[10.1093/annhyg/mel062](#) PMID:[16984946](#)
- de Castilhos Ghisi N, Cestari MM (2013). Genotoxic effects of the herbicide Roundup® in the fish *Corydoras paleatus* (Jenyns 1842) after short-term, environmentally low concentration exposure. *Environ Monit Assess*, 185(4):3201–7. doi:[10.1007/s10661-012-2763-z](#) PMID:[22821326](#)
- De Marco A, De Simone C, Raglione M, Testa A, Trinca S (1992). Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia faba* treated with the herbicides atrazine, glyphosate and maleic hydrazide. *Mutat Res*, 279(1):9–13. doi:[10.1016/0165-1218\(92\)90260-7](#) PMID:[1374535](#)
- deMenezes CC, da Fonseca MB, Loro VL, Santi A, Cattaneo R, Clasen B *et al.* (2011). Roundup effects on oxidative stress parameters and recovery pattern of *Rhamdia quelen*. *Arch Environ Contam Toxicol*, 60(4):665–71. doi:[10.1007/s00244-010-9574-6](#) PMID:[20680259](#)

IARC MONOGRAPHS – 112

- De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M *et al.* (2005a). Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 113(1):49–54. doi:10.1289/ehp.7340 PMID:15626647
- De Roos AJ, Svec MA, Blair A, Rusiecki JA, Dosemeci M, Alavanja MC *et al.* (2005b). Glyphosate results revisited: De Roos *et al.* respond. *Environ Health Perspect*, 113(6):A366–7. doi:10.1289/ehp.113-a366
- De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF *et al.* (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med*, 60(9):E11. doi:10.1136/oem.60.9.e11 PMID:12937207
- De Souza Filho J, Sousa CC, Da Silva CC, De Sabóia-Morais SM, Grisolia CK (2013). Mutagenicity and genotoxicity in gill erythrocyte cells of *Poecilia reticulata* exposed to a glyphosate formulation. *Bull Environ Contam Toxicol*, 91(5):583–7. doi:10.1007/s00128-013-1103-7 PMID:24042842
- Dennis LK, Lynch CF, Sandler DP, Alavanja MC (2010). Pesticide use and cutaneous melanoma in pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 118(6):812–7. doi:10.1289/ehp.0901518 PMID:20164001
- Dill GM, Sammons RD, Feng PCC, Kohn F, Kretzmer K, Mehrsheikh A *et al.* (2010). Chapter 1: Glyphosate: discovery, development, applications, and properties. In: Nandula VK editor. *Glyphosate resistance in crops and weeds: history, development, and management*. Hoboken (NJ): Wiley; pp. 1–33.
- Dimitrov BD, Gadeva PG, Benova DK, Bineva MV (2006). Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis*, 21(6):375–82. doi:10.1093/mutage/gei014 PMID:16998229
- dos Santos KC, Martinez CB (2014). Genotoxic and biochemical effects of atrazine and Roundup(*), alone and in combination, on the Asian clam *Corbicula fluminea*. *Ecotoxicol Environ Saf*, 100:7–14. doi:10.1016/j.ecoenv.2013.11.014 PMID:24433785
- Duke SO, Powles SB (2009). Glyphosate-resistant crops and weeds. Now and in the future. *AgBioForum*, 12(3&4):346–57.
- EFSA (2009). 2007 Annual Report on Pesticide Residues according to Article 32 of Regulation (EC) No 396/2005. Parma: European Food Safety Authority. Available from: <http://www.efsa.europa.eu/en/efsajournal/pub/305r.htm>, accessed 1 November 2014.
- el-Gendy KS, Aly NM, el-Sebae AH (1998). Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of boliti fish (*Tilapia nilotica*). *J Environ Sci Health B*, 33(2):135–49. doi:10.1080/03601239809373135 PMID:9536512
- Elie-Caille C, Heu C, Guyon C, Nicod L (2010). Morphological damages of a glyphosate-treated human keratinocyte cell line revealed by a micro- to nanoscale microscopic investigation. *Cell Biol Toxicol*, 26(4):331–9. doi:10.1007/s10565-009-9146-6 PMID:20043237
- Engel LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J *et al.* (2005). Pesticide use and breast cancer risk among farmers' wives in the Agricultural Health Study. *Am J Epidemiol*, 161(2):121–35. doi:10.1093/aje/kwi022 PMID:15632262
- EPA (1980a). Glyphosate; Submission of rat teratology, rabbit teratology, dominant lethal mutagenicity assay in mice. Washington (DC): United States Environmental Protection Agency, Office of Toxic Substances. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf>, accessed 10 March 2015.
- EPA (1980b). Review of Rodwell DE, Tasker EJ, Blair AM, *et al.* (1980). Teratology study in rats: IRDC No. 401–054. MRID 00046362. Washington (DC): United States Environmental Protection Agency. Available from: <http://www.epa.gov/nct/toxrefdb/>, and from <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-090.pdf>, accessed 10 March 2015.
- EPA (1983). Review of Knezevich A, Hogan G (1983). A chronic feeding study of glyphosate (Roundup Technical) in mice: Project No. 77-2061: Bdn-77- 420. Final Report. MRID 00130406. Washington (DC): United States Environmental Protection Agency. Available from: <http://www.epa.gov/nct/toxrefdb/>, accessed 10 March 2015.
- EPA (1985a). Glyphosate; EPA Reg.#: 524–308; Mouse oncogenicity study. Document No. 004370. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-183.pdf>, accessed 10 March 2015.
- EPA (1985b). EPA Reg.#: 524–308; Roundup; glyphosate; pathology report on additional kidney sections. Document No. 004855. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-206.pdf>, accessed 10 March 2015.
- EPA (1986). Glyphosate; EPA Registration No. 524–308; Roundup; additional histopathological evaluations of kidneys in the chronic feeding study of glyphosate in mice. Document No. 005590. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/>

Glyphosate

- [reviews/103601/103601-211.pdf](#), accessed 10 March 2015.
- EPA (1987). Review of Stout L, Johnson C (1987). 90-Day study of glyphosate administered in feed to Sprague-Dawley rats. Proj. ID ML-86-351/EHL 86128. MRID 40559401. Washington (DC): United States Environmental Protection Agency. Available from: <http://www.epa.gov/ncct/toxrefdb/>, accessed 10 March 2015.
- EPA (1990). Review of Stout L, Ruecker F (1990). Chronic study of glyphosate administered in feed to albino rats: Laboratory Project Number: Msl-10495; RD 1014. MRID 41643801. Washington (DC): United States Environmental Protection Agency. Available from: <http://www.epa.gov/ncct/toxrefdb/>, accessed 10 March 2015.
- EPA (1991a). Second peer review of glyphosate. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-265.pdf>, accessed 10 March 2015.
- EPA (1991b). Glyphosate; 2-year combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats - List A pesticide for reregistration. Document No. 008390. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-263.pdf>, accessed June 2015; see also <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf>, accessed June 2015.
- EPA (1991c). Peer review on glyphosate. Document No. 008527. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency.
- EPA (1991d). Glyphosate - EPA registration No. 524-308 - 2-year chronic feeding/oncogenicity study in rats with technical glyphosate. Document No. 008897. Washington (DC): Office of Pesticides and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/pesticides/chemicalsearch/chemical/foia/cleared-reviews/reviews/103601/103601-268.pdf>, accessed 10 March 2015.
- EPA (1992). Determination of glyphosate in drinking water by direct-aqueous-injection HPLC, post column derivatization, and fluorescence detection. In: Methods for the determination of organic compounds in drinking water - Supplement II (EPA/600/R-92-129). Washington (DC): Environmental Monitoring Systems Laboratory, Office of Research and Development, United States Environmental Protection Agency. Available through NTIS (<http://www.ntis.gov>).
- EPA (1993a). Reregistration Eligibility Decision (RED): Glyphosate. EPA 738-R-93-014. Washington (DC): Office of Prevention, Pesticides And Toxic Substances, Office of Pesticide Programs, United States Environmental Protection Agency. Available from: http://www.epa.gov/oppt00001/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep-93.pdf, accessed 10 March 2015.
- EPA (1993b). RED facts: Glyphosate. EPA-738-F-93-011. Washington (DC): Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency. Available from: <http://www.epa.gov/opptrrd1/reregistration/REDs/factsheets/0178fact.pdf>, accessed 4 May 2015.
- EPA (1997). Pesticides industry sales and usage - 1994 and 1995 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/pesticides/pestsales/95pestsales/market_estimates1995.pdf, accessed 10 March 2015.
- EPA (2011). Pesticides industry sales and usage - 2006 and 2007 market estimates. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Prevention, Pesticides And Toxic Substances, United States Environmental Protection Agency. Available from: http://www.epa.gov/oppt00001/pestsales/07pestsales/market_estimates2007.pdf, accessed 10 March 2015.
- EPA (2015). Toxicity Reference Database (ToxRefDB). Computational Toxicology Research Program, United States Environmental Protection Agency. Available from: <http://www.epa.gov/ncct/toxrefdb/>, accessed 10 March 2015.
- Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer*, 123(7):1657-63. doi:10.1002/ijc.23589 PMID:18623080
- European Commission (2002). Review report for the active substance glyphosate (6511/VI/99-final, 21 January 2002). Brussels: Health and Consumer Protection Directorate-General, European Commission. Available from: http://ec.europa.eu/food/plant/protection/evaluation/existactive/lis11_glyphosate_en.pdf, accessed 29 April 2015.
- Bustis SL, Hailey JR, Boorman GA, Haseman JK (1994). The utility of multiple-section sampling in the histopathological evaluation of the kidney for carcinogenicity studies. *Toxicol Pathol*, 22(5):457-72. doi:10.1177/019262339402200501 PMID:7899775
- FAO (2000). Glyphosate, N-(phosphonomethyl)glycine. Specifications and evaluations for plant protection products. Rome: Food and Agriculture Organization of the United Nations. Available from: <http://www.fao.org>.

IARC MONOGRAPHS – 112

- [org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/glyph01.pdf](http://fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/glyph01.pdf), accessed 28 July 2015.
- Farm Chemicals International (2015). Glyphosate. In: Crop Protection Database. Willoughby (OH): Meister Media Worldwide. Available from: <http://www.farmchemicalsinternational.com/crop-protection-database/#/product/detail/203900/>, accessed 2 February 2015.
- Ferreira D, da Motta AC, Kreutz LC, Toni C, Loro VL, Barcellos LJ (2010). Assessment of oxidative stress in *Rhamdia quelen* exposed to agrichemicals. *Chemosphere*, 79(9):914–21. doi:10.1016/j.chemosphere.2010.03.024 PMID:20371099
- Flower KB, Hoppin JA, Lynch CF, Blair A, Knott C, Shore DL *et al.* (2004). Cancer risk and parental pesticide application in children of Agricultural Health Study participants. *Environ Health Perspect*, 112(5):631–5. doi:10.1289/ehp.6586 PMID:15064173
- Forgacs AL, Ding Q, Jaremba RG, Huhtaniemi IT, Rahman NA, Zacharewski TR (2012). BTK1 murine Leydig cells: a novel steroidogenic model for evaluating the effects of reproductive and developmental toxicants. *Toxicol Sci*, 127(2):391–402. doi:10.1093/toxsci/kfs121 PMID:22461451
- Freedonia (2012). World agricultural pesticides: industry study with forecasts for 2016 & 2021. Study #2902, August 2012. Cleveland (OH): The Freedonia Group. Available from: <http://www.freedoniagroup.com/brochure/29xx/2902smw.pdf>, accessed 10 March 2015.
- Frescura VD, Kuhn AW, Laughinghouse HD 4th, Paranhos JT, Tedesco SB (2013). Post-treatment with plant extracts used in Brazilian folk medicine caused a partial reversal of the antiproliferative effect of glyphosate in the *Allium cepa* test. *Biocell*, 37(2):23–8. PMID:24392578
- Gasnier C, Benachour N, Clair E, Travert C, Langlois E, Laurant C *et al.* (2010). Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. *J Occup Med Toxicol*, 5(1):29. doi:10.1186/1745-6673-5-29 PMID:20979644
- Gasnier C, Dumont C, Benachour N, Clair E, Chagnon MC, Séralini GE (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. *Toxicology*, 262(3):184–91. doi:10.1016/j.tox.2009.06.006 PMID:19539684
- Gehin A, Guillaume YC, Millet J, Guyon C, Nicod L (2005). Vitamins C and E reverse effect of herbicide-induced toxicity on human epidermal cells HaCaT: a biochemometric approach. *Int J Pharm*, 288(2):219–26. doi:10.1016/j.ijpharm.2004.09.024 PMID:15620861
- George J, Prasad S, Mahmood Z, Shukla Y (2010). Studies on glyphosate-induced carcinogenicity in mouse skin: a proteomic approach. *J Proteomics*, 73(5):951–64. doi:10.1016/j.jprot.2009.12.008 PMID:20045496
- George J, Shukla Y (2013). Emptying of intracellular calcium pool and oxidative stress imbalance are associated with the glyphosate-induced proliferation in human skin keratinocytes HaCaT cells. *ISRN Dermatol*, 2013:825180 doi:10.1155/2013/825180 PMID:24073338
- Geret F, Burgeot T, Haure J, Gagnaire B, Renault T, Communal PY *et al.* (2013). Effects of low-dose exposure to pesticide mixture on physiological responses of the Pacific oyster, *Crassostrea gigas*. *Environ Toxicol*, 28(12):689–99. doi:10.1002/tox.20764 PMID:23012874
- Gholami-Seyedkolaei SJ, Mirvaghefi A, Farahmand H, Kosari AA, Gholami-Seyedkolaei SJ, Gholami-Seyedkolaei SJ (2013). Optimization of recovery patterns in common carp exposed to Roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. *Ecotoxicol Environ Saf*, 98:152–61. doi:10.1016/j.ecoenv.2013.09.009 PMID:24094415
- Gluszcak L, Loro VL, Pretto A, Moraes BS, Raabe A, Duarte MF *et al.* (2011). Acute exposure to glyphosate herbicide affects oxidative parameters in piava (*Leporinus obtusidens*). *Arch Environ Contam Toxicol*, 61(4):624–30. doi:10.1007/s00244-011-9652-8 PMID:21465245
- Glyphosate Task Force (2014). How is glyphosate used? Glyphosate facts. Updated 10 March 2014. Darmstadt: Industry Task Force on Glyphosate. Available from: <http://www.glyphosate.eu/how-glyphosate-used>, accessed 21 April 2015.
- Granby K, Vahl M (2001). Investigation of the herbicide glyphosate and the plant growth regulators chlormequat and mepiquat in cereals produced in Denmark. *Food Addit Contam*, 18(10):898–905. doi:10.1080/02652030119594 PMID:11569770
- Greim H, Saltmiras D, Mostert V, Strupp C (2015). Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. *Crit Rev Toxicol*, 45(3):185–208. doi:10.3109/10408444.2014.1003423 PMID:25716480
- Grisolia CK (2002). A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat Res*, 518(2):145–50. doi:10.1016/S1383-5718(02)00086-4 PMID:12113765
- Guha N, Ward MH, Gunier R, Colt JS, Lea CS, Buffler PA *et al.* (2013). Characterization of residential pesticide use and chemical formulations through self-report and household inventory: the Northern California Childhood Leukemia study. *Environ Health Perspect*, 121(2):276–82. PMID:23110983
- Gui YX, Fan XN, Wang HM, Wang G, Chen SD (2012). Glyphosate induced cell death through apoptotic and autophagic mechanisms. *Neurotoxicol Teratol*, 34(3):344–9. doi:10.1016/j.ntt.2012.03.005 PMID:22504123
- Guilherme S, Gaivão I, Santos MA, Pacheco M (2010). European eel (*Anguilla anguilla*) genotoxic and

- pro-oxidant responses following short-term exposure to Roundup—a glyphosate-based herbicide. *Mutagenesis*, 25(5):523–30. doi:10.1093/mutage/gc038 PMID:20643706
- Guilherme S, Gaivão I, Santos MA, Pacheco M (2012a). DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – elucidation of organ-specificity and the role of oxidative stress. *Mutat Res*, 743(1–2):1–9. doi:10.1016/j.mrgento.2011.10.017 PMID:22266476
- Guilherme S, Santos MA, Barroso C, Gaivão I, Pacheco M (2012b). Differential genotoxicity of Roundup® formulation and its constituents in blood cells of fish (*Anguilla anguilla*): considerations on chemical interactions and DNA damaging mechanisms. *Ecotoxicology*, 21(5):1381–90. doi:10.1007/s10646-012-0892-5 PMID:22526921
- Guilherme S, Santos MA, Gaivão I, Pacheco M (2014a). Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible upon cessation of exposure? *Aquat Toxicol*, 155:213–21. doi:10.1016/j.aquatox.2014.06.007 PMID:25058560
- Guilherme S, Santos MA, Gaivão I, Pacheco M (2014b). DNA and chromosomal damage induced in fish (*Anguilla anguilla* L.) by aminomethylphosphonic acid (AMPA)—the major environmental breakdown product of glyphosate. *Environ Sci Pollut Res Int*, 21(14):8730–9. doi:10.1007/s11356-014-2803-1 PMID:24696215
- Hardell L, Eriksson M (1999). A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer*, 85(6):1353–60. doi:10.1002/(SICI)1097-0142(19990315)85:6<1353::AID-CNCR19>3.0.CO;2-1 PMID:10189142
- Hardell L, Eriksson M, Nordstrom M (2002). Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. *Leuk Lymphoma*, 43(5):1043–9. PMID:12148884
- Hayes WJ Jr, Laws ER Jr editors. (1991). *Classes of pesticides*. Handbook of Pesticide Toxicology. Volume 3. New York (NY): Academic Press, Inc.; p. 1340.
- Heu C, Elie-Caille C, Mougey V, Launay S, Nicod I (2012). A step further toward glyphosate-induced epidermal cell death: involvement of mitochondrial and oxidative mechanisms. *Environ Toxicol Pharmacol*, 34(2):144–53. doi:10.1016/j.etap.2012.02.010 PMID:22522424
- Hidalgo C, Rios C, Hidalgo M, Salvadó V, Sancho JV, Hernández F (2004). Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters. *J Chromatogr A*, 1035(1):153–7. doi:10.1016/j.chroma.2004.02.044 PMID:15117086
- Hilton CW (2012). Monsanto & the global glyphosate market: case study. The Wiglaf Journal. June 2012. Available from: <http://www.wiglafjournal.com/pricing/2012/06/monsanto-the-global-glyphosate-market-case-study/>, accessed 28 July 2015.
- Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R *et al.* (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9):1141–7. doi:10.1001/jama.1986.03380090081023 PMID:3801091
- Humphries D, Byrtus G, Anderson AM (2005). Glyphosate residues in Alberta's atmospheric deposition, soils and surface waters. Alberta: Water Research Users Group, Alberta Environment. Available from: <http://environment.gov.ab.ca/info/library/6444.pdf>, accessed 13 November 2014.
- IARC (2006). Data for the Monographs. In: Preamble to the IARC Monographs (amended January 2006). Lyon: International Agency for Research on Cancer. Available from: <http://monographs.iarc.fr/ENG/Preamble/index.php>, accessed 28 July 2015.
- IARC (2014). Table 1. Key characteristics of carcinogens. In: Instructions for authors. Lyon: International Agency for Research on Cancer. Available from: [http://monographs.iarc.fr/ENG/Preamble/previous/Instructions to Authors S4.pdf](http://monographs.iarc.fr/ENG/Preamble/previous/Instructions%20to%20Authors%20S4.pdf), accessed 28 July 2015.
- IPCS (1994). Glyphosate. Environmental Health Criteria 159. Geneva: International Programme on Chemical Safety, World Health Organization. Available from: <http://www.inchem.org/documents/ehc/ehc/ehc159.htm>, accessed 28 July 2015.
- IPCS (1996). Glyphosate. WHO/FAO Data Sheets on Pesticides, No. 91 (WHO/PCS/DS/96.91). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: <http://apps.who.int/iris/handle/10665/63290>.
- IPCS (2005). Glyphosate. International Chemical Safety Card (ICSC 0160). Geneva: International Programme on Chemical Safety, World Health Organization. Available from: <http://www.inchem.org/documents/icsc/icsc/icsc0160.htm>, accessed 2 February 2015.
- Jacob GS, Garbow JR, Hallas LE, Kimack NM, Kishore GM, Schaefer J (1988). Metabolism of glyphosate in *Pseudomonas* sp. strain LBr. *Appl Environ Microbiol*, 54(12):2953–8. PMID:3223761
- Jan MR, Shah J, Muhammad M, Ara B (2009). Glyphosate herbicide residue determination in samples of environmental importance using spectrophotometric method. *J Hazard Mater*, 169(1–3):742–5. doi:10.1016/j.jhazmat.2009.04.003 PMID:19411135
- Jasper R, Locatelli GO, Pilati C, Locatelli C (2012). Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. *Interdiscip Toxicol*, 5(3):133–40. doi:10.2478/v10102-012-0022-5 PMID:23554553
- Jauhainen A, Räsänen K, Sarantila R, Nuutinen J, Kangas J (1991). Occupational exposure of forest workers to glyphosate during brush saw spraying work. *Am Ind Hyg*

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- Assoc J*, 52(2):61–4. doi:10.1080/15298669191564334 PMID:2011980
- JMPR (2006). Glyphosate. In: Joint FAO/WHO Meeting on Pesticide Residues. Pesticide residues in food – 2004: toxicological evaluations. Report No. WHO/PCS/06.1. Geneva: World Health Organization; pp. 95–169. Available from: http://whqlibdoc.who.int/publications/2006/9241665203_eng.pdf?ua=1, accessed 6 March 2015.
- Johnson PD, Rimmer DA, Garrod AN, Helps JE, Mawdsley C (2005). Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. *Ann Occup Hyg*, 49(1):25–32. PMID:15596423
- Kachuri L, Demers PA, Blair A, Spinelli JJ, Pahwa M, McLaughlin JR *et al.* (2013). Multiple pesticide exposures and the risk of multiple myeloma in Canadian men. *Int J Cancer*, 133(8):1846–58. doi:10.1002/ijc.28191 PMID:23564249
- Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S *et al.* (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ Mol Mutagen*, 25(2):148–53. doi:10.1002/em.2850250208 PMID:7698107
- Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB *et al.* (2012). Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med*, 52(1):1–6. doi:10.1016/j.freeradbiomed.2011.09.030 PMID:22027063
- Karunanayake CP, Spinelli JJ, McLaughlin JR, Dosman JA, Pahwa P, McDuffie HH (2012). Hodgkin lymphoma and pesticides exposure in men: a Canadian case-control study. *J Agromed*, 17(1):30–9. doi:10.1080/1059924X.2012.632726 PMID:22191501
- Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N *et al.* (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603
- Kaya B, Creus A, Yanikoğlu A, Cabré O, Marcos R (2000). Use of the *Drosophila* wing spot test in the genotoxicity testing of different herbicides. *Environ Mol Mutagen*, 36(1):40–6. doi:10.1002/1098-2280(200003)36:1<40::AID-EM6>3.0.CO;2-K PMID:10918358
- Kier LD, Kirkland DJ (2013). Review of genotoxicity studies of glyphosate and glyphosate-based formulations. *Crit Rev Toxicol*, 43(4):283–315. doi:10.3109/10408444.2013.770820 PMID:23480780
- Kim YH, Hong JR, Gil HW, Song HY, Hong SY (2013). Mixtures of glyphosate and surfactant TN20 accelerate cell death via mitochondrial damage-induced apoptosis and necrosis. *Toxicol In Vitro*, 27(1):191–7. doi:10.1016/j.tiv.2012.09.021 PMID:23099315
- Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004). Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ Health Perspect*, 112(5):524–31. doi:10.1289/ehp.6649 PMID:15064155
- Kojima H, Takeuchi S, Nagai T (2010). Endocrine-disrupting potential of pesticides via nuclear receptors and aryl hydrocarbon receptor *J Health Sci*, 56(4):374–86. doi:10.1248/jhs.56.374
- Koller VJ, Fürhacker M, Nersesyan A, Mišik M, Eisenbauer M, Knasmueller S (2012). Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. *Arch Toxicol*, 86(5):805–13. doi:10.1007/s00204-012-0804-8 PMID:22331240
- Kolpin DW, Thurman EM, Lee EA, Meyer MT, Furlong ET, Glassmeyer ST (2006). Urban contributions of glyphosate and its degradate AMPA to streams in the United States. *Sci Total Environ*, 354(2–3):191–7. doi:10.1016/j.scitotenv.2005.01.028 PMID:16398995
- Kreutz LC, Gil Barcellos LJ, de Faria Valle S, de Oliveira Silva T, Anziliero D, Davi dos Santos E *et al.* (2011). Altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. *Fish Shellfish Immunol*, 30(1):51–7. doi:10.1016/j.fsi.2010.09.012 PMID:20883798
- Kuang H, Wang L, Xu C (2011). Overview of analytical techniques for herbicides in foods. In: Soloneski S, Larramendy ML, editors. *Herbicides, theory and applications*. Available from: <http://www.intechopen.com/books/herbicides-theory-and-applications>, accessed 28 July 2015.
- Kumar S, Khodoun M, Kettleson EM, McKnight C, Reponen T, Grinshpun SA *et al.* (2014). Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. *Toxicology*, 325:42–51. doi:10.1016/j.tox.2014.08.008 PMID:25172163
- Kwiatkowska M, Huras B, Bukowska B (2014). The effect of metabolites and impurities of glyphosate on human erythrocytes (in vitro). *Pestic Biochem Physiol*, 109:34–43. doi:10.1016/j.pestbp.2014.01.003 PMID:24581382
- Landgren O, Kyle RA, Hoppin JA, Beane Freeman LE, Cerhan JR, Katzmann JA *et al.* (2009). Pesticide exposure and risk of monoclonal gammopathy of undetermined significance in the Agricultural Health Study. *Blood*, 113(25):6386–91. doi:10.1182/blood-2009-07-203471 PMID:19387005
- Larsen K, Najle R, Lifschitz A, Maté ML, Lanusse C, Virkel GL (2014). Effects of sublethal exposure to a glyphosate-based herbicide formulation on metabolic activities of different xenobiotic-metabolizing enzymes in rats. *Int J Toxicol*, 33(4):307–18. doi:10.1177/1091581814540481 PMID:24985121
- Lavy TL, Cowell JE, Steinmetz JR, Massey JH (1992). Conifer seedling nursery worker exposure to

- glyphosate. *Arch Environ Contam Toxicol*, 22(1):6–13. doi:10.1007/BF00213295 PMID:1554254
- Lee EA, Strahan AP, Thurman EM (2001). Methods of analysis by the U.S. Geological Survey Organic Geochemistry Research Group — determination of glyphosate, aminomethylphosphonic acid, and glufosinate in water using online solid-phase extraction and high-performance liquid chromatography/mass spectrometry. Open-File Report 01–454. Lawrence (KS): United States Geological Survey. Available from: <http://ks.water.usgs.gov/pubs/reports/ofr.01-454.pdf>, accessed 28 July 2015.
- Lee WJ, Cantor KP, Berzofsky JA, Zahm SH, Blair A (2004a). Non-Hodgkin's lymphoma among asthmatics exposed to pesticides. *Int J Cancer*, 111(2):298–302. doi:10.1002/ijc.20273 PMID:15197786
- Lee WJ, Colt JS, Heineman EF, McComb R, Weisenburger DD, Lijinsky W *et al.* (2005). Agricultural pesticide use and risk of glioma in Nebraska, United States. *Occup Environ Med*, 62(11):786–92. doi:10.1136/oem.2005.020230 PMID:16234405
- Lee WJ, Lijinsky W, Heineman EF, Markin RS, Weisenburger DD, Ward MH (2004b). Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus. *Occup Environ Med*, 61(9):743–9. doi:10.1136/oem.2003.011858 PMID:15317914
- Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC (2007). Pesticide use and colorectal cancer risk in the Agricultural Health Study. *Int J Cancer*, 121(2):339–46. doi:10.1002/ijc.22635 PMID:17390374
- Li AP, Long TJ (1988). An evaluation of the genotoxic potential of glyphosate. *Fundam Appl Toxicol*, 10(3):537–46. doi:10.1016/0272-0590(88)90300-4 PMID:3286348
- Li Q, Lambrechts MJ, Zhang Q, Liu S, Ge D, Yin R *et al.* (2013). Glyphosate and AMPA inhibit cancer cell growth through inhibiting intracellular glycine synthesis. *Drug Des Dev Ther*, 7:635–43. PMID:23983455
- Lioi MB, Scarfi MR, Santoro A, Barbieri R, Zeni O, Di Berardino D *et al.* (1998). Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutat Res*, 403(1–2):13–20. doi:10.1016/S0027-5107(98)00010-4 PMID:9726001
- Lopes FM, Varela Junior AS, Corcini CD, da Silva AC, Guazzelli VG, Tavares G *et al.* (2014). Effect of glyphosate on the sperm quality of zebrafish *Danio rerio*. *Aquat Toxicol*, 155:322–6. doi:10.1016/j.aquatox.2014.07.006 PMID:25089930
- Lubick N (2009). Environmental impact of cocaine strategy assessed [News] *Nature*. Published online 12 November. doi:10.1038/news.2009.10801
- Lueken A, Juhl-Strauss U, Krieger G, Witte I (2004). Synergistic DNA damage by oxidative stress (induced by H₂O₂) and nongenotoxic environmental chemicals in human fibroblasts. *Toxicol Lett*, 147(1):35–43. doi:10.1016/j.toxlet.2003.10.020 PMID:14700526
- Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI (2009). Low toxic herbicide Roundup induces mild oxidative stress in goldfish tissues. *Chemosphere*, 76(7):932–7. doi:10.1016/j.chemosphere.2009.04.045 PMID:19450865
- Mahendrakar K, Venkatesowda PM, Rao SM, Mutkule DP (2014). Glyphosate surfactant herbicide poisoning and management. *Indian J Crit Care Med*, 18(5):328–30. doi:10.4103/0972-5229.132508 PMID:24914365
- Malatesta M, Perdoni F, Santin G, Battistelli S, Muller S, Biggiogera M (2008). Hepatoma tissue culture (HTC) cells as a model for investigating the effects of low concentrations of herbicide on cell structure and function. *Toxicol In Vitro*, 22(8):1853–60. doi:10.1016/j.tiv.2008.09.006 PMID:18835430
- Mañas F, Peralta L, Raviolo J, García Ovando H, Weyers A, Ugnia L *et al.* (2009b). Genotoxicity of AMPA, the environmental metabolite of glyphosate, assessed by the Comet assay and cytogenetic tests. *Ecotoxicol Environ Saf*, 72(3):834–7. doi:10.1016/j.ecoenv.2008.09.019 PMID:19013644
- Mañas F, Peralta L, Raviolo J, Ovando HG, Weyers A, Ugnia L *et al.* (2009a). Genotoxicity of glyphosate assessed by the comet assay and cytogenetic tests. *Environ Toxicol Pharmacol*, 28(1):37–41. doi:10.1016/j.etap.2009.03.001 PMID:21783980
- Mance D 3rd (2012). The great glyphosate debate. *Northern Woodlands* [online magazine]. 8 March. Available from: <http://northernwoodlands.org/articles/article/the-great-glyphosate-debate>, accessed 28 July 2015.
- Mariager TP, Madsen PV, Ebbelhøj NE, Schmidt B, Juhl A (2013). Severe adverse effects related to dermal exposure to a glyphosate-surfactant herbicide. *Clin Toxicol (Phila)*, 51(2):111–3. doi:10.3109/15563650.2013.763951 PMID:23360343
- Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2014). Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods—insights into the mechanisms of genotoxicity and DNA repair. *Comp Biochem Physiol C Toxicol Pharmacol*, 166:126–33. doi:10.1016/j.cbpc.2014.07.009 PMID:25110831
- Marques A, Guilherme S, Gaivão I, Santos MA, Pacheco M (2015). Erratum to: "Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods - Insights into the mechanisms of genotoxicity and DNA repair" [Comp. Biochem. Physiol. C 166 (2014) 126–133]. *Comp Biochem Physiol C Toxicol Pharmacol*, 168C:1. doi:10.1016/j.cbpc.2014.10.008 PMID:25521452
- Martini CN, Gabrielli M, Vila MC (2012). A commercial formulation of glyphosate inhibits proliferation and differentiation to adipocytes and induces apoptosis in 3T3–L1 fibroblasts. *Toxicol In Vitro*, 26(6):1007–13. doi:10.1016/j.tiv.2012.04.017 PMID:22546541

IARC MONOGRAPHS – 112

- McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA *et al.* (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev*, 10(11):1155–63. PMID:11700263
- McQueen H, Callan AC, Hinwood AL (2012). Estimating maternal and prenatal exposure to glyphosate in the community setting. *Int J Hyg Environ Health*, 215(6):570–6. doi:10.1016/j.ijheh.2011.12.002 PMID:22261298
- Mesnage R, Bernay B, Séralini GE (2013). Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. *Toxicology*, 313(2–3):122–8. doi:10.1016/j.tox.2012.09.006 PMID:23000283
- Meza-Joya FL, Ramírez-Pinilla MP, Fuentes-Lorenzo JL (2013). Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup®SL-Cosmoflux®411F) in the direct-developing frog *Eleutherodactylus johnstonei*. *Environ Mol Mutagen*, 54(5):362–73. doi:10.1002/em.21775 PMID:23625742
- Ministry of Chemicals & Fertilizers (2008). Performance of chemical & petrochemical industry at a glance (2001–2007). New Delhi: Monitoring and Evaluation Division, Department of Chemicals and Petrochemicals, Government of India. Available from: <http://chemicals.mca.gov.in/stat0107.pdf>, accessed February 2015.
- Mladinic M, Berend S, Vrdoljak AL, Kopjar N, Radic B, Zeljezic D (2009b). Evaluation of genome damage and its relation to oxidative stress induced by glyphosate in human lymphocytes in vitro. *Environ Mol Mutagen*, 50(9):800–7. doi:10.1002/em.20495 PMID:19402152
- Mladinic M, Perkovic P, Zeljezic D (2009a). Characterization of chromatin instabilities induced by glyphosate, terbuthylazine and carbofuran using cytome FISH assay. *Toxicol Lett*, 189(2):130–7. doi:10.1016/j.toxlet.2009.05.012 PMID:19477449
- MLHB (2013). Determination of glyphosate residues in human urine samples from 18 European countries. Bremen: Medical Laboratory of Bremen. Available from: https://www.toceurope.org/sites/default/files/glyphosate_study_results_june12.pdf, accessed 24 November 2014.
- Modesto KA, Martinez CB (2010a). Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere*, 81(6):781–7. doi:10.1016/j.chemosphere.2010.07.005 PMID:20684975
- Modesto KA, Martinez CB (2010b). Roundup causes oxidative stress in liver and inhibits acetylcholinesterase in muscle and brain of the fish *Prochilodus lineatus*. *Chemosphere*, 78(3):294–9. doi:10.1016/j.chemosphere.2009.10.047 PMID:19910015
- Mohamed AH (2011). Sublethal toxicity of Roundup to immunological and molecular aspects of *Biomphalaria alexandrina* to *Schistosoma mansoni* infection. *Ecotoxicol Environ Saf*, 74(4):754–60. doi:10.1016/j.ecoenv.2010.10.037 PMID:21126764
- Monge P, Wesseling C, Guardado J, Lundberg I, Ahlbom A, Cantor KP *et al.* (2007). Parental occupational exposure to pesticides and the risk of childhood leukemia in Costa Rica. *Scand J Work Environ Health*, 33(4):293–303. doi:10.5271/sjweh.1146 PMID:17717622
- Monroy CM, Cortés AC, Sicard DM, de Restrepo HG (2005). [Cytotoxicity and genotoxicity of human cells exposed in vitro to glyphosate] *Biomedica*, 25(3):335–45. doi:10.7705/biomedica.v25i3.1358 PMID:16276681
- Moreno NC, Sofia SH, Martinez CB (2014). Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environ Toxicol Pharmacol*, 37(1):448–54. doi:10.1016/j.etap.2013.12.012 PMID:24448465
- Mortensen OS, Sørensen FW, Gregersen M, Jensen K (2000). [Poisonings with the herbicides glyphosate and glyphosate-trimesium] [in Danish] *Ugeskr Laeger*, 162(35):4656–9. PMID:10986892
- Motojyuku M, Saito T, Akieda K, Otsuka H, Yamamoto I, Inokuchi S (2008). Determination of glyphosate, glyphosate metabolites, and glufosinate in human serum by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, 875(2):509–14. doi:10.1016/j.jchromb.2008.10.003 PMID:18945648
- Muangphra P, Kwankua W, Gooneratne R (2014). Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. *Environ Toxicol*, 29(6):612–20. doi:10.1002/tox.21787 PMID:22644885
- Nakashima K, Yoshimura T, Mori H, Kawaguchi M, Adachi S, Nakao T *et al.* (2002). [Effects of pesticides on cytokine production by human peripheral blood mononuclear cells—fenitrothion and glyphosate] *Chudoku Kenkyu*, 15(2):159–65. PMID:12108020
- NCBI (2015). Glyphosate. Compound summary for CID 3496. PubChem Open Chemistry Database. Bethesda (MD): National Center for Biotechnology Information, United States National Library of Medicine. Available from: <http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=3496>, accessed 5 March 2015.
- Nedelkoska TV, Low GKC (2004). High-performance liquid chromatographic determination of glyphosate in water and plant material after pre-column derivatization with 9-fluorenylmethyl chloroformate. *Anal Chim Acta*, 511(1):145–53. doi:10.1016/j.aca.2004.01.027
- NIH (2015). Questionnaires and study data. Agricultural Health Study. National Institutes of Health. Available from: <http://aghealth.nih.gov/collaboration/questionnaires.html>, accessed 12 June 2015.
- Nordström M, Hardell L, Magnuson A, Hagberg H, Rask-Andersen A (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br*

- J Cancer*, 77(11):2048–52. doi:10.1038/hjc.1998.341 PMID:9667691
- NPIC (2010). Glyphosate. General fact sheet. Oregon State University: National Pesticide Information Center. Available from: <http://npic.orst.edu/factsheets/glyphogen.pdf>, accessed June 2015.
- Nwani CD, Nagpure NS, Kumar R, Kushwaha B, Lakra WS (2013). DNA damage and oxidative stress modulatory effects of glyphosate-based herbicide in freshwater fish, *Channa punctatus*. *Environ Toxicol Pharmacol*, 36(2):539–47. doi:10.1016/j.etap.2013.06.001 PMID:23816461
- Omran NE, Salama WM (2013). The endocrine disrupter effect of atrazine and glyphosate on *Biomphalaria alexandrina* snails. *Toxicol Ind Health*, doi:10.1177/0748233713506959 PMID:24215068
- Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P *et al.* (2009). Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. *Occup Environ Med*, 66(5):291–8. doi:10.1136/oem.2008.040972 PMID:19017688
- Ortiz-Ordoñez E, Uriá-Galicia E, Ruiz-Picos RA, Duran AG, Trejo YH, Sedeño-Díaz JE *et al.* (2011). Effect of Yerbimat herbicide on lipid peroxidation, catalase activity, and histological damage in gills and liver of the freshwater fish *Goodea atripinnis*. *Arch Environ Contam Toxicol*, 61(3):443–52. doi:10.1007/s00244-011-9648-0 PMID:21305274
- Paganelli A, Gnazzo V, Acosta H, López SL, Carrasco AE (2010). Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signalling. *Chem Res Toxicol*, 23(10):1586–95. doi:10.1021/tx1001749 PMID:20695457
- Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR, Cross-Canada Group (2011). Soft-tissue sarcoma and pesticides exposure in men: results of a Canadian case-control study. *J Occup Environ Med*, 53(11):1279–86. doi:10.1097/JOM.0b013e3182307845 PMID:22068131
- Park JS, Kwak SJ, Gil HW, Kim SY, Hong SY (2013). Glufosinate herbicide intoxication causing unconsciousness, convulsion, and 6th cranial nerve palsy. *J Korean Med Sci*, 28(11):1687–9. doi:10.3346/jkms.2013.28.11.1687 PMID:24265537
- Paz-y-Miño C, Muñoz MJ, Maldonado A, Valladares C, Cumbal N, Herrera C *et al.* (2011). Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border. *Rev Environ Health*, 26(1):45–51. doi:10.1515/revh.2011.007 PMID:21714381
- Paz-y-Miño C, Sánchez ME, Aréval M, Muñoz MJ, Witte T, De-la-Carrera GO *et al.* (2007). Evaluation of DNA damage in an Ecuadorian population exposed to glyphosate. *Genet Mol Biol*, 30(2):456–60. doi:10.1590/S1415-47572007000300026
- Peluso M, Munnia A, Bolognesi C, Parodi S (1998). ³²P-postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ Mol Mutagen*, 31(1):55–9. doi:10.1002/(SICI)1098-2280(1998)31:1<55::AID-EM8>3.0.CO;2-A PMID:9464316
- Perry L, Adams RD, Bennett AR, Lupton DJ, Jackson G, Good AM *et al.* (2014). National toxicovigilance for pesticide exposures resulting in health care contact - An example from the UK's National Poisons Information Service. *Clin Toxicol (Phila)*, 52(5):549–55. doi:10.3109/15563650.2014.908203 PMID:24735003
- Pesticide Residues Committee (2007). Pesticide residues monitoring report. Fourth quarter report 2006. York: Pesticide Residues Committee. Available from: <http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Committee/PRC-Results-and-Reports/PRC-Reports-by-Year/pesticide-residue-committee-prc-2006>, accessed 2 November 2014.
- Pesticide Residues Committee (2008). Pesticide residues monitoring report. Fourth quarter report 2007. York: Pesticide Residues Committee. Available from: <http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Committee/PRC-Results-and-Reports/PRC-Reports-by-Year/pesticides-residues-committee-prc-reports-2007>, accessed 2 November 2014.
- Pesticide Residues Committee (2009). Pesticide residues monitoring report. Fourth quarter report 2008. York: Pesticide Residues Committee. Available from: <http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Committee/PRC-Results-and-Reports/PRC-Reports-by-Year/pesticide-residues-committee-prc-reports-2009.htm?whc=purpose=Ba>, accessed 2 November 2014.
- Pesticide Residues Committee (2010). Pesticide residues monitoring report. Fourth quarter report 2009. York: Pesticide Residues Committee. Available from: <http://www.pesticides.gov.uk/guidance/industries/pesticides/advisory-groups/PRiF/PRC-Pesticides-Residues-Committee/PRC-Results-and-Reports/PRC-Reports-by-Year/pesticide-residues-committee-prc-reports-2010>, accessed 2 November 2014.
- Piola L, Fuchs J, Oneto ML, Basack S, Kesten E, Casabé N (2013). Comparative toxicity of two glyphosate-based formulations to *Eisenia andrei* under laboratory conditions. *Chemosphere*, 91(4):545–51. doi:10.1016/j.chemosphere.2012.12.036 PMID:23332878
- Poletta GL, Kleinsorge E, Paonessa A, Mudry MD, Larriera A, Siroski PA (2011). Genetic, enzymatic

IARC MONOGRAPHS – 112

- and developmental alterations observed in *Caiman latirostris* exposed in ovo to pesticide formulations and mixtures in an experiment simulating environmental exposure. *Ecotoxicol Environ Saf*, 74(4):852–9. doi:10.1016/j.ecoenv.2010.12.005 PMID:21185601
- Poletta GL, Larriera A, Kleinsorge E, Mudry MD (2009). Genotoxicity of the herbicide formulation Roundup (glyphosate) in broad-snouted caiman (*Caiman latirostris*) evidenced by the Comet assay and the Micronucleus test. *Mutat Res*, 672(2):95–102. doi:10.1016/j.mrgentox.2008.10.007 PMID:19022394
- Prasad S, Srivastava S, Singh M, Shukla Y (2009). Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice. *J Toxicol*, 2009:308985 doi:10.1155/2009/308985 PMID:20107585
- Rank J, Jensen AG, Skov B, Pedersen LH, Jensen K (1993). Genotoxicity testing of the herbicide Roundup and its active ingredient glyphosate isopropylamine using the mouse bone marrow micronucleus test, *Salmonella* mutagenicity test, and *Allium* anaphase-telophase test. *Mutat Res*, 300(1):29–36. doi:10.1016/0165-1218(93)90136-2 PMID:7683765
- República de El Salvador (2013). Asamblea Legislativa aprueba reformas que prohíben pesticidas que dañan la salud, 5 September 2013. Available from: <http://www.asamblea.gob.sv/noticias/archivo-de-noticias/asamblea-legislativa-aprueba-reformas-que-prohiben-pesticidas-que-danan-la-salud>, accessed 28 April 2015. [Spanish]
- Richard S, Moslemi S, Sipahutar H, Benachour N, Seralini GE (2005). Differential effects of glyphosate and Roundup on human placental cells and aromatase. *Environ Health Perspect*, 113(6):716–20. doi:10.1289/ehp.7728 PMID:15929894
- Roberts DM, Buckley NA, Mohamed F, Eddleston M, Goldstein DA, Mehrsheikh A *et al.* (2010). A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. *Clin Toxicol (Phila)*, 48(2):129–36. doi:10.3109/15563650903476491 PMID:20136481
- Roustan A, Aye M, De Meo M, Di Giorgio C (2014). Genotoxicity of mixtures of glyphosate and atrazine and their environmental transformation products before and after photoactivation. *Chemosphere*, 108:93–100. doi:10.1016/j.chemosphere.2014.02.079 PMID:24875917
- Ruder AM, Waters MA, Butler MA, Carreón T, Calvert GM, Davis-King KE *et al.*; Brain Cancer Collaborative Study Group (2004). Gliomas and farm pesticide exposure in men: the Upper Midwest Health Study. *Arch Environ Health*, 59(12):650–7. doi:10.1080/00039890409602949 PMID:16789473
- Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977). Metabolism and degradation of glyphosphate in soil and water. *J Agric Food Chem*, 25(3):517–28. doi:10.1021/jf60211a016 PMID:858844
- Rumack BH (2015). Emergency medical treatment. Glyphosate isopropylamine salt. POISINDEX(R) Information System. CCIS Volume 164, edition expires May, 2015. Available from: <http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?/temp/-M2Dk5e2>.
- Sanchis J, Kantiani L, Llorca M, Rubio F, Ginebreda A, Fraile J *et al.* (2012). Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. *Anal Bioanal Chem*, 402(7):2335–45. doi:10.1007/s00216-011-5541-y PMID:22101424
- Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health*, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670
- Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Manuela Malatesta M *et al.* (2014). Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. *Environmental Sciences Europe*, 26(1):1–14. doi:10.1186/s12302-014-0014-5
- Siddiqui S, Meghvansi MK, Khan SS (2012). Glyphosate, alachor and maleic hydrazide have genotoxic effect on *Trigonella foenum-graecum* L. *Bull Environ Contam Toxicol*, 88(5):659–65. doi:10.1007/s00128-012-0570-6 PMID:22392005
- Simonsen L, Fomsgaard IS, Svensmark B, Spliid NH (2008). Fate and availability of glyphosate and AMPA in agricultural soil. *J Environ Sci Health B*, 43(5):365–75. doi:10.1080/03601230802062000 PMID:18576216
- Sinhorin VD, Sinhorin AP, Teixeira JM, Miléski KM, Hansen PC, Moreira PS *et al.* (2014). Effects of the acute exposition to glyphosate-based herbicide on oxidative stress parameters and antioxidant responses in a hybrid Amazon fish surubim (*Pseudoplatystoma* sp). *Ecotoxicol Environ Saf*, 106:181–7. doi:10.1016/j.ecoenv.2014.04.040 PMID:24840881
- Siviková K, Dianovský J (2006). Cytogenetic effect of technical glyphosate on cultivated bovine peripheral lymphocytes *Int J Hyg Environ Health*, 209(1):15–20. doi:10.1016/j.ijheh.2005.07.005 PMID:16373198
- Slaninova A, Smutna M, Modra H, Svobodova Z (2009). A review: oxidative stress in fish induced by pesticides. *Neuro Endocrinol Lett*, 30(Suppl 1): 2–12. PMID:20027135
- Solomon KR, Anadón A, Carrasquilla G, Cerdeira AL, Marshall J, Sanin LH (2007). Coca and poppy eradication in Colombia: environmental and human health assessment of aerially applied glyphosate. *Rev Environ Contam Toxicol*, 190:43–125. doi:10.1007/978-0-387-36903-7_2 PMID:17432331

- Sorahan T (2015). Multiple myeloma and glyphosate use: a re-analysis of US Agricultural Health Study (AHS) data. *Int J Environ Res Public Health*, 12(2):1548–59. doi:10.3390/ijerph120201548 PMID:25635915
- Sørensen FW, Gregersen M (1999). Rapid lethal intoxication caused by the herbicide glyphosate-trimesium (Touchdown). *Hum Exp Toxicol*, 18(12):735–7. doi:10.1191/096032799678839590 PMID:10627661
- Sribanditmongkol P, Jutavijittum P, Pongraveevongsa P, Wunnapuk K, Durongkadech P (2012). Pathological and toxicological findings in glyphosate-surfactant herbicide fatality: a case report. *Am J Forensic Med Pathol*, 33(3):234–7. doi:10.1097/PAF.0b013e31824b936e PMID:22835958
- Stella J, Ryan M (2004). Glyphosate herbicide formulation: a potentially lethal ingestion. *Emerg Med Australas*, 16(3):235–9. doi:10.1111/j.1742-6723.2004.00593.x PMID:15228468
- Székács A, Darvas B (2012). Forty years with glyphosate. In: Hasaneen MNAE-G, editor. *Herbicides – properties, synthesis and control of weeds*. Croatia: InTech, pp. 247–84. Available from: <http://cdn.intechweb.org/pdfs/25624.pdf>, accessed 28 July 2015.
- Takeuchi S, Iida M, Yabushita H, Matsuda T, Kojima H (2008). In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450-1A induction by propanil, diuron and linuron. *Chemosphere*, 74(1):155–65. doi:10.1016/j.chemosphere.2008.08.015 PMID:18835618
- Temple WA, Smith NA (1992). Glyphosate herbicide poisoning experience in New Zealand. *N Z Med J*, 105(933):173–4. PMID:1589162
- Thongprakaisang S, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J (2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem Toxicol*, 59:129–36. doi:10.1016/j.fct.2013.05.057 PMID:23756174
- Tian J, Shi H, Li X, Yin Y, Chen L (2012). Coupling mass balance analysis and multi-criteria ranking to assess the commercial-scale synthetic alternatives: a case study on glyphosate. *Green Chem*, 14:1990–2000.
- Tice RR, Austin CP, Kavlock RJ, Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect*, 121(7):756–65. doi:10.1289/ehp.1205784 PMID:23603828
- Tomlin CDS, editor (2000). *The pesticide manual: a world compendium*. 12th ed. Croydon: British Crop Protection Council. Available from: <http://trove.nla.gov.au/work/6273016>, accessed 28 July 2015.
- Transparency Market Research (2014). Global glyphosate market expected to reach US\$8.79 billion in 2019. New York: Transparency Market Research. Posted on 9 December 2014. Available from: <http://www.transparencymarketresearch.com/pressrelease/glyphosate-market.htm>, accessed 21 April 2015.
- Truta E, Vochita G, Rosu CM, Zamfirache MM, Olteanu Z (2011). Evaluation of Roundup-induced toxicity on genetic material and on length growth of barley seedlings. *Acta Biol Hung*, 62(3):290–301. doi:10.1556/ABiol.62.2011.3.8 PMID:21840831
- Tu M, Hurd C, Randall JM (2001). *Weed control methods handbook: tools & techniques for use in natural areas*. Version April 2001. Arlington (VA): Wildland Invasive Species Team, The Nature Conservancy. Available from: http://www.invasive.org/gist/products/handbook/01_TitleContents.pdf, accessed 28 July 2015.
- Uren Webster TM, Laing LV, Florance H, Santos EM (2014). Effects of glyphosate and its formulation, Roundup, on reproduction in zebrafish (*Danio rerio*). *Environ Sci Technol*, 48(2):1271–9. doi:10.1021/es404258h PMID:24364672
- Vainio H, Linnainmaa K, Kähönen M, Nickels J, Hietanen E, Marniemi J et al. (1983). Hypolipidemia and peroxisome proliferation induced by phenoxyacetic acid herbicides in rats. *Biochem Pharmacol*, 32(18):2775–9. doi:10.1016/0006-2952(83)90091-6 PMID:6626247
- Varona M, Henao GL, Díaz S, Lancheros A, Murcia A, Rodríguez N et al. (2009). Evaluación de los efectos del glifosato y otros plaguicidas en la salud humana en zonas objeto del programa de erradicación de cultivos ilícitos. [Effects of aerial applications of the herbicide glyphosate and insecticides on human health] *Biomedica*, 29(3):456–75. [Spanish]. doi:10.7705/biomedica.v29i3.16 PMID:20436997
- Vasiluk L, Pinto LJ, Moore MM (2005). Oral bioavailability of glyphosate: studies using two intestinal cell lines. *Environ Toxicol Chem*, 24(1):153–60. doi:10.1897/04-088R.1 PMID:15683179
- Vera-Candioti J, Soloneski S, Larramendy ML (2013). Evaluation of the genotoxic and cytotoxic effects of glyphosate-based herbicides in the ten spotted live-bearer fish *Cnesterodon decemmaculatus* (Jenyns, 1842). *Ecotoxicol Environ Saf*, 89:166–73. doi:10.1016/j.ecoenv.2012.11.028 PMID:23273868
- Vigfusson NV, Vyse ER (1980). The effect of the pesticides, Dexon, Captan and Roundup, on sister-chromatid exchanges in human lymphocytes in vitro. *Mutat Res*, 79(1):53–7. doi:10.1016/0165-1218(80)90147-0 PMID:7432366
- Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF et al. (2001). Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Cancer Causes Control*, 12(6):509–17. doi:10.1023/A:1011293208949 PMID:11519759
- Walsh LP, McCormick C, Martin C, Stocco DM (2000). Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression.

IARC MONOGRAPHS – 112

- Environ Health Perspect*, 108(8):769–76. doi:[10.1289/ehp.00108769](https://doi.org/10.1289/ehp.00108769) PMID:[10964798](https://pubmed.ncbi.nlm.nih.gov/10964798/)
- Wang G, Deng S, Li C, Liu Y, Chen L, Hu C (2012). Damage to DNA caused by UV-B radiation in the desert cyanobacterium *Scytonema javanicum* and the effects of exogenous chemicals on the process. *Chemosphere*, 88(4):413–7. doi:[10.1016/j.chemosphere.2012.02.056](https://doi.org/10.1016/j.chemosphere.2012.02.056) PMID:[22436589](https://pubmed.ncbi.nlm.nih.gov/22436589/)
- Wester RC, Melendres J, Sarason R, McMaster J, Maibach HI (1991). Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. *Fundam Appl Toxicol*, 16(4):725–32. doi:[10.1016/0272-0590\(91\)90158-Z](https://doi.org/10.1016/0272-0590(91)90158-Z) PMID:[1884912](https://pubmed.ncbi.nlm.nih.gov/1884912/)
- Xie L, Thrippleton K, Irwin MA, Siemering GS, Mekebre A, Crane D *et al.* (2005). Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci*, 87(2):391–8. doi:[10.1093/toxsci/kfi249](https://doi.org/10.1093/toxsci/kfi249) PMID:[16049272](https://pubmed.ncbi.nlm.nih.gov/16049272/)
- Yadav SS, Giri S, Singha U, Boro F, Giri A (2013). Toxic and genotoxic effects of Roundup on tadpoles of the Indian skittering frog (*Euflyctis cyanophlyctis*) in the presence and absence of predator stress. *Aquat Toxicol*, 132–133:1–8. doi:[10.1016/j.aquatox.2013.01.016](https://doi.org/10.1016/j.aquatox.2013.01.016) PMID:[23454306](https://pubmed.ncbi.nlm.nih.gov/23454306/)
- Yin G (2011). Glyphosate: There is no substitute. Farm Chemicals International. 3 March 2011. Willoughby (OH): Meister Media Worldwide. Available from: <http://www.farmchemicalsinternational.com/crop-inputs/herbicides/glyphosate-there-is-no-substitute/>, accessed June 2015.
- Yoshioka N, Asano M, Kuse A, Mitsunashi T, Nagasaki Y, Ueno Y (2011). Rapid determination of glyphosate, glufosinate, bialaphos, and their major metabolites in serum by liquid chromatography-tandem mass spectrometry using hydrophilic interaction chromatography. *J Chromatogr A*, 1218(23):3675–80. doi:[10.1016/j.chroma.2011.04.021](https://doi.org/10.1016/j.chroma.2011.04.021) PMID:[21530973](https://pubmed.ncbi.nlm.nih.gov/21530973/)
- Yue Y, Zhang Y, Zhou L, Qin J, Chen X (2008). In vitro study on the binding of herbicide glyphosate to human serum albumin by optical spectroscopy and molecular modeling. *J Photochem Photobiol B*, 90(1):26–32. doi:[10.1016/j.jphotobiol.2007.10.003](https://doi.org/10.1016/j.jphotobiol.2007.10.003) PMID:[18035550](https://pubmed.ncbi.nlm.nih.gov/18035550/)
- Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP *et al.* (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiology*, 1(5):349–56. doi:[10.1097/00001648-199009000-00004](https://doi.org/10.1097/00001648-199009000-00004) PMID:[2078610](https://pubmed.ncbi.nlm.nih.gov/2078610/)
- Zhao W, Yu H, Zhang J, Shu L (2013). [Effects of glyphosate on apoptosis and expressions of androgen-binding protein and vimentin mRNA in mouse Sertoli cells] *Nan Fang Yi Ke Da Xue Xue Bao*, 33(11):1709–13. PMID:[24273285](https://pubmed.ncbi.nlm.nih.gov/24273285/)
- Zouaoui K, Dulaurent S, Gaulier JM, Moesch C, Lachâtre G (2013). Determination of glyphosate and AMPA in blood and urine from humans: about 13 cases of acute intoxication. *Forensic Sci Int*, 226(1–3):e20–5. doi:[10.1016/j.forsciint.2012.12.010](https://doi.org/10.1016/j.forsciint.2012.12.010) PMID:[23291146](https://pubmed.ncbi.nlm.nih.gov/23291146/)



Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate



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Upcoming meetings

June 2–9, 2015, Volume 113:
Some organochlorine insecticides and some chlorophenoxy herbicides
Oct 6–13, 2015, Volume 114:
Red meat and processed meat

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M K Ross; I Rusyn; L Zeise (USA)

In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be published as volume 112 of the IARC Monographs.¹

The insecticides tetrachlorvinphos and parathion were classified as "possibly carcinogenic to humans" (Group 2B). The evidence from human studies was scarce and considered inadequate. Tetrachlorvinphos induced hepatocellular tumours (benign or malignant) in mice, renal tubule tumours (benign or malignant) in male mice,² and spleen haemangioma in male rats. Tetrachlorvinphos is a reactive oxon with affinity for esterases. In experimental animals, tetrachlorvinphos is systemically distributed, metabolised, and eliminated in urine. Although bacterial mutagenesis tests were negative, tetrachlorvinphos induced genotoxicity in some assays (chromosomal damage in rats and in vitro) and increased

cell proliferation (hyperplasia in rodents). Tetrachlorvinphos is banned in the European Union. In the USA, it continues to be used on animals, including in pet flea collars.

For parathion, associations with cancers in several tissues were observed in occupational studies, but the evidence in humans remains sparse. In mice, parathion increased bronchioloalveolar adenoma and/or carcinoma in males, and lymphoma in females. In rats, parathion induced adrenal cortical adenoma or carcinoma (combined),³ malignant pancreatic tumours, and thyroid follicular cell adenoma in males, and mammary gland adenocarcinoma (after subcutaneous injection in females).⁴ Parathion is rapidly absorbed and distributed. Parathion metabolism to the bioactive metabolite, paraoxon, is similar across species. Although bacterial mutagenesis tests were negative, parathion induced DNA and chromosomal damage in human cells in vitro. Parathion markedly increased rat mammary gland terminal end bud density.⁴ Parathion use has been severely restricted since the 1980s.

The insecticides malathion and diazinon were classified as "probably carcinogenic to humans" (Group 2A). Malathion is used in agriculture, public health, and residential insect control. It continues to be produced in substantial volumes throughout the world. There is limited evidence in humans for the carcinogenicity of malathion. Case-control analyses of occupational exposures reported positive associations with non-Hodgkin lymphoma in the USA,⁵ Canada,⁶ and Sweden,⁷ although no increased risk of non-Hodgkin lymphoma was observed in the large Agricultural Health Study cohort (AHS). Occupational use was associated with an increased risk of prostate cancer in a Canadian case-control study⁸ and in the AHS, which reported a significant trend for aggressive cancers after adjustment for other pesticides.⁹ In mice, malathion increased hepatocellular adenoma or carcinoma (combined).¹⁰ In rats, it increased thyroid carcinoma in males, hepatocellular adenoma or carcinoma (combined) in females, and mammary gland adenocarcinoma after subcutaneous injection in females.⁴ Malathion is rapidly absorbed and distributed. Metabolism to the bioactive metabolite, malaaxon, is similar across species. Malaaxon strongly inhibits esterases; atropine reduced carcinogenesis-related effects in one study.⁴ Malathion induced DNA and chromosomal damage in humans, corroborated by studies in animals and in vitro. Bacterial mutagenesis tests were negative. Compelling evidence supported disruption of hormone pathways. Hormonal effects probably mediate rodent thyroid and mammary gland proliferation.

Diazinon has been applied in agriculture and for control of home and garden insects. There was limited evidence for diazinon carcinogenicity

	Activity (current status)	Evidence in humans (cancer sites)	Evidence in animals	Mechanistic evidence	Classification*
Tetrachlorvinphos	Insecticide (restricted in the EU and for most uses in the USA)	Inadequate	Sufficient	—	2B
Parathion	Insecticide (restricted in the USA and EU)	Inadequate	Sufficient	—	2B
Malathion	Insecticide (currently used, high production volume chemical)	Limited (non-Hodgkin lymphoma, prostate)	Sufficient	Genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death	2A†
Diazinon	Insecticide (restricted in the USA and EU)	Limited (non-Hodgkin lymphoma, leukaemia, lung)	Limited	Genotoxicity and oxidative stress	2A†
Glyphosate	Herbicide (currently used; highest global production volume herbicide)	Limited (non-Hodgkin lymphoma)	Sufficient	Genotoxicity and oxidative stress	2A†

EU=European Union. *See the International Agency for Research on Cancer (IARC) preamble for explanation of classification system (amended January, 2006). †The 2A classification of diazinon was based on limited evidence of carcinogenicity in humans and experimental animals, and strong mechanistic evidence; for malathion and glyphosate, the mechanistic evidence provided independent support of the 2A classification based on evidence of carcinogenicity in humans and experimental animals.

Table: IARC classification of some organophosphate pesticides

in humans. Positive associations for non-Hodgkin lymphoma, with indications of exposure-response trends, were reported by two large multicentre case-control studies of occupational exposures.^{5,6} The AHS reported positive associations with specific subtypes, which persisted after adjustment for other pesticides, but no overall increased risk of non-Hodgkin lymphoma.¹¹ Support for an increased risk of leukaemia in the AHS was strengthened by a monotonic increase in risk with cumulative diazinon exposure after adjustment for other pesticides. Multiple updates from the AHS consistently showed an increased risk of lung cancer with an exposure-response association that was not explained by confounding by other pesticides, smoking, or other established lung cancer risk factors.¹² Nonetheless, this finding was not replicated in other populations. In rodents, diazinon increased hepatocellular carcinoma in mice and leukaemia or lymphoma (combined) in rats, but only in males receiving the low dose in each study. Diazinon induced DNA or chromosomal damage in rodents and in human and mammalian cells in vitro. Some additional support for human relevance was provided by a positive study of a small number of volunteers exposed to a diazinon formulation.¹³

Glyphosate is a broad-spectrum herbicide, currently with the highest production volumes of all herbicides. It is used in more than 750 different products for agriculture, forestry, urban, and home applications. Its use has increased sharply with the development of genetically modified glyphosate-resistant crop varieties. Glyphosate has been detected in air during spraying, in water, and in food. There was limited evidence in humans for the carcinogenicity of glyphosate. Case-control studies of occupational exposure in the USA,¹⁴ Canada,⁶ and Sweden⁷ reported increased risks for non-Hodgkin lymphoma that persisted after adjustment for other

pesticides. The AHS cohort did not show a significantly increased risk of non-Hodgkin lymphoma. In male CD-1 mice, glyphosate induced a positive trend in the incidence of a rare tumour, renal tubule carcinoma. A second study reported a positive trend for haemangiosarcoma in male mice.¹⁵ Glyphosate increased pancreatic islet-cell adenoma in male rats in two studies. A glyphosate formulation promoted skin tumours in an initiation-promotion study in mice.

Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Soil microbes degrade glyphosate to aminomethylphosphoric acid (AMPA). Blood AMPA detection after poisonings suggests intestinal microbial metabolism in humans. Glyphosate and glyphosate formulations induced DNA and chromosomal damage in mammals, and in human and animal cells in vitro. One study reported increases in blood markers of chromosomal damage (micronuclei) in residents of several communities after spraying of glyphosate formulations.¹⁶ Bacterial mutagenesis tests were negative. Glyphosate, glyphosate formulations, and AMPA induced oxidative stress in rodents and in vitro. The Working Group classified glyphosate as "probably carcinogenic to humans" (Group 2A).

We declare no competing interests.

Kathryn Z Guyton, Dana Loomis, Yann Grosse, Fatiha El Ghissassi, Lamia Benbrahim-Tallaa, Neela Guha, Chiara Scoccianti, Heidi Mattock, Kurt Straif, on behalf of the International Agency for Research on Cancer Monograph Working Group, IARC, Lyon, France

International Agency for Research on Cancer, Lyon, France

- 1 International Agency for Research on Cancer Volume 112: Some organophosphate insecticides and herbicides: tetrachlorvinphos, parathion, malathion, diazinon and glyphosate. IARC Working Group. Lyon, 3–10 March 2015. IARC Monogr Eval Carcinog Risk Chem Hum (in press).

- 2 Parker CM, Van Gelder GA, Chai EY, et al. Oncogenic evaluation of tetrachlorvinphos in the B6C3F1 mouse. *Fundam Appl Toxicol* 1985; 5: 840–54.
- 3 National Toxicology Program. Bioassay of parathion for possible carcinogenicity. *Natl Cancer Inst Carcinog Tech Rep Ser* 1979; 70: 1–123.
- 4 Cabello G, Valenzuela M, Vilaxa A, et al. A rat mammary tumor model induced by the organophosphorous pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. *Environ Health Perspect* 2001; 109: 471–79.
- 5 Waddell BL, Zahm SH, Baris D, et al. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). *Cancer Causes Control* 2001; 12: 509–17.
- 6 McDuffie HH, Pahwa P, McLaughlin JR, et al. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 1155–63.
- 7 Eriksson M, Hardell L, Carlberg M, Akerman M. Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer* 2008; 123: 1657–63.
- 8 Band PR, Abanto Z, Bert J, et al. Prostate cancer risk and exposure to pesticides in British Columbia farmers. *Prostate* 2011; 71: 168–83.
- 9 Koutros S, Beane, Freeman LE, et al. Risk of total and aggressive prostate cancer and pesticide use in the Agricultural Health Study. *Am J Epidemiol* 2013; 177: 59–74.
- 10 US Environmental Protection Agency. Peer review of malathion: 18-month carcinogenicity study in mice. http://www.epa.gov/opp00001/chem_search/cleared_reviews/csr_PC-057701_updated_004.pdf (accessed March 6, 2015).
- 11 Alavanja MC, Hofmann JN, Lynch CF, et al. Non-Hodgkin lymphoma risk and insecticide, fungicide and fumigant use in the agricultural health study. *PLoS ONE* 2014; 9: e109332.
- 12 Jones RR, Barone-Adesi F, Koutros S, et al. Incidence of solid tumors among pesticide applicators exposed to the organophosphate insecticide diazinon in the Agricultural Health Study: an updated analysis. *Occup Environ Med* 2015 (in press).
- 13 Hatjian BA, Mutch E, Williams FM, Blain PG, Edwards JW. Cytogenetic response without changes in peripheral cholinesterase enzymes following exposure to a sheep dip containing diazinon in vivo and in vitro. *Mutat Res* 2000; 472: 85–92.
- 14 De Roos AJ, Zahm SH, Cantor KP, et al. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med* 2003; 60: E11.
- 15 WHO/FAO. Glyphosate. Pesticides residues in food 2004 Joint FAO/WHO Meeting on Pesticides Residues. Part II Toxicological. IPCS/WHO 2004; 95–162. http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/ (accessed March 6, 2015).
- 16 Bolognesi C, Carrasquilla G, Volpi S, Solomon KR, Marshall EJ. Biomonitoring of genotoxic risk in agricultural workers from five Colombian regions: association to occupational exposure to glyphosate. *J Toxicol Environ Health A* 2009; 72: 986–97.

Invited Specialists

C Portier (Switzerland)

Representatives

M E Gouze, for the French Agency for Food, Environment and Occupational Health and Safety (France); J Rowland, for the US Environmental Protection Agency (USA)

Observers

M K Boye Jensen, for Cheminova (Denmark); B Fervers, for the Léon Bérard Centre (France); E Giroux, for University Jean-Moulin Lyon 3 (France); T Sorahan, for Monsanto Company (USA); C Strupp, for the European Crop Protection Association (Belgium); P Sutton, for the University of California, San Francisco (USA)

IARC/WHO Secretariat

L Benbrahim-Tallaa; R Carel; F El Ghissassi; Sonia El-Zaemey; Y Grosse; N Guha; K Z Guyton; C Le Cornet; M Leon; D Loomis; H Mattock; C Scoccianti; A Shapiro; K Straif; J Zavadil

For the Preamble to the IARC Monographs see <http://monographs.iarc.fr/ENG/Preamble/index.php>

For declarations of interests see <http://monographs.iarc.fr/ENG/Meetings/vol112-participants.pdf>

International Agency for Research on Cancer

World Health
Organization

**Originally prepared as a confidential briefing for Governing Council Members
on IARC evaluation of glyphosate and requests for meetings from CropLife**

1. An IARC Working Group (WG) of 17 international experts evaluated glyphosate in March 2015 as Group 2A, "probably carcinogenic to humans"; IARC scientists are not part of the WG¹. Monsanto and the European Crop Protection Association provided scientific Observers to the meeting¹, who had access to the scientific deliberations and all meeting documents.
2. Monsanto rejected and attacked the IARC findings, calling it "junk science"², and immediately requested that WHO retract the IARC evaluation³ and privately lobbied the US EPA to reject IARC's findings⁴. Monsanto convened their own expert panel (including many past consultants to Monsanto) through Intertek Scientific & Regulatory Consultancy to review the IARC Monograph on glyphosate, finding no evidence of carcinogenicity, and published the results in a sponsored issue of a scientific journal⁵.
3. Coordinated criticisms of IARC by the industry and umbrella organizations such as CropLife and the American Chemistry Council have continued in two broad areas: a) scientific credibility of the programme in general and the evaluation of glyphosate in particular and b) on the continued funding to IARC from US Government sources⁶.
4. On the first point, IARC has been the subject of much misleading reporting. Examples include accusations of:
 - a. Cherry-picking data – for transparency, IARC's procedures⁷ call for systematic evaluation of scientific data in the public domain, but not unpublished studies or industry reports. Of interest, the European Medicines Agency has recently implemented such a procedure and European Food Standards Agency has promised to do so in the future.
 - b. Activist scientists with political agenda – false and defamatory statements have been made concerning IARC scientists; for transparency and public scrutiny IARC disclosed conflicting interests of all participants, including the Secretariat, two months before the meeting; an additional and independent assessment of any potential conflicts of interest was conducted for The Lancet Oncology publication¹.
 - c. Creating needless concerns – IARC's hazard identification has been portrayed as insufficient and the methodology as outdated, in many blogs, news media and articles, including from industry organisations and paid consultants of Monsanto⁵. IARC methodology is published⁷ and constantly evolves to include the latest science; hazard identification is a key foundation for national authorities to make risk assessments; the IARC Monographs do not make any direct public health recommendations, such as limit values.
 - d. The data evaluated do not represent "real world" exposures – this ignores the fact that cancer epidemiology, based on real world exposures associated with cancer risks in humans, is one cornerstone of the IARC Monograph evaluations.

5. IARC Working Group (WG) members and their employers⁸ based in the US have been subject to wide-reaching subpoenas by Monsanto lawyers asking for all draft documents, emails⁹, and other communications on IARC and glyphosate and the WG; these requests are made in the context of US court cases on lymphoma and glyphosate use; arguments from IARC on deliberative documents have been misrepresented as lack of transparency.¹⁰
6. Other members of the WG and IARC Secretariat are also now are being subject to intimidating¹¹ letters from Monsanto lawyers³; WHO and IARC considers the underlying principle of confidentiality of deliberative documents to be fundamental to an open and productive process of scientific deliberation, and that these would be protected from disclosure under UN privileges and immunities.
7. On the second point of IARC funding,
 - a. There has been lobbying of US House of Representatives Committees suggesting that the NIH funding for IARC should be stopped⁴. NIH/NCI USA have been called before the Committees to testify.
 - b. IARC anticipated that the next step for the industry would be to work through the governing body of the Agency: The Netherlands, Australia and Canada have already been approached by CropLife International.
8. CropLife have taken a number of specific actions in relation to the glyphosate evaluation:
 - a. lobbied WHO about the Volume 112 and Volume 113 IARC evaluations⁵;
 - b. misrepresented the Agency in letter to US EPA¹² accusing the Agency of only using partial data and falsely accusing one of our scientists of having a biased view;
 - c. lobbied US EPA about the composition of the expert panel that will consider glyphosate carcinogenicity in Dec 2016.¹²

¹ <http://www.thelancet.com/journals/lanonc/article/PIIS1470-2045%2815%2970134-8/fulltext;http://monographs.iarc.fr/ENG/Meetings/vol112-participants.pdf>

² <http://news.monsanto.com/press-release/research-and-development/monsanto-reinforces-decades-dataand-regulatory-review-clear;http://www.wsj.com/articles/monsanto-bites-back-at-glyphosate-findings-1427147273>

³ see <http://governance.iarc.fr/ENG/infocouncils.php>

⁴ Multiple records were released through a FOIA request to the US EPA: <https://foiaonline.regulations.gov/foia/action/public/view/request?objectId=090004d280904eba> (see releasable Monsanto Summary Comments on IARC Lancet Oncology article as one example.)

⁵ <http://www.tandfonline.com/toc/itxc20/46/sup1?nav=tocList>; see also <https://www.ncbi.nlm.nih.gov/pubmed/27780763>, <https://www.ncbi.nlm.nih.gov/pubmed/27552246>, <https://www.ncbi.nlm.nih.gov/pubmed/7552246>; note that interests are not always disclosed (in early critiques of the IARC evaluation Sir Colin Berry was a vocal critic (e.g. via Science Media Centre) but omitted to declare he had worked as a consultant for Monsanto, whereas <https://www.ncbi.nlm.nih.gov/pubmed/27677669> states "Gary Williams, Sir Colin Berry, João Lauro Viana de Camargo, and Helmut Greim have previously served as independent consultants for the Monsanto Company, some on the European Glyphosate Task Force.")

⁶ The US House Committee on Oversight and Government Reform Chairperson Jason Chaffetz in a Sept. 26 letter to NIH director Francis Collins, Oversight Committee Chairman <https://oversight.house.gov/wp-content/uploads/2016/09/26-JEC-to-Collins-NIH-IARC-Funding-due-10-10.pdf> describes IARC as having "a record of controversy, retractions, and inconsistencies" and asks why the NIH, which has a \$33 billion annual budget, continues to fund it; IARC Director wrote to NIH Director correcting some of the

misrepresentations of the Agency in the letter from Congressman Chaffetz (<http://monographs.iarc.fr/ENG/News/LetterFromDrWild-to-DrCollins.pdf>). The American Chemistry Council issued a statement following Chaffetz's letter accusing IARC of "a long history of passing judgment on substances through a fundamentally-flawed process that yields questionable results". The CEO wrote to Mr Chaffetz along the same lines <https://www.americanchemistry.com/ACC-Letter-to-House-Committee-on-IARC-Monographs.pdf>; Robert Aderholt, chairman of the U.S. congressional Appropriations Subcommittee on Agriculture, wrote in June to NIH director Collins questioning funding of IARC (<http://src.bna.com/fLL>)

⁷ <http://monographs.iarc.fr/ENG/Preamble/currenta4data0706.php>

⁸ search "IARC" at <https://www.nih.gov/sites/default/files/institutes/foia/foia-log-sep2016.pdf>;

⁹ some emails released under subpoena to Monsanto are apparently given to journalists (e.g., <http://reut.rs/2eIowTw>; <https://morningconsult.com/wp-content/uploads/2016/10/MattRossEmail.pdf>) and US House of Representatives

(https://science.house.gov/sites/republicans.science.house.gov/files/documents/201610251122a_Redacted%20n%281.5%29.pdf)

¹⁰ http://www.iarc.fr/en/media-centre/iarcnews/pdf/Reuters_Readmore_Oct2016.pdf;

https://www.iarc.fr/en/media-centre/iarcnews/pdf/Reuters_questions_and_answers_Oct2016.pdf

¹¹ <http://thehill.com/blogs/pundits-blog/healthcare/303597-bully-monsanto-attacks-scientists-who-link-glyphosate-and>

¹² see <https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0385-0005>;

<https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0385-0356>;

<http://191hmt1pr08amfq62276etw2.wpengine.netdna-cdn.com/wp-content/uploads/2016/01/CLA-Comments-on-SAP-Disqualification-10-12-16.pdf>



Poster presentation

0409

**THE NORTH AMERICAN POOLED PROJECT (NAPP):
POOLED ANALYSES OF CASE-CONTROL STUDIES OF
PESTICIDES AND AGRICULTURAL EXPOSURES,
LYMPHOHEMATOPOIETIC CANCERS AND
SARCOMA**

¹Marisha Pahlwa, ²Laura Beane Freeman, ^{3,4}John J. Spinelli, ^{5,6}Aaron Blair, ^{5,6}Punam Pahlwa, ⁷James A. Dosman, ^{7,8}John R. McLaughlin, ^{1,9}Paul A. Demers, ²Shelia Hoar Zahm, ¹Kenneth P. Cantor, ¹⁰Dennis D. Weisenburger, ¹Shelley A. Harris. ¹Occupational Cancer Research Centre, Cancer Care Ontario, Toronto, Ontario, Canada; ²Division of Cancer Epidemiology and Genetics, US National Cancer Institute, Bethesda, Maryland, USA; ³BC Cancer Agency Research Centre, Vancouver, British Columbia, Canada; ⁴School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada; ⁵Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; ⁶Community Health and Epidemiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; ⁷Public Health Ontario, Toronto, Ontario, Canada; ⁸Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; ⁹Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada; ¹⁰University of Nebraska Medical Center, University of Nebraska, Omaha, Nebraska, USA

10.1136/oemed-2014-102362.364

Objectives Previous studies have noted associations between specific pesticides and multiple cancer types. However, assessments for many pesticides have been limited by small numbers of exposed cases. To address this, we established the North American Pooled Project (NAPP), a collaborative effort to evaluate the relationship of pesticide and agricultural exposures to risks of lymphohematopoietic cancers and sarcoma.

Method We harmonised previously collected data from three population-based case-control studies conducted in four American states with a similar Canada-wide study conducted in six provinces. Descriptive analyses of pesticide exposures, personal protective equipment (PPE) use, and demographic data were completed. The prevalence of self-reported pesticide use among cases and controls was determined for specific agents and chemical classes.

Results The NAPP includes 5131 controls and 3274 cases (non-Hodgkin lymphoma [NHL] N=1690; Hodgkin lymphoma [HL] N=507; multiple myeloma [MM] N=587; soft tissue sarcoma N=490). Preliminary descriptive analyses indicate that approximately two-thirds of controls and NHL and MM cases ever lived or worked on a farm or ranch. Nearly half of controls and half of NHL, HL, and MM cases reported using any pesticide. Over 120 different insecticides, herbicides, and fungicides were reported. More than 17% of participants reported using the phenoxy herbicide 2,4-D and over 5% reported DDT, malathion, atrazine, or glyphosate. Around 6% of NHL cases and controls reported ever using PPE.

Conclusions The large number of cases and controls and high frequency of pesticide use in the NAPP will allow us to evaluate less commonly used pesticides, cancer sub-types, and smaller relative risks than previously possible.

0414

**CUMULATIVE MERCURY EXPOSURE AND PERIPHERAL
NERVE FUNCTION IN A SAMPLE OF U.S. DENTAL
PROFESSIONALS**

¹Julia Anglen, ²Leslie Stayner, ²Stephen Gruninger. ¹University of Illinois at Chicago, Chicago, IL, USA; ²American Dental Association, Chicago, IL, USA

10.1136/oemed-2014-102362.365

Objectives To calculate individual cumulative mercury exposures from a convenience sample of dental professionals and measure the effect on peripheral nerve function.

Method Participants attended the American Dental Association's (ADA) conventions held from 1997–2006. Individual surveys were completed and measurements were taken of the median and ulnar sensory nerve amplitude and latency in the dominant hand. The ADA has measured the average urinary mercury concentration of participants since 1977, allowing a cumulative mercury exposure to be estimated for each individual dentist based on the number of years they practiced dentistry. Both fixed and mixed effects (accounting for repeated measures) linear regression models were used.

Results 3923 observations from 2649 dentists were used to perform linear regression using multiple models. Models included individuals with or without imputed BMI, along with either repeated measures or initial observations only. Adjusted covariates included hand temperature, gender, age and BMI. Individuals with rheumatoid arthritis, diabetes, carpal tunnel syndrome (for median nerve models only), or hand temperatures interfering with the accuracy of the instrument were excluded. The main effect of cumulative exposure was found to be significant (p-value <0.05) in median nerve latency and amplitudes but insignificant in ulnar nerve measures. All models but ulnar nerve latency showed a highly significant interaction of cumulative exposure and age (p-value <0.01).

Conclusions Using an estimated cumulative mercury exposure as the measure of effect shows a significant positive association with decreased peripheral nerve function. This study is the first of its kind to estimate dentists' cumulative mercury exposure and its effect on peripheral nerve function.

0415

**WHEN STANDARD OCCUPATIONAL EXPOSURE
ASSESSMENTS ARE UNSUITABLE – EXPOSURE
OBSERVATIONS WITH SOUTH AFRICAN HERBICIDE
SPRAYERS**

Hanna-Andrea Rother, Federico Andrade Rivas, Michelle DeSouza. University of Cape Town, Cape Town, South Africa

10.1136/oemed-2014-102362.366

Objectives South African herbicide sprayers removing alien vegetation are exposed to a myriad of herbicides resulting in acute and chronic health risks. Workers often are not willing to participate in standard biological monitoring assessments through the provision of blood and urine in order to assess these potential risks. Furthermore, laboratory capacity to analyse herbicides residues are limited. The study aim was to document workers' exposure risks in order to develop health interventions using an observation exposure assessment method.

Method Researchers observed three teams, each comprised of 10 workers and one contractor, from February to June 2012. An observational guide was developed and findings were recorded in a field journal. Observations were supported with video and photographic materials.

Results The on-site observations revealed workers lack of PPE compliance, behaviours that increased their exposure risks, and non-compliance with work standards. Workers' exposure risks were compounded by harsh working conditions, high turnover rates of workers, worker's low risk perceptions, power struggles, and gendered beliefs of masculinity being threatened by PPE use.

Conclusions In some circumstances researchers are unable to use biological monitoring methods to establish pesticide exposure risks for workers in developing countries. Observation methods are a viable alternative method, particularly for informing worker risk reduction interventions.



**0409 The North American Pooled Project (NAPP):
Pooled analyses of case-control studies of pesticides
and agricultural exposures, lymphohematopoietic
cancers and sarcoma**

Manisha Pahwa, Laura Beane Freeman, John J Spinelli, Aaron Blair, Punam Pahwa,
James A Dosman, John R McLaughlin, Paul A Demers, Shelia Hoar Zahm, Kenneth P
Cantor, Dennis D Weisenburger and Shelley A Harris

Occup Environ Med 2014 71: A116
doi: 10.1136/oemed-2014-102362.364

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Message

From: John Acquavella [REDACTED]
Sent: 6/1/2016 6:09:01 PM
To: HEYDENS, WILLIAM F [AG/1000] [REDACTED]; Thomas Sorahan [REDACTED]
CC: FARMER, DONNA R [AG/1000] [REDACTED]
Subject: Re: IARC - NAPP Epidemiology Study Abstract re: Glyphosate and NHL



Bill:

In light of the new analysis of the case control studies as, perhaps, part of a strategy to override the AHS findings, I think it is imperative also to request the NCI case control studies via FOI. Also, I do not know much about data sharing or FOI in Canada, but, if possible, it would be helpful to get the McDuffie data.

There are just too many decisions made during analysis that can make an exposure look to be related to a cancer. So, the only way to make sure the truth comes out is to get access to the data and to have fair minded epidemiologists analyze it.

Regards,

John

On 6/1/16, 10:55 AM, "HEYDENS, WILLIAM F [AG/1000]" [REDACTED] wrote:

>Thanks John & Tom.
 >
 >I had forgotten that De Roos 2003 was Cantor + 2 other studies - so John your explanation makes sense.
 >
 >Tom, we all know the answer to the question you pose, right?
 >
 >One way or another, I think all this will lead to analysis of the remaining AHS, and it will be imperative that it's a proper analysis and not an inappropriately adjusted one.
 >
 >Thanks again,
 >
 > Bill
 >
 >-----Original Message-----
 >From: Thomas Sorahan [mailto:[REDACTED]]
 >Sent: Wednesday, June 01, 2016 10:28 AM
 >To: John Acquavella; HEYDENS, WILLIAM F [AG/1000]
 >Cc: FARMER, DONNA R [AG/1000]
 >Subject: RE: IARC - NAPP Epidemiology Study Abstract re: Glyphosate and NHL
 >
 >Hi Bill
 >
 >No I didn't know about this paper. The conference is a 50-year celebration of how great IARC has been. As John has noted the AHS is clearly not included. Why they are bothering with these problem datasets when they have so much unanalysed AHS data is beyond me. If you were ever to contemplate an FOI request, then it might be important to have a copy of the raw entire dataset, if that were possible. When we got the AHS data, it was an analysis file for the De Roos 2005 paper, which limited what could be looked at.
 >
 >Tom
 >
 >
 >From: John Acquavella [REDACTED]
 >Sent: Wednesday, June 01, 2016 3:25 PM
 >To: HEYDENS, WILLIAM F [AG/1000]; Thomas Sorahan
 >Cc: FARMER, DONNA R [AG/1000]
 >Subject: Re: IARC - NAPP Epidemiology Study Abstract re: Glyphosate and NHL
 >
 >Bill:
 >

>It looks like they combined the 3 NCI case control studies (previously combined in 2003 by DeRoos et al.) and the McDuffie study. The epi expert panel manuscript lays out the issues with the individual studies as does the Chang and Delzell meta-analysis. Adding studies together in a meta-analysis helps with statistical power, but it's problematic from a validity standpoint when the underlying studies have systematic errors (recall bias, analytic selection bias, etc.).

>
>It is hard to tell a lot from an abstract about what they actually did in the analysis. Donna might consider a FOI request for the NCI case control studies. A re-analysis would be very helpful in getting to the truth.

>
>Regards,

>
>John

>
>In response to your subsequent email, no problem not including DeRoos since she was not an author of the NCI case control studies. I don't know whether you can read anything in to her exclusion from among the authors.

>
>From: "HEYDENS, WILLIAM F [AG/1000]" <[REDACTED]>
>Date: Wednesday, June 1, 2016 at 4:53 AM
>To: John Acquavella <[REDACTED]>, Thomas Sorahan <[REDACTED]>
>Subject: FW: IARC - NAPP Epidemiology Study Abstract re: Glyphosate and NHL

>
>Hi John & Tom,

>
>See below in case you hadn't already picked up on this. I vaguely remember hearing that this would come out based on comments made in the aftermath of the IARC meeting (Tom, I think you may have heard about this from Aaron Blair?). Right now I don't know where this showed up, it's our understanding that they will present this at an IARC conference June 10th and that they plan to publish it.

>
>I'm obviously not an epidemiologist but my initial thought is that this shows that when you take a hand full of small studies with problems and combine them, you have 1 larger study still with the same problems. That said, I'm sure this will produce more trouble.

>
>I'd appreciate it if you have any thoughts on this.

>
>Thanks,

>
>Bill

>A detailed assessment of glyphosate use and the risks of non-Hodgkin lymphoma overall and by major histological sub-types: findings from the North American Pooled Project Manisha PAHWA, Cancer Care Ontario, Canada BEANE FREEMAN L. 2, SPINELLI J. 3,4, BLAIR A. 2, HOAR ZAHM S. 2, CANTOR K. 2, PAHWA P. 5,6, DOSMAN J. 5, MCLAUGHLIN J. 1,7,8, WEISENBURGER D. 9, DEMERS P. 1,7, HARRIS S. 1,7,10

>
>1 Occupational Cancer Research Centre, Cancer Care Ontario, Toronto, Canada
>2 Division of Cancer Epidemiology and Genetics, U.S. National Cancer Institute, Bethesda, U.S.A.
>3 British Columbia Cancer Agency Research Centre, Vancouver, Canada
>4 School of Population and Public Health, University of British Columbia, Vancouver, Canada
>5 Canadian Centre for Health and Safety in Agriculture, University of Saskatchewan, Saskatoon, Canada
>6 Department of Community Health and Epidemiology, University of Saskatchewan, Saskatoon, Canada
>7 Dalla Lana School of Public Health, University of Toronto, Toronto, Canada
>8 Public Health Ontario, Toronto, Canada
>9 Department of Pathology, City of Hope Medical Center, Duarte, U.S.A
>10 Prevention and Cancer Control, Cancer Care Ontario, Toronto, Canada

>
>Purpose: Glyphosate is the most frequently used herbicide worldwide. The International Agency for Research on Cancer recently classified glyphosate as a probable carcinogen for non-Hodgkin lymphoma (NHL), but the epidemiological studies considered were limited by small sample sizes and a lack of exposure-response data for NHL sub-types. We evaluated potential associations between glyphosate use and NHL risk using detailed information from the North American Pooled Project (NAPP).

>
>Methods: Data from NHL cases (N=1690) and population-based controls (N=5131), recruited from Canada and the Midwest U.S. during the 1980s-1990s for 4 different studies, were recently pooled for the NAPP. Self-reported glyphosate use information was used to assess possible associations with NHL overall and by histological sub-type (follicular lymphoma [FL], diffuse large B-cell lymphoma [DLBCL], small lymphocytic lymphoma [SLL], and other). Odds ratios (OR) and 95% confidence intervals (CI) were estimated with multiple logistic regression models adjusted for demographic and NHL risk factors.

>
>Results: Unadjusted for other pesticides, subjects who ever used glyphosate (N=133) had a significantly elevated NHL risk (OR=1.43, 95% CI: 1.11, 1.83). Glyphosate use for >3.5 years increased SLL risk (OR=1.98, 95% CI: 0.89, 4.39). Handling glyphosate for >2 days/year was associated with significantly higher odds of NHL (OR=2.42, 95% CI: 1.48, 3.96) and DLBCL (OR=2.83, 95% CI: 1.48, 5.41). There were

suggestive risk increases (p-value ≤ 0.02) for NHL, FL, and SLL with greater years*days/year of glyphosate use. Except for SLL, risks attenuated when adjusted for other pesticides.

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>Conclusions: This analysis suggested that glyphosate use was associated with increased NHL risk. Risk differences by histological sub-type were not consistent across glyphosate use metrics and may have been chance findings. Nevertheless, the NAPP's large sample size yielded more precise results than previously possible.

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