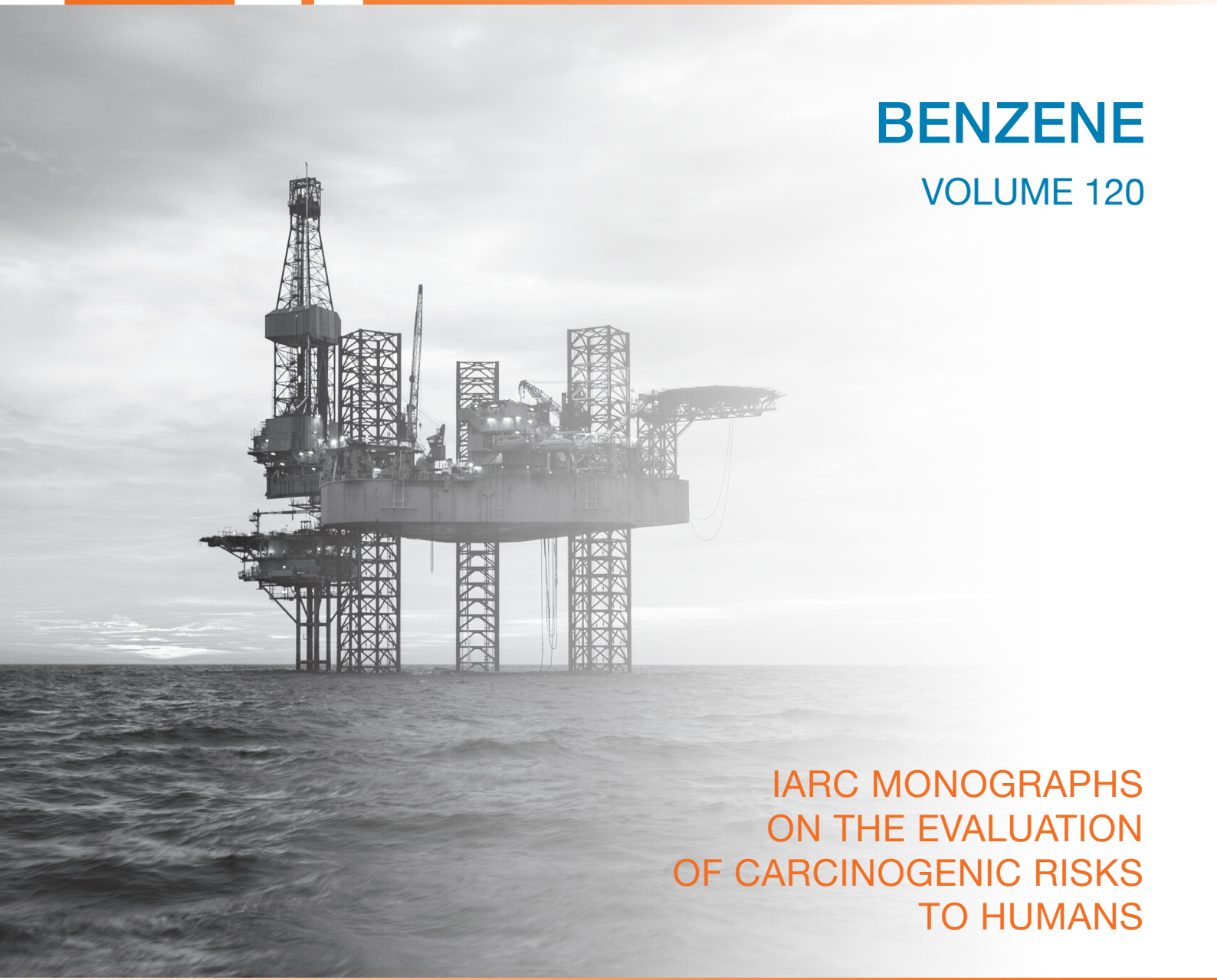
IARC MONOGRAPHS



BENZENE

VOLUME 120

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS

BENZENE

VOLUME 120

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 10–17 October 2017

LYON, FRANCE - 2018

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

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

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

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NOTE TO THE READER

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

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

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

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

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

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[12] Tom K. Grimsrud attended as an Observer for the Cancer Registry of Norway.
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PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

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

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

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

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

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

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

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

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

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

3. Selection of agents for review

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

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

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

4. Data for the *Monographs*

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

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

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

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

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

5. Meeting participants

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

(a) The Working Group

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

(b) Invited Specialists

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

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

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

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

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

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

6. Working procedures

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

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

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

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

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

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

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

B. SCIENTIFIC REVIEW AND EVALUATION

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

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

Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

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

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

1. Exposure data

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

(a) General information on the agent

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

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

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

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

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

(b) Analysis and detection

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

(c) Production and use

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

which may give rise to different impurities, are described.

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

(d) Occurrence and exposure

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

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

(e) Regulations and guidelines

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

2. Studies of cancer in humans

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

(a) Types of study considered

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

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

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

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

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

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

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

(b) Quality of studies considered

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

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

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

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

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

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

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

(c) Meta-analyses and pooled analyses

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

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

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

(d) Temporal effects

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

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

(e) Use of biomarkers in epidemiological studies

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

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

(f) Criteria for causality

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

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

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

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

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

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

3. Studies of cancer in experimental animals

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

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

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

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

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

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

(a) Qualitative aspects

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

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

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

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

(b) Quantitative aspects

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

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

(c) Statistical analyses

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

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

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

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

4. Mechanistic and other relevant data

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

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

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

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

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

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

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

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

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

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

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

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

(c) Other data relevant to mechanisms

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

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

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

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

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

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

6. Evaluation and rationale

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

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

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

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

(a) Carcinogenicity in humans

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

***Sufficient evidence of carcinogenicity*:**

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

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

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

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

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

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

(b) Carcinogenicity in experimental animals

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

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

***Sufficient evidence of carcinogenicity*:**

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

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

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

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

(c) Mechanistic and other relevant data

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

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

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

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

(d) Overall evaluation

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

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

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

Group 1: The agent is carcinogenic to humans.

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

Group 2.

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

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

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

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

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

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

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

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

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

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

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

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

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

(e) Rationale

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

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GENERAL REMARKS

This one-hundred-and-twentieth volume of the *IARC Monographs* presents an evaluation of the carcinogenic hazard to humans of exposure to benzene.

The conclusions of this volume represent the sixth evaluation of the carcinogenicity of benzene by an *IARC Monographs* Working Group. Successive evaluations published in Volumes 7 (IARC, 1974), 29 (IARC, 1982), and 100F (IARC, 2012) and Supplements 1 (IARC, 1979) and 7 (IARC, 1987) considered progressively larger and more complex volumes of data and yielded consistent, yet steadily broader and more compelling, conclusions about the carcinogenicity of benzene.

The available data were sparse at the time of the first evaluation (IARC, 1974). The Working Group determined that the available evidence from studies of experimental animals did not permit a conclusion to be drawn, but found suggestive evidence from epidemiological case reports and one case–control study that benzene causes leukaemia in humans; the current system of formal classifications of evidence had not yet been introduced at that time (it was introduced in Volume 17). Benzene was reviewed again in Supplement 1, which updated Volumes 1–20. With formal classifications then in place, the evidence in experimental animals was found to be *inadequate*, and the human epidemiological evidence, now supplemented by several occupational cohort studies and case–control studies in addition to case reports, was found to be *sufficient*. In the overall evaluation, benzene was found to be *carcinogenic to humans* (Group 1), a finding that has stood since that time.

Additional data had become available when benzene was reviewed again for Volume 29 (IARC, 1982). The Working Group now found the evidence in experimental animals to be *limited* and concluded that the modestly expanded epidemiological evidence established a causal relationship between exposure to benzene and development of acute myeloid leukaemia.

With further growth of the database during the 1980s, the evidence in experimental animals was found to be *sufficient* when benzene was evaluated again for Supplement 7 (IARC, 1987). Although mechanistic evidence was not yet formally incorporated into overall evaluations at that time, induction of chromosomal aberrations in exposed humans and of chromosomal aberrations and micronuclei in rodents was also noted in the summary report.

The volume of evidence had grown substantially larger and more complex by 2009, when the evaluation of benzene was updated for Volume 100F (IARC, 2012). The Working Group confirmed the previous findings of *sufficient evidence* of carcinogenicity in humans and experimental animals and, for the first time, presented *strong evidence* of multiple genotoxic effects based on a review of extensive mechanistic data. In humans, the Working Group concluded

that benzene causes acute myeloid leukaemia/acute non-lymphocytic leukaemia (both terms were used in epidemiological studies reviewed in that volume) and found *limited* evidence that benzene causes acute lymphocytic leukaemia, chronic lymphocytic leukaemia, non-Hodgkin lymphoma, and multiple myeloma.

The current evaluation was undertaken with two principal goals: (i) to incorporate new epidemiological and experimental evidence, including a large number of mechanistic studies in exposed humans, and (ii) to assess quantitative exposure–response relationships of exposure to benzene with both human cancer risks and relevant biological end-points in exposed humans. Such quantitative evaluations were recommended as an adjunct to future *Monographs* by an Advisory Group on quantitative risk characterization (IARC, 2014).

In the current evaluation, the Working Group again confirmed the carcinogenicity of benzene based on *sufficient evidence* of carcinogenicity in humans, *sufficient evidence* of carcinogenicity in experimental animals, and *strong* mechanistic evidence. The Working Group's evaluation of the accumulated evidence from human epidemiological studies focused on studies in which occupational or environmental exposure to benzene was specifically identified. The findings fully supported the previous conclusion that benzene causes acute non-lymphocytic leukaemia – including acute myeloid leukaemia – in adults, as well as the previous observations of *limited evidence* for chronic lymphocytic leukaemia, non-Hodgkin lymphoma, and multiple myeloma. On the basis of new data available since the last review, the Working Group also found *limited* evidence that benzene causes chronic myeloid leukaemia and lung cancer, and acute myeloid leukaemia in children. The Working Group's review of the large body of mechanistic studies took into account the key characteristics of carcinogens (Smith et al., 2016). The Working Group affirmed the *strong evidence* that benzene is genotoxic, and found that it also exhibits many other key characteristics of carcinogens, including in exposed humans. In particular, benzene is metabolically activated to electrophilic metabolites; induces oxidative stress and associated oxidative damage to DNA; is genotoxic; alters DNA repair or causes genomic instability; is immunosuppressive; alters cell proliferation, cell death, or nutrient supply; and modulates receptor-mediated effects.

The evidence reviewed for this evaluation, the Working Group's conclusions, and their analysis of exposure–response relationships are detailed in this volume. A summary of the key findings has appeared in *The Lancet Oncology* (Loomis et al., 2017).

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1. EXPOSURE DATA

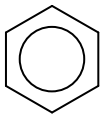
1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 71-43-2
Primary name: benzene
IUPAC systematic name: benzene

1.1.2 Structural and molecular formulae, and relative molecular mass

Structural formula:



From O'Neil (2006) and Lide (2008)

Molecular formula: C_6H_6
Relative molecular mass: 78.1

1.1.3 Chemical and physical properties of the pure substance

From HSDB (2018)

Description: clear, colourless, volatile, highly flammable liquid

Boiling point: 80.1 °C

Melting point: 5.558 °C

Density: 0.8756 g/cm^3

Refractive index: 1.5011 at 20 °C

Solubility: slightly soluble in water (1.8 g/L at 25 °C); miscible with acetic acid, acetone, chloroform, ethyl ether, and ethanol

Viscosity: 0.604 mPa at 25 °C

Vapour pressure: 94.8 mmHg at 25 °C

Stability: benzene is a very stable molecule due to its aromaticity, that is, the delocalization of pi electrons in the benzene molecule creating a resonance; catalysts are often needed to make benzene undergo a chemical reaction; benzene is volatile with a boiling point of 80 °C, and is highly flammable

Flash point: –11.1 °C

Octanol/water partition coefficient: log K_{ow}, 2.13; conversion factor (20 °C, 101 kPa): 1 ppm = 3.19 mg/m^3.

1.1.4 Technical products and impurities

The impurities found in commercial products are toluene, xylene, phenol, thiophene, carbon disulfide, acetylnitrile, and pyridine. Thiophene-free benzene has been specially treated to avoid destroying the catalysts used in reactions with benzene. Refined nitration-grade benzene is free of hydrogen sulfide and sulfur dioxide (HSDB, 2018).

1.2 Production and use

1.2.1 Production

(a) Production process

Benzene was first isolated by Faraday in 1825 from a liquid condensed by compressing oil gas; Mitscherlich first synthesized it in 1833 by distilling benzoic acid with lime. Benzene was first recovered commercially from light oil derived from coal tar in 1849, and from petroleum in 1941 (IARC, 1982).

Benzene can be produced in several ways. One method is by catalytic reforming, which involves the dehydrogenation of cycloparaffins, dehydroisomerization of alkyl cyclopentanes, and the cyclization and subsequent dehydrogenation of paraffins. The feed to the catalytic reformer (platinum-rhenium on an alumina support of high surface area) for benzene is thermally cracked naphtha cut at 71–104 °C. The benzene product is most often recovered from the reformate by solvent extraction techniques (Fruscella, 2002).

Benzene can also be prepared by cracking, a multistep process where crude oil is heated, steam is added, and the gaseous mixture is then briefly passed through a furnace at temperatures of 700–900 °C. The dissolved compounds undergo fractional distillation, which separates out the different components, including benzene (Fruscella, 2002).

Alternatively, benzene can be prepared from toluene by hydrodealkylation. In the presence of a catalyst (chromium, molybdenum, and/or platinum), toluene and hydrogen are compressed to pressures of 20–60 atmospheres and the mixture is heated to temperatures of 500–660 °C. This reaction converts the mixture to benzene and methane, and benzene is separated out by distillation (Fruscella, 2002).

(b) Production volume

Benzene is listed as a high production volume chemical by the Organisation for Economic Co-operation and Development (OECD, 2009). In 2012, global benzene production was approximately 42.9 million tonnes. In the USA, production volumes during 1986–2002 were more than 1 billion pounds [> 450 000 tonnes] (HSDB, 2018). In order of volume produced, the five countries producing the greatest quantities of benzene in 2012 were China, the USA, the Republic of Korea, Japan, and Germany (Merchant Research & Consulting Ltd, 2014). In 2014, the industry reported benzene production and consumption in western Europe (Germany, Belgium, France, Italy, Luxembourg, the Netherlands, Denmark, Ireland, the United Kingdom, Greece, Spain, Portugal, Austria, Finland, and Sweden – the EU-15 – plus Norway and Switzerland) of 6.7 and 7.5 million tonnes, respectively (PetroChemicals Europe, 2015).

The use of benzene for the production of ethylbenzene, cumene, cyclohexane, and nitrobenzene accounts for 90% of annual benzene consumption. In order of volume consumed, China, the USA, and western Europe consume about half of the total benzene produced (IHS Markit, 2017).

The United States Environmental Protection Agency (EPA) report published in February 2017 (Report No. 17-P-0249) reports a total benzene consumption of 57 701 737 237 gallons (equivalent to 1.9×10^8 tonnes; 1 gallon = 3.7858 L, benzene density of 0.879 g/cm^3) for 84 facilities in the USA in 2014 (EPA, 2017).

1.2.2 Uses

Historically, benzene was used as a degreaser of metals, a solvent for organic materials, a starting and intermediate material in the chemical and drug industries (e.g. to manufacture rubbers, lubricants, dyes, detergents, and pesticides), and an additive to unleaded gasoline

(ATSDR, 2007; Williams et al., 2008; NTP, 2016). Benzene use has diminished since its carcinogenic properties became widely publicized (IARC, 1982); however, some countries have continued to use benzene in specific products such as glue (Vermeulen et al., 2004).

Benzene occurs naturally in petroleum products (e.g. crude oil and gasoline), and is also added to unleaded gasoline for its octane-enhancing and anti-knock properties. Typically, the concentration of benzene in these fuels is 1–2% by volume (ATSDR, 2007). Benzene concentration in fuels sold in the European Union must be less than 1.0% by volume (European Commission, 2009).

The percentage of benzene in gasoline has varied with the refinery and time period from which it originated. Until 1931, the benzene content of the gasoline imported into the United Kingdom was 1% v/v (Lewis et al., 1997). In 1971, Parkinson reported that gasoline in the United Kingdom contained 2.8–5.8% benzene v/v (Parkinson, 1971). In Canada in the 1970s and the 1980s, benzene content in fuel was reported as 0.7–3.7% (Armstrong et al., 1996); in Australia, benzene content of 1–5% by weight during 1950–1990 was reported (Glass et al., 2000).

Gasoline can be enriched with benzene by adding benzene-toluene-xylene, which is generated during coke making. Where necessary, sidestream petroleum is added to adjust the octane rating; for example, reformate includes 5–12% benzene (Glass et al., 2000). Before 1950, a small proportion of gasoline enriched with benzene sold in the United Kingdom included up to 36% benzene (Lewis et al., 1997). Gasoline enriched with benzene included up to approximately 10% benzene in Canada during 1914–1938 (Armstrong et al., 1996) and in Australia until around 1970 (Glass et al., 2000).

The primary use of benzene today is in the manufacture of organic chemicals. In Europe, benzene is mainly used to make styrene, phenol, cyclohexane, aniline, maleic anhydride, alkylbenzenes, and chlorobenzenes. It is an intermediate in the production of anthraquinone, hydroquinone, benzene hexachloride, benzene sulfonic acid, and other products used in drugs, dyes, insecticides, and plastics (ICIS, 2010). In the USA, the primary use of benzene is in the production of ethylbenzene, accounting for 52% of the total benzene demand in 2008. Most ethylbenzene is consumed in the manufacture of styrene, which is used in turn in polystyrene and various styrene copolymers, latexes, and resins. The second-largest use of benzene in the USA (accounting for 22% of demand) is in the manufacture of cumene (isopropylbenzene), nearly all of which is consumed in phenol production. Benzene is also used to make chemical intermediates, including cyclohexane, used in making certain nylon monomers (15%); nitrobenzene, an intermediate for aniline and other products (7%); alkylbenzene, used in detergents (2%); chlorobenzenes, used in engineering polymers (1%); and miscellaneous other uses (1%) (Kirschner, 2009).

1.3 Measurement and analysis

1.3.1 Detection and quantification

Common standard methods to assay benzene in air are presented in Table 1.1, along with selected methods for measuring some biomarkers of exposure in urine.

Assays to monitor benzene in air were first developed to measure air concentration in the workplace, including personal exposure of workers, and to assess compliance with occupational limits. Typically, to measure 8-hour exposure, air is pumped through cartridges containing charcoal or other suitable sorbents for the duration of the entire work shift. In the laboratory, benzene is desorbed from sorbent using solvents such as carbon disulfide (NIOSH, 2003, method 1501) or high-temperature thermal desorption (NIOSH, 1996, method 2549), and analysed with either a gas chromatograph equipped with a flame ionization

Table 1.1 Representative methods for the analysis of benzene in air and its main urinary biomarkers

Sample matrix	Analyte	Assay procedure	Limit of detection	Reference
Air	Benzene	Pumping air through solid sorbent tube, solvent desorption, and GC-FID	0.5 µg/sample (sample volume 5–30 L)	NIOSH (2003), method 1501
	Benzene	Pumping air through solid sorbent tube, thermal desorption, and GC-MS	100 ng per tube or less (sample volume 1–6 L)	NIOSH (1996), method 2549
	Benzene	Real-time monitor with FTIR detector	0.32 ppm for a 10 m absorption pathlength	NIOSH(2002), method 3800
	Benzene	Portable GC-PID	0.02 ppm	NIOSH (1994), method 3700
	Benzene	Passive sampling [with solid sorbent device], solvent/thermal desorption, and GC-MS	Variable depending on geometry of sampler and sampling time	EPA (2014)
Urine	t,t-MA	HPLC-UV analysis	5 µg/L	Lee et al. (2005)
	SPMA	SPE LC-MS/MS analysis	0.2 µg/L	NIOSH (2014), method 8326
	Benzene	HS GC-MS analysis	0.025 µg/L	Fustinoni et al. (1999)

FTIR, Fourier-transform infrared spectroscopy; FID, flame ionization detection; GC, gas chromatography; HPLC-UV, high-pressure liquid chromatography, ultraviolet spectroscopy; HS, head space; LC-MS/MS, liquid chromatography, tandem mass spectrometry; MS, mass spectrometry; PID, photoionization detector; SPE, solid phase extraction; SPMA, *S*-phenylmercapturic acid; t,t-MA, *trans*,*trans*-muconic acid

detector (NIOSH, 2003, method 1501) or a mass spectrometer (NIOSH, 1996, method 2549). As an alternative, passive samplers do not need a pump and allow benzene sampling via air diffusion through them; see EPA (2014) for a review of different assays using passive samplers for the determination of volatile organic compounds, including benzene. The sensitivity of both active and passive assays depends on sample volume, desorption method, and instrumental analysis; a higher sampling volume, the use of thermal desorption, and detection by mass spectrometer are associated with greater sensitivity (detection by mass spectrometer also offers high specificity). The design determines the sampling rate for passive samplers; radial geometry warrants a high flow rate and therefore larger sampling volume over a specific sampling time (Cocheo et al., 2000).

A real-time monitor can be used to check for benzene leaks and to measure short-term exposure, especially during critical operations, allowing the simultaneous sampling of air and detection of benzene. Benzene can be separated from other chemicals by portable gas chromatography and detected by photoionization detector (NIOSH, 1994, method 3700), or can be measured by extractive Fourier-transform infrared spectrometry (NIOSH, 2002, method 3800).

The alternative method of measuring benzene exposure by biomonitoring dates to the 1980s (Lauwerys, 1983); the first biomarkers, such as phenol, have been progressively abandoned in favour of biomarkers that are less abundant but more specific. The currently recommended biomarkers for assessment of benzene exposure in the workplace include urinary *trans*,*trans*-muconic acid (t,t-MA), urinary *S*-phenylmercapturic acid (SPMA), and urinary benzene (INRS, 2017).

t,t-MA is a urinary metabolite of benzene accounting for about 4% of the absorbed dose. Formed and excreted in urine with rapid kinetics with a half-life of about 5 hours (Boogaard & van

Sittert, 1995), it is useful for assessment of recent exposure. It is measured using high-performance liquid chromatography with an ultraviolet detector (Lee et al., 2005), and standardized assays are present on the market. Its limitation is poor specificity, as t,t-MA is also produced by the metabolism of the preservative sorbic acid or sorbates contained in food and beverages (Ruppert et al., 1997; Weaver et al., 2000). t,t-MA is recommended when exposure is higher than 0.2 ppm (Kim et al., 2006a), depending on the amount of sorbic acid preservatives in the diet.

SPMA is a urinary metabolite of benzene accounting for less than 1% of the absorbed dose; it is formed and excreted in urine with rapid kinetics (half-life of ~9 hours; Boogaard & van Sittert, 1995). SPMA in urine is a specific biomarker, and is assayed using solid phase extraction followed by liquid chromatography coupled with tandem mass spectrometry (NIOSH, 2014, method 8326). The limitations of the use of this biomarker are the few standardized assays available and the high cost of the equipment to perform the assay. The variability associated with genetic polymorphism of glutathione *S*-transferase enzymes also affects urinary levels of SPMA (see Section 4.1).

Unmetabolized benzene is excreted in urine in a tiny proportion (< 0.1%) and with rapid kinetics (a half-life of a few hours). It is a specific biomarker, being uniquely indicative of exposure to benzene. It is assayed using online headspace sampling followed by gas chromatography or mass spectrometry (Fustinoni et al., 1999). A limitation in the use of urinary unmetabolized benzene is the lack of standardized assays; in addition, the volatility of benzene in urine may cause the loss of the analyte if no precautions are taken during sampling and in the storage of samples.

Both SPMA and urinary benzene are currently the biomarkers of choice to assess exposure to benzene in studies involving the general population (Fustinoni et al., 2005; Lovreglio et al., 2011; Andreoli et al., 2015).

1.3.2 Assessment of occupational exposure in epidemiological studies

A variety of exposure assessment methods have been used in epidemiological studies of workers potentially exposed to benzene; methods are summarized in the following sections. Additional details on exposure assessment methods used in key epidemiological studies evaluated by the Working Group are provided in Section 1.6.

(a) Occupational cohorts compared with the general population

Many early studies of chemical and petroleum industry workers compared mortality and cancer incidence in the workers and in the general population (e.g. Decouflé et al., 1983; Consonni et al., 1999; Divine et al., 1999; Koh et al., 2014) in terms of either standardized mortality ratios and/or standardized incidence ratios. Benzene was known to be present at such facilities, but benzene exposure estimates were not provided and benzene may not have been specifically mentioned in such studies. Where benzene is mentioned, the metrics are usually expressed as exposed/not exposed, sometimes with the duration or era of the exposed job included. In all cases, there could have been individuals occupationally exposed to benzene in the general population (comparison group).

(b) Expert assessment using interviews, personal questionnaires, or job-specific modules

In occupational studies, some investigators have classified workers with respect to benzene exposure from questionnaires, including those that probe for specific determinants of exposure, such as job-specific modules (e.g. Reid et al., 2011). Benzene exposure may be categorized

semiquantitatively, for example, "no exposure" versus "probable exposure", or "high" versus "medium" versus "low" exposure (e.g. Adegoke et al., 2003; Black et al., 2004; Miligi et al., 2006; Krishnadasan et al., 2007; Seidler et al., 2007). The interpretation of such exposure categories varies from one study to another, depending on the era, country, and industry sectors evaluated, for example.

In population-based studies, exposure must be assessed across a range of occupations and industries by evaluating the type and duration of jobs reported by study participants.

(c) Expert assessment using job characteristics with no individual-level measurements

In some studies, experts classify workers within certain employment start-date periods, industry sectors, and/or job or task categories as exposed or not exposed to benzene (e.g. Koh et al., 2011; Linet et al., 2015). These experts are usually from the specific facility, or at least from the industry sector, and are often occupational hygienists. In most studies the exposure groupings appeared to be performed before case identification, for example in cohort studies, or the assessors were case-blind for case–control studies. This methodology can be used for cohort studies (Infante et al., 1977; Wong, 1987a; Koh et al., 2011), or in case–control studies (e.g. Wong et al., 2006). Duration of exposure is a common metric in these types of studies, and provides a semiquantitative dimension to the exposure assessment. The metrics commonly used in these analyses are exposure category (where provided) and duration of exposed job. Broad exposure groupings were based on employment structure in several studies, for example hourly (potentially higher risk of exposure) versus salaried (potentially lower risk of exposure) workers (e.g. Wen et al., 1983; Wongsrichanalai et al., 1989; Honda et al., 1995). Some similar exposure assessments have a semiquantitative element, for example providing an exposure dimension of high, medium, or low for the work area (McMichael et al., 1975; Rushton & Alderson, 1981).

(d) Exposure assessment using quantitative measurements grouped by job characteristics

The strongest exposure estimates are those where measured benzene exposure data from relevant facilities were attributed by experts to individual job titles or work areas (e.g. Dosemeci et al., 1994). Exposure data may have been collected on an industry- or cohort-wide basis and then applied to specific individual participants, notably in nested case–control studies. This methodology has been applied in China in population-based case–control studies (Bassig et al., 2015), where measured exposure data from many industries has been available since the 1950s (e.g. Wong et al., 2010; Friesen et al., 2012).

There will be some imprecision in the application of a (usually) limited number of data points to other individuals, perhaps employed at other facilities or over different timeframes. Exposure may vary between facilities, between workers, and between days for the same worker, regardless of how average exposure data are assigned. It is important to ensure that the measurement data are representative of usual exposure (normal working circumstances), and include jobs for which lower and higher levels of exposure have been measured. The exposure estimates are quantitative and usually expressed as averaged mean benzene intensity (ppm or mg/m^3) or cumulative exposure (ppm-years or (mg/m^3)-years). The exposure grouping may take into account measured exposure data from multiple sites across a range of industry sectors (e.g. Portengen et al., 2016).

Data on personal exposure to benzene were not usually available before 1970, so extrapolations back in time may be needed. Exposure modifiers, for example, historical changes in work processes, percentage of benzene in petrol, or the presence of ventilation, may have been

used to estimate exposure for jobs and for eras where measured data may not be available or applicable (Armstrong et al., 1996; Lewis et al., 1997; Glass et al., 2000). These exposures were usually estimated with the aid of occupational hygienists from within the industry, and are discussed in more detail in Section 1.6.1. Smith et al. (1993) used such methodology to estimate total hydrocarbon exposure, from which Wong et al. (1999) estimated benzene exposure.

1.3.3 Exposure assessment for molecular epidemiology

Several factors should be considered in the design of epidemiological mechanistic studies. These include the congruency in the time period of effect or disease onset relative to exposure, the magnitude of effects observed, and inter- and intraindividual variability in the response.

For studies on cancer, long-term average exposure is relevant. The latency for leukaemia can be relatively short, for example less than 10 years (Finkelstein, 2000; Richardson, 2008), so exposure during this period should be characterized.

Shorter periods of more recent exposure should be considered for other end-points such as leukopenia (Lan et al., 2004), or chromosomal aberrations (Zhang et al., 1998; Marchetti et al., 2012) including genetic damage (Liu et al., 1996; Zhang et al., 2016). To identify changes in leukocyte numbers, for example, exposure to benzene in the 180 days before blood collection is relevant (Ward et al., 1996).

In a cross-sectional study, it is important to collect both exposure and outcome data for the same individuals to account for inter- and intraindividual variability associated with relevant parameters, for example, diet, smoking, shift work, and time-of-day effects. Data describing these factors should be collected systematically and incorporated within the analyses.

In assessing the exposure, a sufficient number of participants are needed to account for the variability in uptake and human metabolism, particularly where the biomarker of effect is labile (e.g. oxidative stress). In addition, repeated measurements to estimate average exposure are advisable to account for day-to-day variability in exposure.

Investigators should use recognized and validated methods of collection and analysis, ensuring quality by taking into account the most relevant parameters, including the limit of detection.

1.4 Occurrence and exposure

1.4.1 Occupational exposure

Benzene is a ubiquitous pollutant that is present in several industries and occupations, including the production and refining of oil and gas, the distribution, sale, and use of petroleum products, coke production, the manufacture and use of chemical products, automobile repair, shoe production, firefighting, and various operations related to engine exhaust. Due to the high volatility of benzene, occupational exposure to benzene mainly occurs via inhalation. Benzene also penetrates skin, but the degree of dermal absorption of benzene will depend upon the exposure scenario. Dermal absorption will vary according to the tasks being performed (e.g. dipping machinery parts, immersion of hands, or using petroleum-based products as degreasing agents), the benzene content of the product, the composition of the product containing benzene, contact time, and the area of the body on which the chemical resides (Kalnas & Teitelbaum, 2000; Williams et al., 2011; Jakasa et al., 2015). In these scenarios, the exposure will not usually be to pure benzene.

The major industries and occupations in which workers are potentially exposed to benzene are reviewed in the following sections. This

summary is not exhaustive, and the interested reader is referred to several reviews of occupational exposure to benzene across industries that have been published for Europe and North America (Runion & Scott, 1985; Nordlinder & Ramnäs, 1987; van Wijngaarden & Stewart, 2003; Capleton & Levy, 2005; Williams et al., 2008) and Asia (Kang et al., 2005; Liang et al., 2005; Navasumrit et al., 2005; Liu et al., 2009; Park et al., 2015). For some industries or applications, information in the literature is limited. For example, the use of pure benzene as a solvent and reagent in chemical laboratories is well known, but no report on exposure level of benzene was found for laboratory technicians apart from in the petroleum industry.

Although not exhaustive, Table 1.2 gives a summary of reported personal full-shift airborne benzene concentrations, while Table 1.3 summarizes biomonitoring data for the industries.

(a) Production, refining, and distribution of petroleum and petroleum-derived products

The petroleum industry can be divided into upstream and downstream segments. The upstream segment refers to conventional exploration, extraction, and production of crude oil and natural gas, described in the following section, as well as unconventional oil and gas development (UOGD). UOGD involves high-volume hydraulic fracturing, commonly referred to as "fracking", which is coupled to (vertical or horizontal) drilling to extract oil and gas from shale formations (i.e. extraction of materials other than crude oil and natural gas). UOGD includes the process of injecting large volumes of water, proppants (often sand), and potentially hazardous chemicals into wellbores at high pressure, fracturing the rock and enabling the outflow of trapped oil or gas from shale formations (EPA, 2013). The downstream segment consists of refinery operations (production and ancillary operations within the refinery and distribution depots, e.g. tank dipping, pump repairs, filter cleaning), distribution (loading of ships, railcars and road tankers, delivery to service stations), and retail of the petroleum fractions (attendant or self-service filling of customer vehicles).

(i) Upstream petroleum industry (conventional oil and gas extraction)

During drilling, the revolving steel bit must be lubricated and cooled, the well requires pressure support, and the rock cuttings must be transported to the surface. Drilling fluid, a complex oil- or water-based mixture, is used for these purposes. The characteristics of the hydrocarbon base oils in the drilling fluids have changed over time. Diesel as a base oil for drilling was gradually replaced in the early 1980s in the United Kingdom and Norway by petroleum-mineral oils with a reduced aromatic content; non-aromatic mineral oils (aromatic content < 0.01%) were used after 1998 (Gardner, 2003; Steinsvåg et al., 2006, 2007; Bråtveit et al., 2012). The mud-handling areas were originally designed for water-based mud that did not generate vapours, with open flow lines and mud pits. Other than measurements of oil mist and oil vapour, there have been very limited attempts to characterize the exposure regarding its composition. Theoretically, however, hydrocarbon and benzene exposure can occur through contamination of the drilling fluid from the geological formation in which it is drilled, or from hydrocarbons that are added to the drilling fluid to improve drilling properties, as in diesel and drilling fluids containing aromatics in the 1980s (Verma et al., 2000; Steinsvåg et al., 2007). With the exception of eight area measurements made during drilling in Canada showing a full-shift concentration of 0.006 mg/m^3 (with a highest measurement of 0.019 mg/m^3 and one personal measurement of < 0.010 mg/m^3), no information on this exposure scenario was available (Verma et al., 2000).

Table 1.2 Occupational exposure to benzene in air: personal measurements

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m^3)[a]	Exposure range (mg/m^3)[a]	Comments/additional data[a]
Upstream petroleum industry, unconventional							
Esswein et al. (2014)	USA, 2013	Flowback operations, workers gauging tanks	17	Full shift (typically 12 h)	AM (SD), 0.25 (0.16) ppm [0.8 (0.51)]	0.01–0.37 ppm [0.032–1.18]	Task-based short-term (2.5–30 min)
		Flowback operations, workers not gauging tanks	18	Full shift (typically 12 h)	AM (SD), 0.04 (0.03) ppm [0.13 (0.096)]	0.004–0.05 ppm [0.013–0.16]	
Upstream petroleum industry, conventional							
Bråtveit et al. (2007)	Norway, 2005	Process operators	35	657 min (range, 450–730 min)	AM (SD), 0.042 (0.132) ppm [0.13 (0.42)] GM, 0.005 ppm [0.016]	< 0.001–0.69 ppm [< 0.003–2.2]	Exposure varied according to tasks performed
		Flotation work	6		AM (SD), 0.221 (0.267) ppm [0.71 (0.85)] GM, 0.114 ppm [0.360]	0.030–0.688 ppm [0.095–2.2]	
		Sampling	11		AM (SD), 0.005 (0.005) ppm [0.16 (0.16)] GM, 0.003 ppm [0.096]	< 0.001–0.014 ppm [< 0.003–0.04]	
		Miscellaneous	18		AM (SD), 0.005 (0.01) ppm [0.16 (0.03)] GM, 0.003 ppm [0.096]	< 0.0010.023 ppm [< 0.003–0.07]	
Steinsvåg et al. (2007)	Norway, 1994–2003	Process and drilling operations (12 installations)	367	12 h	AM (SD), 0.037 (0.099) ppm [0.12 (0.32)] GM (GSD), 0.007 (5.7) ppm [0.22 (18.21)]	< LOD–2.6 ppm [< LOD–8.31]	165 measurements < LOD were set to LOD/√2

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Steinsvåg et al. (2007) (cont.)		Deck workers	29		AM (SD), 0.17 (0.51) ppm [0.54 (1.63)] GM (GSD), 0.010 (14) ppm [0.03 (44.7)]	< LOD–2.6 ppm [< LOD–8.31]	> LOD = 10
		Process operators	204		AM (SD), 0.036 (0.097) ppm [1.15 (0.31)] GM (GSD), 0.008 (5.3) ppm [0.026 (16.93)]	< LOD–0.97 ppm [< LOD–3.1]	> LOD = 101
		Laboratory	40		AM (SD), 0.012 (0.019) ppm [0.038 (0.06)] GM (GSD), 0.006 (3.7) ppm [0.019 (11.82)]	< LOD–0.11 ppm [< LOD–0.35]	> LOD = 13
		Mechanics	78		AM (SD), 0.006 (0.011) ppm [0.019 (0.035)] GM (GSD), 0.002 (4.5) ppm [0.006 (14.37)]	< LOD–0.08 ppm [< LOD–0.26]	> LOD = 37
		Electricians	16		AM (SD), 0.015 (0.017) ppm [0.048 (0.05)] GM (GSD), 0.007 (5.7) ppm [0.019 (18.85)]	< LOD–0.05 ppm [< LOD–0.16]	> LOD = 4
Kirkeleit et al. (2006b)	Norway, 2004	Crude oil production, vessel	139	592 min (range, 43–931 min)	AM, 0.43 ppm [1.37] GM (GSD), 0.02 (12.42) ppm [0.06 (39.7)]	< 0.001–16.75 ppm [< 0.003–53.5]	LOD, 0.001 ppm [0.003]

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Kirkeleit et al. (2006b) (cont.)		Process operators	30	669 min (range, 182–915 min)	AM, 0.39 ppm [1.25] GM (GSD), 0.01 (9.68) ppm [0.03 (30.92)]	< 0.001–7.3 ppm [< 0.003–23.32]	
		Deck workers	47	564 min (range, 43–866 min)	AM, 0.89 ppm [2.84] GM (GSD), 0.02 (19.11) ppm [0.06 (61.04)]	< 0.001–16.75 ppm [< 0.003–53.5]	The high exposure levels represent cleaning and maintenance of crude oil cargo tanks
		Mechanics	31	632 min (range, 257–705 min)	AM, 0.07 ppm [0.22] GM (GSD), 0.007 (12.04) ppm [0.02 (38.4)]	< 0.001–0.51 ppm [< 0.003–1.63]	
		Contractors	31	518 min (range, 190–931 min)	AM, 0.11 ppm [0.35] GM (GSD), 0.05 (4.90) ppm [0.16 (15.65)]	< 0.001–0.42 ppm [< 0.003–1.34]	
Verma et al. (2000)	Canada, 1985–1996	Conventional oil/gas	198	Long-term	AM, 0.206 GM, 0.036	0.003–7.78	For occupational groups see paper
		Conventional gas	838		GM, 0.010	0.006–57.6	
		Pipeline	8		AM, 0.392 GM, 0.350	0.16–1.54	
		Heavy oil processing	236		AM, 0.112 GM, 0.051	< 0.003–1.60	
Oil spill clean-up operations							
Gjesteland et al. (2017)	Norway, 2016	Sampling boats	21	10.8 h (range, 5.2–14.3 h)	AM, 0.43 ppm [1.37] GM (GSD), 0.20 (4.52) ppm [0.64 (14.44)]	0.01–1.52 ppm [0.03–4.86]	Field trial with spill of two fresh oils (22 workers, > 2 d)
		Workers on release ship and oil recovery ship	11	9.8 h (range, 5.2–12.5 h)	AM, 0.05 ppm [0.16] GM (GSD), 0.02 (0.02) ppm [0.064 (0.064)]	0.002–0.10 ppm [0.006–0.32]	
Downstream, petroleum refinery industry							
Almerud et al. (2017)	Sweden, 2009–2011	Process technicians, refinery I	132	Full shift (8 or 12 h)	AM, 0.153	95% CI, 0.01–0.022; maximum, 3.77	
		Maintenance workers, refinery I	67		AM, 0.0059	95% CI, 0.004–0.009; maximum, 1.32	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Almerud et al. (2017) (cont.)		Process technicians outdoor, refinery II	66		AM, 0.0137	95% CI, 0.0083–0.023; maximum, 0.27	
		Laboratory workers, refinery I	25		AM, 0.0046	95% CI, 0.0034–0.0062; maximum, 0.0154	
		Laboratory workers, refinery II	11		AM, 0.0084	95% CI, 0.0034–0.021; maximum, 0.02	
Akerstrom et al. (2016)	Sweden, 2011–2013	Turnarounds, refinery I	43	Full shift (8 or 12 h)	AM, 0.61	95% CI, 0.23–1.60 µg/m³	
		Turnarounds, refinery II	26		AM (SD), 0.96 (1.3) GM (GSD), 0.23 (0.0075)	0.007–4.5	
		Oil harbour workers (jetty workers and dock workers)	34		AM, 0.31	95% CI, 0.08–1.2	
		Sewage tanker drivers	16		AM, 0.36	95% CI, 0.068–1.9	
Widner et al. (2011)	USA, 1977–2005	Refinery and dock workers	406	480–661 min	NR	0.006–15 ppm [0.19–47.9]	GM not calculated because > 50% of measurements < LOD
		Dock connecting crew	179	535–664 min	GM (GSD), 0.023 (11) ppm [0.073 (35.1)]	0.010–15 ppm [0.03–47.9]	
		Contractor–tankerman	38	326–463 min	GM (GSD), 0.25 (8.8) ppm [0.8 (28.1)]	0.010–9.8 ppm [0.03–31.3]	
Kreider et al. (2010)	USA, 1977–2006	Routine operation, all areas and job titles	624	> 180 min	AM, 0.091 ppm [0.29]	Minimum–maximum detected, 0.004–6.0 ppm [0.013–19.2] 75th, 95th percentile, 0.043, 0.31 ppm [0.14–0.99]	GM not calculated because > 50% of measurements < LOD

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Kreider et al. (2010) (cont.)		Start up	50		AM, 0.046 ppm [0.15]	Minimum–maximum detected, 0.015–0.29 ppm [0.048–0.93] 75th, 95th percentile, 0.05, 0.17 ppm [0.16, 0.54]	
		Turnaround	471		AM, 0.17 ppm [0.54] GM (GSD), 0.032 (6.7) ppm [0.1 (21.4)]	Minimum–maximum detected, 0.004–9.200 ppm [0.013–29.4] 75th, 95th percentile, 0.12, 0.68 ppm [0.38, 2.17]	
CONCAWE (2002)	Europe, 1999–2001	Offsite refinery operator	6	451–498 min	AM, 0.3 GM, 0.2	10–90th percentiles, 0.1–0.5	
		Laboratory technician blending test gasoline for research	7	215–487 min	AM, 3.7 GM, 1.6	10–90th percentiles, 0.2–8.3	
CONCAWE (2000)	Europe, 1993–1998	Onsite operators (including catalytic reformer, gasoline blending)	97	Full shift	AM, 0.22	0.008–7.88	91% corresponding to full shift (8 or 12 h)

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
CONCAWE (2000) (cont.)		Refinery offsite operators (tank farm, including dipping, sampling, valve operation, dewatering, loading rail cars)	321		AM, 0.32	0.008–23.3	
		Refinery maintenance workers (pump maintenance, instrument calibration, enclosed equipment)	373		AM, 0.41	0.008–18.1	
		Refinery laboratory technicians (including product analysis, octane rating testing)	628		AM, 0.30	0.0015–5.0	
		Tank cleaners (including sludge cleaning)	49		AM, 2.10	0.008–38.7	
Downstream, distribution							
Lovreglio et al. (2016)	Italy, NR	Fuel tanker drivers	17	8 h	AM (SD), 0.28 (0.248) Median, 0.246	0.0074–1.017	
CONCAWE (2002)	Europe, 1999–2001	Rail car operators, top loading with vapour recovery)	21	64–363 min	AM, 0.5 GM, 0.4	10–90th percentiles, 0.2–0.7	
		Rail car operators, top loading without vapour recovery	16	165–450 min	AM, 4.0 GM, 1.4	10–90th percentiles, 0.3–10	
CONCAWE (2002)	Europe, 1999–2001	Road tanker distribution; drivers, bottom loading with vapour recovery	33	185–555 min	AM, 0.6 GM, 0.4	10–90th percentiles, 0.2–1.2	Pre-2000 specification gasoline

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
CONCAWE (2000)	Europe, 1993–1998	Marine and rail loading; ship deck crew, open loading	41	Full shift	AM, 0.56	0.08–5.4	91% corresponding to full shift (8 or 12 h)
		Ship deck crew, closed loading	2		AM, 0.56	0.51–0.6	
		Ship deck crew, unloading	32		AM, 0.51	0.023–3.7	
		Jetty staff	46		AM, 0.37	0.023–1.7	
CONCAWE (2000)	Europe, 1993–1998	Road tanker distribution Road tanker drivers, top loading	69	Full shift	AM, 2.07	0.04–48.2	
		Road tanker drivers, bottom loading (without vapour recovery)	223		AM, 0.82	0.008–15	
		Road tanker drivers, bottom loading (with vapour recovery)	137		AM, 0.37	0.03–1.99	
		Drivers, other category or unspecified	56		AM, 1.26	0.07–19.2	
		Road tanker terminal rack operators	126		AM, 0.64	0.003–4.2	
		Road tanker terminal supervisors/ operators	151		AM, 0.36	0.001–3.1	
		Road tanker terminal maintenance	52		AM, 0.52	0.001–7.9	
Foo (1991)	Singapore	Petroleum delivery tanker drivers	14	Full shift	AM, 1.10 ppm [3.51] GM, 0.81 ppm [2.59]	0.08–2.37 ppm [0.26–7.57]	21 gasoline stations Short-term exposure (n = 7): AM, 3.1 ppm [9.9], range 0.07–11.6 ppm [0.22–37.05]

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Petrochemical manufacturing							
Sahmel et al. (2013)	USA, 1974–1999	Use of petroleum-based raw materials;	2359	8 h	AM (SD), 0.54 (5.0) ppm [1.72 (15.97)] Median, 0.042 ppm [0.13]	NR	
		Routine employee exposure (all) 1974–1986	1289		AM (SD), 0.885 (6.72) ppm [2.83 (21.47)] Median, 0.12 ppm [0.38]	NR	Median exposure during time periods corresponding to year when OEL changed
		1987–1999	1070		AM (SD), 0.125 (0.676) ppm [0.4 (2.16)] Median, 0.016 ppm [0.051]	NR	
		1974–1983	916		AM (SD), 1.103 (7.739) ppm [3.52 (24.72)] Median, 0.19 ppm [0.61]	NR	Median exposure during time periods stratified according to key process changes
		1984–1991	865		AM (SD), 0.206 (2.024) ppm [0.66 (6.47)] Median, 0.01 ppm [0.03]	NR	
		1992–1999	578		AM (SD), 0.148 (0.578) ppm [0.47 (1.85)] Median, 0.021 ppm [0.067]	NR	
Williams & Paustenbach (2005)	USA, 1976–1987	Petrochemical manufacturing facility (acetic acid); mainly process operators	749	4–10 h	AM (SD), 1.75 (3.8) ppm [5.59 (12.14)]	NR	See paper for mean exposure levels for various production processes/areas
Coke production							
He et al. (2015)	China, NR	Topside, plant A	27	8 h	AM (SD), 0.705 (0.259)	0.268–1.197	Plant A: top charging of coal; no air pollution control
		Topside, plant B	28		AM (SD), 0.290 (0.11)	0.085–0.489	Plant B: stamp charging of coal; bag house for air pollution control

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Bieniek & Łusiak (2012)	Poland, 2005–2010	Cokery workers	202	Full shift	0.15	0.01–1.79	
		Coke oven workers	122		Median, 0.16	5–95th percentile, 0.04–0.60	
		Coke by-products	37		Median, 0.37	5–95th percentile, 0.061–1.39	
		Other workers in the coke plant	43		Median, 0.09	5–95th percentile, 0.011–0.292	Electricians and supervising personnel
Kivistö et al. (1997)	Estonia, 1994	Cokery workers	18	Full shift	AM (SD), 1.3 (2.7) ppm [4.15 (8.62)] Median, 0.4 ppm [1.28]	0.09–11.7 ppm [0.29–37.37]	
		Benzene factory workers	20		AM (SD), 1.6 (3.3) ppm [5.11 (10.54)] Median, 0.6 ppm [1.92]	0.06–14.7 ppm [0.19–46.96]	
Drummond et al. (1988)	UK, 1986	Battery workers	NR	Full shift	AM, 0.31 ppm [0.99]	NR	Each worker measured for 3–5 consecutive shifts
		Refining process of benzene	NR		AM, 1.32 ppm [4.22]	Maximum, 4.3 ppm [13.74]	
Petrol stations							
Campo et al. (2016)	Italy, 2008–2009	Petrol station attendants	89	~5 h	Median, 0.059	5–95% CI, 0.005–0.284	
Lovreglio et al. (2016)	Italy, NR	Filling station attendants	13	8 h	AM (SD), 0.02 (0.015) Median, 0.0138	0.0045–0.0534	
Lovreglio et al. (2014)	Italy, NR	Filling station attendants	24	8 h	AM (SD), 0.023 (0.017) Median, 0.02	0.0045–0.0663	
Bahrami et al. (2007)	Islamic Republic of Iran, NR	Petrol station workers	25	2–4 h	AM (SD), 1.40 (0.80) ppm [4.47 (2.56)]	0.2–3.1 ppm [0.64–9.9]	
Navasumrit et al. (2005)	Thailand, NR	Petrol station attendants	50	8 h	AM (SD), 121.67 (14.37) ppb [0.39 (0.046)] GM, 86.4 ppb [0.28]	2.80–439.9 ppb [0.0089–1.42]	
CONCAWE (2002)	Europe, 1993–1998	Service station attendants, without vapour recovery	26	189–465 min	AM, 0.3 GM, 0.3	10–90th percentile, 0.2–0.5	Pre-2000 specification gasoline

Table 1.2 Occupational (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
CONCAWE (2002) (cont.)		Service station attendants, with vapour recovery	7	288–437 min	AM, 0.1 GM, 0.1	10–90th percentile, 0.1–0.1	
		Service station cashiers	13	235–490 min	AM, 0.2 GM, 0.2	0.1–0.2	
		Service station workers, miscellaneous	6	237–280 min	AM, 0.2 GM, 0.1	0.1–0.2	
CONCAWE (2000)	Europe, 1993–1998	Service station attendants, without vapour recovery	417	Full shift	AM, 0.25	0.001–1.9	91% corresponding to full shift (8 or 12 h)
		Service station cashiers	268		AM, 0.05	0.001–1.92	
		Petrol pump maintenance workers	2		AM, 0.55	0.16–0.93	
		Service station workers, miscellaneous	5		AM, 0.03	0.01–0.10	
Lagorio et al. (1994)	Italy, 1991–1992	Petrol station attendants	27	8 h	AM (SD), 1.73 (5.53)	NR	Alkylated and lead-free gasoline: 2.86% and 2.65% benzene by volume, respectively
Lagorio et al. (1993)	Italy, 1992	Filling station attendants	111	8 h	AM (SD), 0.55 (2.46) GM (GSD), 0.12 (3.82)	0.001–28.02	111 filling stations
Foo (1991)	Singapore, NR	Gasoline kiosk attendants	54	Full shift	AM, 0.20 ppm [0.64] GM, 0.16 ppm [0.51]	0.028–0.71 ppm [0.89–2.27]	21 gasoline stations Short-term exposure (n = 49): AM, 6.6 ppm [21.08]; GM, 1.0 ppm [3.19]; range, 0.064–179 ppm [0.20–571.78]
Runion & Scott (1985)	USA, 1978–1783	Retail service stations	1478	Full shift	AM (SD), 0.06 (0.02) ppm [0.19 (0.06)] GM (GSD), 0.02 (5.4) ppm [0.06 (17.25)]	< 1.0 to > 10 ppm [< 3.19 to > 31.9] Range, NR	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Automobile repair							
Egeghy et al. (2002)	USA, 1998–1999	Mechanics	197	4 h	AM (SD), 0.118 (0.166) Median, 0.0597	< 0.009–1.14	Self-administered sampling; benzene content of gasoline < 1%
Javelaud et al. (1998)	France, 1996	Mechanics	65	8 h	AM (SD), 0.48 (1.49) GM, 0.06 Median, 0.14	< 0.005–9.31	23 garages
Hotz et al. (1997)	Country NR, 1994–1995	Mechanics	156	8 h	Median, 0.01 ppm [0.032]	5–95th percentile, < LOD–0.14 ppm [< LOD–0.45]	
Foo (1991)	Singapore, NR	Motorcar service mechanics	54	Full shift	AM, 0.17 ppm [0.54] GM, 0.10 ppm [0.32]	0.014–1.7 ppm [0.045–5.43]	21 gasoline stations
Nordlinder & Ramnäs (1987)	Sweden, NR	Mechanics, small garage (summer)	> 100	Full shift	AM, 1.6	NR	
		Mechanics, small garage (winter)			AM, 6.8	NR	
		Mechanics, medium and large garages (summer)			AM, 0.4	NR	
		Electricians, medium and large garages (summer)			AM, 1.0	NR	
		Mechanics, medium and large garages (winter)			AM, 0.8	NR	
		Electricians, medium and large garages (winter)			AM, 1.4	NR	
Exposure from engine exhaust							
Arayasiri et al. (2010)	Thailand, 2006	Traffic police	24	8 h	AM (SD, 0.0382 (0.0027) Median, 0.039	(0.0155–0.069)	
		Office police	24		AM (SE), 0.007 (0.0005) Median, 0.0062	0.0036–0.014	
Manini et al. (2008)	Italy, 2005	Traffic police	19	6 h	AM, 0.0061	0.0003–0.012	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Bahrami et al. (2007)	Islamic Republic of Iran, NR	Taxi drivers	60	2–4 h	AM (SD), 0.31 (0.22) ppm [0.99 (0.7)]	0.07–0.95 ppm [0.22–3.03]	
Manini et al. (2006)	Italy, 2004	Taxi drivers	37	24 h	AM (SD), 0.006 (0.0017)	NR	Non-smokers, ambient concentration in taxi during the 12 h shift, 0.0075 (0.0019)
Crebelli et al. (2001)	Italy, 1998–1999	Traffic police	139	7 h	AM (SD), 0.009 (0.011) GM (GSD), 0.0068 (0.002)	0.0013–0.0767	
		Office police	63		AM (SD), 0.0038 (0.0015) GM (GSD), 0.0035 (0.0015)	0.0011–0.0083	
Fustinoni et al. (1995)	Italy, 1994	Traffic wardens, urban and outdoors	20	5 h	AM (SD), 0.053 (0.03)	0.02–0.108	
		Traffic wardens, indoors (clerks)	19		AM (SD), 0.029 (0.008)	0.017–0.044	
Navasumrit et al. (2005)	Thailand, NR	Cloth vendors	22	8 h	AM (SD), 22.61 (1.32) ppb [0.073 (0.004)] Median, 21.1 ppb [0.067]	13.9–40.7 ppb [0.044–0.13]	
		Grilled-meat vendors	21		AM (SD), 28.19 (2.23) ppb [0.09 (0.007)] Median, 24.61 ppb [0.078]	16.8–52.0 ppb [0.054–0.17]	
Shoemaking							
Azari et al. (2012)	Islamic Republic of Iran, NR	Shoemakers, 12 workshops (October)	48	8 h	Mean (SE), 1.10 (0.11) ppm [3.51 (0.35)]	NR	Three consecutive months (October–December), examined effects of climate change and restriction of air flow due to closure of windows and shutdown of general ventilation systems
		Shoemakers, 12 workshops (November)			Mean (SE), 1.37 (0.14) ppm [4.38 (0.45)]	NR	
		Shoemakers, 12 workshops (December)			Mean (SE), 1.52 (0.18) ppm [4.86 (0.57)]	NR	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Estevan et al. (2012)	Spain, 2002–2007	Shoemakers: 2002–2003	329	NR	AM (SD), 0.05 (0.15)	NR	18–26% of samples were ≥ LOD (0.01)
		Shoemakers: 2004–2005	218		AM (SD), 0.07 (0.14)	NR	
		Shoemakers: 2006–2007	302		AM (SD), 0.05 (0.14)	NR	
Zhang et al. (2011)	China, NR	Shoemakers	44	8 h	AM (SD), 44.81 (33.59) GM (GSD), 27.91 (3.29)	2.57–146.11	
Vermeulen et al. (2004)	China, 2000–2001	Large shoe factory (safety shoes): all workers	2667	8 h	AM, 3.46 ppm [11.05] GM (GSD), 1.28 (3.64) ppm [4.09 (11.63)]	10–90th percentiles, 0.20–7.00 ppm [0.64–22.4]	No glues reported to contain benzene
		Large shoe factory (safety shoes): cutting	427		AM, 0.45 ppm [1.44] GM (GSD), 0.34 (2.05) ppm [1.09 (6.55)]	0.17–0.15 ppm [0.54–3.67]	
		Large shoe factory (safety shoes): modelling	735		AM, 2.74 ppm [8.75] GM (GSD), 1.71 (2.81) ppm [5.46 (8.98)]	0.38–6.04 ppm [1.21–19.29]	
		Large shoe factory (safety shoes): fitting	1096		AM, 2.19 ppm [7] GM (GSD), 1.12 (2.98) ppm [3.58 (9.52)]	0.26–4.68 ppm [0.83–14.95]	
		Large shoe factory (safety shoes): finishing	241		AM, 8.35 ppm [26.67] GM (GSD), 2.91 (3.33) ppm [9.3 (10.64)]	0.65–11.69 ppm [2.08–37.34]	
		Large shoe factory (safety shoes): packing	168		AM, 15.55 ppm [49.67] GM (GSD), 7.60 (3.47) ppm [24.28 (11.08)]	1.43–43.06 ppm [4.57–137.55]	
		Small shoe factory (luxury shoes): all workers	116	8 h	AM, 21.86 ppm [69.83] GM (GSD), 14.4 (2.31) ppm [46 (7.38)]	10–90th percentiles, 5.23–50.63 ppm [16.71–161.73]	6 of 7 glues contained benzene (0.60–34% benzene)

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Vermeulen et al. (2004) (cont.)		Small shoe factory (luxury shoes): cutting	41		AM:10.96 ppm [35.01] GM (GSD), 10.24 (1.45) ppm [32.71 (4.63)]	6.53–16.26 ppm [20.86–51.94]	
		Small shoe factory (luxury shoes): modelling	18		AM, 9.04 ppm [28.88] GM (GSD), 7.75 (1.75) ppm [24.76 (5.59)]	4.45–18.69 ppm [14.21–59.7]	
		Small shoe factory (luxury shoes): fitting	47		AM, 29.31 ppm [93.62] GM (GSD), 21.34 (2.34) ppm [68.17 (7.47)]	7.06–65.17 ppm [22.55–208.17]	
		Small shoe factory (luxury shoes): finishing	10		AM, 54.64 ppm [174.54] GM (GSD), 28.03 (3.30) ppm [89.54 (10.54)]	7.62–179.60 ppm [24.34–573.69]	
Printing							
Portengen et al. (2016)	China, 1949 to after 2000	Printing	232	NR	AM, 94.1 GM (GSD), 8.2 (13.0)	NR	40% of measurements < LOD (3.19)
Kang et al. (2005)	Republic of Korea, 1992–2000	Offset printing	4	NR	AM (SD), 0.017 (0.012) ppm [0.0543 (0.038)] GM (GSD), 0.014 ppm [0.0447]	0.008–0.034 ppm [0.0255–0.11]	
Handling of jet fuel							
Smith et al. (2010)	USA, NR	US Air Force personnel					LOD, 0.9 µg/m³ Jet fuel JP-8 (0.004–0.007% benzene)
		All	69	Full shift	GM (GSD), 0.0016 (0.0035)	< LOD–0.0364	
		Group assumed exposed to concentration	25	Full shift	GM (GSD), 0.0029 (0.0034)	< LOD–0.0364	
Egeghy et al. (2003)	USA, NR	US Air Force personnel					Jet fuel JP-8 (0.0002–0.0123 weight% benzene)
		Group exposed to low concentration	140	4 h	Median, 0.0031	< 0.001–0.0613	
		Group exposed to moderate concentration	38	4 h	Median, 0.0074	0.0014–1.85	
		Group exposed to high concentration	114	4 h	Median, 0.252	0.0061–6.63	

Table 1.2 (continued)

Reference	Location, collection year	Occupational description, setting	*n*	Sampling time	Exposure concentration (mg/m³)[a]	Exposure range (mg/m³)[a]	Comments/additional data[a]
Holm et al. (1987)	Sweden, 1983–1984	Swedish National Defence	92	12 h	GM (GSD), 0.06 (4.0)	Maximum, 7.2	Jet fuel MC-77 (equivalent to JP4 (< 1% benzene)
		All samples	46	8 h (TWA)	GM (GSD), 0.06 (4.1)	Maximum, 4.1	
		Jet fuel handling	6		GM (GSD), 0.03 (2.6)	Maximum, 0.1	
		Flight service	28		GM (GSD), 0.08 (3.6)	Maximum, 1.2	
		Workshop service	12		GM (GSD), 0.05 (5.7)	Maximum, 4.1	
		Pure jet fuel exposure	38		GM (GSD), 0.06 (4.2)	Maximum, 4.1	
		Mixed solvents exposure	8		GM (GSD), 0.11 (3.1)	Maximum, 0.5	
Firefighting							
Reinhardt & Ottmar (2004)	USA, 1992–1995	Initial attack (full shift)	45	13.3 h (range, 12–18 h)	GM, 3 ppb [0.096]	Maximum, 24 ppb [0.077]	13 d of initial attack incidents
		Initial attack (at fires)		3.3 h (range, 2–10 h)	GM, 14 ppb [0.045]	Maximum, 43 ppb [0.14]	
		Project wildfires (full shift)	84	13.9 h (range, 4–24 h)	GM, 4 ppb [0.013]	Maximum, 249 ppb [0.8]	17 d at eight separate project wildfires
		Project wildfires (at fires)		10.4 h (range, 2–24 h)	GM, 6 ppb [0.019]	Maximum, 384 ppb [1.23]	
		Prescribed burns (full shift)	200	11.5 h (range, 6–18 h)	GM, 16 ppb [0.051]	Maximum, 58 ppb [0.19]	39 prescribed burns
		Prescribed burns (at fires)		7 h (range, 2–13 h)	GM, 28 ppb [0.089]	Maximum, 88 ppb [0.28]	
Austin et al. (2001)	Canada, NR	Structural fires	9	Short-term	AM (SD), 3.38 (3.45) ppm [10.8 (11.02)]	0.12–10.76 ppm [0.38–34.37]	Area samples (not personal)
Bolstad-Johnson et al. (2000)	USA, 1998	Structural fires	95	Short-term	AM (SD), 0.383 (0.425) ppm [1.22 (1.36)]	0.07–1.99 ppm [0.22– 6.36]	25 fires

AM, arithmetic mean; CI, confidence interval; d, day(s); GM, geometric mean; GSD, geometric standard deviation; h, hour(s); LOD, limit of detection; min, minute(s); *n*, number of measurements; NR, not reported; OEL, occupational exposure limit; ppb, parts per billion; ppm, parts per million; SD, standard deviation; SE, standard error; TWA, time-weighted average

[a] Exposure concentrations and range given in mg/m³, unless indicated otherwise; if published in another unit, the concentration in mg/m³ is given in square brackets

Table 1.3 Summary of selected studies on the biological monitoring of occupational exposure to benzene

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (μg/m³)[a, b]	Urinary t,t-MA (μg/g creatinine)[a,c]	Urinary SPMA (μg/g creatinine)[a,c]	Urinary benzene (μg/L)[a,d]	Other biomarkers[a]
Campo et al. (2016)	Italy, NR	Filling station attendants	89	Median, 59 (5–284)[e]	Median, 127 (27–522)[e] μg/L	Median, 0.19 (< 0.1–1.28)[e] μg/L	Median, 0.339 (0.090–2.749)[e]	NR
		Unexposed workers	90	4 (1–18)[e]	Median, 117 (< 20–509)[e] μg/L	Median, < 0.1 (< 0.1–0.99)[e] μg/L	Median, 0.157 (0.054–2.554)[e]	NR
Lv et al. (2014)	China, NR	Shoe manufacturing workers	55	GM, 6980	NR	GM, 99[e]	NR	NR
Fustinoni et al. (2011)	Poland, NR	Petrochemical refinery workers	71	Median, 190 (50–2310)[e]	NR	Median, 0.65 (0.12–5.3)[e]	Median, 0.55 (0.117–7.487)[e]	NR
		Petrochemical office workers	97	NR	NR	Median, 0.40 (< 0.10–2.29)[e]	Median, 0.32 (0.083–2.316)[e]	NR
Carrieri et al. (2010)	Italy, 2006	Petrochemical workers	29	0.014 (< 0.001–0.280) ppm [45 (< 3–890)]	101 (< 6.86–746)	2.8 (< 0.06–38.59)	NR	NR
Lovreglio et al. (2010)	Italy, NR	Fuel tanker drivers	18	307 (7.4–1017)	134 (16–400)	2.94 (0.25–12.13)	2.96 (0.16–10.4)	Urinary phenol, 19 (5.0–33.0) mg/L
		Filling station attendants	23	23.5 (4.5–66.3)	86 (11–157)	0.79 (0.05–3.33)	0.62 (0.04–2.87)	Urinary phenol, 17.1 (8.0–29.0) mg/L
		Controls	31	4.6 (< 3.0–11.5)	93 (13–734)	0.65 (0.03–4.48)	1.23 (< 0.02–11.4)	Urinary phenol, 18.6 (3.0–36.0) mg/L
Hoet et al. (2009)	NR	Petrochemical workers	110	< 0.1 ppm [< 320]	50 (< 20–980)	0.97 (0.21–12.78)	0.270 (< 0.10–5.35)	Blood benzene, 0.405 (< 0.10–13.58) μg/L
Bråtveit et al. (2007)	Norway, 2004–2005	Petrochemical workers	12	0.042 (< 0.001–0.69) ppm [130 (< 3–2200)]	NR	NR	3.9 (0.5–34) nmol/L	Post shift; blood benzene, 1.8 (1.0–4.0) nmol/L
		Catering operator and office employees	9	NR	NR	NR	1.6 (0.5–4.0) nmol/L	Blood benzene, 1.8 (1.0–4.0) nmol/L

Table 1.3 (continued)

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (μg/m³)[a, b]	Urinary t,t-MA (μg/g creatinine)[a,c]	Urinary SPMA (μg/g creatinine)[a,c]	Urinary benzene (μg/L)[a,d]	Other biomarkers[a]
Bahrami et al. (2007)	Islamic Republic of Iran, NR	Taxi drivers	60	0.31 (0.07–0.95) ppm [990 (220–3030)]	310 (90–1270)	NR	NR	NR
		Petrol station workers	9	1.40 (0.2–3.1) ppm [4470 (640–9900)]	2640 (1200–3280)	NR	NR	NR
		Controls	18	ND	170 (10–350)	NR	NR	NR
Manini et al. (2006)	Italy, 2004	Taxi drivers	21 NS	7.5	122	GM, 2.14	GM, 0.44	NR
			16 S	8.1	154	GM, 3.79	GM, 2.58	NR
Kim et al. (2006a)	China, 2000–2001	Shoemaking factory workers	164 women	Median, 1.28 (0.017–88.9) ppm [4090 (54–284 000)]	Median, 13.5 (0.644–426) μmol/L	Median, 262 (1.50–29 400) nmol/L	Median, 283 (6.21–53 900) nmol/L	NR
			86 men	Median, 1.05 (0.122–50.2) ppm [3350 (390–160 350)]	Median, 10.3 (1.50–370) μmol/L	Median, 137 (3.68–33 000) nmol/L	Median, 216 (19.4–42 600) nmol/L	NR
		Clothes manufacturing workers (controls)	87 women	Median, 3.40 (0.146–21.2) ppb [10.86 (0.47–67.72)]	Median, 1.06 (0.152–6.17) μmol/L	Median, 1.94 (0.591–86.4) nmol/L	Median, 1.48 (0.091–7.47) nmol/L	NR
			52 men	Median, 3.71 (0.146–533) ppb [11.85 (0.47–1702.55)]	Median, 1.09 (0.132–5.78) μmol/L	Median, 3.24 (0.591–68.1) nmol/L	Median, 1.59 (0.091–130) nmol/L	NR
Fustinoni et al. (2005)	Italy, 1999–2000	Filling station attendants	78	Median, 61 (11–478)	NS: Median, 49 (< 10–581) μg/L	Median, 5.8 (0.2–10.9) μg/L	Median, 0.342 (0.042–2.836)	NR
					S: Median,144 (15–321) μg/L	Median, 7.5 (0.2–24.8) μg/L	Median, 1.168 (0.055–5.111)	Section 1.01 NR
		Traffic police	77	Median, 22 (9–316)	NS: Median, 82 (< 10–416) μg/L	NS: Median, 5.3 (0.2–13.8) μg/L	NS: Median, 0.151 (0.025–0.943)	NR
					S: Median, 213 (52–909) μg/L	S: Median, 9.1 (2.4–13.8) μg/L	S: Median, 0.753 (0.054–4.246)	NR
		Office workers	58	Median, 6 (< 6–115)	NS: Median, 33 (< 10–1089) μg/L	NS: Median, 4.1 (0.2–12.5) μg/L	NS: Median, 0.133 (< 0.015–0.409)	NR
					S: Median, 71 (< 10–270) μg/L	S: Median, 8.0 (0.2–13.9) μg/L	S: Median, 0.331 (0.064–4.615)	NR

Table 1.3 (continued)

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (μg/m³)[a, b]	Urinary t,t-MA (μg/g creatinine)[a,c]	Urinary SPMA (μg/g creatinine)[a,c]	Urinary benzene (μg/L)[a,d]	Other biomarkers[a]
Fustinoni et al. (2005) (cont.)		Bus drivers	152	Median, 21 (< 6–92)	NS: Median, 57 (< 10–536) μg/L	NS: Median, 5.6 (0.2–13.3) μg/L	NR	NR
					S: Median, 174 (< 10–695) μg/L	S: Median, 9.3 (0.2–65.9) μg/L	NR	NR
		Researchers	49	Median, 9 (< 6–46)	NS: Median, 51 (< 10–181) μg/L	NS: Median, 9.0 (0.2 – 182.2) μg/L	NR	NR
					S: Median, 195 (< 10–444) μg/L	S: Median, 13.7 (3.0–19.9) μg/L	NR	NR
Chakroun et al. (2002)	Tunisia, NR	Tanker fillers	20	0.16 (0.02–0.42) ppm [510 (63.89–1340)]	350 (80–1110)	NR	NR	NR
		Filling station attendants	10	0.20 (0.09–0.52) ppm [640 (290–1660)]	310 (150–590)	NR	NR	NR
		Controls	20	ND	110 (20–390)	NR	NR	NR
Waidyanatha et al. (2001, 2004)	China, ~1995	Rubber, adhesive, and paint manufacturers	42	14.5 (1.65–30.6) ppm [46 320 (5270–97 740)]	16 200 (1140–77 800) μg/L	712 (050–5890) μg/L	8.42 (0.837–27.9)	NR
				109 (31.5–329) ppm [348 180 (100 620–1 050 920)]	51 300 (7250–133 000) μg/L	9420 (123–27 500) μg/L	50.2 (1.30–284)	NR
		Sewing machine manufacturing workers (controls)	41	0.015 (0.0–0.11) ppm [48 (0.0–350)]	108 (020–338) μg/L	21 (2–79) μg/L	0.145 (0.027–2.06)	NR
Kivistö et al. (1997)	Estonia, 1994	Benzene production (in winter)	25	1.6 (0.06–14.7) ppm [5110 (190–46 960)]	38 (< 0.2–210) μmol/L	99 (< 0.3–1030)	965 (10–6250) nmol/L	Benzene in blood, 174 (8–1160) nmol/L
		Cokery workers (in winter)	27	1.3 (0.09–11.7) ppm [4150 (290–37 370)]	11 (< 0.2–35) μmol/L	73 (< 0.3–1020)	372 (22–1750) nmol/L	Benzene in blood, 160 (18–1690) nmol/L
		Rural controls (in winter)	10	0.009 ppm [28.75]	0.8 (< 0.2–8.1) μmol/L	2.1 (< 0.3–18)	12 (2–45) nmol/L	Benzene in blood, 7 (< 3–22) nmol/L

Table 1.3 (continued)

Reference	Country, year	Occupational description	No. of participants	Benzene exposure in air (µg/m³)[a, b]	Urinary t,t-MA (µg/g creatinine)[a,c]	Urinary SPMA (µg/g creatinine)[a,c]	Urinary benzene (µg/L)[a,d]	Other biomarkers[a]
Boogaard & van Sittert (1995, 1996)	Several countries including Belgium, Germany, and the Netherlands, 1992–1994	Natural gas production platforms	24	< 100–19 200	< 10–9920 µmol/mol creatinine	< 0.5–378 µmol/mol creatinine	NR	NR
		Chemical manufacturing	130	< 10–100 000	< 10–31 300 µmol/mol creatinine	< 1–1096 µmol/mol creatinine	NR	NR
		Oil refineries with aromatic plants	16	110–3300	8–1200 µmol/mol creatinine	0.9–46.4 µmol/mol creatinine	NR	NR
		Fuel tanker drivers and gasoline attendants	14	NR	9–830 µmol/mol creatinine	0.5–8. µmol/mol creatinine	NR	NR
		Employees without potential benzene exposure	38 NS	NR	29 µmol/mol creatinine	0. 94 µmol/mol creatinine	NR	NR
			14 S	NR	46 µmol/mol creatinine	1.71 µmol/mol creatinine	NR	NR

GM, geometric mean; ND, not detected; NR, not reported; NS, non-smokers; ppb, parts per billion; ppm, parts per million; S, smokers; SPMA, *S*-phenylmercapturic acid; t,t-MA, *trans*,*trans*-muconic acid

[a] Benzene exposure and biomarker concentrations are reported as mean (minimum–maximum), if not indicated otherwise

[b] Exposure concentration and range given in µg/m³ unless indicated otherwise; if published in another unit, the conversion to µg/m³ is given in square brackets

[c] Exposure concentration and range given in µg/g creatinine unless indicated otherwise; if published in another unit, the conversion to µg/g creatinine is given in square brackets

[d] Exposure concentration and range given in µg/L unless indicated otherwise; if published in another unit, the conversion to µg/L is given in square brackets

[e] 5–95th percentile

The separation and processing of crude oil and natural gas into crude oil, condensate, gas, and produced water before transport to shore via pipelines or tank ships takes place in a closed processing equipment and pipeline system. All four petroleum streams contain benzene, however, and the likelihood of exposure to benzene increases whenever the system is opened. The composition of crude oil and gas condensate varies between oil and gas fields and depends upon several factors, such as geological conditions in the reservoirs and the production age of the oil field, but typically lies within the range of < 0.01 and 3.0% by weight (Verma & des Tombe, 1999; Verma et al., 2000; Kirkeleit et al., 2006a), with benzene content in condensate being higher. The full-shift mean exposure in the production of oil and natural gas is usually well below 1 ppm [3.19 mg/m^3] benzene, the 8-hour permissible exposure limit set by the Occupational Safety and Health Administration (OSHA, 2017), during ordinary activity (Glass et al., 2000; Verma et al., 2000; Kirkeleit et al., 2006a; Bråtveit et al., 2007; Steinsvåg et al., 2007) (Table 1.2). However, some specific tasks, such as cleaning and maintenance of tanks and separators, pipeline pigging operations, and storage tank gauging, may cause short-term exposures in excess of this (Runion, 1988; CONCAWE, 2000; Glass et al., 2000; Verma et al., 2000; Kirkeleit et al., 2006a; Esswein et al., 2014).

With technological advances and more efficient reservoir completion techniques, UOGD has grown in the past decades. The only study available for this segment indicates that the potential for exposure is higher than for conventional oil and gas extraction (Esswein et al., 2014; Table 1.2).

(ii) Downstream petroleum industry: refining

The full-shift exposure to benzene during ordinary activity in the refining petroleum industry tends to be higher than for upstream activities, but still with average values well below 1 ppm [3.19 mg/m^3] (Nordlinder & Ramnäs, 1987; Verma et al., 1992, 2001; CONCAWE, 2000, 2002; Glass et al., 2000; Akerstrom et al., 2016; Almerud et al., 2017) (see Table 1.2). However, the range of exposure indicates potential for exceeding 1 ppm [3.19 mg/m^3]; this is particularly true for refinery maintenance, laboratory technicians, and dock workers. Specific tasks such as sampling, opening of vessels for maintenance and cleaning, and loading of petrol may cause high short-term exposure (Runion, 1988; Hakkola & Saarinen, 1996; Vainiotalo & Ruonakangas, 1999; Davenport et al., 2000; Verma et al., 2001; Kreider et al., 2010; Widner et al., 2011). However, while workers before 2000 were likely to have been exposed to higher concentrations of benzene because of a higher content of benzene in reformate stream (Burns et al., 2017), the range of benzene exposures reported in recent studies is considerably reduced (Campagna et al., 2012; Akerstrom et al., 2016; Almerud et al., 2017; Burns et al., 2017). Some of the reported exposure levels are given in Table 1.2.

(iii) Downstream petroleum industry: distribution

In the petroleum transport chain there is a potential for exposure at each point where the products are stored and transferred, and the reported exposures tend to be higher than for production and refinery workers (Halder et al., 1986; Javelaud et al., 1998; CONCAWE, 2000, 2002; Glass et al., 2000). However, because of a lowered content of benzene in petrol (Verma & des Tombe, 2002; Williams & Mani, 2015), as well as the recent introduction of vapour recovery systems in the petroleum distribution chain in at least developed countries, the exposure to benzene for these groups of workers has declined over the years. Some of the reported exposure levels are given in Table 1.2.

Williams et al. (2005) reviewed the available industrial hygiene data describing exposure during the marine transport of products

containing benzene (1975–2000). Although there were differences in sampling strategies and in the benzene content of the liquids being transported, air monitoring data revealed concentrations of 0.2–2.0 ppm [0.64–6.4 mg/m³] during closed-loading and 2–10 ppm [6.4–31.9 mg/m³] during open-loading operations. These estimates are somewhat higher than average values, but in line with the range of exposures reported in other reviews (CONCAWE, 2000, 2002; Verma et al., 2001).

(iv) Oil spill clean-up operations

The petroleum production and distribution scenario for which there is a lack of knowledge on exposure levels is the clean-up of an oil spill. In an oil spill field trial in the North Sea in 2016, full-shift measurements of benzene for personnel closest to the slick yielded a geometric mean exposure of 0.2 ppm benzene [0.64 mg/m³] (Gjesteland et al., 2017). No exposure to benzene was detected in personal samples collected during the Deepwater Horizon spill of light crude oil (Ahrenholz & Sylvain, 2011). In the Prestige and Nakhodka spills of heavy fuel oil, the measured benzene exposure was low because of the low content of volatile organic compounds (Morita et al., 1999; Pérez-Cadahía et al., 2007).

(v) Retail petrol stations

Averaged full-shift exposures of up to 0.65 mg/m³ (McDermott & Vos, 1979; Runion & Scott, 1985; Foo, 1991; Lagorio et al., 1993; CONCAWE, 2000, 2002; Verma et al., 2001; Chakroun et al., 2002; van Wijngaarden & Stewart, 2003; Fustinoni et al., 2005) and 59 µg/m³ [0.059 mg/m³] (Carrieri et al., 2006; Lovreglio et al., 2010, 2014; Campo et al., 2016) have been measured before and after 2000, respectively. Reported benzene exposure levels (Table 1.2 and Table 1.3) suggest that, in higher-income countries, at least, they have decreased with time. The decline is mainly ascribed to a decrease in benzene content in gasoline, as well as the installation of vapour recovery systems at retail gas stations capturing vapours during vehicle fuelling. The information on exposure for petrol station attendants in low- and middle-income countries is scarce, but available studies indicate somewhat higher concentrations of benzene for these workers compared with those reported from more developed countries (Navasumrit et al., 2005; Bahrami et al., 2007).

(b) Exposure from engine exhaust

Benzene from engine exhaust represents a potential exposure for professional drivers and urban workers, including taxi drivers, police, street workers, and others employed at workplaces with exposure to exhaust gases from motor vehicles (Nordlinder & Ramnäs, 1987). Reported exposure concentrations for these workers differ with region (Table 1.2). Reported median exposures for traffic police in Italy (2005) and Thailand (2006) were 6.1 µg/m³ and 38.2 µg/m³, respectively (Manini et al., 2008; Arayasiri et al., 2010). Cloth vendors and grilled-meat vendors in Thailand have been reported to have experienced mean exposures of 22.61 ppb [73 µg/m³] and 28.19 ppb [90 µg/m³], respectively (Navasumrit et al., 2005). Although the data for urban workers in low- and middle-income countries are scarce, the available information on both workers and outdoor air concentrations (see Section 1.4.2) indicates exposure to higher concentrations for these workers relative to the levels typical of higher-income countries.

(c) Automobile repair

Workers employed in automobile repair shops and recycling are potentially exposed to benzene through contact with gasoline vapour and engine products. Measured mean exposures before 2000 are typically less than 1 ppm (Nordlinder & Ramnäs, 1987; Foo, 1991; Hotz et al., 1997; Javelaud et al., 1998; Egeghy et al., 2002) (Table 1.2).

(d) Coke production

Benzene exposure is a potential hazard in the carbonization of coal to form coke used in the manufacture of steel, produced in the refining of the crude coke fractions and the by-products. China is currently the largest coke producer globally; average benzene exposure concentrations of 0.705 and 0.290 mg/m^3 were measured during a survey of two plants, where charging and pushing activities accounted for almost 70% of the exposure at the topside (He et al., 2015). During the period 2005–2010 in Poland, median exposures of 0.09–0.37 mg/m^3 according to job category were reported (Bieniek & Łusiak, 2012). Median exposure at a cokery in a shale oil petrochemical plant in Estonia was reported as 0.4 ppm [1.28 mg/m^3] one decade earlier (Kivistö et al., 1997). Average exposure for coke oven workers in the USA during 1978–1983 was reported as 8.46 ppm [27.02 mg/m^3] (van Wijngaarden & Stewart, 2003), while reported mean levels in the United Kingdom in 1986 ranged from 0.31 ppm [0.99 mg/m^3] in coke oven workers to 1.32 ppm [4.22 mg/m^3] in by-product workers (refining process of benzene) (Drummond et al., 1988).

(e) Rubber manufacturing

Benzene has historically been used in the manufacture of rubber, including the production of tyres and general rubber goods, and the process of retreading. It has also been used as a component in cement, glue, binding agents, and release agents, but has mainly been replaced by other agents (IARC, 2012). Some of the solvents used today still contain low benzene concentrations, however. From a pooled dataset on rubber manufacture workers in China used in a nested case–cohort study within the National Cancer Institute-Chinese Academy of Preventive Medicine (NCI-CAPM) cohort (n = 585), arithmetic and geometric means of 157.3 mg/m^3 and 45.6 mg/m^3 (geometric standard deviation, GSD, 6.4 mg/m^3) were reported from 1949 until after 2000 (Portengen et al., 2016). Averages of 1.42 ppm [4.54 mg/m^3] (n = 179) and 0.34 ppm [1.09 mg/m^3] (n = 4358) for rubber manufacture and production of tyres and inner tubes, respectively, have been reported from the USA and Canada (Runion & Scott, 1985; van Wijngaarden & Stewart, 2003).

The exposure levels in a cohort of workers producing rubberized food-coating materials have been estimated several times (Rinsky et al., 1981, 1987; Paustenbach et al., 1992; Crump, 1994; Utterback & Rinsky, 1995). In the latest retrospective assessment, the highest exposures (involving the jobs of neutralizer, quencher, knifeman, and spreader) were typically 50–90 ppm during 1939–1946 (lower during 1942–1945) and 10–40 ppm during 1947–1976 at the 50th percentile (Williams & Paustenbach, 2003). These estimated exposure levels were two to four times as great as for other jobs in this same cohort.

Kromhout et al. (1994) performed an exposure assessment of solvents in 10 rubber-manufacturing plants in the Netherlands in 1988. The use of particular solvents varied widely, and those selected for the quantitative assessment of exposure were based on the individual solvents, cements, and release and bonding agents used in the plants included in the study. The final assessment was restricted to paraffins, aromatic compounds, chlorinated hydrocarbons, ketones, alcohols, and esters. Benzene was not included, suggesting that the products used in the European rubber industry did not contain benzene from the late 1980s (Kromhout et al., 1994).

(f) Shoemaking

Shoemaking consists of several steps, including: the cutting of the material (leather, rubber, plastic, etc.), fitting of parts, sewing and gluing the various parts together, and finally the trimming and buffing of the shoes (Wang et al., 2006). Benzene is used as a solvent in glues, adhesives, and paint in the shoe-manufacturing process. Dermal exposure to benzene has

been reported as low, and does not significantly contribute to systemic exposure of benzene (Vermeulen et al., 2004). Although no longer as relevant in Europe and North America as in the past, this source of occupational benzene exposure is still of importance in some countries, notably in Asia.

In a recent Chinese study of shoe factory workers, mean exposures of 21.86 ppm [69.83 mg/m^3] and 3.46 ppm [11.05 mg/m^3] were reported for a small and large factory, respectively (Vermeulen et al., 2004). Benzene and toluene exposures were partly determined by the degree of contact with glues, the benzene and toluene content of each glue, air movement, and ventilation patterns. From a pooled dataset on workers in the shoemaking industry in China used in a nested case–cohort study within the NCI-CAPM cohort (n = 635), arithmetic and geometric means of 69.2 mg/m^3 and 8.1 mg/m^3 (GSD, 10.8 mg/m^3) were reported for the period from 1949 to after 2000 (Portengen et al., 2016).

In a benzene exposure assessment in 12 Iranian shoemaking workshops (semiautomated, year not given) mean exposures (standard error) for three consecutive months were 1.10 (0.11) ppm [3.51 (0.35) mg/m^3], 1.37 (0.14) ppm [4.38 (0.45) mg/m^3], and 1.52 (0.18) ppm [4.86 (0.57) mg/m^3] (Azari et al., 2012).

In the shoemaking industry in Spain, where benzene was unintentionally present in the adhesive as a contamination, the mean benzene exposure concentrations for the periods 2002–2003, 2004–2005, and 2006–2007 were 0.05 mg/m^3, 0.07 mg/m^3, and 0.05 mg/m^3, respectively (Estevan et al., 2012).

(g) Firefighting

Because of the incomplete combustion and pyrolysis of organic and synthetic materials, respectively, firefighters are potentially exposed to benzene during firefighting (municipal and wildfire), overhaul, and training. The heterogeneity of types of fires, time spent at fires, and types of structure or material burning, as well as the limited collection of data due to the extreme conditions, have hampered the characterization of exposure to benzene by firefighters and data are scarce. However, the few reported data suggest that the full-shift exposure is much less than 0.5 ppm, and is higher for the knockdown of wildfires compared with structure fires (Reinhardt & Ottmar, 2004); the potential for short-term exposure much higher than 1 ppm [3.19 mg/m^3] has also been reported (Bolstad-Johnson et al., 2000; Austin et al., 2001).

(h) Occupational use of products containing benzene

Benzene was formerly a common solvent and ingredient in a variety of products, including paint, printing inks, and glues, and is a natural component in products derived from petroleum. However, the benzene content in these products has either been replaced or reduced following regulations and other initiatives in the 1980s and 1990s.

(i) Application of paint

Benzene has been largely replaced as a solvent in paint, but is still used in some countries. Although this was a significant source of benzene exposure historically, data are lacking on benzene exposure during the use of paint that contains benzene as a constituent or contamination. In a review of benzene exposure in industries using paint in China, combining all the years during 1956–2005, relevant median exposures were reported for many activities, including: spray painting, 43.9 mg/m^3 (maximum, 3212 mg/m^3); brush painting, 58.2 mg/m^3 (maximum, 3373.5 mg/m^3); mixing, 53.6 mg/m^3 (maximum, 139.4 mg/m^3); immersion, 27.4 mg/m^3 (maximum, 540.0 mg/m^3); and paint manufacturing, 15.08 mg/m^3 (maximum, 344.0 mg/m^3) (Liu et al., 2009). From a pooled dataset on spray painting in China (n = 3754) used in a nested case–cohort study within the NCI-CAPM

cohort, arithmetic and geometric means of 62.5 mg/m^3 and 9.4 (GSD, 8.9) mg/m^3 averaged over the period from 1949 to after 2000 were reported (Portengen et al., 2016). The corresponding exposure concentrations for painting (n = 1099) were 115.3 mg/m^3 and 17.1 (GSD, 10.2) mg/m^3. In a pilot study, eight painters in small car repair shops in Italy were reported to have experienced an arithmetic mean exposure of 9.8 mg/m^3 (range, 0.4–53 mg/m^3) over a period of 236–323 min (Vitali et al., 2006). The authors ascribed the benzene exposure mainly to fuel vapour and gasoline used for degreasing and paint dilution.

(ii) Printing industry

Benzene was withdrawn from its significant use as a solvent of printing inks in Europe in the 1950s, but was used in the USA in the rotogravure processes from the 1930s until the beginning of the 1960s (IARC, 1996). Reported mean exposures from the printing industry are 0.58 ppm [1.85 mg/m^3] in the USA (van Wijngaarden & Stewart, 2003), and 0.017 ppm [0.0543 mg/m^3] in the Republic of Korea (Kang et al., 2005), but it is still a concern in some low- and middle-income countries. From the pooled dataset from the NCI-CAPM cohort (n = 232), arithmetic and geometric means of 94.1 mg/m^3 and 8.2 (GSD, 13.0) mg/m^3 averaged over the period from 1949 until after 2000 were reported (Portengen et al., 2016).

(iii) Use of petroleum-based products containing benzene in small amounts

Benzene is a residual component (< 0.1%) in petroleum-based products such as mineral spirit, jet fuel, degreasing agents, and other solvents. There are insufficient data to draw any conclusions on air concentrations generated when using these products, but estimations and reported exposure after simulations and controlled testing performed in relation to lawsuits can be found in several publications (Fedoruk et al., 2003; Williams et al., 2008; Hollins et al., 2013). There have been some reports on exposure to benzene during handling of various types of jet fuel; although exposure concentrations vary between the studies, work tasks, and circumstances, the reported values indicate a potential for exceeding exposures of 1 ppm [3.19 mg/m^3] (Holm et al., 1987; Egeghy et al., 2003; Smith et al., 2010).

(i) Biological monitoring of occupational exposure to benzene

Although the measurement of benzene in air is the most common method of investigating exposure in occupational settings, biomonitoring is considered the best technique as the characteristics of the individual and the use of protective equipment are taken into account. Moreover, when dermal exposure is a consideration, biological monitoring is the only system that can integrate both exposure routes.

A summary of selected studies on occupational exposure to benzene using biological monitoring is provided in Table 1.3. Investigated occupational settings include: the petrochemical industry (Boogaard & van Sittert, 1995, 1996; Kirkeleit et al., 2006b; Bråtveit et al., 2007; Hoet et al., 2009; Carrieri et al., 2010; Fustinoni et al., 2011; Hopf et al., 2012); cookery (Kivistö et al., 1997); and manufacturing, including chemical manufacturing (Boogaard & van Sittert, 1995, 1996; Kivistö et al., 1997), shoemaking (Kim et al., 2006a; Lv et al., 2014), adhesive production, and rubber and paint manufacturing (Waidyanatha et al., 2001, 2004). Exposure to gasoline vapours encountered by filling station attendants, tanker fillers, and fuel tanker drivers (Boogaard & van Sittert, 1995, 1996; Chakroun et al., 2002; Fustinoni et al., 2005; Bahrami et al., 2007; Lovreglio et al., 2010; Campo et al., 2016) and traffic exhaust exposure, such as that incurred by traffic police, and taxi and bus drivers (Fustinoni et al., 2005; Manini et al., 2006; Bahrami et al., 2007), were also investigated. A few studies have

investigated exposure to benzene encountered by firefighters (Caux et al., 2002; Fent et al., 2014).

Benzene is present in a complex mixture of chemicals in the large majority of these settings, although this percentage can be small in the case of gasoline vapours and traffic exhaust fumes, for example.

In 1995 and 1996, Boogaard and van Sittert investigated 184 workers exposed to benzene in various occupational settings (natural gas production platforms, chemical manufacturing, oil refineries, fuel tank drivers, and gasoline attendants), measuring personal benzene exposure and two minor urinary metabolites (t,t-MA and SPMA) in urine samples collected at the end of shifts. Personal exposure ranged from less than 0.01 to 100 mg/m^3. A group of 52 unexposed employees was also investigated as controls. It was estimated that about 4% and 0.1% of the inhaled dose was excreted in urine as t,t-MA and SPMA, respectively, with half-lives of about 5 hours and 9 hours. The correlation between personal benzene exposure and both biomarkers was very good, demonstrating their utility as biomarkers of exposure. Owing to the presence of background levels of t,t-MA in the urine of workers not exposed to benzene, this biomarker would be of limited use for assessing low benzene concentrations (Boogaard & van Sittert, 1995, 1996).

In later years, other studies in China investigated manufacturing workers exposed to high benzene concentrations in the rubber, adhesive, and paint production industries (up to 329 ppm [1051 mg/m^3]) (Waidyanatha et al., 2004) and in factories manufacturing glue, shoes, and sporting goods (up to 107 ppm [342 mg/m^3]) (Qu et al., 2003). Several benzene metabolites, such as urinary phenol, catechol, hydroquinone, t,t-MA, and SPMA, were investigated and all found to be correlated with personal benzene exposure. SPMA and t,t-MA demonstrated their superior ability as biomarkers of recent exposure, however; they were present in lower background concentrations in workers not exposed to benzene and they revealed a higher sensitivity in correlating with lower concentrations of occupational benzene. Urinary unmetabolized benzene was also measured, and demonstrated a very good correlation with personal benzene exposure and with the other urinary biomarkers (Waidyanatha et al., 2001).

Another study in China in 2000 applied urinary biomarkers to assess exposure in 250 shoemaking workers, using 139 clothes manufacturing workers as controls. Biomarkers were consistently elevated when the median benzene exposure level of the group was at or above 0.2 ppm for t,t-MA and SPMA, 0.5 ppm for phenol and hydroquinone, and 2 ppm for catechol (Kim et al., 2006a).

Much lower occupational exposures in fuel tanker drivers, filling station attendants, taxi and bus drivers, and traffic police were reported in Italy, with levels of up to 1017 µg/m^3 [1.017 mg/m^3] (Fustinoni et al., 2005; Manini et al., 2006; Lovreglio et al., 2010; Campo et al., 2016). Only the most specific biomarkers were measured in these studies, including urinary t,t-MA, SPMA, and unmetabolized benzene. These studies reported on the possibility of correlating very low benzene concentrations with both SPMA and urinary benzene, but not with t,t-MA. Moreover, these studies demonstrated the impact of tobacco smoking on the levels of biomarkers; smokers without occupational exposure to benzene had higher levels of benzene biomarkers than non-smoking filling station attendants (Fustinoni et al., 2005).

[The Working Group noted that, considered together, these studies showed that urinary SPMA and unmetabolized benzene are the most specific and sensitive biomarkers for the investigation of low occupational exposures, such as those found in most work settings. They are short-term biomarkers of exposure, and the best sampling time is at the end of the exposure or shift.]

1.4.2 General population exposure

Benzene is present ubiquitously in the environment, for example as a result of emissions from forest fires and volcanoes. However, the major environmental sources of benzene are anthropogenic. Such sources include industrial emissions, the burning of coal and oil, motor vehicle exhaust, and fuel evaporation. The primary route of environmental exposure to benzene is through inhalation, although exposure from ingestion of water and foods contaminated with benzene can also occur (ATSDR, 2007). Exposure to benzene can occur in microenvironments due to the evaporation of gasoline from parked cars in attached garages, while driving, or while pumping gasoline, or by spending time outdoors in close proximity to heavily trafficked areas or gasoline service stations. Benzene is a component of tobacco smoke; exposure therefore occurs when smoking or inhaling sidestream smoke (environmental tobacco smoke) (IARC, 2004).

(a) Outdoor air levels of benzene

Outdoor air concentrations of benzene vary widely throughout the world (see Table 1.4). In a review of air quality data from 42 European countries in 2014, the European Environment Agency reported no exceedances of the annual limit for benzene (5 μg/m^3) (European Environment Agency, 2016). Earlier, Guerreiro et al. (2014) reported that very few (0.9%) monitoring stations in Europe in 2011 exceeded this annual guideline for benzene. Over the period from mid-2009 to November 2012, mean and median benzene levels in northern Italy (Mestre) averaged 1.8 and 1.1 μg/m^3, respectively (Masiol et al., 2014). For a 5-year period from 2009 to 2013, benzene levels as measured at a single monitoring station in Edmonton, Canada, averaged 0.72 μg/m^3 (Bari & Kindzierski, 2017). In 2013, average benzene levels across 343 monitoring stations in the USA ranged from 0 ppb carbon (equivalent to ppb multiplied by the number of carbon atoms) in Queen Valley, a sparsely populated town in southern Arizona, to 8.27 ppb carbon [~1.38 ppb = 4.41 μg/m^3] in Steubenville, an industrial city in eastern Ohio (ATSDR, 2015). Based on data from seven continuous monitors in Tehran, Islamic Republic of Iran, in 2012 and 2013, annual benzene concentrations of 3.444 μg/m^3 were reported (Miri et al., 2016). The highest reported levels were in China, where benzene levels averaged 6.81 ppb [21.75 μg/m^3] over approximately 20 years, with city-specific averages from 0.73 ppb [2.33 μg/m^3] (Hong Kong Special Administrative Region) to 20.47 ppb [65.39 μg/m^3] (Ji'nan) (Zhang et al., 2017).

There is evidence that benzene outdoor air concentrations have declined significantly over time in Europe (> 70% decline during 2000–2014) (European Environment Agency, 2016) and the USA (66% decline during 1994–2009) (EPA, 2010). In addition to long-term trends, levels may vary seasonally. Jiang et al. (2017) reported average benzene concentrations in outdoor air of 502.5, 116.8, 111.21, and 294.8 parts per trillion [1.61, 0.37, 0.36, and 0.94 μg/m^3] in the spring, summer, autumn, and winter, respectively in Orleans, France. Similarly, outdoor air concentrations of benzene in the United Kingdom were reported to vary over the calendar year, with higher levels in the winter than during the summer (Duarte-Davidson et al., 2001).

Disasters may affect short-term air quality. After the Deepwater Horizon oil spill in the Gulf of Mexico in April 2010, mean benzene concentrations in air over the ensuing 5 months averaged 4.83 μg/m^3 (min., 0.12 μg/m^3; max., 81.89 μg/m^3) and 2.96 μg/m^3 (min., 0.14 μg/m^3; max., 290 μg/m^3) in regional and coastal areas of Louisiana, USA, respectively. These concentrations were higher than those measured from six urban areas in the state over the same period, which averaged 0.86 μg/m^3 (min., 0.51 μg/m^3; max., 2.33 μg/m^3) (Nance et al., 2016).

Table 1.4 Environmental monitoring of benzene

Reference	Country, year	No. of samplings	Sampling matrix	Exposure concentration (mean)[a]	Range[a]	Comments
Bruinen de Bruin et al. (2008)	European Union, from 2003	11 13 8 10 7 9 6 17 11 12 3 8	Personal exposures	5.1 (average overall) 2.0 (Helsinki) 2.3 (Leipzig) 3.2 (Brussels) 3.3 (Arnhem) 3.3 (Budapest) 4.1 (Dublin) 4.2 (Nijmegen) 5.2 (Catania) 7.5 (Athens) 8.0 (Nicosia) 8.5 (Milan) 9.4 (Thessaloniki)	NR	7 d average levels of benzene (μg/m^3) measured in indoor work (12 cities; n = 150; AM, 5.1); indoor home (9 cities; n = 59; AM, 3.2); and outdoor work (12 cities; n = 91; AM, 2.7) environments
Masiol et al. (2014)	Italy, 2000–2013	102 074	Outdoor air	1.8	0–10.2	
Miri et al. (2016)	Islamic Republic of Iran, March 2012–March 2013	NR	Outdoor air	3.444	NR	
Bari & Kindzierski (2017)	Canada, 2009–2013	NR	Outdoor air	0.72	Maximum, 3.31	
Jiang et al. (2017)	France, Oct 2010–Aug 2011	49 30 30 56	Outdoor air	502.50 ppt (spring) [1.61] 116.80 ppt (summer) [0.37] 111.21 ppt (fall) [0.36] 294.80 ppt (winter) [0.94]	16.8–2296 ppt [0.05–7.33] 16.6–674.4 ppt [0.05–2.15] 14.6–431.5 ppt [0.046–1.38] 49.8–1163.3 ppt [0.16–3.72]	2 h samples
McMahon et al. (2017)	USA, 2015–2016	116	Drinking-water wells	NR	< 0.026–0.127 μg/L	Benzene detection frequencies (≥ 0.013 μg/L) were 9.3%, 13.3%, and 2.4% in Eagle Ford (Texas), Fayetteville (Arkansas), and Haynesville (Texas) shale hydrocarbon production areas, respectively

Table 1.4 (continued)

Reference	Country, year	No. of samplings	Sampling matrix	Exposure concentration (mean)[a]	Range[a]	Comments
Zhang et al. (2017)	China, 1990–2014	NR	Outdoor air	4.42 ppb (Beijing) [14.12] 14.16 ppb (Guangzhou) [45.23] 6.95 ppb (Shanghai) [22.2] 3.94 ppb (Jiaxing) [12.59] 3.76 ppb (Nanjing) [12.01] 6.30 ppb (Hangzhou) [20.12] 6.61 ppb (Macau) [21.11] 13.09 ppb (Changchun) [41.81] 20.10 ppb (Changzhou) [64.2] 20.47 ppb (Ji'nan) [65.39] 11.36 ppb (Lianyungang) [36.29] 4.71 ppb (Nanning) [15.05] 6.47 ppb (Zhengzhou) [20.67] 6.45 ppb (Dongguan) [20.6] 0.92 ppb (Tianjin) [2.94] 1.89 ppb (Anshan) [6.04] 1.61 ppb (Shenyang) [5.14] 1.42 ppb (Shaoxing) [4.54] 0.94 ppb (Tai'an) [3.00] 0.73 ppb (Hong Kong SAR) [2.33]	NR	

AM, arithmetic mean; d, day(s); NR, not reported; ppb, parts per billion; ppt, parts per trillion; SAR, Special Administrative Region

[a] Exposure concentration and range given in μg/m^3; if published in another unit, the conversion to μg/m^3 is given in square brackets.

(b) Personal exposures to benzene

A study published in 2008 reported on personal monitoring data for benzene collected in 12 European cities, with the lowest arithmetic mean concentration reported for residents of Helsinki, Finland (2.0 µg/m^3), and the highest for residents of Thessaloniki, Greece (9.4 µg/m^3) (Bruinen de Bruin et al., 2008).

(c) Benzene in drinking-water and food

Benzene exposure can occur due to ingestion of water and food contaminated with benzene (ATSDR, 2007). During 1985–2002, the United States Geological Survey detected benzene in 37 of 1208 (3.1%) domestic water well samples that were collected at sites across the country; all but one sample had concentrations that were less than 1 µg/L (Rowe et al., 2007). In 2015 and 2016, a small proportion of the samples from 116 drinking-water (domestic and public supply) wells in the Eagle Ford (9.3%), Fayetteville (13.3%), and Haynesville (2.4%) shale hydrocarbon production areas in Texas and Arkansas, USA, had detectable levels, and all concentrations were less than 0.15 µg/L (McMahon et al., 2017).

Based on a review of studies published during 1996–2013, relatively low concentrations were reported in carbonated beverages and other foodstuffs (< 1 ppb); the highest levels (18 ppb) were found in organ meats (Salviano Dos Santos et al., 2015). Over a 5-year period (1996–2006), the United States Food and Drug Administration evaluated 70 "table-ready" foods. Benzene was found in all of them except American cheese and vanilla ice cream; levels ranged from 1 ppb (in milk-based infant formula and raw strawberries) to 190 ppb (fully cooked ground beef) (Fleming-Jones & Smith, 2003). Medeiros Vinci et al. (2012) detected benzene in 58% of 455 food samples purchased and analysed from four supermarkets in Belgium in 2010, with the highest mean levels found in smoked (18.90 µg/kg) and canned (7.40 µg/kg) fish, as well as in fatty fish (3.1 µg/kg) and ready-to-eat salads (2.79 µg/kg). Mean levels were much lower in non-fatty (0.52 µg/kg) fish, raw meat (0.31 µg/kg), and eggs (below the limit of detection).

(d) Biomonitoring of benzene exposure

Nationally conducted surveys that include a biomonitoring component have documented benzene exposures in the general population (see Table 1.5). Based on data collected as part of the Canadian Health Measures Survey during 2012–2013 for people aged 12–79 years (n = 2488), geometric mean blood benzene concentrations were 0.036 µg/L (Haines et al., 2017). Based on the United States National Health and Nutrition Examination Survey (NHANES) in 2001–2002, 2003–2004, 2005–2006, and 2007–2008, median benzene blood concentrations for the United States population were 0.03 µg/L (n = 837), 0.027 µg/L (n = 1345), 0.026 µg/L (n = 3091), and less than the limit of detection (n = 2685), respectively (US Department of Health and Human Services, 2018). Using NHANES biomonitoring data, Arnold et al. (2013) reported differences in median blood benzene concentrations between those individuals who had pumped gasoline into a car or motor vehicle during the previous 3 days (0.029 µg/L) and those who had not (0.025 µg/L). Benzene concentrations were also higher for individuals who reported having inhaled diesel exhaust during the previous 3 days (0.039 µg/L) compared with those who had not (0.027 µg/L).

Biomonitoring studies have also documented environmental exposure to benzene by measuring metabolites of benzene in urine. The Korean National Environmental Health Survey, which was conducted among adults aged 19 years and older during 2012–2014 (n = 6376), reported geometric mean levels of urinary t,t-MA of 58.8 µg/L (Choi et al., 2017). Among 336 adults (age, 35–69 years) living in central Italy who had cotinine levels less than 100 µg/g creatinine (the cut-off value above that was used to define a smoker), reported median

Table 1.5 Summary of selected studies with biological monitoring of environmental exposure to benzene[a]

Reference	Country, year	Population	*n*	Biomarker concentration (μg/L)[a, b]					Comments
				Urinary t,t-MA	Urinary SPMA	Urinary benzene	Blood benzene	Breast milk	
Haines et al. (2017)	Canada, 2012–2013	Adults (12–79 yr)	2488	NR	NR	NR	GM, 0.036 (0.020–0.067)[c]	NR	CHMS uses a stratified, multistage household-based sampling strategy; sample size indicated is the number of unweighted participants
US Department of Health and Human Services (2018)	USA, 2001–2002	Adults (≥ 12 yr)	837	NR	NR	NR	Median, 0.030 (0.100–0.190)[d]	NR	NHANES uses a complex multistage probability design; sample size indicated is the number of unweighted participants; 25th percentiles not provided
	2003–2004		1345	NR	NR	NR	Median, 0.027 (0.064–0.170)[d]	NR	
	2005–2006		3091	NR	NR	NR	Median, 0.026 (0.056–0.220)[d]	NR	
	2007–2008		2685	NR	NR	NR	Median, < LOD (0.041–0.198)[d]	NR	
	2011–2012		2466	NR	Median, < LOD (1.07–1.95)[d]	NR	NR	NR	
Choi et al. (2017)	Republic of Korea, 2012–2014	Adults (≥ 19 yr)	6376	GM, 58.8 (30.2–118)[c]	NR	NR	NR	NR	
Schoeters et al. (2017)	Belgium, 2003–2004	Adolescents (14–15 yr)	1586	GM, 99 (92–107)	NR	NR	NR	NR	FLEHS uses a stratified clustered multistage design; geometric mean concentrations are adjusted for age, sex, smoking, and creatinine levels

Table 1.5 (continued)

Reference	Country, year	Population	*n*	Biomarker concentration (µg/L)[a, b]					Comments
				Urinary t,t-MA	Urinary SPMA	Urinary benzene	Blood benzene	Breast milk	
Tranfo et al. (2017)	Italy, NR	Adults (35–69 yr) with cotinine < 100 µg/g creatinine	336	85.48 µg/g creatinine	0.23 µg/g creatinine	NR	NR	NR	
Blount et al. (2010)	USA (Baltimore, Maryland)	Women	12	NR	NR	NR	NR	Median, 0.080	Convenience sample via announcements and word of mouth
Protano et al. (2012)	Italy, NR	Children (5–11 yr)	396	127.59 (13.76–972.918) µg/g creatinine	0.62 (0.06–4.35) µg/g creatinine	NR	NR	NR	
Lovreglio et al. (2011)	Italy (Puglia), 2009	Adult men	137	52.0 (< 20 to 734) µg/g creatinine	< 0.03 (< 0.03–5.22) µg/g creatinine	0.08 (< 0.02 to 11.40)	NR	NR	
Fustinoni et al. (2010)	Italy, 2007–2008	Adults (19–75 yr)	108	NR	NR	0.122 (0.083–0.294)[c]	NR	NR	
Fabietti et al. (2004)	Italy (Rome), NR	Women	23	NR	NR	NR	NR	0.06 (0.01–0.18) µg/kg	

CHMS, Canadian Health Measures Survey; FLEHS, Flemish Environment and Health Study; GM, geometric mean; LOD, limit of detection; NHANES, US National Health and Nutrition Examination Survey; NR, not reported; yr, year(s)

[a] Biomarker concentrations are reported as arithmetic mean levels (minimum–maximum) unless indicated otherwise.

[b] Concentrations are given in µg/L (micrograms/L) unless indicated otherwise.

[c] 25–75th percentile

[d] 75–95th percentile

urinary levels of t,t-MA and SPMA were 85.48 and 0.23 µg/g creatinine, respectively (Tranfo et al., 2017). Fustinoni et al. (2010) reported a urinary benzene level of 0.122 µg/L (median) in 108 Italian men and women.

A few studies have examined the exposure of adolescents and children to benzene using biomonitoring data. Geometric mean concentrations of urinary t,t-MA, adjusted for age, sex, smoking status, and creatinine concentrations in adolescents aged 14 and 15 years, were reported by the Flemish Environment and Health Study of 99 µg/L in 2003–2004 (n = 1586), 94 µg/L in 2007–2008 (n = 206), and 61 µg/L in 2013 (n = 204) (Schoeters et al., 2017). Based on urine samples collected from 396 Italian children (age, 5–11 years), Protano et al. (2012) reported mean levels of 127.59 and 0.62 µg/g creatinine for t,t-MA and SPMA, respectively.

In workers who are not exposed to benzene through their occupation, the combined effects of smoking and environmental tobacco smoke contribute, on average, 85% and 23% to total benzene exposure among smokers and non-smokers, respectively (Weisel, 2010). In a 2009–2011 nationally representative study of exposure to volatile organic compounds in Canada, statistically significant differences in indoor residential concentrations of benzene were detected between homes with and without smokers (difference, 1.12 µg/m^3) (Zhu et al., 2013). Geometric mean benzene concentrations in blood were 0.136 and 0.024 µg/L for smokers and non-smokers, respectively, as assessed using biomonitoring data from the 2003–2004 NHANES survey (Kirman et al., 2012). Similarly, Tranfo et al. (2017) reported urinary levels of t,t-MA and SPMA of 141.32 and 1.83 µg/g creatinine in smokers, compared with 90.68 and 0.20 µg/g creatinine in non-smokers, respectively.

1.5 Regulations and guidelines

The International Labour Organization Benzene Convention (C136) Article 2(1) states: "Whenever harmless or less harmful substitute products are available, they shall be used instead of benzene or products containing benzene." This convention was passed in 1971 and ratified by 38 countries (ILO, 1971). The European Union classified benzene as a category I carcinogen under Directive 67/548/EEC (European Commission, 1967). Benzene is not allowed to be placed on the market with the exception of fuel, or used as a substance or as a constituent of mixtures in concentration greater than 0.1% by weight (EU-OSHA, 2006). The USA withdrew benzene from consumer products in 1978 (IARC, 1982).

1.5.1 Occupational exposure limits

(a) USA

The 8-hour permissible exposure and short-term limits set by the Occupational Safety and Health Administration are 1 ppm [3.19 mg/m^3] and 5 ppm [15.95 mg/m^3], respectively (CFR 1910.1028) (OSHA, 2017) (Table 1.6).

Occupational exposure limit (OEL) recommendations for benzene have been made by the American Conference of Governmental Industrial Hygienists (ACGIH). ACGIH recommends a threshold limit value (TLV) during an 8-hour work shift of 0.5 ppm [1.6 mg/m^3] and a short-term exposure limit (STEL) of 2.5 ppm [~8 mg/m^3]. ACGIH also recommends a biological exposure index (BEI) for t,t-MA in urine of 500 µg/g creatinine and for SPMA in urine of 25 µg/g creatinine (ACGIH, 2012). The United States National Institute for Occupational Safety and Health (NIOSH) recommended exposure level (REL) for the time-weighted average is 0.1 ppm [0.32 mg/m^3] (NIOSH, 2010) and the short-term limit value is 1 ppm [3.2 mg/m^3].

Table 1.6 International occupational exposure limits for benzene

Country	Limit value – 8 h		Limit value – short-term		Remarks
	ppm	mg/m³	ppm	mg/m³	
Australia	1	3.2			
Austria	1	3.2	4	12.8	TRK value (based on technical feasibility)
Belgium	1	3.25			
Canada – Ontario	0.5		2.5		
Canada – Quebec	1	3	5	15.5	
China		6		10 (1)	(1) 15 min average value
Denmark	0.5	1.6	1.0	3.2	
European Union	1	3.25			
Finland	1 (1)	3.25 (1)			(1) Binding limit value
France	1	3.25			
Germany (AGS)	0.6 (1) 0.06 (2)	1.9 (1) 0.2 (2)	4.8 (1)(3)	15.2 (1)(3)	(1) Workplace exposure concentration corresponding to the proposed tolerable cancer risk (2) Workplace exposure concentration corresponding to the proposed preliminary acceptable cancer risk (3) 15 min average value
Hungary				3	
Ireland	1	3			
Israel	0.5	1.6	2.5 (1)	8 (1)	(1) 15 min average value
Italy	1	3.25			skin
Japan	10				
Japan – JSOH	1 (1)(2) 0.1 (1)(3)				(1) Reference value corresponding to an individual excess lifetime risk of cancer (2) Individual excess lifetime risk of cancer 10^{-3} (3) Individual excess lifetime risk of cancer 10^{-4}
Latvia	1	3.25			
New Zealand	1		2.5		
Poland		1.6			
Romania	1	3.25			
Singapore	1	3.18			
Republic of Korea	1	3	5	16	
Spain	1	3.25			Skin
Sweden	0.5	1.5	3 (1)	9 (1)	(1) 15 min average value
Switzerland	0.5	1.6			
The Netherlands		3.25			
Turkey	1	3.25			

Table 1.6 (continued)

Country	Limit value – 8 h		Limit value – short-term		Remarks
	ppm	mg/m³	ppm	mg/m³	
USA – NIOSH	0.1	0.32	1 (1)	3.2	(1) Ceiling limit value (15 min)
USA – OSHA	1		5		
United Kingdom	1				

Current OELs are reported here but are subject to revisions over time

AGS, German Committee on Hazardous Substances; h, hour(s); JSOH, Japan Society for Occupational Health; min, minute(s); NIOSH, National Institute for Occupational Safety and Health; NR, not reported; OEL, occupational exposure limit; OSHA, Occupational Safety and Health Administration; ppm, parts per million; TRK, technical guidance concentration

From GESTIS (2017)

(b) Europe

The European Union and most European countries have an OEL of 1 ppm, as does the Scientific Committee on Occupational Exposure Limits (SCOEL) (from 1991), but a few countries have opted for lower values (Table 1.6). The biological exposure limits set by the committee are 28 µg of benzene per litre of blood and 46 µg SPMA per gram of creatinine (SCOEL, 2014). The OEL set by the European Chemicals Agency (ECHA) is 1 ppm (3.25 mg/m³) (Annex III of Directive 2004/37/EC, European Commission, 2004).

In Germany, the Committee for Hazardous Substances has proposed a tolerable risk of 4 : 1000 and an acceptable risk of 4 : 10 000 (changing to 4 : 100 000), applicable over a working lifetime of 40 years with continuous exposure every working day. For benzene, the tolerable and acceptable risks correspond to 8-hour concentrations of 1.9 mg/m³ and 0.2 mg/m³ (0.02 mg/m³ by 2018), respectively (Bau, 2013).

1.5.2 Environmental exposure limits

(a) Air

The World Health Organization (WHO) states that there is no safe level of exposure to benzene; for general guidance, the concentrations of airborne benzene associated with excess lifetime risks of leukaemia of 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} are 17, 1.7, and 0.17 µg/m³, respectively (WHO, 2000). The benzene air concentration limit in Europe since 1 January 2010 is 5 µg/m³ averaged over 1 year (European Commission, 2008). The maximum limit value for benzene in petrol (gasoline) is 1.0% v/v limit (Directive 2009/30/EC, European Commission, 2009).

The United States EPA has specified cancer risk levels: 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} risk, corresponding to concentrations of 13–45, 1.3–4.5, and 0.13–0.45 µg/m³, respectively. The EPA reference concentration, the estimated continuous inhalation exposure without risk to health, is 3×10^{-2} mg/m³ (EPA, 2000).

The United States Agency for Toxic Substances and Disease Registry has derived minimal risk levels for acute duration (≤ 14 days) of 0.009 ppm, intermediate duration (15–364 days) of 0.006 ppm, and chronic duration (≥ 365 days) of 0.003 ppm (ATSDR, 2007).

WHO guidelines for indoor air recommend reducing indoor benzene concentrations to the lowest achievable level by eliminating indoor sources of benzene and adjusting ventilation (WHO, 2010).

(b) Water

WHO guidelines for drinking-water recommend a maximum concentration of benzene of 0.01 mg/L (WHO, 2003, 2008). The European Council Directive 98/83/EC on the quality of water intended for human consumption (adopted in 1998) has set the benzene limit to 0.001 mg/L water (European Commission, 1998).

The United States EPA sets regulatory limits for the amount of benzene contaminants in water provided by public water systems: specified cancer risk levels of 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} correspond to drinking-water concentrations of 100–1000, 10–100, and 1–10 µg/L, respectively. The EPA reference dose is 4×10^{-3} mg/kg per day (EPA, 2000).

1.6 Exposure assessment methods in epidemiological studies of cancer

1.6.1 Industry-based studies of occupational exposure

Selected epidemiological studies of cancer and occupational exposure are summarized in Table 1.7. The most common metrics of benzene exposure in these studies are the presumption of occupational exposure by duration (years),

Table 1.7 Exposure assessment method for selected occupational epidemiological studies of exposure to benzene

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert exposure estimation for individual participants' work histories based on measured benzene exposure data	Distribution workers in Canadian petroleum industry; retrospective estimates account for job, site, and era in participants' work histories; base estimates from exposure measurements	Mean intensity 0.0–6.16 ppm [0.0–19.6 mg/m^3]; cumulative exposure 0.0–219.8 ppm-yr [0.0–702.1 (mg/m^3)-yr]; dermal exposure ranking	Work histories and site information well characterized; exposure estimates based on personal measurements collected from 1970 onwards; some task-based hydrocarbon measurements used to validate estimates	Relatively few data points for some base estimates; extrapolation back to as early as 1910 increases uncertainty; potential for other hydrocarbon exposures	Armstrong et al. (1996)	Schnatter et al. (1996)
	Marketing and distribution workers in UK petroleum industry; retrospective estimates of each job or task in the participants' work histories; base estimates developed from exposure measurements adjusted using modifying factors (e.g. job activity, % benzene in fuel, loading technology)	Mean intensity < 0.02 to ≥ 0.4 ppm [< 0.06 to ≥ 1.28 ppm]; cumulative exposure < 0.45 to ≥ 45 ppm-yr [< 1.44 to ≥ 143 (mg/m^3)-yr] Peaks by frequency: daily, weekly, monthly; intensity: 1–3 ppm [3–10 mg/m^3], > 3 ppm [> 10 mg/m^3]; peaks by duration: 1–15 min, 15–60 min Potential for skin exposure (none, low medium, high)	Based on measured exposure data; background exposure assigned for 40% work histories less likely to need extrapolation	Limited job history information for participants pre-1975; extrapolation back to as early as 1910 increases uncertainty; relatively few data points for some base estimates; potential for other hydrocarbon exposures	Lewis et al. (1997)	Rushton & Romaniuk (1997)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert exposure estimation for individual participants' work histories based on measured benzene exposure data (cont.)	Upstream, refinery, and distribution workers in Australian petroleum industry; retrospective estimates account for each job or task in the participants' work histories; base estimates from exposure measurements adjusted with modifying factors (e.g. exposure differences over time or between worksites)	Intensity group range ≤ 0.1 to > 3.2 ppm [≤ 0.32 to > 10.2 mg/m^3]; cumulative exposure mean and range 4.7 (0.01–57.3) ppm-yr [15.0 (0.03–183) (mg/m^3)-yr]; peak as exposure to products with > 70% benzene	Work histories and site information from companies well established; estimates based on measured exposure data; the majority of participants' exposure in 1970s, so less extrapolation needed	Relatively few data points for some estimates; extrapolation back to as early as 1955 increases uncertainty; potential for other hydrocarbon exposures	Glass et al. (2000)	Glass et al. (2003)
	Petroleum industry workers from Canada, UK, and Australia (see Armstrong et al., 1996; Lewis et al., 1997; Glass et al., 2003); exposure assessment by individual study; pooled data compared and adjusted by country	Mean average intensity (SD) 0.22 (0.7) ppm [0.7 (2.24) mg/m^3], mean maximum intensity (SD) 0.41 (1.3) ppm [1.31 (4.2) mg/m^3]; median cumulative exposure (SD) 5.2 (17.0) ppm-yr [16.6 (54.3) (mg/m^3)-yr]; peaks > 3 ppm [10 mg/m^3] for 15–60 min; dermal exposure likelihood; exposure certainty ranking	All studies used measured exposure data; exposure estimation quality scores allowed sensitivity analyses	Relatively few data points for some base estimates; some extrapolation back to pre-1920; different countries, industry sectors, and eras may limit comparability of exposure estimates; potential for other hydrocarbon exposures	Armstrong et al. (1996), Lewis et al. (1997), Glass et al. (2003, 2010, 2017)	Schnatter et al. (2012), Rushton et al. (2014)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert exposure assessment for individual participants' work histories based on measured benzene exposure data, taking account of job title, site, and era	Workers in two USA waterproof cloth manufacturing facilities; JEMs based on air sampling data and detailed work histories to provide individual time-specific exposure estimates; Rhomberg et al. (2016) used Monte Carlo techniques to estimate exposures used in tertile, quartiles, and quintiles	Cumulative exposure 0.001 to > 400 ppm-yr [0.003 to > 1280 (mg/m^3)-yr] (Rinsky et al., 1987), 6.64 ppm [21.2 mg/m^3] platform, 10.46 ppm [33.4 mg/m^3] scrap area (Utterback & Rinsky, 1995)	Occupational hygiene measurements for some sites; exposure estimates adjusted to era; compared exposure estimates with contemporary TLVs; no other exposures	Limited occupational hygiene measurements at some sites; some measurements are spot samples and area samples (not personal); accuracy of detector tube and combustible gas indicator measurements unclear	Rinsky et al. (1981), Paustenbach et al. (1992), Crump (1994), Utterback & Rinsky (1995)	Schnatter et al. (1996), Rinsky et al. (2002), Rhomberg et al. (2016)
Expert assessment using exposure measurements grouped by work characteristic (e.g. job title, work area, industry)	Workers in USA chemical plant; job titles of workers in three areas of a chemical plant assigned to four exposure categories based on measured data	Cumulative exposure groups: 0–3.9, 4.0–24.9, and > 25 ppm-yr [0–12, 13–79, and > 80 (mg/m^3)-yr] (Collins et al., 2015)	Measured exposure data available, adjusted for time period, department, and job	Some estimates based on few personal measurements per job (extent of personal data unclear); most exposures before 1980 estimated without personal exposure data (Collins et al., 2003); limited detail on exposure estimation methods; potential exposure to other known or suspected human carcinogens	Bloemen et al. (2004)	Collins et al. (2003), Bloemen et al. (2004), Collins et al. (2015)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Categoric expert assessment of exposure to form industry JEM by era (1970–1979, 1980–1989, 1990–1999, 2000–2009)	Workers in Norwegian offshore petroleum industry; industry experts coded workers' job histories into 27 job categories in five job sections; exposure burden score created for each job by summing task scores (multiplying categoric scores from intensity, duration, and frequency); job-STEL scores created by summing task-STEL scores (adjusting categoric scores for frequency of task and peak/task)	Intensity 0–0.040 ppm [0–0.130 mg/m^3]; cumulative exposure 0–0.948 ppm-yr [0–3.03 (mg/m^3)-yr] (Stenehjem et al., 2015); STEL probability score	Expert job grouping, some personal measurement data; limited range of tasks and exposures all likely low; extrapolation back to 1970 only	Limited benzene exposure data available (most post-2000); personal measurement data did not cover all jobs and time periods; potential exposure to other known and suspected human carcinogens	Steinsvåg et al. (2007, 2008), Bråtveit et al. (2011, 2012)	Stenehjem et al. (2015)
Expert assessment of job title/area use of JEM to derive exposure estimates	Workers in 672 Chinese facilities in range of industries in 12 cities (712 factories in Yin et al., 1994); estimated using ambient exposure measurements, and production and process information for seven calendar periods for each job title; individual work histories linked to measured exposure data	For leukaemia cases intensity 6.5–487 mg/m^3, cumulative exposure 37.7–5438.4 (mg/m^3)-yr (Yin et al., 1989)	Individualized exposure assessments based on participants' work histories; exposure estimates predictive of benzene poisonings in a validation paper (Dosemeci et al., 1997)	Comparatively few measured data points in pre-1975 period; most measurements based on short-term ambient samples, not personal measurements; overall, 22% of estimates have a high confidence rating	Dosemeci et al. (1994, 1997), Yin et al. (1994), Portengen et al. (2016)	Yin et al. (1996b), Hayes et al. (1997), Linet et al. (2015)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert assessment of job title and allocation of ppm exposure estimates, then grouped into categories	Chemical workers from seven USA plants; jobs classified as continuous, intermittent, or no exposure; exposure in ppm assigned to tasks in jobs; estimates varied by era	Cumulative exposure presented in three exposure categories: < 180, 180–719, ≥ 720 ppm-mo [< 575, 575–2297, ≥ 2300 (mg/m^3)-mo] (Wong, 1987b)	Exposure based on measured data adjusted for production and process changes for five of the seven plants	Exposure data sparse before 1970, requiring extrapolation to pre-1910; proportion of exposure data obtained via personal versus fixed ambient sampling unclear; limited employment records for two of the seven plants; potential for other unspecified chemical exposures	Wong (1987a,b)	Wong (1987a,b)
Expert assessment from job title based on measured hydrocarbon exposure data and extrapolation for era	Distribution workers in USA petroleum industry; 8-h TWA THC exposure (for each task and summed) and annual frequency of peak exposures estimated for eight job categories in case–control study (four for cohort study) for four eras (pre-1950, 1950–1964, 1965–1974, 1975–1985)	Cumulative exposure in ppm-yr, peak of at least 500 ppm [1600 mg/m^3]; THC averaged over 15–90 min	Individualized exposure assessments based on participant work histories using measured personal and ambient sample data	Based on THC, not benzene; few data for certain jobs; measured data from 1975–1980 extrapolated to earlier periods back to pre-1950	Smith et al. (1993)	Wong et al. (1993, 1999)
Expert assessment of workplace to form JEM, then applied to participants' job histories	Workers in Italian shoe factory; questionnaires used to gather data on determinants of exposure (e.g. amount of glue, % benzene, production rate, work process changes over time); modelled exposure estimates used to form JEM	Intensity 0–92 ppm [0–294 mg/m^3], mean cumulative exposure (SD) 58.4 (93.9), range 0–522.4 ppm-yr [0–1670 (mg/m^3)-yr] (Seniori Costantini et al., 2003) calculated; dichotomization at 40 ppm-yr [128 (mg/m^3)-yr] used (Costantini et al., 2009)	Model validated using measured exposure data; historical changes in benzene concentration in glue and work processes characterized	Complete job history data available only for 16% cohort; no measured personal exposure data; potential for other exposures (i.e. solvents in glues) unspecified	Seniori Costantini et al. (2003)	Costantini et al. (2009)

Table 1.7 (continued)

Exposure assessment method	Description of population and exposure assessment	Exposure metrics reported	Strengths of specific study	Limitations of specific study	Exposure assessment reference	Epidemiology Reference
Expert assessment of job title/area; industry JEM converted to ppm exposure estimates	Gas and electric utility workers in France; TWA estimations (expressed in units of exposure) based on JEM	Cumulative unit-yr (conversion to ppm-yr); range 0 to ≥ 1.98 ppm-yr [0–6.32 (mg/m^3)-yr])	Estimates considered measured occupational data from other studies; exposure estimates accounted for changes in % benzene in petrol	Relative exposures between groups only (e.g. "use of solvents" for cleaning or degreasing and "exposure to gasoline"); no measured data from the population of interest; potential for other exposures (e.g. herbicides, chlorinated solvents, styrene, ionizing radiation)	Guénel et al. (2002)	Guénel et al. (2002)

h, hour(s); JEM, job–exposure matrix; min, minute(s); mo, month(s); ppm, parts per million; SD, standard deviation; STEL, short-term exposure limit; THC, total hydrocarbons; TLV, threshold limit value; TWA, time-weighted average; yr, year(s)

average exposure intensity (ppm or mg/m^3), or cumulative exposure, which is the intensity of exposure multiplied by the number of years exposed (ppm-years or (mg/m^3)-years). These metrics indicate that inhalation is the major route of entry, although some studies have also considered dermal exposure (e.g. Lewis et al., 1997; Schnatter et al., 2012; Rushton et al., 2014). The likelihood of peak exposure (using various definitions of peak) has also been examined in some studies (e.g. Lewis et al., 1997; Schnatter et al., 2012; Stenehjem et al., 2015).

The main sectors where exposure assessment for benzene has been carried out for epidemiological studies are the petroleum industry (e.g. Wong et al., 1993 ; Armstrong et al., 1996 ; Lewis et al., 1997 ; Glass et al., 2000; Steinsvåg et al., 2007, 2008; Bråtveit et al., 2011), the chemical industry (e.g. Wong, 1987a, b), and industries that use benzene in manufacturing processes (e.g. Rinsky et al., 1981; Yin et al., 1994; Utterback & Rinsky, 1995), including shoemaking (Seniori Costantini et al., 2003).

Exposure to benzene is often assessed by experts who group workers by job or facility (where appropriate) and then assign exposure to each person using a job–exposure matrix, which may have a time dimension. The exposure estimates in the job–exposure matrix may be quantitative, that is, based on personal and/or area benzene sampling (Rinsky et al., 1981), or they may be semiquantitative. Relative measures can later be translated into benzene concentration (e.g. ppm or mg/m^3) (Guénel et al., 2002; Bråtveit et al., 2012). Very large studies where multiple experts examine different facilities can increase variability in assessments, but this can be mitigated by standardization across facilities (e.g. Portengen et al., 2016).

Most cohort studies and their nested case–control studies are retrospective, with some including participants from as early as 1910 (Wong, 1987a, b; Armstrong et al., 1996; Lewis et al., 1997). Because exposure data were sparse before 1970, the validity of exposure estimates extrapolated to earlier time periods may be uncertain (e.g. Rinsky et al., 1981; Utterback & Rinsky, 1995; Collins et al., 2015). Even for recent time periods, measured data may not be available or may be inadequate to describe exposures from all jobs. In some studies, data from one facility may be attributed to workers at a similar facility, for example offshore workers on different platforms (Bråtveit et al., 2012; Stenehjem et al., 2015). These differences in data availability may result in varying exposure assessments and outcomes (see Section 2.1.1).

Personal sampling data became more common from the 1970s onwards. Recent studies are therefore more likely to assess exposure using personal measurement data, from which more robust exposure estimates can be derived. It is preferable to assess a high proportion of the participants' time at risk of exposure with contemporary exposure measurement data (Glass et al., 2000; Vlaanderen et al., 2010). When personal measurement data are available, temporal and between-worker exposure variability should be considered (Kromhout et al., 1993).

Changes in facilities over time have been considered in some studies listed in Table 1.7; for example, Dosemeci et al. (1994) and Wong (1987a) took production rate into account. Portengen et al. (2016) used a modelling process to consider several factors affecting exposure. Some studies incorporated factors to account for changes over time and between sites, for example due to changing technology and variations in products handled (Armstrong et al., 1996; Lewis et al., 1997; Glass et al., 2000).

Uncertainty is also introduced when exposure to benzene is based on modelling from total hydrocarbon exposure, as the proportion of benzene may vary with the source of the hydrocarbons (e.g. Smith et al., 1993).

Studies based mainly on grab or area sampling data (e.g. Rinsky et al., 1981; Dosemeci

et al., 1994; Yin et al., 1994) have been used to derive average long-term exposure estimates, which can be less certain than those based on individual-level measurement data collected over longer periods (e.g. full work shifts).

Other exposures may have been incurred by participants in the studies listed in Table 1.7, for example, from other hydrocarbons for petroleum industry workers. Coexposures identified in these studies are listed in the limitations column. Some coexposures, for example styrene, have been associated with an increased risk of leukaemia (e.g. Guénel et al., 2002). Other exposures may not have been described, including low exposure to X-rays for some petroleum industry workers and possibly 1,3-butadiene for some refinery workers (Akerstrom et al., 2016; Almerud et al., 2017).

The application of validation methods can increase confidence in the exposure estimates. Such methods include the use of exposure estimation quality scores (e.g. Schnatter et al., 2012) and the assessment of interrater agreement (e.g. Steinsvåg et al., 2008).

1.6.2 General population studies

(a) Childhood cancer

Epidemiological studies focused on associations between benzene in outdoor air pollution and risks of childhood cancer in Denmark, France, Italy, and the USA. Primary methods to assess exposure to benzene are summarized for selected studies in Table 1.8, which provides a summary of the general approach and metric(s) that were used, along with strengths and limitations. All the studies used a geographical information system (GIS) to manage spatially referenced data from different sources in their benzene exposure assessments.

One investigation (Heck et al., 2014) used routine air monitoring data from 1990 to 2007 (collected every 12 days) from 39 monitors in the state of California (163 696 square miles or 423 970 km^2), USA, and developed exposure estimates by linking maternal residences to the closest outdoor air monitor. However, not all monitors were operating throughout the study period; for example, in 2008 there were only 17 benzene monitors in operation (Cox et al., 2008). In addition, stationary monitors were often sited near heavy industry, busy freeways, or in agriculturally rich areas (Heck et al., 2014).

All other key studies relied on Gaussian dispersion models to predict outdoor benzene concentrations in air, for example: the California Line Source Dispersion model, version 4 (CALINE4) (Vinceti et al., 2012), the Danish Operational Street Pollution Model (Raaschou-Nielsen et al., 2001), or the EPA Assessment System for Population Exposure Nationwide (ASPEN) (Symanski et al., 2016; Janitz et al., 2017). Developed by the Department of Transportation in California, USA, CALINE4 is an air dispersion model for roads (and other linear air pollutant sources) used to estimate outdoor air concentrations of benzene and other contaminants at defined locations in a given area. The National-Scale Air Toxics Assessment (NATA) uses ASPEN, a dispersion model that relies upon a national inventory of emissions data for hazardous air pollutants, as well as other characteristics that affect the fate and transport of pollutants in the environment (e.g. the rate, location, and height of release of pollutants, and wind speed and direction).

The CALINE4 model used in the Italian study by Vinceti et al. (2012) used locally collected traffic flow data for a single year, but relied on vehicular emission factors over a longer period (1990–2007). One drawback in using the ASPEN model is that modelled estimates are only available for selected years (i.e. 1996, 1999, 2002, 2005, and 2011). Symanski et al. (2016) used all available estimates at the time of their study (until 2005) whereas Janitz et al. (2017) relied on data for a single year (2005). Because the NATA model inputs change over time, Symanski et al. (2016)

Table 1.8 Exposure assessment from selected environmental epidemiological studies of benzene exposure

Exposure assessment method	Location	Exposure metrics reported	Strengths[a]	Limitations[a]	Reference
Childhood cancers: routine air monitoring data					
Outdoor air measurements of benzene obtained from stationary (state) regulatory monitors that collected 24-h samples every 12 d; linked to geocoded participant residences (buffer of 2 km for ALL and 6 km for AML)	California, USA	Residential benzene levels (μg/m^3) calculated for each maternal trimester of pregnancy, entire pregnancy period, and child's first year of life	Exposure estimates available for pregnancy and childhood (first year of life) periods	Variable distances between residences and closest monitor; unable to account for residential mobility during pregnancy or the first year of life	Heck et al. (2014)
Childhood cancers: Gaussian dispersion models					
Modelling outdoor air concentrations of benzene from vehicular emissions at geocoded residential addresses using CALINE4 (considers traffic flow, vehicle emissions factors, and meteorological data)	Northern Italy	Quartiles of average annual residential benzene levels < 0.10, ≥ 0.10–0.25, ≥ 0.25–0.50, and ≥ 0.50 μg/m^3; quartiles of maximum hourly residential benzene levels < 2, ≥ 2–4, ≥ 4–6, and ≥ 6 μg/m^3	Validation conducted with air measurements from monitoring stations; considered coexposures to PM_{10}	Uncertainty associated with emissions and traffic data sources, and use of a single calendar year to estimate exposures; limited validation due to small number of air monitoring stations; Pearson's correlation coefficient of 0.43 between modelled and monitored (n = 6 monitors) data; unable to account for residential mobility during childhood (up to 14 yr from birth)	Vinceti et al. (2012)
Modelled annual census tract level estimates of outdoor benzene levels for 1996, 1999, 2002, and 2005 from the US EPA NATA linked to geocoded maternal addresses at birth of infants	Texas, USA	Quartile estimates of outdoor benzene levels (based on the distribution in the controls for each NATA year): low, medium, medium–high, and high	NATA estimates account for point, mobile, and area sources of benzene emissions; considered coexposures to 1,3-butadiene and PAHs	Modelled annual estimates available only at the census tract level for specific year (1996, 1999, 2002, and 2005); unable to account for residential mobility during pregnancy and early childhood (up to 4 yr from birth)	Symanski et al. (2016)
Address at birth linked to the census tract concentration for benzene using the 2005 US EPA NATA database (see Symanski et al., 2016)	Oklahoma, USA	Quartiles of estimated outdoor benzene levels: 0.11 to < 0.39, 0.39 to < 0.67, 0.67 to < 0.91, and 0.91–2.03 μg/m^3	NATA estimates account for point, mobile, and area sources of benzene emissions	Modelled annual estimates of outdoor benzene levels for 1 yr only (2005) to assess exposure at birth; unable to account for residential mobility during pregnancy and childhood (up to 19 yr from birth)	Janitz et al. (2017)

Table 1.8 (continued)

Exposure assessment method	Location	Exposure metrics reported	Strengths[a]	Limitations[a]	Reference
Annual benzene concentrations for grids of area 10 m^2 (Paris), 25 m^2 (inner suburbs), or 50 m^2 (outer suburbs), estimated from a dispersion model linked to air monitoring data; benzene estimates linked to geocoded addresses at the time of diagnosis (cases) or inclusion (controls), and data on proximal roadways using the Navteq database	Paris and surrounding areas, France	Subjects were classified based on whether estimated annual benzene concentration at their residence was < 1.3 μg/m^3 (median exposure for the controls) or ≥ 1.3 μg/m^3; major road length classified as low, medium, and high	Use of an air dispersion model to account for vehicular emissions, meteorology, and information on fate, transport, and transformation of pollutants; inclusion of a term to account for local traffic in the exposure metric	Unable to account for residential mobility before time of diagnosis (up to 14 yr from birth)	Houot et al. (2015)
Modified version of the Operational Street Pollution Model used to estimate average residential exposure to benzene; estimated air concentrations based on measurements made during 1994–1995 at four sites; included other info (e.g. traffic pattern, vehicle emission factors, meteorology); residential history ascertained from the Danish population registry	Denmark	Tertiles of cumulative exposure to benzene (in 1000 ppb-d): < 0.5, 0.5 to < 1.3, and ≥ 1.3	Temporally resolved estimates during pregnancy and during the child's life; accounted for residential mobility; validation conducted with 204 air measurements in urban and rural locations	Did not account for sources of pollution other than traffic; benzene exposure estimated using measurements taken after case diagnosis (1968–1991); validation results indicate poor correlation in rural areas between modelled and monitored results	Raaschou-Nielsen et al. (2001)
Cancer in adults					
Self-reported via completed questionnaires	Rochester, Minnesota, USA	Ever exposed regularly to benzene or derivative (yes/no)	May capture exposure to benzene in work and non-work environments	Little contrast in exposure metric; recall of exposures may not be accurate	Antwi et al. (2015)
Address at baseline was linked to the census tract concentration for benzene using the 2005 US EPA NATA database (see Symanski et al., 2016)	California, USA	Quintile estimates of outdoor benzene levels	NATA estimates account for point, mobile, and area sources of benzene emissions	Use of modelled annual estimates of outdoor benzene levels for 1 yr (2005) to assess exposures at baseline; unable to account for residential mobility during follow-up period; no information about exposure in non-residential environments, exposure to cigarette smoke, and housing characteristics that may influence exposures at home	Garcia et al. (2014)

Table 1.8 (continued)

Exposure assessment method	Location	Exposure metrics reported	Strengths[a]	Limitations[a]	Reference
Reconstructed levels of benzene (from multiple sources of contamination) in the water distribution system using fate and transport models linked to residential histories of navy and marine personnel stationed at the base	US Marine Corps Base, Camp Lejeune, North Carolina	Quartiles of cumulative exposure (μg/L-mo) to benzene: < 2, 2–45, > 45–110, and > 110–601	Rigorous methods used to reconstruct solvent contamination of drinking-water sources	Inaccuracies in residential histories likely; did not account for time spent away from the base for training or deployment	Bove et al. (2014)

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CALINE4, California Line Source Dispersion Model, version 4; d, day(s); h, hour(s); mo, month(s); NATA, National-Scale Air Toxics Assessment; PAHs, polycyclic aromatic hydrocarbons; PM_{10}, particulate matter of diameter < 10 μm; ppb, parts per billion; US EPA, United States Environmental Protection Agency; yr, year(s)

[a] List not exhaustive

conducted a sensitivity analysis by limiting the study population to cases and controls born within 1 year of a NATA release; estimated odds ratios were similar in magnitude, but less precise. Two investigations focused their assessments on exposures due to emissions from vehicular traffic: Raaschou-Nielsen et al. (2001) and Vinceti et al. (2012).

Houot et al. (2015) derived final estimates at geocoded locations using geostatistical methods that combined the dispersion modelled data for a 10 m^2 grid in the city of Paris, a 25 m^2 grid in the inner suburb, and a 50 m^2 grid in the outer suburb with available air monitoring data.

Studies based in the USA (Symanski et al., 2016; Janitz et al., 2017) used NATA estimates that were generated for all census tracts within the continent of North America. Census tract boundaries are drawn based on population size (average population size, 4000 people) and therefore vary by size and shape.

Because the exposure assessments in the reviewed studies relied on a records-based linkage to develop the exposure metrics, there was no response or recall bias in the exposure assessments. The study by Raaschou-Nielsen et al. (2001) offered an advantage over other studies because it addressed residential mobility in estimates of cumulative exposure; residential histories obtained from a national database, from 9 months before birth to the time of diagnosis, were used. All other studies relied on a single residence (either at birth or at the time of diagnosis) upon which to base the exposure assessment. The use of a single residence may have increased uncertainty in the exposure assessments, particularly in studies that included children diagnosed at older ages (e.g. 14–19 years) (Vinceti et al., 2012; Houot et al., 2015; Janitz et al., 2017).

A strength of the studies by Raaschou-Nielsen et al. (2001) and Heck et al. (2014) was their ability to construct temporally resolved estimates of exposure during pregnancy and childhood that allowed for an assessment of exposure at different life stages. However, none of the studies incorporated information on time spent away from the residence for the mother or the child and, by not accounting for exposures in other environments (e.g. maternal exposures at work), uncertainty in the exposure assessments was likely introduced.

Outdoor air includes multiple pollutants from diverse natural and anthropogenic sources; the air pollutant mixture can therefore vary both locally and regionally. Methods for addressing multiple exposures included the application of co-pollutant models (Symanski et al., 2016) and factor analysis (Heck et al., 2014). Information on indoor air sources of benzene (e.g. environmental tobacco smoke) was unavailable in all studies, as was information on housing characteristics (e.g. living in a residence with an attached garage); only one investigation had information about maternal smoking (Symanski et al., 2016).

In most of the studies, the control population (all of the investigations in Table 1.8 used a case–control study design) represented the source population and was therefore unlikely to be affected by exposure-related selection bias. However, some bias may have been introduced in the investigation by Heck et al. (2014) who excluded 2978 cases and 142 188 controls from the parent study because residences were not within defined buffers around a stationary air monitor (2 km for acute lymphoblastic leukaemia and 6 km for acute myeloid leukaemia). Vinceti et al. (2012) also excluded individuals living in mountainous areas (< 10% of the total population in the study area) because the CALINE4 dispersion model was not developed to incorporate rocky terrain in predicting air pollutant concentrations near roadways.

Vinceti et al. (2012) presented results from a validation study and, based on measurements collected at six monitoring stations, reported a modest correlation (Pearson correlation coefficient, 0.43) between the CALINE4 modelled

estimates and outdoor air benzene levels. Raaschou-Nielsen et al. (2001) compared the results from their dispersion model with passive sampler measurements of benzene at various street locations in Copenhagen, Denmark and in rural areas. Pearson correlation coefficients of 0.62–0.68 were reported for urban locations (range in values based on differences in meteorological inputs); correlations were much lower for rural locations (0.15–0.19) where there is little variation in traffic levels. Regarding the NATA data, previous studies reported good agreement between the ASPEN modelled estimates and monitored levels of benzene in ambient air (Symanski et al., 2016).

(b) Cancer in adults

Studies on cancer risks associated with environmental benzene exposure have used a variety of approaches in their exposure assessments (see Table 1.8 for a summary).

In a nested case–control study of 82 cases and 83 controls among lifelong never-smokers of the Shanghai Cohort Study (a prospective cohort of 18 244 Chinese men, aged 45–64 years at enrolment) (Yuan et al., 2014), exposures to benzene were assessed using SPMA based on measured concentrations of stored urine samples collected at baseline. While SPMA is a specific biomarker for benzene exposure, its half-life in the body is relatively short; relying on a single urinary measurement of SPMA is problematic as it is not representative of average exposure.

Two drinking-water systems at the United States Marine Corps Base, Camp Lejeune, North Carolina were contaminated with tetrachloroethylene and other solvents, including benzene, from 1975 until February 1985. Bove et al. (2014) reconstructed monthly contaminant levels in the water distribution system using fate and transport models; these were linked to residential histories of marine and navy personnel living at the base to generate lagged (10-, 15-, and 20-year) and unlagged estimates of cumulative exposure. Exposures may have been misclassified due to errors in the reconstructed levels of benzene in the water distribution system, as well as inaccuracies in identifying units assigned to the base, in determining the location of the barracks or housing for marine/navy personnel with families, or in accounting for time spent away from the base for training or deployment.

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2. CANCER IN HUMANS

The published evidence on the association between benzene exposure and cancers of the lymphatic and haematopoietic system was last reviewed in *IARC Monographs* Volume 100F (IARC, 2012a), when it was concluded that there was *sufficient evidence* in humans for acute myeloid leukaemia (AML)/acute non-lymphocytic leukaemia (ANLL) and *limited evidence* for acute lymphocytic leukaemia (ALL), chronic lymphocytic leukaemia (CLL), multiple myeloma (MM), and non-Hodgkin lymphoma (NHL).

This Working Group reviewed the association between benzene exposure and cancers of the lymphatic and haematopoietic system again, including those studies considered in *IARC Monographs* Volume 100F as well as studies published since that review in 2009. According to the 2017 *WHO Classification of Tumours of Haematopoietic and Lymphatic Tissues* (Swerdlow et al., 2017), the Working Group considered AML and myelodysplastic syndrome (MDS) as well as chronic myeloid leukaemia (CML) and myeloproliferative disorder (MPD) in the broader category of leukaemia; the category of lymphomas was considered to include NHL as well as its various subtypes (e.g. MM, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and hairy cell leukaemia (HCL)), CLL, ALL, and Hodgkin lymphoma (HL). These studies are reviewed in Sections 2.1 and 2.2, respectively.

The Working Group also reviewed all available studies of the association between benzene exposure and other cancers in children and adults published before and after *IARC Monographs* Volume 100F. These reviews are presented in Sections 2.3 and 2.4, respectively.

Studies of adult cancers in occupational cohorts and in the general population are considered separately in Sections 2.1, 2.2, and 2.4, due to differences in approaches to the assessment of benzene exposure and the analysis of data according to the study setting.

Although tobacco smoke is an important source of benzene exposure for the population at large, accounting for half of population exposure to benzene in the USA (American Cancer Society, 2016), the Working Group did not review studies of smoking-related exposures, because tobacco smoke contains numerous correlated components that could confound the effects of benzene. Studies of tobacco smoking and exposure to secondhand tobacco smoke are reviewed in *IARC Monographs* Volume 100E (IARC, 2012b).

2.1 Adult leukaemia

2.1.1 Occupational cohort studies

(a) Introduction

This section reviews epidemiological studies of leukaemia in occupational cohorts, including occupational cohort studies and nested case–control analyses of such studies. Data on adult leukaemia in non-occupational cohort studies

and population-based case–control studies are reviewed in Section 2.1.2.

Benzene was first classified as a human carcinogen with *sufficient evidence* in *IARC Monographs* Supplement 1 and Volume 29 (IARC, 1982). Substantial support for this classification has since come from associations between exposure to benzene and leukaemia, particularly AML/ANLL, in several occupational cohorts described in *IARC Monographs* Supplement 7 (IARC, 1987) and later in *IARC Monographs* Volume 100F, compiled in 2009 (Baan et al., 2009; IARC, 2012a).

Among the studies that were published after the period covered by *IARC Monographs* Volume 100F, the Working Group chose not to consider results for broad aggregations of different cancer types, including "haematopoietic cancers", "leukaemia", or "myelogenous leukaemia" (Richardson, 2009; Merlo et al., 2010; Koh et al., 2011, 2014; Bonneterre et al., 2012); these diagnostic categories are not specific enough or sufficiently informative. Studies of occupational groups where exposure to benzene was not clearly documented and characterized were also excluded (Gudzenko et al., 2015). First, the main features of occupational cohort studies considered in this chapter are described (Table 2.1). Leukaemia risks associated with benzene exposure by histological type are described in the following sections for each of the cohort studies.

(b) Studies published since IARC Monographs Volume 100F

(i) Petroleum distribution workers

Three cohort studies of petroleum distribution workers conducted in Australia (Glass et al., 2003), Canada (Schnatter et al., 1996), and the United Kingdom (Rushton & Romaniuk, 1997) were updated with new cases of cancers of the lymphatic and haematopoietic system diagnosed up until December 2006 (Australia), 1994 (Canada), and 2005 (United Kingdom), and were pooled for reanalysis using a nested case–control study design (Schnatter et al., 2012). Only male cases and matched controls were included in the analysis (370 leukaemia cases and 1587 controls). All leukaemia diagnoses were reviewed by haematopathologists, who reclassified 8 leukaemia cases of the original publications to MDS or MPD. Benzene exposure was reassessed to allow comparability among the three studies, using exposure measurement data and individual work histories obtained from company records in Canada and the United Kingdom, or from trained interviewers in Australia. Six exposure metrics were derived: cumulative exposure (ppm-years), average intensity (ppm), maximum intensity (ppm, i.e. the highest job-specific exposure estimate), duration of employment (years), peak exposure (yes/no, when employed in a particular job for at least 1 year and having experienced > 3 ppm exposure for 15–60 minutes at least weekly), and dermal exposure (no, low, medium, high; defined as the highest job-specific probability of skin contact for at least 1 year). [The strengths of this study included the high quality of the assessment of benzene exposure and of diagnostic classification. The size of the study was relatively large, but small numbers were available in some subgroup analyses. Scarce or no information on potential confounders (e.g. smoking or multiple exposures other than benzene at the workplace) was available.]

(ii) Dow Chemical workers, Midland, Michigan

A retrospective cohort mortality study of 2266 workers exposed to benzene at Dow Chemical plant in Michigan (USA) (Bloemen et al., 2004) (included in *IARC Monographs* Volume 100F, Table 2.1, available at: http://publications.iarc.fr/123) was later updated (Collins et al., 2015). Vital status and cause of death were derived from the company's research database, regularly updated from several sources including the National Death Index. The follow-up, starting in 1940, was extended by

Table 2.1 Occupational cohort studies of exposure to benzene and leukaemia subtypes in adults

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schnatter et al. (2012) Australia, Canada, UK 1981–2006 (Australia), 1964–1994 (Canada), 1950–2005 (UK) Nested case–control	Cases: 370 diagnoses based on incidence and mortality data (hospital records, cancer registries, death certificates) Controls: 1587, 5 age-matched (Australia) or 4 age- and company-matched (Canada and UK) controls selected using incidence density-based sampling Exposure assessment method: quantitative measurements; exposure assessment was conducted at the job/ worksite/era level, based on routinely collected industry exposure measurements; work history was collected from company records (Canada and UK) or through interview and company records (Australia)	Leukaemia (AML)	Cumulative exposure tertiles (ppm-yr)			NR	Exposures are relatively low; MDS (potentially previously reported as AML) may be the more relevant health risk for such low exposure; strongest suggestion of a risk of MPD is for the exposure time window 2–20 yr (reported in Glass et al., 2014); based on limited data, smoking was unlikely to be a confounder Strengths: large study size; review of diagnosis by haematopathologists; re-assessment of exposure across the three studies Limitations: smoking data were incomplete
			≤ 0.348	20	1.00		
			0.348–2.93	19	1.04 (0.50–2.19)		
			> 2.93	21	1.39 (0.68–2.85)		
		Leukaemia (CML)	Cumulative exposure tertiles (ppm-yr)			NR	
			≤ 0.348	4	1.00		
			0.348–2.93	16	5.04 (1.45–17.50)		
			> 2.93	8	2.20 (0.63–7.68)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 workers exposed to benzene at a chemical plant Exposure assessment method: quantitative measurements; job titles were assigned to exposure categories by an industrial hygienist, based on IH measurements (JEM)	Leukaemia (AML): C92.0	Cumulative exposure (ppm-yr) 0–3.9 4.0–24.9 ≥ 25 Trend test *P* value, 0.88	 0 3 2	 0 (0–2.50) 1.87 (0.39–5.47) 1.39 (0.17–5.03)	Age, race, sex	Third update of the Dow Chemical plant retrospective cohort; one death for MDS, which was reported from the high-exposure group (SMR, 25.05; 95% CI, 0.63–139.58) Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: mortality study based on death certificates

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Stenehjem et al. (2015) Norway 1965–1999/1999–2011 Cohort	24 917 male petroleum workers; offshore oil industry workers for at least 20 days during 1965–1999, all men, extracted from a cohort who responded to a survey conducted with postal questionnaires Exposure assessment method: quantitative measurements; a JEM was developed using monitoring data and job-specific information, giving semiquantitative estimates; JEM scores converted into corresponding ppm values	Leukaemia (myeloid): ICD-10 (codes C92, D45–7)	Cumulative exposure tertiles (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	Nested case–cohort study based on an updated cohort of Norwegian offshore workers; evidence of dose-related patterns for cumulative exposure, exposure intensity and peak exposures for AML; weak links with duration; risks are higher for those with first exposure before 1980 Strengths: prospective case–cohort design; data from Cancer Registry of Norway ensure a high degree of completeness; independent exposure estimates developed for this cohort; analyses adjusted for some confounders Limitations: potential recall bias for distant occupations (non-differential); individual differences in exposure within each occupational group could not be taken into account
			T1 (< 0.001–0.037)	5	1.12 (0.31–4.01)		
			T2 (> 0.037–0.123)	4	1.12 (0.30–4.23)		
			T3 (0.124–0.948)	6	2.24 (0.65–7.71)		
			Trend test *P* value, 0.188				
		Leukaemia (AML): ICD-10 (code C92.0)	Cumulative exposure tertiles (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	
			T1 (< 0.001–0.037)	2	1.40 (0.18–11.00)		
			T2 (> 0.037–0.123)	1	0.85 (0.08–9.29)		
			T3 (0.124–0.948)	5	4.85 (0.88–27.00)		
			Trend test *P* value, 0.052				
		NHL (CLL): ICD-10 (codes C83.0, C91.1)	Cumulative exposure tertiles (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	
			T1 (< 0.001–0.037)	4	6.23 (0.71–54.00)		
			T2 (> 0.037–0.123)	2	3.08 (0.28–34.00)		
			T3 (0.124–0.948)	5	6.74 (0.75–60.00)		
			Trend test *P* value, 0.212				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rhomberg et al. (2016) USA 1940–1996 Cohort	1696 workers from three rubber manufacturing plants (Pliofilm) for at least 1 d Exposure assessment method: quantitative measurements; updated benzene exposure estimates based on job classifications, reconstructed by additional interviews of former workers	Leukaemia (AML)	Cumulative exposure quintiles (ppm-yr)			NR	One of many re-evaluations of the Pliofilm cohort; evidence of a threshold effect and relevant exposure window (exposure within 10 yr of cancer onset appeared to be most relevant) Strengths: re-evaluated benzene exposure estimates based on quintiles Limitations: mortality-based; no control for potential confounders; low number of cases; no new cases; exposure reassessment for this cohort was based on few additional data and was supported by the chemical industry; elevated estimates increase the likelihood of observing an apparent threshold
			< 1.55	0	0 (0–8.88)		
			1.55–6.33	0	0 (0–8.68)		
			6.34–20.24	0	0 (0–8.57)		
			20.25–80.10	0	0 (0–7.53)		
			> 80.11	6	10.11 (3.71–22.01)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ireland et al. (1997) USA 1940–1977/ through 1991 Cohort	4172 hourly male chemical plant workers who began employment during 1940–1977 Exposure assessment method: expert judgement; benzene-using departments: nitrobenzene, phenol, chlorobenzene, muriatic acid, and alkylbenzene production; most exposures estimated by IH judgement with information on process changes	Leukaemia: all (AML, ALL, CML, CLL) ICD-8 (codes 204–207)	Cumulative exposure (ppm-mo)			Age	Cumulative exposures were low compared with rubber hydrochloride cohort Strengths: examined exposure categories and number of days with peak exposures Limitations: collection of exposure data began in 1980 when only chlorobenzene and muriatic acid departments were still running, so most exposure assignments were estimated by industrial hygienists (including during 1940s–1950s, when exposure data were very sparse); death certificates were the primary ascertainment source; some leukaemias likely missed or misclassified; possibility of exposure to contaminants in coal-tar-derived benzene used at facility; benzene exposures for maintenance workers could not be estimated
			Unexposed	5	1.1 (0.4–2.6)		
			< 12	2	2.5 (0.3–8.9)		
			12–72	0	0 (0–5.4)		
			≥ 72	3	4.6 (0.9–13.4)		
		Leukaemia: acute nonlymphatic	Cumulative exposure (ppm-mo)			Age	
			Unexposed	2	1.4 (0.2–5.0)		
			< 12	1	3.7 (0.1–20.6)		
			12–72	0	0 (0–44.1)		
			≥ 72	1	4.5 (0.1–25.3)		
		NHL (CLL)	Cumulative exposure (ppm-mo)			Age	
			Unexposed	1	1.0 (0–5.5)		
			< 12	1	5.9 (0.1–32.6)		
			12–72	0	0 (0–24.7)		
			≥ 72	1	6.7 (0.2–37.7)		
		Multiple myeloma	Cumulative exposure (ppm-mo)			Age	
			Unexposed	1	0.5 (0–2.8)		
			< 12	0	0 (0–10.1)		
			12–72	2	6.8 (0.8–2.5)		
			≥ 72	1	3.7 (0.1–20.1)		
		Hodgkin lymphoma	Cumulative exposure (ppm-mo)			Age	
			Unexposed	0	0 (0–3.3)		
			< 12	0	0 (0–16.8)		
			12–72	0	0 (0–21.4)		
			≥ 72	0	0 (0–27.4)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Linet et al. (2015) China, 12 cities 1972–1987/1972–1999 Cohort	73 789 benzene-exposed and 35 504 unexposed Chinese workers; spray and brush painting (coatings), rubber, chemical (including pharmaceutical manufacturing), shoemaking, and other (including printing and insulation) industries Exposure assessment method: records; workers dichotomized (benzene-exposed/unexposed) based on job titles and factory records of use of benzene-containing materials	Pharynx (nasopharynx): ICD-8 (code 147)	Exposed	29	1.9 (0.9–4.3)	Sex, attained age, attained calendar year	Update of the NCI-CAPM cohort; supersedes Yin et al. (1996a), Hayes et al. (1996); lag 2 yr for HLD, 10 yr for all other outcomes; no unexposed incident cases available for CLL Strengths: large sample size; follow-up of 28 yr Limitations: exposure dichotomized to exposed/unexposed only (no further classification); wide range of industrial processes included; limited control for confounders
		Stomach/gastric cancer	Exposed	211	1.0 (0.8–1.3)	Sex, attained age, attained calendar year	
		NHL (B-cell lymphoma): ICD-8 (codes 202–202); lymphomas and Hodgkin lymphoma	Exposed	31	4.0 (1.6–13.4)	Sex, attained age, attained calendar year	
		NHL (B-cell lymphoma): ICD-9 (codes 202–202); lymphomas and Hodgkin lymphoma	Exposed	31	3.2 (1.4–9.4)	Sex, attained age, attained calendar year	
		NHL (B-cell lymphoma): ICD-9 (codes 202, 202)	Exposed	30	3.9 (1.5–13.2)	Sex, attained age, attained calendar year	
		Multiple myeloma: ICD-9 (code 20)	Exposed	1	0.12 (0.01–0.96)	Sex, attained age, attained calendar year	
		Leukaemia: ICD-9 (codes 204–208)	Exposed	60	2.5 (1.4–4.9)	Sex, attained age, attained calendar year	
		Leukaemia (lymphoid): ICD-9 (codes 204.0, 204.1, 204.2)	Exposed	10	5.4 (1.0–99.3)	Sex, attained age, attained calendar year	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Linet et al. (2015) (cont.)		Leukaemia (ALL): ICD-9 (code 204.0)	Exposed	18	4.5 (0.8–83.9)	Sex, attained age, attained calendar year	
		Leukaemia (myeloid): ICD-9 (codes 205, 206)	Exposed	39	2.2 (1.1–4.6)	Sex, attained age, attained calendar year	
		Leukaemia (AML): ICD-9 (codes 205.0, 206.0, 207.0, 207.1, 207.2)	Exposed	26	2.1 (0.9–5.2)	Sex, attained age, attained calendar year	
		Leukaemia (CML): ICD-9 (codes 205.1, 205.2)	Exposed	13	2.5 (0.8–10.7)	Sex, attained age, attained calendar year	
		Leukaemia: acute, NOS, ICD-9 (code 208.0)	Exposed	6	3.5 (0.6–66.1)	Sex, attained age, attained calendar year	
		Leukaemia: NOS, ICD-9 (codes 208.8, 208.9)	Exposed	5	2.4 (0.4–44.4)	Sex, attained age, attained calendar year	
		NHL (CLL): ICD-9 (codes 204.1, 204.2)	Exposed	2	NR	Sex, attained age, attained calendar year	
		NHL (CLL): ICD-9 (codes 204.1, 204.2)	Exposed	2	NR	Sex, attained age, attained calendar year	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kirkeleit et al. (2008) Norway 1981–2003 Cohort	27 919 offshore petroleum workers registered to the Norwegian registry of employers and employees, and 366 114 matched controls from the general working population Exposure assessment method: other; location of work and job category	Leukaemia (ALL): ICD-9 (code 204.0)	Exposed in upstream offshore workers	1	2.20 (0.30–16.60)	Sex, age, year of first exposure, education	
		Leukaemia (AML)	Exposed in upstream offshore workers	6	2.89 (1.25–6.67)	Sex, age, year of first exposure, education	
		Leukaemia (CML)	Exposed in upstream offshore workers	1	1.44 (0.19–10.70)	Sex, age, year of first exposure, education	
Guénel et al. (2002) France 1978–1989 Nested case–control	Cases: 72 identified among male workers Controls: 285 controls matched to the cases by year of birth Exposure assessment method: expert judgement; JEM developed from expert judgement	Leukaemia (ALL): ICD-9 (code 204.0)	Exposure (benzene unit-yr)			Age matched	
			Never	9	1.0		
			> 0 to < 5.5	1	0.6 (0.1–5.3)		
			> 5.5	2	3.3 (0.3–43.3)		
			Trend test *P* value, 0.16				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wong et al. (1993) USA 1946–1985 Cohort	18 135 employees with potential exposure to gasoline for at least 1 yr at land-based terminals (n = 9026) or on marine vessels (n = 9109) Exposure assessment method: questionnaire	Leukaemia (ALL)	Land-based employees exposed to gasoline	2	1.3 (0.1–4.5)	NR	
			Marine-based employees exposed to gasoline	1	0.8 (0–4.4)		

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; d, day(s); HLD, haematopoietic, lymphoproliferative, and related disorders; ICD, International Statistical Classification of Diseases and Related Health Problems; IH, industrial hygiene; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; mo, month(s); MPD, myeloproliferative disorder; NCI-CAPM, National Cancer Institute-Chinese Academy of Preventive Medicine; NHL, non-Hodgkin lymphoma;
NOS, not otherwise specified; NR, not reported; ppm, parts per million; SMR, standardized mortality ratio; yr, year(s)

13 years to the end of 2009. Industrial hygiene measurements of benzene were used to estimate job-specific exposures over time. The average exposure duration of cohort members was 4.9 years (range, 30 days–44.7 years), and cumulative exposure of the subjects was divided into three categories (0–3.9, 4.0–24.9, and ≥ 25 ppm-years). [The strengths of this study included its long and complete follow-up and comprehensive exposure assessment. However, it was based on mortality rather than incidence, there was no control for potential confounders, and the number of cases was small.]

(iii) Chinese workers

The incidence of and mortality from cancers of the lymphatic and haematopoietic system were studied in a large cohort of Chinese workers comprising 74 828 workers exposed to benzene and 35 805 unexposed workers (National Cancer Institute-Chinese Academy of Preventive Medicine (NCI-CAPM) cohort). The initial follow-up period of 1972–1987, which had a quantitative assessment for exposure to benzene (Hayes et al., 1997), was extended to 1999 using factory records, hospital records, and death certificates (Linet et al., 2015). Benzene exposure assessment was based on factory and job-specific information on the use of material containing benzene, and was limited to classification as ever (for at least 6 months) versus never exposed, preventing any dose–response evaluation. The study included 60 and 13 incident cases of leukaemia of all types in exposed and unexposed workers, respectively. [The strengths of this study included the large size of the cohort, which included both sexes and covered several different industries, and the long follow-up, with small numbers lost to follow-up. Control for potential confounders was limited to sex, age, and calendar period. The numbers of cases were relatively small in some subgroups, particularly among unexposed workers.]

(iv) Norwegian offshore oil workers

Kirkeleit et al. reported on a prospective cohort study of 27 919 workers listed as having been employed in the offshore oil industry in the Norwegian Registry of Employers and Employees between 1981 and 2003, and followed up for cancer incidence in the Cancer Registry of Norway until the end of December 2003 (Kirkeleit et al., 2008). No quantitative estimates of benzene exposure were derived.

Stenehjem et al. (2015) reported on 24 917 male petroleum workers with at least 20 days employment offshore between 1965 and 1999. The cohort was established by means of a postal questionnaire in 1998, asking participants to report on occupational history and potential confounding factors. About 50% of the offshore workers overlapped with the register-based cohort of male and female offshore workers followed up by Kirkeleit et al. (2008). The follow-up periods of the two studies overlapped by only 5 years out of a total of 31 years of observation; Kirkeleit et al. (2008) covered 1981–2003 and Stenehjem et al. (2015) covered 1999–2011. The overlap is described in (Stenehjem et al., 2014). Incident cancers were identified prospectively by linkage with the Cancer Registry of Norway (Stenehjem et al., 2015). A total of 112 cases of cancers of the lymphatic and haematopoietic system diagnosed during 1999–2011 were identified and compared with a reference subcohort of 1661 workers using a nested case–cohort design (Stenehjem et al., 2015). A job-exposure matrix (JEM) was developed to assess exposure to benzene. The JEM scores were then translated into corresponding ppm values estimated on the basis of industrial benzene measurement data in Norway (Steinsvåg et al., 2007; Bratveit et al., 2011). In all analyses, adjustment was made for benzene exposure from other work (coded as yes or no, depending on the self-reported job titles and/or industry sector where the worker had ever been employed, e.g. shipping, chemical industry,

painting and surface treatment, farming and forestry, or other industry) and smoking status (yes, no, unknown). [The main strengths of this study were the prospective design, the reliability of incidence data, and detailed exposure estimates (Steinsvåg et al., 2007).]

(v) Reassessment of the Pliofilm cohort study

The cohort of workers at three Pliofilm (rubber hydrochloride) manufacturing plants in Ohio (USA) consisted of 1696 workers followed up for mortality between 1940 and 1996 (Wong, 1995; Rinsky et al., 2002) included in *IARC Monographs* Volume 100F, Table 2.1 (available at: http://publications.iarc.fr/123). Methods of exposure assessment differed between investigators, leading to different distributions of benzene exposure in the cohort and different risk values depending on the exposure levels assigned to the cases. In a recent publication, Rhomberg and collaborators reassessed exposure to benzene using a probabilistic approach based on air sampling data and assumptions about how workplace concentrations decreased over time (Rhomberg et al., 2016). The uptake of benzene from dermal exposures was also estimated, and new exposure information was obtained through additional interviews of former workers (Williams & Paustenbach, 2003). Using these new estimates, the authors divided cohort members according to quantiles of benzene exposure distribution; about 20% of the cohort members were found to have cumulative exposures of more than 80.11 ppm-years. Previous investigators (Wong, 1995; Rinsky et al., 2002) had both used fixed cut-offs of 40, 200, and 400 ppm-years. [The Working Group noted that both the outcome categorization (leukaemia subtypes) and the exposure assessment methods and cut-offs were revised from multiple analyses reported from this cohort, and that this had an important impact on different risk estimates reported for the same set of study participants.]

(c) Acute non-lymphocytic leukaemia/acute myeloid leukaemia and myelodysplastic syndrome

Studies of AML and ANLL were reviewed by a previous Working Group in *IARC Monographs* Volume 100F. That review included studies also present in previous evaluations for Volume 29 (IARC, 1982) and Supplement 7 (IARC, 1987). The data reviewed in *IARC Monographs* Volume 100F (IARC, 2012a) were described as follows by that Working Group: "...analyses of cohort studies (e.g. results in Crump (1994) and Wong (1995), based on the cohort study described in Infante et al. (1977) and Rinsky et al. (1981, 1987), which reported an excess risk for combined (mostly acute) myelogenous and monocytic leukaemia) and new cohort studies with quantitative data on benzene exposure have shown evidence of a dose–response relationship between exposure to benzene and risk for ANLL/AML in various industries and in several countries (Hayes et al., 1997; Rushton & Romaniuk, 1997; Divine et al., 1999b; Guénel et al., 2002; Collins et al., 2003; Glass et al., 2003; Bloemen et al., 2004; Gun et al., 2006; Kirkeleit et al., 2008). It was also noted that the NCI-CAPM cohort study [of Chinese workers exposed to benzene] found evidence of an increased risk for the combined category of ANLL and myelodysplastic syndromes (Hayes et al., 1997)".

New results on AML/ANLL and CML published since that time are described in the following and summarized in Table 2.1. Results regarding myelodysplastic syndromes (MDS) are also described in the text (not included in the table), as some cases of MDS can progress to AML and may have been classified in this way in earlier publications.

(i) Petroleum distribution workers

In the pooled analysis of three updated nested case–control studies of petroleum distribution workers from Australia, Canada, and the United Kingdom, 60 cases were classified as AML

(241 matched controls) and 29 as MDS (129 matched controls) (Schnatter et al., 2012).

Conditional logistic odds ratios (ORs) for AML were above unity for most exposure metrics, although none reached statistical significance (highest vs lowest cumulative exposure tertiles OR, 1.39; 95% confidence interval (CI), 0.68–2.85; average exposure intensity OR, 1.90; 95% CI, 0.86–4.18; maximum exposure intensity OR, 1.65; 95% CI, 0.75–3.73; duration of employment OR, 1.70; 95% CI, 0.75–3.87; peak exposure OR, 1.50; 95% CI, 0.82–2.75; dermal exposure OR, 1.15; 95% CI, 0.60–2.22), but no clear dose–response relationship could be demonstrated. In a further analysis of the same AML data, these associations were found to be more consistent in the subgroup of terminal workers who experienced higher exposure levels (Rushton et al., 2014). Finally, MDS showed a consistent monotonic trend for all benzene exposure metrics (e.g. for cumulative exposure, highest vs lowest tertile OR, 4.33; 95% CI, 1.31–14.3; *P* for trend, 0.01; based on 29 cases) (Schnatter et al., 2012).

[Quantitative exposure assessment and ascertainment of leukaemia subtypes were conducted carefully in this pooled analysis. The average exposure to benzene was found to be much lower than in studies of other populations exposed at higher levels, possibly explaining the non-statistically significant associations with AML. A monotonic trend was observed between benzene exposure and MDS. Previous studies relied upon an outcome classification where MDS was typically not identified (e.g. from death certificate). Some cases classified as AML in the original cohort studies were reclassified as MDS in the pooled analysis, leading to a more precise definition of outcomes, and therefore also likely contributing to the lack of associations with AML.]

(ii) Dow Chemical workers, Midland, Michigan

There were five deaths from AML in the cohort of 2266 workers exposed to benzene at a Dow Chemical plant, giving a standardized mortality ratio (SMR) of 1.11 (95% CI, 0.36–2.58) in the total population (*P* for trend, 0.88) (Collins et al., 2015). Standardized mortality ratios were similar when considering the whole ANLL subgroup (five deaths) or taking account of a latency period of more than 30 years (four deaths). No associations with AML were observed by tertiles of cumulative benzene exposure (in ppm-years). There was one MDS death in the group exposed to the highest concentrations of benzene. [This study had important limitations in terms of the small number of leukaemia cases, the use of mortality rather than incidence data, and the absence of an internal reference group.]

(iii) Chinese workers

Previously published results of ANLL incidence in this cohort of Chinese workers revealed statistically significantly elevated relative risks (RRs) for cumulative benzene exposure of 40 ppm-years or more (*P* for trend, 0.06). In analyses of ANLL/MDS, a significant positive trend was also observed (*P* for trend, 0.01) (Hayes et al., 1997). This updated study confirmed previous results, with more precise estimates (Linet et al., 2015). A total of 26 AML cases were ascertained among the subjects exposed to benzene and 7 among the unexposed, resulting in a relative risk of 2.1 (95% CI, 0.9–5.2). In addition, there were 8 MDS cases among the exposed and none among the unexposed group. Relative risks for AML/MDS were lower in 1988–1999 (RR, 1.3; 95% CI, 0.4–5.9) compared with 1972–1987 (RR, 3.7; 95% CI, 1.5–12.8), but the difference was not significant. [The strengths of this study included the large size of the cohort and the long and complete follow-up, with small numbers of subjects lost to follow-up. The main limitation was the lack of analysis of quantitative exposure

to benzene, as workers were simply categorized as exposed or not exposed.]

(iv) Norwegian offshore oil workers

A study based on a Norwegian cohort of offshore oil industry workers (included in *IARC Monographs* Volume 100F, Table 2.1, available at: http://publications.iarc.fr/123) showed an increased risk of AML (RR, 2.89; 95% CI, 1.25–6.67) compared with the general working population (Kirkeleit et al., 2008).

In a later, partially overlapping cohort study analysed using a case–cohort approach (Stenehjem et al., 2015), the hazard ratio (HR) of AML for offshore workers ever exposed versus never exposed to benzene was 2.18 (95% CI, 0.47–10.00). The risk estimate was substantially higher in the highest tertile of cumulative exposure (0.124–0.948 ppm-years) compared with the lowest tertile (< 0.001–0.037 ppm-years), with a hazard ratio of 4.85 (95% CI, 0.88–27.00; *P* for trend, 0.052). Regarding other metrics evaluated, hazard ratios were greatest in the highest tertile of average intensity (HR, 3.21; 95% CI, 0.63–19; *P* for trend, 0.092), cumulative peak (HR, 3.61; 95% CI, 0.59–26.00; *P* for trend, 0.166), and average peak (HR, 4.87; 95% CI, 0.90–26.00; *P* for trend, 0.056). No clear pattern was observed for duration of exposure in years.

[The main strengths of these studies included the prospective design, the reliability of incidence data, and the detailed exposure estimates. Stenehjem et al. (2015) included new cases of AML diagnosed during 1999–2011 but not the cases included in the earlier follow-up; this led to a relatively small number of cases. The narrow distribution of benzene exposure was an important limitation.]

(v) Reassessment of the Pliofilm cohort study

After reassessment of exposure to benzene in the Pliofilm cohort study in Ohio, all six deaths from AML were observed in the highest quintile of benzene exposure (SMR, 10.11; 95% CI, 3.71–22.01), possibly indicating a threshold effect of benzene exposure of more than 80.11 ppm-years (Rhomberg et al., 2016). By contrast, using fixed cut-offs for categories of benzene exposure (based on a balanced distribution of cases), Rinsky et al. (2002) classified four deaths from exposure to benzene at more than 400 ppm-years, giving an unstable standardized mortality ratio of 34.79 (95% CI, 9.48–89.09) in this exposure category. In the analysis using lag times of 0, 5, 10, 15, or 20 years, Rhomberg et al. (2016) found that the highest risk of AML mortality remained in the highest category of exposure, and the observations were consistent with an association with benzene exposure within the past 10 years. [The elevated exposure estimates increased the likelihood of observing an apparent threshold by assigning exposed workers to a higher exposure category; these results were questioned by the Working Group, however, due to the retrospective reassessment of exposure and the use of simulation methods.]

(d) Chronic myeloid leukaemia and myeloproliferative disorder

Studies of CML and occupational exposure to benzene were also reviewed in *IARC Monographs* Volume 100F. Occupational cohort studies available at that time were described as follows: "Several studies in the petroleum industry and in other settings show non-significantly increased risks for CML, whereas other studies show no evidence of an association, including two that had quantitative estimates of exposure to benzene but no dose–response relationship (Rushton & Romaniuk, 1997; Guénel et al., 2002)".

Additional data for CML/MPD in occupational cohorts that have become available since that time are described here and summarized in Table 2.1.

(i) Petroleum distribution workers

The pooled analysis of updated case–control studies nested within three occupational cohorts of petroleum distribution workers from Australia, Canada, and the United Kingdom exposed to low concentrations of benzene included 28 cases of CML (characterized by the presence of the Philadelphia chromosome, a specific genetic abnormality in chromosome 22) and 30 cases of MPD (Schnatter et al., 2012). Matched controls included 122 and 124 men for CML and MPD, respectively. For CML, compared with the lowest tertile, the odds ratio for cumulative exposure was 5.04 (95% CI, 1.45–17.50) in the second tertile (exposure of 0.34–2.93 ppm-years) and 2.20 (95% CI, 0.63–7.68) in the highest tertile (*P* for trend, 0.02). No clear indication of the existence of a monotonic dose–response relationship emerged when incorporating the additional exposure metrics considered in the study (see Section 2.1.1(b)(i)).

For MPD, odds ratios for cumulative exposure were 1.28 (95% CI, 0.47–3.98) in the second tertile and 1.79 (95% CI, 0.68–4.74) in the upper tertile; the trend was not significant (*P* for trend, 0.49). No strong relationship was shown with any other metrics for the whole exposure period. After restricting the exposure window to 2–20 years before diagnosis, statistically or borderline significant dose–response trends were found for cumulative exposure, dermal exposure, maximum intensity, and average intensity. An odds ratio of 3.81 (95% CI, 1.36–10.70) was reported for peak exposure, based on 18 cases ever exposed to more than 3 ppm for 1 year or more (Schnatter et al., 2012; Glass et al., 2014). [This study was the first to examine CML and MPD as separate entities. The Working Group noted that exposure to benzene was relatively low in these cohorts.]

(ii) Chinese workers

The incidence of CML in the NCI-CAPM cohort of Chinese workers was non-significantly elevated in exposed workers compared with non-exposed workers (13 exposed cases; OR, 2.5; 95% CI, 0.8–10.7) (Linet et al., 2015). Results for mortality were almost identical (not reported). [No dose–response relationship was reported, because workers were simply classified as exposed or unexposed to benzene.]

2.1.2 General-population studies

See Table 2.2

General-population studies of leukaemia in adults and exposure to benzene were also reviewed in *IARC Monographs* Volume 100F, reporting the following for ANLL: "In one case–control study an increased risk for childhood ANLL was found for maternal self-reported occupational exposure to benzene (Shu et al., 1988; see Table 2.1, available at: http://publications.iarc.fr/123). One case–control study of childhood cancer in Denmark did not find an association of estimates of environmental benzene exposure from air pollution with an increased risk for ANLL (Raaschou-Nielsen et al., 2001)." Regarding CML, Volume 100F reported: "Case–control studies have shown inconsistent results, with both increased risks (exposure for > 15 years was associated with an OR of 5.0 (1.8–13.9; Adegoke et al., 2003)) and no increase in risk (Björk et al., 2001) reported (see Table 2.6, available at: http://publications.iarc.fr/123)".

For the current evaluation, the Working Group included all general-population cohort studies and case–control studies published in 2009 or later that examined the relationship between benzene exposure (assessed quantitatively or qualitatively) and AML or CML. Studies were excluded if they did not specifically address benzene exposure, but instead used other indicators of traffic-related air pollution (Raaschou-Nielsen et al., 2016) or residential proximity to

Table 2.2 General-population studies of exposure to benzene and leukaemia in adults

Reference, location, follow-up/ enrolment period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kaufman et al. (2009) Bangkok, Thailand 1997–2003 Case–control	Cases: 87 incident cases at Siriraj Hospital, Bangkok Controls: 756 initially age- and sex-matched hospital patients with diagnoses "considered generally unrelated to the exposures of interest" Exposure assessment method: questionnaire	Leukaemia (AML)	Ever exposed Unexposed Exposed	 81 6	 1.0 4.9 (1.4–17.0)	Age, sex, income, use of cellphones, occupational and nonoccupational pesticide exposure, pesticides used near the home, working with powerlines, living near powerlines	Strengths: high response (100%) Limitations: small study; self-reported ever/never exposure; potentially substantial selection and/ or recall bias
Wong et al. (2010a) Shanghai 2003–2007 Case–control	Cases: 722 newly diagnosed AML cases in 29 hospitals; response 94.6% Controls: 1444 patients without malignant diseases and without diseases of the lymphatic and haematopoietic system admitted to the same hospital as the individually matched case (2 controls per case); response 99.0% Exposure assessment method: expert judgement; exposure classification carried out on a job-by-job basis (jobs identified by questionnaire) by an expert committee (blind for case–control status)	Leukaemia (AML)	Benzene exposure (yr) Never (reference) Ever ≤ 10 > 10 to < 20 > 20 Benzene exposure (mg/m³) Group 1: < 1 Group 2: 1–10 Groups 3, 4: >> 10 Period of first exposure 1940–1959 1960–1979 1980–1999 after 2000 Trend test *P* value, 0084 (length of exposed job); 0.01 (maximum exposure)	 644 78 43 21 14 40 20 18 8 22 36 12	 1.00 1.43 (1.05–1.93) 1.99 (1.29–3.07) 1.44 (0.82–2.51) 0.74 (0.39–1.39) 1.18 (0.79–1.76) 1.63 (0.90–2.94) 2.05 (1.05–3.98) 1.33 (0.54–3.26) 0.97 (0.57–1.62) 1.57 (1.00–2.46) 4.18 (1.56–11.15)	Age, sex, hospital	Funding: Benzene Health Effects Consortium Strengths: large study; complete occupational histories with expert assessment Limitations: hospital-based study including potential for selection bias; expert assessment of benzene exposure based on self-reported questionnaire data

Table 2.2 (continued)

Reference, location, follow-up/ enrolment period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Saberi Hosnijeh et al. (2013) 23 centres in 10 European countries 1992–2000 Cohort	241 465 men and women aged 35–70 yr at recruitment, with no prevalent cancer Exposure assessment method: expert judgement; occupational exposures of high-risk occupations estimated by linking them to a general-population JEM originally developed for another study; exposure to benzene classified as "high", "low", and "no exposure" based on job code; 113 cases of AML, but not specified by exposure	Leukaemia (AML)	No exposure	NR	1.00	Sex, smoking status, alcohol intake, age at recruitment, country	Strengths: large cohort with long follow-up; detailed information on confounders Limitations: lack of occupational histories in large number of participants; different procedures to identify cases; exposure classification not very detailed
			Low exposure	NR	1.06 (0.63–1.81)		
			High exposure	NR	1.52 (0.78–2.98)		
			Trend test *P* value, 0.28				
		Leukaemia (CML)	No exposure	NR	1.00	Sex, smoking status, alcohol intake, age at recruitment, country	
			Low exposure	NR	1.00 (0.45–2.22)		
			High exposure	NR	1.97 (0.75–5.19)		
			Trend test *P* value, 0.30				
		NHL (CLL)	No exposure	NR	1.00	Sex, smoking status, alcohol intake, age at recruitment, country	
			Low exposure	NR	1.11 (0.78–1.58)		
			High exposure	NR	0.56 (0.27–1.14)		
			Trend test *P* value, 0.37				
Talibov et al. (2014) Finland, Iceland, Norway, Sweden 1961–2005 Nested case–control	Cases: 15 332 incident cases Controls: 76 660 randomly selected among cohort members who were alive and free from AML on the date of diagnosis of the matched index case (5 controls per case) Exposure assessment method: other; NOCCA JEM based on FINJEM; quantitative assessment (ppm-yr)	Leukaemia (AML)	Cumulative exposure (ppm-yr)			Year of birth, sex, country	The study was funded by Doctoral Programs in Public Health (DPPH)/ Academy of Finland Strengths: very large nested study; selection bias improbable Limitations: exposure classification by JEM relatively unprecise; "cross-sectional" information on jobs held (based on census records); no adjustment for smoking or genetic factors
			50th and 90th percentiles: unexposed	NR	1.00		
			≤ 3.7	430	1.02 (0.84–1.24)		
			3.7–13.6	310	0.88 (0.71–1.11)		
			> 13.6	68	0.80 (0.56–1.15)		
			Trend test *P* value, 0.33				

AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; FINJEM, Finnish job-exposure matrix; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NOCCA, Nordic Occupational Cancer Study; NR, not reported; ppm, parts per million; yr, year(s)

gasoline plume (Talbott et al., 2011), or if they only combined benzene exposure with exposure to other solvents (Poynter et al., 2017), even if the text explicitly referred to "benzene exposure".

Since 2009, one new cohort study in the general population (European Prospective Investigation into Cancer and Nutrition study by Saberi Hosnijeh et al., 2013), one nested case–control study in the Nordic Occupational Cancer Study cohort (Talibov et al., 2014), and two new case–control studies (Kaufman et al., 2009; Wong et al., 2010a) have investigated the relationship between occupational benzene exposure and adult leukaemia.

A large cohort study with 241 465 participants covering 23 centres in 10 European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and the United Kingdom) identified 113 AML cases by either population cancer registries, health insurance records, pathology registries, or active contact with study subjects or next of kin (Saberi Hosnijeh et al., 2013). Occupational exposure to benzene was assessed through a general-population JEM based on self-reported occupations of high risk. Exposure to benzene was classified as either "no exposure", "low exposure", or "high exposure". In the high-exposure category, the hazard ratio for AML was 1.52 (95% CI, 0.78–2.98; *P* for trend, 0.28). The same study reported on CML (46 cases in total) and found an increased hazard ratio of 1.97 in the high-exposure group (95% CI, 0.75–5.19; *P* for trend, 0.37). [The strengths of this study included its large size, its long follow-up, and the detailed information about confounders. The limitations included the lack of complete occupational histories in large numbers of participants, different procedures for case identification, and the lack of specificity in the exposure classification.]

Talibov et al. (2014) conducted a very large case–control study nested within the Nordic Occupational Cancer Study cohort. The study in Finland, Iceland, Norway, and Sweden comprised 15 332 AML cases and 76 660 control subjects. The authors did not find an association between occupational benzene exposure, as assessed by a JEM, and AML. With occupational unexposed workers as a reference, the hazard ratios of those exposed to benzene at 3.7 or less, 3.7–13.6, and more than 13.6 ppm-years was 1.02 (95% CI, 0.84–1.24), 0.88 (95% CI, 0.71–1.11), and 0.80 (95% CI, 0.56–1.15), respectively (*P* for trend, 0.33). [The strengths of this study included its very large size and its nested design, making selection bias improbable. The limitations included incomplete work histories for many participants and the imprecise exposure classification by JEM.]

In a hospital-based case–control study in Shanghai, China, Wong et al. (2010a) compared 722 newly diagnosed AML cases with 1444 control subjects without malignant diseases or diseases of the lymphatic and haematopoietic system. The authors found a monotonic exposure–response relationship between maximum occupational benzene exposure and AML (*P* for trend, 0.01). The odds ratios were 1.18 (95% CI, 0.79–1.76), 1.63 (95% CI, 0.90–2.94), and 2.05 (95% CI, 1.05–3.98) for maximum exposure to benzene at less than 1, 1–10, and more than 10 mg/m^3, respectively. Individuals with a first diagnosis after the year 2000 had a higher risk than individuals with an earlier date of first diagnosis. [The strengths of this study included its large size, as well as complete occupational history with job-specific questions, and expert assessment of exposures. The limitations included the potential for selection bias as a consequence of the hospital-based control selection.]

In a small hospital-based case–control study in Bangkok, Thailand, 87 AML cases were compared with 756 patients of the same hospital (Kaufman et al., 2009). For self-reported occupational benzene exposure, an elevated odds ratio of 4.9 (95% CI, 1.4–17.0) was found. [The high response rate was a strength of this study. Limitations included the potential for selection and recall bias as a consequence of the

hospital-based control selection and the use of self-reported benzene exposure (ever vs never).]

2.2 Adult lymphoma

This section presents the Working Group's review of studies of NHL and HL in adults. Because most of the available studies did not group the entities now included within NHL according to the current WHO classification (Swerdlow et al., 2017), the disease entities presented here are those used in the original publications. For occupational cohort studies, which were more numerous, data are presented for total NHL as defined in the original studies and MM when separate risk data were reported (in the same subsection), for CLL, for ALL, and for HL.

2.2.1 Occupational cohort studies

(a) Non-Hodgkin lymphoma and multiple myeloma

Twenty-one studies on the association between NHL, including MM, and exposure to benzene in occupational cohorts were included in *IARC Monographs* Volume 100F (see Table 2.9, available at: http://publications.iarc.fr/123). The purpose of the current update is to establish whether new studies contribute to the causal assessment of the overall evidence. Several articles on adult lymphomas included in *IARC Monographs* Volume 100F or published later were excluded by the Working Group either because the exposure assessment was considered inadequate to determine whether workers were exposed to benzene (Guberan & Raymond, 1985; Cuzick & De Stavola, 1988; La Vecchia et al., 1989; Blair et al., 1993; Walker et al., 1993; Lagorio et al., 1994; Satin et al., 1996; Lynge et al., 1997; Anttila et al., 1998; Gérin et al., 1998; Lundberg & Milatou-Smith, 1998; Divine et al., 1999b; Persson & Fredrikson, 1999; Mao et al., 2000; Wong et al., 2001a, b; Sorahan et al., 2002; Kauppinen et al., 2003; Xu et al., 2003; Dryver et al., 2004; Huebner et al., 2004; Punjindasup et al., 2015), or because these were either methodological articles or focused on mechanisms (Vineis et al., 2007; Barry et al., 2011; Faisandier et al., 2011).

Studies in occupational cohorts published after the compilation of *IARC Monographs* Volume 100F that are included for evaluation here are those published by Koh et al. (2011, 2014), Collins et al. (2015), Linet et al. (2015), and Stenehjem et al. (2015). These studies are summarized in Table 2.3.

Most studies reported a small number of NHL cases as a result of exposure to benzene, usually less than 20, and generally presented mortality as an outcome, leading to low sensitivity of ascertainment for NHL. The exceptions are the studies by Hayes et al. (1997), Nilsson et al. (1998), Glass et al. (2003), Sorahan et al. (2005), Kirkeleit et al. (2008), Koh et al. (2011, 2014), Linet et al. (2015), and Stenehjem et al. (2015), which identified incident cases. To broadly characterize the available studies, exposure contexts included a variety of manufacturing processes including the petroleum industry, chemical plants, or others, as well as different exposure assessment methods (see Section 1.4.1 on Occupational exposure). Among the studies published before the previous evaluation in *IARC Monographs* Volume 100F, the current Working Group considered those with high-quality exposure assessment, case ascertainment, and follow-up, as well as a large sample size and adjustment for confounders, to be the most informative. None of the studies in the previous Monograph fulfilled all these criteria. All the studies considered in the evaluation are described below (chronologically), but only studies published after *IARC Monographs* Volume 100F are included in Table 2.3.

Wong (1987a) studied male workers from seven chemical plants in the USA, where jobs were classified based on past quantitative measurements. An apparent dose–response

Table 2.3 Occupational cohort studies of exposure to benzene and lymphoma in adults

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 chemical industry workers exposed to benzene as solvent and raw material Exposure assessment method: quantitative measurements; job-specific exposure estimated from measurements taken from 1944 to the late 1970s	NHL: ICD-10 (codes C82–C85.9)	Cumulative exposure (ppm-yr)			Age, race, sex	Third update of the Dow Chemical plant retrospective cohort Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: small cohort; mortality data (based on death certificates) for a period over which diagnosis and classification were uncertain
			> 30 yr latency	12	1.02 (0.53–1.78)		
			0–3.9	6	1.23 (0.45–2.69)		
			4–24.9	6	1.10 (0.41–2.40)		
			≥ 25	3	0.58 (0.12–1.69)		
			Trend test *P* value, 0.26				
		Hodgkin lymphoma: ICD-10 (code C81)	Cumulative exposure (ppm-yr)			Age, race, sex	
			> 30 years latency	1	1.32 (0.03–7.36)		
			0–3.9	0	0 (0–5.28)		
			4–24.9	2	2.63 (0.32–9.51)		
			≥ 25	0	0 (0–4.85)		
			Trend test *P* value, 0.35				
Linet et al. (2015) China 1972–1987/1972–1999 Cohort	35 804 benzene-exposed workers in 672 factories (spray and brush painting, rubber, chemical, shoemaking, and other) Exposure assessment method: records; factory and job title-specific information on the use of benzene-containing materials formed the basis for determining benzene-exposed or unexposed jobs; no quantitative assessment	Multiple myeloma: ICD-9 (code 203)	Exposed/unexposed			Sex, age, calendar year	Strengths: very large cohort; few losses to follow-up; long follow-up (28 yr); very careful ascertainment of haematolymphopoietic malignancies Limitations: no quantitative assessment of exposure; wide range of industrial processes included; coexposures vary and were not addressed in the analyses; very small numbers for CLL (zero unexposed cases)
			Mortality	1	0.10 (0.01–1.00)		
			Incidence	1	0.12 (0.01–0.96)		
		NHL: ICD-9 (codes 200, 202)	Exposed/unexposed			Sex, age, calendar year	
			Mortality	31	4.0 (1.6–13.4)		
			Incidence	30	3.9 (1.5–13.2)		
		NHL (CLL): ICD-9 (codes 204.1, 204.2)	Exposed/unexposed			Sex, age, calendar year	
			Incidence	2	NR		
		Leukaemia (ALL): ICD-9 (code C204.0)	Exposed/unexposed			Sex, age, calendar year	
			Incidence	8	4.5 (0.8–83.9)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koh et al. (2011) Korea 1960–2007 Cohort	8866 male workers in refinery/petrochemical complex in Korea producing benzene or using benzene as a raw material Exposure assessment method: other; job title	NHL	Subgroups of workers (exposure-based) in the petrochemical complex			Age, calendar period	Strengths: incidence data Limitations: small number of cases; exposure assessment based on job title
			Manufacturing workers	2	0.70 (0.08–2.52)		
Koh et al. (2014) Korea 2002–2007 (2002–2005 for incidence) Cohort	14 698 male workers registered in a regional petrochemical plant maintenance workers union Exposure assessment method: none; job title	NHL: ICD-10 (codes C82–C85)	Maintenance workers, incidence	3	1.83 (0.38–5.34)	Age	Limitations: very small number of cases
		NHL: ICD-10 (codes C82–C85)	Maintenance workers, mortality	2	1.24 (0.15–4.47)	Age	
Stenehjem et al. (2015) Norway 1965–1999/1999–2011 Cohort	24 917 male petroleum workers; offshore oil industry workers for at least 20 d during 1965–1999, extracted from a cohort who responded to a survey conducted with postal questionnaires Exposure assessment method: quantitative measurements; a JEM was developed using monitoring data and job-specific information, giving semiquantitative estimates; JEM scores then translated into corresponding ppm values	NHL (B-cell lymphoma): ICD-10 (codes C82–C91)	Exposed/unexposed			Age, benzene exposure from other work, ever daily smoker	Nested case–cohort study based on an updated cohort of Norwegian offshore workers Strengths: prospective case–cohort design; data from Norway cancer registry ensure a high degree of completeness; independent exposure estimates developed for this cohort; analyses adjusted for some confounders Limitations: potential recall bias for distant occupations (non-differential); individual differences in exposure within each occupational group could not be taken into account
			Exposed	61	1.49 (0.90–2.48)		
			Trend test *P* value, 0.245				
		Multiple myeloma: ICD-10 (code C90.0)	Exposed/unexposed			Age, benzene exposure from other work, ever daily smoker	
			Exposed	13	1.64 (0.55–4.89)		
			Trend test *P* value, 0.024				
		NHL (CLL): ICD-10 (codes C83.0, C91.1)	Cumulative exposure tertile (ppm-yr)			Age, benzene exposure from other work, ever daily smoker	
			T1 (< 0.001–0.037)	4	6.23 (0.71–54.00)		
			T2 (> 0.037–0.123)	2	3.08 (0.28–34.00)		
			T3 (0.124–0.948)	5	6.74 (0.75–60.00)		
			Trend test *P* value, 0.212				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 workers exposed to benzene at a chemical plant Exposure assessment method: quantitative measurements; job titles were assigned to exposure categories by an industrial hygienist, based on IH measurements (JEM)	Leukaemia: ICD-10 (codes C91–C95)	Cumulative exposure (ppm-yr)			NR	Third update of the Dow Chemical plant retrospective cohort; one death for MDS, and it was in the high-exposure group (SMR 25.05; 95% CI: 0.63–139.58) Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: mortality study based on death certificates; no evaluation of possible confounders
			0–3.9	3	0.60 (0.12–1.76)		
			4.0–24.9	7	1.23 (0.49–2.53)		
			≥ 25	10	1.72 (0.86–3.17)		
			Trend test *P* value, 0.15				
		Leukaemia (myeloid): ICD-10 (code C92)	Cumulative exposure (ppm-yr)			NR	
			0–3.9	0	0 (0–1.79)		
			4.0–24.9	4	1.78 (0.48–4.54)		
			≥ 25	4	1.93 (0.53–4.94)		
			Trend test *P* value, 0.24				
		Leukaemia (AML): ICD-10 (code C92.0)	Cumulative exposure (ppm-yr)			NR	
			0–3.9	0	0 (0–2.50)		
			4.0–24.9	3	1.87 (0.39–5.47)		
			≥ 25	2	1.39 (0.17–5.03)		
			Trend test *P* value, 0.88				
		Leukaemia (lymphoid): ICD-10 (code C91)	Cumulative exposure (ppm-yr)			NR	
			0–3.9	1	0.78 (0.02–4.36)		
			4.0–24.9	1	0.68 (0.02–3.78)		
			≥ 25	2	1.31 (0.16–4.72)		
			Trend test *P* value, 0.53				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Schnatter et al. (2012) Australia, Canada, UK 1981–2006 (Australia), 1964–1994 (Canada), 1950–2005 (UK) Nested case–control	Cases: 370 diagnoses based on incidence and mortality data (hospital records, cancer registries, death certificates) Controls: 1587; 5 age-matched (Australia) or 4 age- and company-matched (Canada and UK) controls selected using incidence density-based sampling Exposure assessment method: quantitative measurements; conducted at the job/ worksite/era level, based on routinely collected industry exposure measurements; work history was collected from company records (Canada and UK) or through interview and company records (Australia)	NHL (CLL)	Cumulative exposure (ppm-yr) ≤ 0.348 0.348–2.93 > 2.93 Trend test *P* value, 0.9	 24 32 24	 1.00 1.49 (0.81–2.76) 1.05 (0.56–1.98)	NR	Exposures are relatively low; based on limited data, smoking was unlikely to be a confounder Strengths: large study size; review of diagnosis by haematopathologists; reassessment of exposure across the three studies Limitations: Smoking data were incomplete

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; ICD, International Statistical Classification of Diseases and Related Health Problems; IH, industrial hygiene; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million;
SMR, standardized mortality ratio; yr, year(s)

relationship between NHL and cumulative exposure to benzene was observed, with a relative risk of 3.7 (*P* value, < 0.04) for continuous or intermittent benzene exposure, and one of 3.8 (*P* value, < 0.04) for continuous benzene exposure compared with no exposure. [The Working Group noted the very small number of subjects in this study and that 95% confidence intervals were not reported.] Wong & Raabe (1997) conducted a nested case–control study on MM in gasoline distribution workers (17 MM deaths among the exposed). Total hydrocarbon concentrations in air were used as a surrogate measure of exposure to benzene. *P* values for trend were 0.06 for exposure duration, 0.77 for cumulative exposure to total, and 0.08 for peak exposure. [The Working Group considered the use of indirect estimates of exposure to benzene and the very small number of MM deaths to be strong limitations of this study.]

Schnatter et al. (1996) and Glass et al. (2003) both report on NHL and MM or NHL/MM, and were later included in a pooled analysis (Schnatter et al., 2012). Schnatter et al. (1996) conducted a nested case–control study of petroleum distribution workers in Canada where benzene exposure was quantitatively assessed and standardized mortality ratios were reported. Eight deaths from NHL were observed, and exposure–response analyses based on these showed no consistent pattern. Similarly, the study found non-significant standardized mortality ratios for MM based on seven deaths only (Schnatter et al., 1996). [The Working Group noted the adjustment for several potential confounders, but the very small sample size of the study.] Glass et al. (2003) conducted a nested case–control study in the petroleum industry in Australia, and did not observe a relationship between cumulative exposure to benzene and NHL/MM.

Consonni et al. (1999) report on a cohort of 1583 male oil refinery workers employed during 1949–1982 and followed up to May 1991. Comparing exposed with non-exposed workers, a standardized mortality ratio of 2.12 (95% CI, 0.68–4.95) was found based on 5 exposed NHL cases. The excess risk was significantly increased among workers with 15 years or more of employment, and 30 years or more since first employment.

Using a JEM based on air sampling data, Rinsky et al. (2002) assessed quantitative exposure to benzene for a cohort of workers manufacturing Pliofilm in the USA; the number of NHL or MM cases was very limited, however (5 for each, based on death certificates). The standardized mortality ratio for white males was 1.00 (95% CI, 0.32–2.33) for NHL and 2.12 (95% CI, 0.69–4.96) for MM (reference group unexposed). [The Working Group noted that exposure–response for NHL was not modelled because of the small numbers of cases.] Using the same Pliofilm cohort reported by Rinsky et al. (2002), Wong (1995) focused specifically on MM. Results were reported in terms of levels of cumulative exposure, and no increased mortality risk was observed. [The Working Group noted that results were based on extremely small numbers; 4 MM cases in total were categorized across four exposure strata.]

Collins et al. (2003) reported on NHL and MM in a small study with long follow-up (from 1940 to 1997) at a single chemical plant that was studied previously by Bond et al. (1986). The study was based on individual exposure measurements, and no increased risks for NHL mortality were observed (25 exposed cases in total). An increased standardized mortality ratio was reported for MM, but with a dose–response relationship that did not reach statistical significance (reference group unexposed). [The Working Group noted the generally low exposure levels ranging from less than 1 ppm-years to 632 ppm-years, but with a median of 3 ppm-years.]

Sorahan et al. (2005) reported on a cohort of workers considered occupationally exposed to benzene based on records of the Factory Inspectorate in the United Kingdom. A total of

15 NHL deaths (SMR, 0.94; 95% CI, 0.53–1.56) and 24 incident NHL cases (standardized incidence ratio (SIR), 1.00; 95% CI, 0.64–1.49) occurred in the exposed workers compared with the unexposed. Additionally, based on 8 incident cases and six deaths, the same study found no increased risk of MM. [The Working Group noted the small numbers for MM and that the exposure assessment was limited, although exposure levels were historically high.]

Kirkeleit et al. (2008) reported on a large cohort of more than 27 000 offshore petroleum workers in Norway (see Section 2.1.1(b)(iv) for further details). Compared with the reference unexposed group, the overall relative risk was 1.01 for incident NHL (95% CI, 0.58–1.75) and 2.49 for incident MM (95% CI, 1.21–5.13). [This study overlaps partially with Stenehjem et al. (2015), described below. No quantitative assessment of benzene exposure was available.]

Several new studies reporting pertinent data for NHL or MM and benzene exposure in occupational settings have been published since the previous *IARC Monographs* Volume 100F (Table 2.3).

Stenehjem et al. (2015) studied 61 NHL incident cases. Overall, compared with a reference unexposed group, there was a slight excess of NHL cases among the exposed subjects (RR, 1.49; 95% CI, 0.9–2.48), and a stronger but not statistically significant association with specific histological type. No *P* trend was observed for NHL (*P* trend of 0.245 based on tertiles of exposure). The incidence of MM (13 exposed cases) was increased among exposed workers (RR, 1.64; 95% CI, 0.55–4.89), and a significant dose–response relationship with exposure tertiles was found (*P* for trend, 0.024). [The Working Group noted the very low levels of exposure in these workers: the upper values of average intensity and cumulative exposure were estimated to be 0.040 ppm and 0.948 ppm-years, respectively.]

Linet et al. (2015) updated the study by Hayes et al. (1997) that was based on 35 804 male and female workers exposed to benzene in 672 factories in China, with a long follow-up (28 years) and good case ascertainment. This study did not assess benzene exposure quantitatively. A total of 31 NHL deaths (RR, 4.0; 95% CI, 1.6–13.4) and 30 incident cases (RR, 3.9; 95% CI, 1.5–13.2) were reported. Only one death and 1 incident case of MM were recorded. [The Working Group considered this a strong study due to the robust case ascertainment and long follow-up.] Data on exposure–response relationships in the same cohort were reported earlier by Hayes et al. (1996) (see *IARC Monographs* Volume 100F, Table 2.9, available at: http://publications.iarc.fr/123). The relative risk of mortality from NHL for those exposed to benzene for more than 10 years, compared with a reference unexposed group, was 4.2 (95% CI, 1.1–15.9) based on 11 exposed cases. A non-monotonic dose–response relationship was observed with average (*P* for trend, 0.04) and cumulative (*P* for trend, 0.02) benzene exposure.

Collins et al. (2015) updated a cohort study previously reported by Bloemen et al. (2004) on mortality among 2266 chemical workers in the USA. Controlled for age, sex, and gender, the standardized mortality ratio for NHL observed in workers with more than 30 years latency (n = 12) was 1.02 (95% CI, 0.53–1.78). [The Working Group noted the robust exposure assessment but very small numbers, particularly in analyses by cumulative exposure.]

(b) Chronic lymphocytic leukaemia

The data on the association between CLL and exposure to benzene that were available at the time (until 2009) were reviewed in *IARC Monographs* Volume 100F and described as follows: "Several cohort studies in the petroleum industry [subsequently included in a pooled analysis by Schnatter et al. (2012)] showed mixed results, with some non-significantly increased risks reported and other studies showing no association (see Table 2.7, available at http://publications.iarc.fr/123). In a nested

case–control study in the Australian petroleum industry an increasing risk for CLL was detected with increasing exposure to benzene over a relatively small range of ppm–years, but the increase was not significant (Glass et al., 2003). Similarly, in a nested case–control study within a cohort of French gas and electrical utility workers, a non-significant increase in risk with increasing years of benzene exposure was detected (Guénel et al., 2002). Some evidence of risk with increasing benzene exposure was also found in a cohort study among petroleum workers in the United Kingdom, but the trends were not clear and interpretation is difficult as white- and blue-collar workers were mixed in the analysis and interactions may have been present (Rushton & Romaniuk, 1997)".

The current Working Group reviewed these studies and determined that several did not meet the criteria established for inclusion (see Section 2.1.1(a)) (McCraw et al., 1985; Satin et al., 1996; Lynge et al., 1997; Divine et al., 1999b; Divine & Hartman, 2000; Wong et al., 2001a; Lewis et al., 2003; Bloemen et al., 2004; Huebner et al., 2004). One study reviewed previously was superseded by later updates (Glass et al., 2003).

Three of the five occupational cohort studies published after *IARC Monographs* Volume 100F and described in Section 2.2.1(b) (petroleum distribution workers in Australia, Canada, and the United Kingdom; Chinese workers; and Norwegian offshore oil workers) presented data on CLL and benzene exposure (Table 2.3), as described in the following sections.

(i) Petroleum distribution workers in Australia, Canada, and the United Kingdom

Exposure to benzene was compared between 80 cases of CLL and 345 matched controls in the pooled analysis of updated case–control studies nested in occupational cohorts of petroleum distribution workers (Schnatter et al., 2012; Rushton et al., 2014). When compared with subjects in the lowest exposure tertile of cumulative exposure (< 0.348 ppm-years), the odds ratio of CLL was more elevated in the intermediate exposure tertile (0.348–2.93 ppm-years; OR, 1.49; 95% CI, 0.81–2.76; 32 cases) than in the highest exposure tertile (> 2.93 ppm-years; OR, 1.05; 95% CI, 0.56–1.98; 24 cases). No clear indication of an association was shown with the other exposure metrics reported in this study (see Section 2.1.1(b)). No dose–response relationship was observed for CLL, except with duration of employment (*P* for spline, < 0.03). Refinery workers (mainly from the Australian study) showed a higher risk of CLL compared with subjects who had never worked as a refinery operator or craftsman (RR, 1.99; 95% CI, 0.87–4.57).

(ii) Chinese workers exposed to benzene

The large NCI-CAPM cohort of Chinese workers included only two CLL cases among the workers exposed to benzene and none among the unexposed; no relative risk could be computed (Linet et al., 2015).

(iii) Norwegian offshore oil workers

In the nested case–cohort study on Norwegian offshore oil industry workers, 12 cases of CLL were compared with 1661 reference workers from the same cohort (Stenehjem et al., 2015). A five-fold hazard ratio of CLL for workers ever versus never exposed to benzene was reported (HR, 5.4; 95% CI, 0.7–41.0). The risk estimates for cumulative exposure were substantially higher in the exposed subjects with respect to the unexposed (HR in the upper tertile, 6.74; 95% CI, 0.75–60.00; 5 cases), but no exposure–response relationship was found (*P* for trend, 0.212). Hazard ratios were consistently elevated when considering all the other metrics reported in the study (see Section 2.1.1(b)), although the highest risks were often in the intermediate tertiles of exposure (e.g. the HR for the middle tertile of average peak exposure was 6.66; 95% CI, 1.32–34.00; 6 exposed cases) and no statistically significant dose–response trend was observed for any of the

metrics. [The Working Group noted that exposure levels in the study were generally low.]

(c) Acute lymphocytic leukaemia

ALL is a rare cancer in adults, and this makes it difficult to study its association with exposure to benzene. The maximum number of exposed cases in the studies included in *IARC Monographs* Volume 100F was 8 (IARC, 2012a; Table 2.3, available at: http://publications.iarc.fr/123). The evidence for the association between ALL in adults and benzene exposure that was available at the time of the previous evaluation was described as follows: "In multiple cohorts there was a non-significantly increased risk for ALL, but the numbers of cases were small (Rushton, 1993; Wong et al., 1993; Satin et al., 1996; Yin et al., 1996a; Divine et al., 1999b; Guénel et al., 2002; Lewis et al., 2003; Gun et al., 2006; Kirkeleit et al., 2008)".

The Working Group reviewed these studies and determined that most (Rushton, 1993; Satin et al., 1996; Divine et al., 1999b; Lewis et al., 2003; Gun et al., 2006) did not meet the criteria for inclusion (see Section 2.1.1 (a)) in the current evaluation.

The Working Group identified one earlier study that met the inclusion criteria, but had not been reviewed in *IARC Monographs* Volume 100F: Sorahan et al. (2005). In this study, which was conducted in the United Kingdom, no cases of ALL were observed (0.83 expected) in a cohort of 5514 male and female workers exposed to benzene.

Only one new study with pertinent data for adult ALL and benzene exposure has been published since the previous IARC review. Eight cases of incident ALL were ascertained in the NCI-CAPM cohort of Chinese workers among those who held jobs entailing exposure to benzene, and one among the unexposed (Linet et al., 2015). An elevated relative risk for incidence of 4.5 (95% CI, 0.8–83.9) was found. Formal statistical significance was reached in the "all lymphoid leukaemia" group (9th International Statistical Classification of Diseases and Related Health Problems (ICD-9), code 204), that is, after the addition of 2 cases of CLL among the exposed (RR, 5.4; 95% CI, 1.0–99.3) based on a total of 10 cases. A dose–response evaluation was not conducted, because benzene exposure assessment was limited to a categorization of ever versus never exposed.

Among the included studies (except Sorahan et al., 2005, which had no ALL cases), the risk estimates for ALL as a result of benzene exposure ranged from 0.8 to 4.5, and all the 95% confidence intervals included the null.

(d) Hodgkin lymphoma

The evidence for HL was reviewed in *IARC Monographs* Volume 100F. At that time the Working Group noted that the data on HL in studies of cohorts exposed to benzene were sparse, with most studies having very small numbers of cases and reporting no association (see Table 2.13, available at: http://publications.iarc.fr/123). The evidence from these studies was judged to be *inadequate*.

With the exception of the study by Collins et al. (2015) (described in Section 2.1.1 (b) and summarized in Table 2.3), which found no association between HL mortality and cumulative exposure to benzene based on only 2 cases of HL, no additional data have been reported on the association between HL and exposure to benzene.

2.2.2 General-population studies

This review included all published, peer-reviewed epidemiological studies reporting a risk estimate for the association between exposure to benzene and NHL, CLL, DLBCL, follicular lymphoma, HCL, MM, ALL, or HL in study populations enrolled from the population at large, distinct from industry-based cohorts. Relevant studies by Clavel et al. (1996), Orsi et al.

(2010), and Wong et al. (2010b) were not included in *IARC Monographs* Volume 100F but have been added to this chapter, in addition to Bassig et al. (2015) (all summarized in Table 2.4). One study by Jiao et al. (2012) on a gene-environment interaction with a BRCA2 variant was excluded as it did not report overall risks for benzene exposure. All the studies were of a case–control design, with the exception of a cohort study from Shanghai (Bassig et al., 2015). The case–control studies were a mixture of hospital-based and population-based designs. The quality of the population controls varied extensively; studies conducted within the USA were often based on random digit dialling (Wang et al., 2009) or driving licence rosters (Kato et al., 2005), often obtaining low response rates. All studies included newly diagnosed incident cases, usually with a re-examination of diagnoses. Histological reviews were performed in the studies by Scherr et al. (1992), Fritschi et al. (2005), and Miligi et al. (2006) (see *IARC Monographs* Volume 100F, Table 2.10, for details of studies included, available at: http://publications.iarc.fr/123). Exposure contexts for the studies reviewed here mainly included occupational exposure of the subjects. The assessment of benzene exposure ranged from a self-report ascertained by questionnaire [which the Working Group did not deem to be of sufficient quality for assessing exposure to benzene] to expert judgement based on quantitative measurements in factories (Bassig et al., 2015), although most studies used a JEM (see Section 1.3 and Section 1.6 for further information). The studies judged most informative by the Working Group were those that scored high for exposure assessment features, with a large sample size and high-quality design (including a histological review of cases and high response rates). Several studies were excluded due to small sample size (Linet et al., 1987; Kato et al., 2005; Ruckart et al., 2013) or because the exposure assessment was very limited (Micheli et al., 2014).

Clavel et al. (1996) found no association between exposure to benzene and HCL [currently classified as a subtype of NHL]. Exposure assessment was based on a JEM. [The Working Group noted that the response rate was low among controls, at around only 57%.]

The large European multicentre Epilymph study (Cocco et al., 2010), which included population- and/or hospital-based controls depending on the areas and used a JEM to assess benzene exposure, was previously included in *IARC Monographs* Volume 100F. Cocco et al. (2010) found no association between exposure to benzene and NHL, DLBCL, follicular lymphoma, or MM; a positive association between CLL and benzene exposure was observed, but there was no evidence of a dose–response relationship (OR of exposed versus never exposed to benzene isolated from other organic solvents, 1.8; 95% CI, 1.0–3.2; *P* for trend, 0.14). [The Working Group noted that the response rate was low in the population controls, at around only 52%. An earlier study by Seidler et al. (2007), which was already included in the Epilymph analysis, reported no association with NHL.]

Fritschi et al. (2005) found no association between NHL and exposure to benzene in a population-based study in Australia that included 68 exposed cases. Exposure to benzene was assessed by a JEM. [The Working Group noted the relatively low response rate among the population-based controls, at around only 61%.]

Miligi et al. (2006) describe a well-conducted population-based study on more than 1400 NHL cases and 1500 controls from the general population, with high response rates of 79%. Cases were examined by a panel of pathologists. Exposure assessment was based on detailed questionnaires, expert judgement, and a JEM (with assessment of probability and intensity of exposure). Positive associations were found for medium and high benzene exposure versus very low and low benzene exposure (OR, 1.6; 95% CI; 1.0–2.4) based on 58 cases, as well as a non-significant

Table 2.4 Epidemiological studies of exposure to benzene and adult lymphoma in the general population

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bassig et al. (2015) China (Shanghai) 1996–2000/until 2009 Cohort	73 087 women only, aged 40–70 yr; Shanghai Women's Study (population-based), with 92.7% response rate Exposure assessment method: quantitative measurements; JEM that combined benzene measurements in factories (since 1954) plus questionnaire data and other information; probability and intensity of exposure assigned	NHL	Cumulative exposure (mg/m^3)-yr) Unexposed (reference) Ever exposed Tertile 1: ≤ 35.2 Tertile 2: 35.21–102.4 Tertile 3: > 102.4 Trend test *P* value, 0.006 for duration, 0.005 for cumulative exposure	 78 24 3 9 12	 1.00 1.86 (1.17–2.96) 0.92 (0.29–2.94) 2.20 (1.10–4.41) 2.16 (1.17–4.00)	Ever smoking, alcohol intake, BMI, education, age	Strengths: highly representative of the general female population in Shanghai; accurate data on exposure; accurate data from cancer registry on incident cancers (very low losses to follow-up) Limitations: only 24 NHL among the exposed
Clavel et al. (1996) France 1980–1990 Case–control	Cases: 226 hairy cell leukaemia patients recruited in 18 French hospitals; only living cases included (60% of 368 eligible) Controls: 425 hospital-based, matched to cases by sex, birth date, admission date, residence; mainly from orthopaedic and rheumatology departments; response rate, 57% Exposure assessment method: expert judgement; JEM that assessed a score for ppm of exposure to benzene; exposure blindly assigned to cases and controls	NHL (HCL)	Unexposed Cumulative benzene exposure (ppm-yr) < 1 (score) 1–5 (score) ≥ 5	189 15 10 7	1.0 0.7 (0.4–1.3) 0.7 (0.3–1.4) 0.5 (0.2–1.2)	Matching variables age and sex, smoking status, residence, admission date	Strengths: large series of rare tumour; good exposure assessment Limitations: low response rate among controls; only living cases (prevalent) included, meaning potential source of bias

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Orsi et al. (2010) France 2000–2004 Case–control	Cases: 244 NHL, 87 HD, 56 MM; hospital-based, men only (aged 20–75 yr); incident cases; response rate, 95% Controls: 456 mainly from orthopaedic and rheumatology departments, residing in hospital catchment area; matched by age, sex, centre; cancers excluded, as well as diseases related to occupation, alcohol, or smoking; response rate, 91.2% Exposure assessment method: expert judgement; job-specific questionnaires evaluated by chemical engineer; experts derived ppm estimates from previously published intensity measurement campaigns	NHL	All benzene, exposed vs unexposed	94	1.0 (0.7–1.5)	Age, centre, socioeconomic status	Strengths: good exposure assessment by expert; very high response rate Limitations: hospital-based study
			Benzene > 1 ppm-yr	70	1.4 (0.9–2.1)		
			Pure benzene	6	3.0 (0.8–11.2)		
			Pure benzene, definite exposure	5	3.4 (0.8–15.0)		
			Latency 30 yr, pure benzene	5	5.5 (1.0–30.7)		
			High intensity of exposure	4	2.6 (0.6–11.2)		
			Same, diffuse large cell lymphoma	4	7.2 (1.6–33.2)		

Table 2.4 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Wong et al. (2010b) Shanghai 2003–2008 Case–control	Cases: 649 hospital-based from 25 hospitals; response rate, 76% Controls: 1298 hospital-based controls matched by age and sex, with exclusion of blood malignancies; response rate, NR Exposure assessment method: expert judgement; exposure assessment conducted by experts by estimating ppm of exposure	NHL	Maximum exposure to benzene			Age, sex, hospital	Limitations: hospital-based study
			Score 1	32	1.14 (0.73–1.78)		
			Score 2	9	0.75 (0.35–1.64)		
			Score 3–4	9	1.21 (0.53–2.76)		
			Trend test *P* value, 0.76 for maximum exposure, 0.80 for duration of exposure				

BMI, body mass index; CI, confidence interval; HCL, hairy cell leukaemia; HD, Hodgkin disease; JEM, job-exposure matrix; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million; vs, versus; yr, year(s)

threefold increased risk for exposure duration of more than 15 years (OR, 2.9; 95% CI, 0.9–9.0) based on 14 cases.

In a hospital-based case–control study of men only with very high response rates (83%) conducted in France, Orsi et al. (2010) reported no increased NHL risk with exposure to benzene compared with no exposure (OR, 1.0; 95% CI, 0.7–1.5), and a positive association for those exposed to "pure benzene" (OR, 3.0; 95% CI, 0.8–11.2) based on 6 exposed cases. No associations were observed for HL and MM. [The Working Group noted an unclear choice of controls and the small numbers for the different exposure metrics, for example, "pure benzene".]

A population-based case–control study in the Boston metropolitan area by Scherr et al. (1992), with high response rates of 80% and including a review of pathological material (slides), found no significant association with NHL in ever versus never exposed cases (OR, 1.2; 95% CI, 0.5–2.6). [The Working Group noted the potential for information bias due to the use of self-reported exposure via questionnaires, as well as the small sample size.]

In a population-based case–control study of women aged 21–84 years, Wang et al. (2009) used a JEM (with assessment of probability and intensity of exposure) to report slightly elevated, non-statistically significant risks for NHL in the group exposed to medium to high concentrations of benzene, particularly for specific histological types (*P* for trend, 0.04 for DLBCL, 0.08 for CLL, and 0.18 for follicular lymphoma). [The Working Group noted generally low response rates.]

A hospital-based study by Wong et al. (2010b), including 649 cases and 1298 matched controls, found no association between NHL and benzene exposure assessed by experts based on job questionnaires, and a significant association for follicular lymphoma (based on 7 cases only).

A population-based cohort study conducted after the publication of *IARC Monographs* Volume 100F evaluated the association between benzene exposure and NHL: Bassig et al. (2015) followed up 73 087 women in the Shanghai general population for NHL incidence through the Cancer Registry, and used a quantitative JEM based on actual benzene measurements (with assessment of probability and intensity of exposure to benzene). Response rates were very high (93%). The overall hazard ratio for the ever exposed versus the unexposed group was 1.86 (95% CI, 1.17–2.96) based on 24 exposed cases, and an exposure–response relationship was reported with both duration of exposure to benzene (*P* for trend, < 0.006) as well as cumulative exposure (*P* for trend, < 0.005). A case–control study in Italy showed evidence of a dose–response relationship between exposure to benzene for a duration of more than 15 years and CLL (*P* for trend, 0.05) (Costantini et al., 2008).

In a case–control study in residents in Shanghai (532 cases and 502 controls from the general population), a significant 3.9-fold increased risk for ALL was reported for the group with 15 years or more of self-reported occupational exposure to benzene, based on 5 exposed cases in this category (Adegoke et al., 2003); no association was observed in another study in the USA, with only 3 cases in the highest exposure group (see *IARC Monographs* Volume 100F, Table 2.4, available at: http://publications.iarc.fr/123).

2.3 Childhood cancer

Age-specific incidence rates for several types of childhood cancer peak at ages < 5 years, indicating that risk factors exist in the early life environment or might be inherited. Few risk factors have been identified, with the exception of ionizing radiation and chemotherapy, meaning that the majority of cases are unexplained.

It is known that benzene causes AML/ANLL in adults. Positive associations have also been observed between exposure to benzene and ALL, CLL, MM, and NHL (IARC, 2012a). Leukaemia

is the most common type of childhood cancer, leading to the hypothesis that benzene could also cause leukaemia in children. Benzene often occurs as part of mixed exposures, such as in utero in pregnant women who smoke, or from second-hand smoke and traffic exhaust in ambient air. Studies of childhood cancers have used various indicators of such mixtures, for example, traffic (von Behren et al., 2008; Amigou et al., 2011), petrol stations, and automotive repair garages near the residence (Steffen et al., 2004; Brosselin et al., 2009). Studies of parental occupational exposures and childhood cancer have also used indicators for mixed exposure which may include benzene, such as "solvent use" (van Steensel-Moll et al., 1985; Carlos-Wallace et al., 2016). [The Working Group is aware of these studies but decided to review only those specifically assessing exposure to benzene. The Working Group noted that, even in studies where benzene is specifically assessed, benzene is often one of many correlated air pollutants; confounding from such correlated air pollutants can rarely be excluded.]

Ecological studies have compared incidence rates of childhood leukaemia (Whitworth et al., 2008; Senkayi et al., 2014) and tumours of the central nervous system (CNS) (Danysh et al., 2015) with benzene levels assessed at census tracts or county level. These studies were not reviewed by the current Working Group because of the usual limitations of the ecological design for causal inference.

The Working Group reviewed a series of case–control studies that quantified ambient benzene levels, either assessed at the exact address or as a mean for the area where childhood cancer cases and controls lived. A case–control study that assessed exposure by measuring a benzene metabolite in urine from childhood cases and controls (Jiang et al., 2016) was also reviewed. One additional case–control study (Ruckart et al., 2013) was not reviewed, because the information that could be extracted was limited because the study included only 13 verified childhood cases of cancer of the haematopoietic system (11 leukaemia and 2 NHL) and because exposure to benzene was not quantified. Another case–control study that investigated the distance from the residence to industries emitting benzene was not reviewed because benzene concentrations were not quantified (García-Pérez et al., 2015).

The Working Group also reviewed a series of case–control studies and two cohort studies that compared the occupational exposure to benzene of the parents of childhood cancer cases and controls. Table 2.5 includes only relevant studies of cancer sites with sufficient or limited evidence that were either not included in, or published after, *IARC Monographs* Volume 100F (IARC, 2012a).

2.3.1 Childhood exposure to benzene in outdoor air

Four case–control studies assessed benzene concentrations at the exact address(es) where childhood cancer cases and controls lived (see Table 2.5).

In a population-based study included in *IARC Monographs* Volume 100F, Raaschou-Nielsen et al. (2001) identified 1989 cases (age, 0–14 years) of leukaemia, lymphoma, and tumours of the CNS in the Danish Cancer Registry, and selected 5506 controls at random among the whole Danish childhood population using the Danish Population Registry. Controls were matched to cases by sex, age, and calendar time. The residential history of each child was traced from 9 months before birth to the time of diagnosis. Benzene exposure was calculated from a dispersion model based on traffic and the configuration of the street and buildings at the address. The analyses adjusted for urban development, geographical region, type of residence, low-frequency electromagnetic fields (power lines and transformer stations), mother's age, and birth order. For exposure to benzene during childhood between the 90th and 99th percentile

Table 2.5 Epidemiological studies of exposure to benzene and childhood leukaemia in the general population

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Crosignani et al. (2004) Province of Varese, Italy 1978–1997 Case–control	Cases: 120 from cancer registry Controls: 480 population-based from Health Service Archives Exposure assessment method: other; modelled concentration of benzene outside the residence at time of diagnosis	Leukaemia: ICD-9 (codes 204.0–208.9), lymphoid leukaemia, myeloid leukaemia, monocytic leukaemia, other specified and unspecified leukaemia	Benzene concentration (μg/m³) < 0.1 0.1–10 > 10 Trend test *P* value, 0.005	 88 25 7	 1.0 1.5 (0.9–2.5) 3.9 (1.4–11.3)	Sex, age, SES of municipality	Incidence, ages 0–14 yr Strengths: population-based; exposure model Limitations: small number of cases; only address at diagnosis; non-differential misclassification of exposure

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vinceti et al. (2012) Emilia-Romagna region, northern Italy 1998–2009 Case–control	Cases: 83 from cancer registry Controls: 332 population-based from Health Service Archives Exposure assessment method: other; CALINE4 dispersion model estimating exposure to benzene from road traffic; based on address at diagnosis; exposure categories are approximate quartiles of annual average benzene concentrations	Leukaemia: acute leukaemia	Benzene concentration (μg/m³)			Sex, age, province of residence, PM_{10} concentration	Incidence within previous 0–14 yr; validation of dispersion model reported; linear trends were not statistically significant; stronger and statistically significant associations with AML for children < 5 yr (OR, 5.46; 95% CI, 1.12–26.51; 11 cases) Strengths: population-based, exposure model Limitations: small number of cases; only address at diagnosis; limited confounder adjustment; non-differential misclassification of exposure
			< 0.10	16	1.0		
			0.10 to < 0.25	18	0.8 (0.5–2.6)		
			0.25 to < 0.50	17	1.1 (0.5–2.6)		
			≥ 0.50	32	1.7 (0.8–3.6)		
		Leukaemia (ALL)	OR per 1 μg/m³ increase in average benzene concentration and with a 10 μg/m³ increase in average PM_{10} concentration	64	0.97 (0.49–1.93)	Sex, age, province of residence, PM_{10} concentration	
		Leukaemia (AML)	OR per 1 μg/m³ increase in average benzene concentration and with a 10 μg/m³ increase in average PM_{10} concentration	19	1.92 (0.64–5.78)	Sex, age, province of residence, PM_{10} concentration	

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Houot et al. (2015) Île-de-France, France 2002–2007 Case–control	Cases: 517 from cancer registry Controls: 6147 population-based, from tax databases Exposure assessment method: records; modelled concentration of benzene outside the residence at time of diagnosis	Leukaemia (ALL)	Benzene concentration (μg/m³)			Age	Incidence within previous 0–14 yr Strengths: population-based; exposure model Limitations: small number of AML cases; only address at diagnosis; limited confounder adjustment; non-differential misclassification of exposure
			< 1.3	215	1.0		
			≥ 1.3	210	0.9 (0.7–1.0)		
		Leukaemia (AML)	Benzene concentration (μg/m³)			Age	
			< 1.3	33	1.0		
			≥ 1.3	59	1.6 (1.0–2.4)		
Heck et al. (2014) California, USA 1990–2007 Case–control	Cases: 66 ALL and 41 AML from cancer registry Controls: 2627 for ALL and 17 299 for AML, randomly from population with California birth certificates Exposure assessment method: other; air toxics measured at monitoring station nearest to home address at time of birth (ALL, ≤ 2 km; AML, ≤ 6 km)	Leukaemia (ALL)	OR per IQR increase in benzene concentration (1.2 ppb) during pregnancy			Year of birth, mother's birth place, parity, neighbourhood SES, mother's race/ethnicity	Incidence within previous 0–5 yr; exploratory study of 22 air toxics Strengths: population-based; monitoring-based exposure assessment Limitations: small number of cases; no address history; multiple testing; non-differential misclassification of exposure
			1st trimester	66	0.85 (0.58–1.26)		
			2nd trimester	66	1.16 (0.80–1.67)		
			3rd trimester	66	1.50 (1.08–2.09)		
			Entire pregnancy	66	1.44 (0.84–2.48)		
			1st year of life	61	1.23 (0.62–2.43)		
		Leukaemia (AML)	OR per IQR increase in benzene concentration (1.2 ppb) during pregnancy			See above	
			1st trimester	41	1.13 (0.64–2.01)		
			2nd trimester	41	1.30 (0.74–2.28)		
			3rd trimester	41	1.75 (1.04–2.93)		
			Entire pregnancy	41	1.94 (0.89–4.19)		
			1st year of life	25	2.61 (0.97–6.99)		

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Janitz et al. (2017) Oklahoma, USA 1997–2012 Case–control	Cases: acute leukaemia, 307; ALL, 228; AML, 79; cancer registry Controls: 1013 population with Oklahoma birth certificates Exposure assessment method: other; USEPA National Air Toxics Assessment database modelled benzene 2005 concentration of the census tract where living at time of birth; exposure level cut-points were based on quartiles of benzene concentration (μg/m³) among controls	Leukaemia: ALL and AML combined	Benzene concentration (μg/m³)			Week of birth, race/ ethnicity, age at diagnosis, sex, birth order, electromagnetic fields, urbanization, maternal education, smoking during pregnancy	Incidence within previous 0–19 yr; an alternative exposure categorization showed substantially lower ORs in association with the very highest exposures (above 95th percentile, 1.33–2.03 μg/m³) Strengths: population-based exposure model Limitations: small number of cases; no address history; exposure assessment for only 1 yr, non-differential misclassification of exposure
			0.11 to < 0.39	73	1.00		
			0.39 to < 0.67	71	1.06 (0.71–1.58)		
			0.67 to < 0.91	77	1.21 (0.79–1.87)		
			0.91–2.03	86	1.28 (0.83–1.97)		
		Leukaemia (ALL): childhood ALL	Benzene concentration (μg/m³)			Week of birth, race/ ethnicity, age at diagnosis, sex, birth order, electromagnetic fields, urbanization, maternal education, smoking during pregnancy	
			0.11 to < 0.39	NR	1.00		
			0.39 to < 0.67	NR	0.91 (0.58–1.44)		
			0.67 to < 0.91	NR	1.07 (0.66–1.76)		
			0.91–2.03	NR	1.06 (0.65–1.74)		
		Leukaemia (AML): childhood AML	Benzene concentration (μg/m³)			Week of birth, race/ ethnicity, age at diagnosis, sex, birth order, electromagnetic fields, urbanization, maternal education, smoking during pregnancy	
			0.11 to < 0.39	NR	1.00		
			0.39 to < 0.67	NR	1.60 (0.67–3.82)		
			0.67 to < 0.91	NR	1.92 (0.77–4.74)		
			0.91–2.03	NR	2.42 (0.98–5.96)		

Table 2.5 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Spycher et al. (2017) Switzerland 1990 or 2000/ until end of 2008 Cohort	1 664 801 children aged < 16 yr Exposure assessment method: other; census data on parental occupation and JEM (based on ISCO-88) to categorize potential for benzene exposure	Leukaemia (ALL) Leukaemia (AML)	Maternal occupational exposure Ever exposed Maternal occupational exposure Ever exposed	19 3	1.92 (1.18–3.13) 1.05 (0.32–3.48)	Sex; year of birth; census; education; household crowding; neighbourhood SES; background ionizing radiation and electromagnetic fields from radio and TV transmitters; distance to nearest highway, petroleum refinery, petrol station, motor vehicle service station	No associations with AML or ALL and paternal exposure were observed Strengths: population-based study with data on incidence; models adjusted for a range of socioeconomic, perinatal, and environmental factors; accurate and complete outcome ascertainment Limitations: no adjustment for maternal smoking; small number of exposed AML cases

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CALINE4, California Line Source Dispersion model, version 4; CI, confidence interval; ICD, International Statistical Classification of Diseases and Related Health Problems; IQR, interquartile range; ISCO, International Standard Classification of Occupations; JEM, job-exposure matrix; NR, not reported; OR, odds ratio; PM, particulate matter; ppb, parts per billion; SES, socioeconomic status; USEPA, United States Environmental Protection Agency; yr, year(s)

of the distribution of exposure, or above the 99th percentile compared with below the 50th percentile, the respective adjusted relative risks were 0.9 (95% CI, 0.6–1.5) and 0.4 (95% CI, 0.1–1.6) for leukaemia, 1.1 (95% CI, 0.7–1.8) and 0.6 (95% CI, 0.2–1.7) for tumours of the CNS, and 0.8 (95% CI, 0.4–1.8) and 0.4 (95% CI, 0.1–2.0) for lymphomas. The results also indicated no associations between benzene concentrations at the address(es) during pregnancy and cancer risk, although the group exposed to the second-highest levels of benzene was associated with a relative risk for lymphomas of 2.2 (95% CI, 1.2–3.9). This higher risk for lymphomas in association with benzene concentrations during pregnancy was restricted to HL; based on 19 exposed cases, a relative risk of 4.3 (95% CI, 1.5–12.4) was observed in association with exposure above the 90th percentile compared with below the 50th percentile (see *IARC Monographs* Volume 100F, Table 2.14, available at: http://publications.iarc.fr/123). [The strengths of this study included the population-based design, the large sample size, the assessment of cumulative exposure over all addresses during pregnancy and childhood, and the objective, model-based exposure assessment method. However, the study was limited by the non-differential misclassification of exposure.]

A small study of leukaemia incidence was undertaken in Varese, Italy (Crosignani et al., 2004). A total of 120 incident cases were identified from the population-based Lombardy Cancer Registry, and 480 population controls, matched to cases by age and sex, were selected from the population-based Health Service Archives. Benzene concentration at the address at diagnosis was calculated on the basis of traffic density on surrounding roads and distances from the home address to roads with heavy traffic. When comparing children exposed to high concentrations of benzene (estimated annual average, benzene at > 10 μg/m^3) with children not exposed to road traffic emissions (estimated annual average, benzene at < 0.1 μg/m^3), the relative risk was 3.9 (95% CI, 1.4–11.3) based on 7 exposed cases, and 4.3 (95% CI, 1.5–12.6) after adjustment for socioeconomic status of the municipality. There was a trend across the three exposure categories (*P* for trend, < 0.005). [The strengths of the study included the population-based design and the objective, model-based, exposure assessment method. The limitations included the small number of cases, the use of only the address at diagnosis for exposure assessment, and the non-differential misclassification of exposure.]

In the Emilia-Romagna region, northern Italy, Vinceti et al. (2012) identified 83 incident cases of acute leukaemia among children (age, 0–14 years) in the population-based Italian Association of Paediatric Haematology and Oncology cancer registry. A total of 332 population controls, individually matched to cases by sex, year of birth, and province of residence during the diagnosis year, were selected. Traffic-related benzene and PM_{10} (particulate matter of diameter ≤ 10 μm) concentrations were estimated by the California Line Source Dispersion model, version 4 (CALINE4), at the address at time of diagnosis. Modelled benzene concentrations were validated against those measured at fixed-site monitoring stations, showing a correlation coefficient of 0.43 (95% CI, −0.48–0.89) for annual mean values. The odds ratio for acute leukaemia was 1.7 (95% CI, 0.8–3.6) for the highest (≥ 0.50 μg/m^3) compared with the lowest (< 0.10 μg/m^3) category of benzene exposure, after adjustment for PM_{10}. Linear analyses adjusted for PM_{10} showed odds ratios of 0.97 (95% CI, 0.49–1.93; 64 cases) and 1.92 (95% CI, 0.64–5.78; 19 cases) for ALL and AML, respectively, in association with a 1 μg/m^3 increase in average benzene concentration. Restricting the linear analyses to children diagnosed when aged 0–4 years yielded odds ratios of 1.95 (95% CI, 0.58–6.50; 27 cases) and 5.46 (95% CI, 1.12–26.51; 11 cases) for ALL and AML, respectively. [A strength of this study was the population-based design and the objective exposure assessment by

use of a validated exposure model, although the correlation between calculated and measured benzene was relatively low. Limitations included the small sample size resulting in wide confidence intervals, the use of only the address at the time of diagnosis for exposure assessment, and non-differential misclassification of exposure.]

Houot et al. (2015) estimated benzene concentrations at the addresses at the time of diagnosis of 517 incident acute leukaemia cases (age, 0–14 years) and 6147 control children living in the Île-de-France region in France. Cases were identified from the National Registry of Childhood Haematopoietic Malignancies, and control children were selected from a population-based tax database. The benzene modelling took into account contributions from both urban background pollution and local traffic. The subjects were classified on the basis of the benzene exposure estimate at their home being either less than 1.3 μg/m^3 (median exposure for the controls) or 1.3 μg/m^3 or more. When comparing the two groups, the odds ratio for ALL was 0.9 (95% CI, 0.7–1.0; based on 210 exposed cases) and for AML was 1.6 (95% CI, 1.0–2.4; $P < 0.05$, based on 59 exposed cases). The analyses were adjusted for age. [Strengths included the population-based design and the objective, model-based exposure assessment method. Limitations included the limited adjustment for potential confounders, the use of only the address at the time of diagnosis for exposure assessment, the non-differential misclassification of exposure, and the limited contrast in the analysis of exposures to above versus below the median.]

A series of studies from California, USA, studied the association between a wide range of air toxics (including benzene) measured at 39 different sites and different types of childhood cancers. Cases (age, 0–5 years) were identified from the California Cancer Registry, and population controls were selected randomly from the California birth records. Birth addresses of cases and control children were allocated to 1 of the 39 monitoring stations if the address was within a certain distance, and the measured air toxic concentration was averaged over certain time periods; both the distance and the time period differed between the studies. Odds ratios were calculated for one interquartile range (IQR) increase in benzene concentration, corresponding to 1.2 ppb (3.8 μg/m^3). Heck et al. (2013) compared benzene concentrations during pregnancy for 74 neuroblastoma cases and 13 115 control children, and observed an odds ratio of 1.36 (95% CI, 0.82–2.25) per IQR increase of benzene concentration after adjustment for year of birth, maternal race/ethnicity, maternal age, and method of payment for prenatal care. Shrestha et al. (2014) studied 337 cases of Wilms tumour and 96 514 control children, and found an odds ratio of 1.07 (95% CI, 0.84–1.36) per IQR of benzene concentration during pregnancy after adjustment for birth year, maternal age, maternal race/ethnicity, parity, and census-based socioeconomic status. Heck et al. (2014) included 66 cases of ALL and 41 cases of AML, and found odds ratios of 1.44 (95% CI, 0.84–2.48) for ALL and 1.94 (95% CI, 0.89–4.19) for AML per IQR increase of benzene concentration during the entire pregnancy. Results were adjusted for year of birth, maternal race/ethnicity, mother's birthplace, parity, and neighbourhood socioeconomic status. Exposure during the third trimester was associated with statistically significant odds ratios. Odds ratios in association with benzene concentration during the first year of life were similar to those reported for exposure during pregnancy. Heck et al. (2015) included 88 retinoblastoma cases and 25 144 control children, and found a significant odds ratio of 1.67 (95% CI, 1.06–2.64) per IQR increase of benzene concentration during pregnancy after adjustment for year of birth, maternal race and birthplace, paternal age, and method of payment for prenatal care. Von Ehrenstein et al. (2016) investigated the risk for tumours of the CNS based on 168 cases and 27 199 control children; odds

ratios of 2.14 (95% CI, 1.12–4.06; 38 cases) for primitive neuroectodermal tumour, 0.82 (95% CI, 0.36–1.87; 30 cases) for medulloblastoma, and 0.83 (95% CI, 0.53–1.29; 100 cases) for astrocytoma per IQR increase of benzene concentration during pregnancy were reported. Results were adjusted for year of birth, maternal race/ethnicity, maternal age, birthplace, and education. [The strengths of these studies included the population-based design and the objective, monitoring-based exposure assessment method. Limitations included the small sample sizes, the assessment of exposure at only one address, the non-differential misclassification of exposure, and the explorative nature including analyses of many pollutants. Not adjusting for smoking at home, and especially maternal smoking during pregnancy, were other common limitations of many studies. The results for neuroblastoma and retinoblastoma appear to be generating, rather than testing, hypotheses.]

Symanski et al. (2016) identified 1248 cases (age, 0–4 years) of ALL from the Texas Cancer Registry and selected 12 172 population-based control children from birth certificates. The address at birth was used to allocate each child to a census tract. Concentrations of benzene, 1,3-butadiene and polycyclic organic matter at census tract level were extracted from the United States Environmental Protection Agency National-Scale Air Toxics Assessment (NATA), which provided modelled concentrations for years 1996, 1999, 2002, and 2005; each address was allocated to an exposure quartile for the year closest in time. The statistical models adjusted for time of birth, census tract (random effect), maternal age, infant birth weight, sex, and maternal race/ethnicity, and reported odds ratios of 1.19 (95% CI, 1.00–1.41), 1.16 (95% CI, 0.98–1.38), and 1.17 (95% CI, 0.98–1.39) for the second, third, and fourth exposure quartiles compared with the first. Models including both benzene and 1,3-butadiene showed associations between childhood cancer and exposure to 1,3-butadiene, but not to benzene. [The Working Group noted that it is difficult to disentangle the effect of correlated pollutants. The strengths of this study included: the population-based design; the large sample size; the objective, model-based exposure assessment method; and the mutual adjustment for other air pollutants. Limitations included the lack of information about address history and non-differential misclassification of exposure. Further, the transformation of absolute exposure concentrations into quartiles based on 4 different years makes a quantitative interpretation of the results difficult: for example, the third exposure quartile for benzene in 1996 has a lower range of benzene levels than the second exposure quartile in 1999.]

Janitz et al. (2017) studied benzene and acute leukaemia including 228 cases of ALL and 79 of AML (age, 0–19 years) from the Oklahoma Central Cancer Registry, USA. A total of 28% of identified cases were excluded, however, because they could not be linked to birth certificates. Population controls (n = 1013) were selected from birth certificates, matched by week of birth. Address at birth was allocated to the census tract, and benzene concentrations for 2005 for each census tract were extracted from the NATA database. Children were divided into quartiles of exposure and, in a secondary analysis, the cut-off point at the 40th (0.53 μg/m^3), 60th (0.78 μg/m^3), and 95th (1.33 μg/m^3) percentiles were used to form exposure categories. The results indicated no association between benzene and ALL, with an odds ratio for the highest quartile compared with the lowest of 1.06 (95% CI, 0.65–1.74) and that for above the 95th percentile compared with below the 40th percentile being 0.67 (95% CI, 0.28–1.62). In contrast, the two corresponding odds ratios for AML were 2.42 (95% CI, 0.98–5.96) and 1.58 (95% CI, 0.53–4.69), with an indication of an exposure–response relationship over quartiles but not over the alternative exposure categorization. The analyses adjusted for time of birth, race/ethnicity, age at diagnosis, sex, birth order,

exposure to electromagnetic fields, urbanization, and maternal education and smoking during pregnancy. [The strengths included the population-based design and the objective, model-based exposure assessment method. Limitations included the limited sample size, the lack of information about address history, the exposure assessment being based on only 1 year, and the non-differential misclassification of exposure. The Working Group noted the exclusion of cases that could not be linked to a birth certificate as a potential source of selection bias.]

Jiang et al. (2016) measured the benzene metabolite *trans*,*trans*-muconic acid (t,t-MA) in urine samples from 71 cases of ALL identified at the Shenzhen Children's Hospital, China, and from those of 142 control children selected from the orthopaedics section and matched to cases by sex and age. A higher proportion was above the detection limit among cases compared with controls, and higher t,t-MA concentration was associated with an increased risk (OR, 1.09; 95% CI, 1.00–1.19). [The Working Group noted that the related exposure contrast was not reported in the article. Other limitations included: the risk for reverse causation because urine was collected after the ALL diagnosis; a lack of translation between concentrations of t,t-MA in urine (which only reflect exposure during the few hours before urine collection) and exposure to benzene, meaning that the use of t,t-MA in this context did not provide a good context for exposure to benzene and the validity of this exposure assessment method is low (see Section 1.3); and the non-differential misclassification of exposure.]

2.3.2 Parental occupational exposure to benzene

Nine case–control and two cohort studies assessed the occupational exposure to benzene of parents of childhood cancer cases and controls. In 8 of the 11 studies, the exposure assessment was based on information about parental occupation, industry, or exposure collected by interviews with parents after their child received a diagnosis of cancer. Three other studies used information from birth certificates (Shaw et al., 1984) or census data (Feychting et al., 2001; Spycher et al., 2017), which could not be influenced differently by parents of cases and controls. Exposure was assessed in two (yes/no) or three (e.g. no/possible/probable) categories either by parents themselves or by experts or JEM, but was never quantified. [The Working Group noted that the interview-based exposure assessment in 8 of the 11 studies implied a risk for recall and interviewer bias. Differential participation among parents of cases and controls may also have biased the results. Other general limitations were the low numbers of exposed parents, leading to imprecise risk estimates, and the lack of quantification of benzene exposure. For these reasons, the Working Group gave little weight to this group of studies when evaluating benzene as a potential cause of childhood cancer.]

The results from the 11 studies are inconsistent. Shaw et al. (1984) used information about job from birth certificates in a case–control study in California, USA, and reported no difference in childhood leukaemia risk between case and control groups for paternal occupation with potential for benzene exposure. In a cohort study in Sweden, Feychting et al. (2001) reported no association between parental job (2–26 months before the child's birth, obtained from census data) with the potential for benzene exposure (possible and probable exposure combined) and the risk for leukaemia (RR, 1.23; 95% CI, 0.39–3.85; 3 cases) or tumours of the CNS (RR, 0.91; 95% CI, 0.23–3.70; 2 cases) in their children. The risk of leukaemia in children younger than 5 years was 2.0 (95% CI, 0.6–6.3) based on 3 exposed cases [although benzene exposure was too rare for a meaningful analysis].

In a nationwide cohort study in Switzerland, Spycher et al. (2017) used census data about parental occupation and a JEM to categorize

potential for benzene exposure (Table 2.5). In association with maternal exposure to benzene, the study showed a hazard ratio for ALL of 1.92 (95% CI, 1.18–3.13; 19 exposed cases) using the partially adjusted model and 2.63 (95% CI, 1.58–4.38) using the fully adjusted model, which included 14 variables but not maternal smoking. No association between maternal exposure to benzene and risk for AML (3 exposed cases only), lymphoma, NHL, tumours of the CNS, or glioma (Table 2.5) was found. The study also found no association between paternal benzene exposure and risk for ALL or AML, although an increased non-significant risk was observed for AML (HR, 2.66; 95% CI, 0.79–9.00; 3 exposed cases in the upper exposure category). [The Working Group considered this study to be informative because of: the lack of potential for participation, recall, or interviewer bias; the objective assessment of benzene exposure using a JEM; the proper adjustment for potential confounders; and the greater number of cases with exposed parents compared with most other studies. No adjustment was made for parental smoking, however, and there was potential for exposure misclassification.]

Among the eight case–control studies with subjective (interview-based) information about benzene exposure, three found statistically significant associations, two found increased odds ratios without statistical significance, and three found no association.

Shu et al. (1988) (see Table 2.4 in *IARC Monographs* Volume 100F, available at: http://publications.iarc.fr/123) investigated parental exposures in relation to childhood leukaemia in Shanghai and found an association between maternal exposure during pregnancy and risk for ANLL (OR, 4.0; 95% CI, 1.8–9.3; 11 cases) but not for ALL (OR, 1.3; 95% CI, 0.5–3.0; 8 cases).

McKinney et al. (1991), evaluated in *IARC Monographs* Volume 100F, investigated the associations between parental exposure to benzene and risk for leukaemia and NHL (combined) in north England; statistically significant associations with paternal preconceptional exposure (OR, 5.8; 95% CI, 1.7–26.4; 12 cases) and with maternal preconceptional exposure (OR, 4.0; 95% CI, 0.3–118.0; 2 cases) were found.

Castro-Jiménez & Orozco-Vargas (2011) investigated parental benzene exposure and risk for ALL in Columbia, and reported unadjusted odds ratios of 3.0 (95% CI, 1.3–7.1) and 1.6 (95% CI, 0.8–3.1) in association with maternal and paternal exposure, respectively, before conception, and an unadjusted odds ratio of 1.9 (95% CI, 0.8–4.2) in association with maternal exposure during pregnancy. After adjustment for maternal age, parental preconception smoking status, and maternal socioeconomic status during pregnancy, odds ratios of 5.50 (95% CI, 1.38–21.92) and 11.65 (95% CI, 2.98–45.59) were observed for exposure to benzene by mother only and by both parents, respectively.

Feingold et al. (1992) investigated childhood cancer in Denver, Colorado, USA, and reported odds ratios in association with paternal occupational benzene exposure during the year before the child's birth of 0.7 (95% CI, 0.1–3.1) for tumours of the CNS and 1.6 (95% CI, 0.5–5.8) for ALL. Peters et al. (2014) investigated childhood tumours of the brain in Australia, and reported odds ratios of 2.4 (95% CI, 0.2–25.7) and 2.7 (95% CI, 0.9–7.9) in association with maternal exposure in the year before birth and paternal exposure in the year before conception.

Kaletsch et al. (1997) studied childhood leukaemia and lymphoma in Germany, and found no association with parental occupational exposure to benzene.

Shu et al. (1999) investigated parental occupational exposures and risk of ALL in offspring (see Table 2.4 in *IARC Monographs* Volume 100F, available at: http://publications.iarc.fr/123). The authors reported odds ratios of 0.7 (95% CI, 0.3–1.8), 0.5 (95% CI, 0.1–1.6), and 0.6 (95% CI, 0.2–1.6) for maternal exposure to benzene before conception, during pregnancy, and after birth, respectively. Corresponding odds ratios for

paternal occupational exposure to benzene were 1.2 (95% CI, 0.8–1.2), 1.0 (95% CI, 0.6–1.7), and 1.2 (95% CI, 0.7–1.9).

Infante-Rivard et al. (2005) studied childhood ALL in Canada and reported an odds ratio of 0.8 (95% CI, 0.2–3.1) in association with maternal benzene exposure during the 2 years before birth, and one of 1.4 (95% CI, 0.3–6.3) in association with maternal exposure during pregnancy.

[Treating all leukaemias as a single entity is a limitation of several of the preceding studies, given the evidence of etiological heterogeneity.]

2.4 Other cancers

This section describes epidemiological studies on benzene exposure and cancer in adults in sites other than in the lymphohaematopoietic system. The tables (Table 2.6 and Table 2.7) include only relevant studies of cancer sites with sufficient or limited evidence that either were not included in *IARC Monographs* Volume 100F (IARC, 2012a) or were published later.

2.4.1 Occupational cohort studies

Several occupational cohort studies have reported results for multiple solid tumour sites. Some earlier studies were updated for specific outcomes, most often leukaemia or lymphomas, but results for other cancers were not reported in the updates.

The Working Group included occupational cohort studies that reported risk estimates specifically for benzene, based on either individual estimates of exposure or identification of subcohorts exposed to benzene. Studies were excluded if they did not meet these inclusion criteria (Guberan & Raymond, 1985; Dagg et al., 1992; Rushton, 1993; Schnatter et al., 1993; Tsai et al., 1993, 1996, 2003; Walker et al., 1993; Honda et al., 1995; Collingwood et al., 1996; Fu et al., 1996; Satin et al., 1996; Järvholm et al., 1997; Lynge et al., 1997; Lundberg & Milatou-Smith, 1998; Pukkala, 1998; Consonni et al., 1999; Divine et al., 1999a, b; Wong et al., 2001a, b; Sorahan et al., 2002; Kauppinen et al., 2003; Lewis et al., 2003; Huebner et al., 2004; Gun et al., 2006; Bonneterre et al., 2012), were superseded by updates (Ott et al., 1978), presented results only for excessively broad outcome groupings, or had very small study populations (Decouflé et al., 1983).

Studies publicly available at the time were reviewed in *IARC Monographs* Volume 100F, and a new search of the literature for this review identified a few additional studies in occupational cohorts. The following studies available at the time of the previous IARC review are included in the current evaluation: Tsai et al. (1983), Wilcosky et al. (1984), Bond et al. (1986), Wong (1987a, b), Wong et al. (1993), Greenland et al. (1994), Hayes et al. (1996), Bulbulyan et al. (1999), and Sorahan et al. (2005). A brief description of these cohorts is provided in the following section.

(a) Description of occupational cohorts

Bond et al. (1986) studied mortality among 956 workers from a chemicals production plant in Michigan, USA, exposed to benzene (see Section 2.1.1). Industrial hygiene data were used to weight jobs as incurring very low, low, moderate, or high levels of exposure, a representative time-weighted average exposure value was assigned to each level, and cumulative dose indices were calculated for each worker by summing daily time-weighted average values over the work history. Exposure–response analyses were made both including and excluding workers who were exposed to arsenic, asbestos, or high levels of vinyl chloride. Updated results for this cohort were reported by Collins et al. (2015) (see Section 2.1.1(b)), but data were not provided for all cancer sites.

Bulbulyan et al. (1999) reported cancer mortality among women in the Russian printing

Table 2.6 Occupational cohort studies of exposure to benzene and cancer of the lung

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Linet et al. (2015) China, 12 cities 1972–1987/1972–1999 Cohort	74 827 benzene-exposed and 35 504 unexposed Chinese workers; spray and brush painting (coatings), rubber, chemical (including pharmaceutical manufacturing), shoemaking, and other (including printing and insulation) industries Exposure assessment method: records; workers dichotomized (benzene exposed/unexposed) based on job titles and factory records on use of benzene-containing materials	Lung: ICD-9 (code 162)	Exposed	351	1.5 (1.2–1.9)	Sex, attained age, attained calendar year	Supersedes Yin et al. (1996a), Hayes et al. (1996); lag of 2 yr for HLD and 10 yr for all other outcomes; MDS RR mortality, infinity (1.5–infinity), $n = 7$; incidence, infinity (1.9–infinity), $n = 8$ Strengths: large sample size, 28-yr follow-up Limitations: exposure dichotomized to exposed/unexposed only (no further classification); wide range of industrial processes included; coexposures vary and were not addressed in the analyses
Koh et al. (2014) Republic of Korea 2002–2007 Cohort	14 698 male workers registered in a regional petrochemical plant maintenance workers union 2002–2007 Exposure assessment method: none; benzene-exposed workers	Lung: ICD-10 (codes C33–34) Lung: ICD-10 (codes C33–34)	SMR exposed SIR exposed	9 5	0.68 (0.31–1.29) 0.73 (0.24–1.71)	Age Age	Strengths: good coverage of target population Limitations: short follow-up time; no quantitative exposure assessment; occupational histories and specific tasks not available; tobacco exposure history not available
Koh et al. (2011) Republic of Korea 1992–2007 follow-up (16 yr) Cohort	8866 male workers at seven petrochemical plants producing or using benzene Exposure assessment method: none; classified by job	Lung (mortality) Lung (incidence) 	All workers Manufacturing workers Office workers All workers Manufacturing workers Office workers	5 3 2 8 2 6	0.35 (0.11–0.83) 0.31 (0.06–0.91) 0.44 (0.05–1.95) 0.60 (0.26–1.17) 0.22 (0.03–0.78) 1.42 (0.52–3.09)	Age and calendar period Age and calendar period 	Strengths: the first investigation of cancer risk of workers in a refinery/petrochemical complex in the Republic of Korea; data from cancer registry; ICD-10 coding Limitations: no control for smoking; short follow-up; small number of cases; healthy worker effect

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Collins et al. (2015) USA 1940–2009 Cohort	2266 workers exposed to benzene at a chemical plant Exposure assessment method: quantitative measurements; job titles were assigned to exposure categories by an industrial hygienist, based on IH measurements (JEM)	Lung: ICD-10 (codes C33–C34)	Ever exposed 0–3.9 ppm-yr	146	1.05 (0.89–1.24)	Age, race, sex	Third update of the Dow Chemical plant retrospective cohort Strengths: extensive benzene exposure monitoring; complete work history information; periodic medical examination at workplace; long and complete follow-up Limitations: mortality study based on death certificates

CI, confidence interval; HLD, haematopoietic, lymphoproliferative, and related disorders; ICD, International Statistical Classification of Diseases and Related Health Problems; IH, industrial hygiene; JEM, job-exposure matrix; MDS, myelodysplastic syndrome; ppm, parts per million; RR, relative risk; SIR, standard incidence ratio; SMR, standardized mortality ratio; yr, year(s)

Table 2.7 Epidemiological studies of exposure to benzene and cancer at other sites in adults in the general population

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Bove et al. (2014) USA (California) 1975–1985/1979–2008 Cohort	309 901 marine and naval personnel who began service during 1975–1985 and were stationed anytime during this period in one of two camps, one of them having contaminated drinking-water and the other not Exposure assessment method: records; monthly average estimates of contaminant concentrations in drinking-water, published in peer-reviewed agency record; each individual was assigned estimated exposure based on residence	Lung: trachea, bronchus, and lung; ICD-9 (code 162)	Camp Pendleton (referent, non-contaminated water)	216	0.81 (0.71–0.93)	Age, sex, race, rank, education	Despite the possible healthy veteran effect bias, elevations in SMR were observed in the exposed camp Strengths: large cohorts; low loss to follow-up Limitations: exposure misclassification; for a mortality endpoint a longer follow-up is necessary; 97% of the Camp Lejeune cohort was of age < 55 yr and < 6% had died by the end of the study
			Camp Lejeune (exposure to contaminated drinking-water)	237	0.92 (0.80–1.04)		
Yuan et al. (2014) China, Shanghai 1986–1989 Nested case–control	Cases: 82 men, lifelong non-smokers aged 45–64 yr at enrolment Controls: 83 members of the Shanghai Cohort study without cancer, non-smokers and alive at the time of cancer diagnosis of the case; matched by age at enrolment (±2 yr), year, month of urine sample collection (±1 month), and neighbourhood of residence at recruitment Exposure assessment method: other; levels of urinary PAH and VOCs (SPMA for benzene) prospectively analysed	Lung	Quartiles of SPMA (metabolite of benzene)			Age at baseline, neighbourhood of residence at enrolment, years of sample storage, urinary cotinine level	Strengths: active follow-up with annual in-person interviews; after 22 yr loss of follow-up low (only 5%); urinary cotinine was also quantified to confirm non-smoking status Limitations: relatively small sample size; 26% of cases not histologically confirmed; small number of cases of SCC (n = 16); urinary samples were only collected at baseline
			1st quartile (ref)	17	1.00		
			2nd quartile	18	1.03 (0.39–2.69)		
			3rd quartile	19	1.10 (0.44–2.78)		
			4th quartile	20	1.57 (0.65–3.80)		
			Trend test P value, 0.31				
		Lung (SCC)	Tertiles of SPMA			Age at baseline, neighbourhood of residence at enrolment, years of sample storage, urinary cotinine level	
			1st tertile (ref)	NR	1.00		
			2nd tertile	NR	1.97 (0.31–12.65)		
			3rd tertile	NR	5.76 (1.11–28.96)		
			Trend test P value, 0.023				

Table 2.7 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Villeneuve et al. (2014) Toronto, Canada 1997–2002 Case–control	Cases: 445 incident cases of cancer of the trachea, bronchus, or lung; men and women aged 20–84 yr; non-smokers were oversampled (35%); recruited from four tertiary-care hospitals in Toronto Controls: 948 (425 population and 523 hospital); one control series was population-based (from tax assessment files), the other control series was recruited from a nonspecialized family medicine clinic Exposure assessment method: other; land-use regression models linked to residential addresses; questionnaire on exposures	Lung: trachea, bronchus, and lung; ICD-9 (code 162) Lung: trachea, bronchus, and lung; ICD-9 (code 162)	IQR increase in time-weighted average benzene concentration IQR increase ($0.15\ \mu g/m^3$) Residential benzene exposure, 10 yr before interview IQR increase ($0.15\ \mu g/m^3$)	NR NR	1.84 (1.26–2.68) 1.58 (1.15–2.16)	Controls frequency-matched to cases by ethnicity, age, sex, pack-years of smoking (continuous variable, summed over pipe, cigar, and cigarette use), exposure to second-hand smoke, BMI, family history of cancer, neighbourhood measures of unemployment, median family income	The exposure profile among hospital controls would be expected to be higher than that in population controls (because of residential location of the hospital) Strengths: good exposure assessment including smoking history Limitations: low participation rate of population controls (59%) and lung cancer cases (62%)

BMI, body mass index; CI, confidence interval; ICD, International Statistical Classification of Diseases and Related Health Problems; IQR, interquartile range; NR, not reported; PAH, polycyclic aromatic hydrocarbon; SCC, squamous cell cancer; SMR, standardized mortality ratio; SPMA, *S*-phenylmercapturic acid; VOCs, volatile organic compounds; yr, year(s)

industry; the occupation of bookbinding was known to have used benzene until 1958. [The Working Group noted that many women in the bookbinding group probably did not work during the time when benzene was used.]

Greenland et al. (1994) followed up 1821 male workers at a transformer assembly facility from 1969 to 1984. Interviews with long-term employees were used to develop JEMs for seven types of exposure, including benzene, classified as none, indirect (nearby), or direct. [The notable limitations of this study included work history records not being available for many (34%) of the workers, so the size of the underlying cohort was unknown.]

Hayes et al. (1996) examined mortality among a cohort of Chinese workers from multiple industries, of whom 74 828 were exposed to benzene and 35 805 were unexposed. The study provided relative risk estimates and assessed trends across several cumulative exposure categories (0, < 10, 10–39, 40–99, 100–400, and > 400 ppm-years) for a subset of outcomes.

A total of 5514 workers from 233 facilities in the United Kingdom who were judged by their employers to be exposed to benzene were followed up for cancer incidence (1971–2001) and mortality (1968–2002) by Sorahan et al. (2005). No exposure assessment was included in the analyses because air sampling data were from 1966/1967 and only available from 130 of the facilities. [The study was retained by the Working Group as each cohort member was reported as exposed. Some under-ascertainment in the incidence component of the study was reported.]

Tsai et al. (1983) reported on 454 male benzene workers ever employed at a refinery in Texas, USA. Exposure evaluation used 1973–1982 air sampling data to determine which employees worked in benzene areas and for what duration.

Wilcosky et al. (1984) examined 6678 active and retired rubber industry workers from Akron, Ohio, USA. The study assigned worker exposures based on employment in a process area where a specific solvent was authorized for use, but the authors acknowledged that the solvents may not have been used in some of the areas.

Wong (1987a, b) reported on a study of 4602 workers exposed to benzene and 3074 unexposed workers in seven chemical plants in the USA. Wong (1987a) reported results for exposure characterized as intermittent or continuous. A companion paper presented results for this same population by cumulative exposure category for select outcomes, in which Mantel–Haenszel extension χ^2 trend test results were given (Wong, 1987b).

Wong et al. (1993) reported data for a cohort of gasoline distribution workers in the USA, 9026 of whom were based on land and 9109 who operated on marine vessels between 1946 and 1985, who were followed up for mortality outcomes until 1989. The exposure assessment for this study developed metrics for cumulative and peak exposures to total hydrocarbons as a surrogate for benzene exposure to components of gasoline.

Many of these cohort studies had limitations. In some of the occupational settings, all or subsets of workers had potential exposure to other substances such as asbestos, dust, other solvents, and industrial chemicals. Bond et al. (1986) addressed some of these additional exposure types in the analysis, in this case by removing workers exposed to arsenic, asbestos, or high levels of vinyl chloride. None of these studies adjusted for covariates other than demographic factors. The studies were also inconsistent in addressing latency; some presented results by time since first exposed or employed.

Of the more recent studies included in this evaluation, Stenehjem et al. (2017) prospectively followed up a cohort of 24 917 offshore petroleum workers in Norway (see Section 2.2.1(b)) for 13.5 years for incident cases of cancer, including cancer of the skin (melanoma and squamous cell carcinoma of the forearm and hand). An update of a cohort of Chinese workers from multiple industries (see Section 2.2.1(b)) (Linet et al.,

2015) provided rate ratios comparing outcomes between ever- and never-exposed workers. Koh et al. (2014) followed up 14 698 temporary maintenance workers in a refinery/petrochemical complex in the Republic of Korea for mortality from 2002 to 2007 and for incidence from 2002 to 2005; the workers' exposure to benzene was characterized by Chung et al. (2010). [The Working Group noted the short follow-up time as a limitation of this study.] Koh et al. (2011) also examined cancer mortality during 1992–2007 and incidence during 1997–2005 among 8866 male workers in units of a refinery/petrochemical complex in the Republic of Korea that produced or used benzene. Results were given separately for production, maintenance, laboratory, and office workers, but the authors were not able to quantify exposures because they lacked adequate job history and exposure records.

(b) Cancer sites reviewed in IARC Monographs Volume 100F

(i) Cancer of the lung

The evidence from occupational cohort studies of an association between exposure to benzene and cancer of the lung that was available at the time of publication of *IARC Monographs* Volume 100F was judged to be *inadequate*. Cohort studies available at that time, with information on potential or estimated benzene exposure and cancer of the lung, are described in Table 2.15 of Volume 100F (available at: http://publications.iarc.fr/123).

Bond et al. (1986) reported that among 956 chemical manufacturing plant workers in the USA, who had been employed for 1 month or longer during 1938–1978 and followed up through 1982, overall mortality from cancer of the lung was not increased (SMR, 0.99; 95% CI, 0.59–1.57; 18 deaths). Among cumulative exposure categories, an excess risk (SMR, 2.04; 5 deaths) was observed for the category of 500–599 ppm-months, while no excess risk was observed for the categories of 0–499 ppm-months (SMR, 0.62; 6 deaths) and 1000 ppm-months or more (SMR, 0.49; 2 deaths) [95% CIs were not reported]. In an update of this cohort (see Table 2.6), Collins et al. (2015) reported that among 2266 United States chemical manufacturing plant workers beginning employment during 1940–1982 and followed up through 2009, no excess risk of cancer of the lung (including lung, trachea, and bronchus; ICD-10, codes C33–C34) was observed (SMR, 1.05; 95% CI, 0.89–1.24; 146 deaths).

Collins et al. (2003) reported that among 4417 chemical manufacturing hourly workers in Illinois, USA, who began employment during 1940–1977 and were followed up through 1997, a 60% excess risk of cancer of the lung was observed among those with cumulative exposure to benzene at more than 6 ppm-years (SMR, 1.6; 95% CI, 1.2–2.1; based on 55 deaths). There was a monotonic trend in standardized mortality ratios across cumulative exposure groups exposed to benzene at less than 1 ppm-years (SMR, 1.1; 95% CI, 0.7–1.5), 1–6 ppm-years (SMR, 1.3; 95% CI, 1.0–1.8), and more than 6 ppm-years (SMR, 1.6; 95% CI, 1.2–2.1), although the reference group also presented an elevated risk for cancer of the lung (SMR, 1.3; 95% CI, 1.1–1.5).

Sorahan et al. (2005) reported that among 5514 workers exposed to benzene in 233 factories in the United Kingdom during 1966/1967 or earlier, followed up for mortality during 1968–2002, there was a significant increase in mortality from cancer of the lung (SMR, 1.21; 95% CI, 1.07–1.35; based on 294 deaths) and in incidence of cancer of the lung (SIR, 1.19; 95% CI, 1.06–1.34; based on 293 cases). There was no clear evidence of heterogeneity by type of industry, despite exposure of some of these workers to other carcinogens such as asbestos and polycyclic aromatic amines. [The Working Group noted that some of the deaths coded to cancer of the lung may actually have been due to mesothelioma. Some cancer cases may have been missed or misclassified.]

Wong (1987a, b) reported a standardized mortality ratio of 1.12 (95% CI, 0.90–1.39; *n* = 86), but no exposure–response relation was observed. Among land-based gasoline distribution and marine distribution workers, Wong et al. (1993) reported standardized mortality ratios of 0.66 (95% CI, 0.57–0.77; *n* = 165) and 1.07 (95% CI, 0.94–1.21; *n* = 208), respectively.

The Working Group identified several other pertinent studies that were available at the time, but not included in the previous review (see Table 2.6). Tsai et al. (1983) reported a standardized mortality ratio of 0.52 (95% CI, 0.06–1.86; *n* = 2) in a study of refinery workers. Greenland et al. (1994) observed an odds ratio of 0.58 (95% CI, 0.31–1.07) when directly comparing exposed transformer repair workers with their indirectly exposed or unexposed counterparts. Bulbulyan et al. (1999) reported a standardized mortality ratio of 0.7 (95% CI, 0.1–2.0; *n* = 3) among female bookbinders.

Additional data were available for several more recent studies. Linet et al. (2015) (see Section 2.1.1(b) for details) updated data on cancer among Chinese workers exposed to benzene, studied previously by Hayes et al. (1996). Workers exposed to benzene demonstrated a significant excess of mortality from cancer of the lung (RR, 1.5; 95% CI, 1.2–1.9; *n* = 351). The highest relative risk for mortality from cancer of the lung was for workers in the rubber and coatings industries. Relative risks for death from cancer of the lung were significantly elevated and of the same magnitude in the early (1972–1987) and later (1988–1999) follow-up periods. All analyses were stratified according to sex, attained age, and attained calendar year. [The Working Group noted that there was no control for potential confounding by smoking or other occupational exposures. However, the authors noted that associations were similar in women and men, although the prevalence of smoking is generally much lower among Chinese women.] Hayes et al. (1996) and Yin et al. (1996b) previously reported data for this cohort. Mortality from cancer of the lung (also including trachea and bronchus; ICD-9, code 162) was in excess in the cohort overall (RR, 1.4; 95% CI, 1.0–2.0) due to men (RR, 1.5; 95% CI, 1.0–2.2; 109 cases) but not women (RR, 1.0; 95% CI, 0.4–2.9; 16 cases), and it was increased among workers with greater estimated cumulative benzene exposure (RR, 1.7 for those with ≥ 400 ppm-years exposure vs no exposure; *P* value for trend, 0.01).

Koh et al. (2011) reported on a study of cancer mortality and incidence among petrochemical workers at plants producing or using benzene; no excess in cancer of the lung mortality (SMR, 0.31; 95% CI, 0.06–0.91) or incidence (SIR, 0.22; 95% CI, 0.03–0.78) was reported in manufacturing workers. [The Working Group noted the presence of a healthy worker effect and the short follow-up time.]

Koh et al. (2014) reported on a study of cancer mortality and incidence among temporary maintenance workers at a refinery/petrochemical complex in the Republic of Korea; no excess in cancer of the lung mortality (SMR, 0.68; 95% CI, 0.31–1.29) or incidence (SIR, 0.73; 95% CI, 0.24–1.71) was observed.

(ii) Cancer of the kidney

The evidence available on the association between occupational exposure to benzene and cancer of the kidney was reviewed in *IARC Monographs* Volume 100F, and judged to be *inadequate* at that time; pertinent occupational cohort studies reviewed in Volume 100F are described in Table 2.17 (available at: http://publications.iarc.fr/123). The results of these studies generally do not show a consistent association, although several studies did report elevated but not statistically significant risks for cancer of the kidney (Wong, 1987a, b; Tsai et al., 1993; Sorahan et al., 2005).

Wong et al. (1993) reported on a nested case–control study of United States land-based or marine petroleum distribution workers for

exposure to gasoline containing 2–3% benzene (Wong et al., 1999); several quantitative indices of gasoline exposure (duration of exposure, cumulative exposure, frequency of peak exposure, age at first exposure, and year of first exposure) were used for analysis, but an excess mortality risk was not found for cancer of the kidney. [The Working Group noted potential exposure misclassification and a healthy worker effect.]

Wong (1987a, b) observed a standardized mortality ratio of 0.85 (95% CI, 0.27–1.98; $n = 5$), and did not observe any association between level of exposure and increased risk of cancer of the kidney: no cases were reported in the highest cumulative exposure category.

An odds ratio of 4.29 (95% CI, 1.33–13.8) for death from cancer of the kidney was reported for transformer manufacturing facility workers directly exposed to benzene compared with workers who were unexposed or indirectly exposed (Greenland et al., 1994).

Bulbulyan et al. (1999) reported a standardized mortality ratio of 1.9 (95% CI, 0.4–5.6; $n = 3$) among female bookbinders exposed to benzene.

Collins et al. (2015) reported no excess of death from cancer of the kidney (SMR, 0.78; 95% CI, 0.34–1.55; $n = 8$).

(c) Other cancer sites

Data for other cancer sites were not reviewed in detail in *IARC Monographs* Volume 100F. The key findings of pertinent studies that reported data for several additional types of cancer are reported in the following sections.

(i) Cancers of the nasal cavity, pharynx, larynx, and other respiratory sites

Several studies have reported data on other cancers of the respiratory tract, including the nasal cavity, buccal cavity, pharynx, and larynx (Tsai et al., 1983; Wong, 1987b; Greenland et al., 1994; Hayes et al., 1996; Bulbulyan et al., 1999; Sorahan et al., 2005; Koh et al., 2011, 2014; Linet et al., 2015). Results were based on small numbers of cases or deaths and were generally close to expectation. Two studies reported increased, albeit non-significant, relative risks for cancer of the nasopharynx. Among temporary maintenance workers at a refinery/petrochemical complex in the Republic of Korea, excess mortality (SMR, 5.88; 95% CI, 1.21–17.2; based on 3 deaths) and incidence (SIR, 8.33; 95% CI, 1.72–24.50; based on 3 cases) of cancer of the nasopharynx were reported. The relative risk of death from cancer of the nasopharynx was also elevated (RR, 1.9; 95% CI, 0.9–4.3; $n = 29$) in the Chinese cohort of workers exposed to benzene (Linet et al., 2015), although there was no evidence of a trend with cumulative benzene exposure (Hayes et al., 1996).

(ii) Cancer of the oesophagus

Chinese worker cohorts reported a P value for trend of 0.09 for the association between mortality from cancer of the oesophagus and cumulative exposure to benzene (Hayes et al., 1996). An elevated risk (RR, 1.6; 95% CI, 1.0–2.5; 70 exposed deaths) was reported when comparing workers exposed to benzene with unexposed workers in an updated analysis of the Chinese worker cohort (Linet et al., 2015). Greenland et al. (1994) reported an odds ratio of 1.23 (95% CI, 0.26–5.72) for mortality from cancer of the oesophagus among directly exposed workers compared with indirectly exposed or unexposed workers. Bulbulyan et al. (1999) observed an increase in female bookbinders (SMR, 4.1; 95% CI, 1.0–10.4; $n = 4$). Wong (1987a) observed no excess mortality from cancer of the oesophagus in workers continuously exposed to benzene, and no dose–response relationship was detected for the four deaths observed (Wong, 1987b). Sorahan et al. (2005) reported incidence and mortality results that were not statistically significant and were near or below expectation. Koh et al. (2014) observed a standardized mortality ratio of 0.51 (95% CI, 0.01–2.85; $n = 1$) and no incident cases.

(iii) Cancer of the stomach

The results from most studies of cancer of the stomach are generally at or below expectation. Hayes et al. (1996) reported a *P* value for trend of 0.63 for the association between mortality from cancer of the stomach and cumulative benzene exposure. Greenland et al. (1994) observed an odds ratio of 0.32 (95% CI, 0.04–2.42) for directly exposed workers compared with indirectly exposed or unexposed workers. A cancer of the stomach mortality deficit was reported by Wong (1987a) for workers exposed to benzene (SMR, 0.43; 95% CI, 0.16–0.94; $n = 6$). Tsai et al. (1983) reported a non-significantly elevated standardized mortality ratio of 2.32 for men working 1 year or more in areas exposed to benzene, based on a single case. Sorahan et al. (2005) reported a standardized mortality ratio of 1.06 (95% CI, 0.80–1.37; $n = 57$) and a similar standardized incidence ratio. Bulbulyan et al. (1999) reported a significantly elevated mortality from cancer of the stomach among press operators (SMR, 2.2; 95% CI, 1.0–4.2; $n = 9$) but no elevation among bookbinders (SMR, 1.0; 95% CI, 0.5–1.8; $n = 12$). Wilcosky et al. (1984) reported an odds ratio of 1.3 (based on 12 exposed cases) comparing male workers with potential cumulative benzene exposure of more than 1 year with those with no benzene exposure. Linet et al. (2015) reported no excess risk (RR, 1.0; 95% CI, 0.8–1.3; 211 exposed deaths) for mortality from cancer of the stomach when comparing exposed workers with those not exposed. Koh et al. (2014) reported a standardized mortality ratio of 0.83 (95% CI, 0.41–1.48; $n = 11$) and a standardized incidence ratio of 0.99 (95% CI, 0.56–1.64; $n = 15$). Koh et al. (2011) found significant deficits of mortality from cancer of the stomach in all workers (SMR, 0.24; 95% CI, 0.06–0.60; $n = 4$) and in manufacturing workers (SMR, 0.25; 95% CI, 0.05–0.74; 3 cases), as well as in all-cause mortality and all-cancer mortality among all workers and manufacturing workers.

(iv) Cancers of the colon, rectum, and anus

A study of the Chinese worker cohort reported no evidence (*P* for trend, 0.91) of an exposure–response relationship between exposure to benzene and cancers of the colon and rectum (Hayes et al., 1996). In the update of that study, Linet et al. (2015) observed a relative risk of 1.5 (95% CI, 1.0–2.3; 79 exposed deaths) for cancers of the colon and rectum. Greenland et al. (1994) reported deficits in odds ratios for mortality from cancers of the colon (OR, 0.74; 95% CI, 0.33–1.66) and rectum (OR, 0.85; 95% CI, 0.29–2.47) in directly exposed workers compared with those who were indirectly or not exposed. Wong (1987a) reported a standardized mortality ratio of 1.08 (95% CI, 0.52–1.98; $n = 10$) in continuously exposed workers; no significant exposure–response relationships for cancer of the colon were reported (Wong, 1987b). Sorahan et al. (2005) reported a deficit for mortality (SMR, 0.81; 95% CI, 0.57–1.11; 38 cases) from and incidence (SIR, 0.86; 95% CI, 0.65–1.10; 60 cases) of cancer of the colon. However, for cancer of the rectum increased risks for mortality (SMR, 1.05; 95% CI, 0.71–1.48; $n = 31$) and incidence (SIR, 1.13; 95% CI, 0.86–1.45; $n = 61$) were reported. Among women exposed to benzene while employed as bookbinders, Bulbulyan et al. (1999) reported standardized mortality ratios of 1.3 (95% CI, 0.6–2.6; $n = 8$) for cancer of the colon and 1.3 (95% CI, 0.4–3.1; $n = 5$) for cancer of the rectum. Tsai et al. (1993) reported a standardized mortality ratio for cancer of the colon of 0.94 (95% CI, 0.60–1.40; $n = 24$). For cancers of the intestine and anus, Koh et al. (2014) reported a standardized mortality ratio of 0.33 (95% CI, 0.04–1.20; $n = 2$) and a standardized incidence ratio of 0.91 (95% CI, 0.37–1.88; $n = 7$) among temporary maintenance workers. Koh et al. (2011) reported a standardized mortality ratio of 0.49 (95% CI, 0.06–1.78; $n = 2$) for cancers of the colon and anus among manufacturing workers.

(v) Cancers of the liver and biliary tract

When analysing mortality from cancers of the liver and gall bladder among Chinese workers by cumulative benzene exposure, no notable exposure–response relationships or elevations in exposed versus unexposed workers were observed by Hayes et al. (1996) (*P* for trend, 0.16). Linet et al. (2015) reported a relative risk for cancers of the liver, gallbladder, and bile duct of 1.2 (95% CI, 0.9–1.4; 286 exposed deaths) in the updated study of Chinese benzene workers. Greenland et al. (1994) reported a non-significant odds ratio of 2.76 (95% CI, 0.68–11.20; $n = 9$) for cancers of the liver, gallbladder, and biliary tract combined among workers directly exposed to benzene compared with their unexposed or indirectly exposed counterparts. Elevations in mortality (SMR, 1.54; 95% CI, 0.74–2.84; $n = 10$) and morbidity (SIR, 1.31; 95% CI, 0.57–2.59; $n = 8$) from cancer of the liver were reported by Sorahan et al. (2005); regarding cancer of the gallbladder, the same study reported deficits in mortality (SMR, 0.60; 95% CI, 0.08–2.26; two deaths) and morbidity (SIR, 0.66; 95% CI, 0.14–1.92; three deaths). Bulbulyan et al. (1999) reported 1 observed death from liver cancer, compared with 1.2 cases expected in bookbinders. Wong (1987b) reported no excess risk or exposure–response trends among chemical workers in the USA. Koh et al. (2014) reported a standardized mortality ratio of 0.82 (95% CI, 0.51–1.25; $n = 21$) and a standardized incidence ratio of 1.07 (95% CI, 0.58–1.79; $n = 14$) for cancer of the liver. Koh et al. (2011) reported a standardized mortality ratio of 0.64 (95% CI, 0.34–1.09; $n = 13$) for cancers of the liver and biliary tract among manufacturing workers.

(vi) Cancer of the prostate

Greenland et al. (1994) reported an odds ratio of 1.02 (95% CI, 0.49–2.12) for directly exposed workers compared with indirectly exposed or unexposed workers. No associations were seen by Wong (1987a) (SMR, 0.93; 95% CI, 0.34–2.03; 6 deaths) or Wilcosky et al. (1984) (OR, 0.73; 11 deaths; CI not reported). Sorahan et al. (2005) reported a standardized mortality ratio of 0.94 (95% CI, 0.70–1.24; $n = 50$) and a standardized incidence ratio of 1.10 (95% CI, 0.91–1.32; $n = 121$). Koh et al. (2014) reported a standardized mortality ratio of 2.51 (95% CI, 0.06–14.00; $n = 1$) and a standardized incidence ratio of 1.20 (95% CI, 0.03–6.71; $n = 1$). Koh et al. (2011) observed no deaths from cancer of the prostate in manufacturing workers.

(vii) Cancer of the bladder

No association between exposure to benzene and cancer of the bladder was seen by Greenland et al. (1994) or Wong (1987a). Sorahan et al. (2005) reported a standardized mortality ratio of 1.00 (95% CI, 0.66–1.46; 27 cases) and a standardized incidence ratio of 1.04 (95% CI, 0.81–1.31; 69 cases). Bulbulyan et al. (1999) observed 1 death from cancer of the bladder in bookbinders, where 0.5 was expected. Among press operators, standardized mortality ratio was 12.5 (95% CI, 1.5–45.1; $n = 2$). Linet et al. (2015) reported a relative risk for cancer of the bladder for exposed versus unexposed workers of 0.9 (95% CI, 0.4–2.2; 18 exposed deaths). Koh et al. (2011) observed no deaths from cancer of the bladder.

(viii) Cancer of the skin

Bond et al. (1986) found four deaths from cancer of the skin, all in the lowest exposure category (0–499 ppm-months). The overall standardized mortality ratio for cancer of the skin was 4.41 unlagged and 6.22 with a 15-year lag. Wong (1987a) observed a non-significant deficit in workers continuously exposed to benzene, with the single exposed case (of 3 cases in total) occurring in the lowest exposure category (Wong, 1987b). For all cancers of the skin, Koh et al. (2014) reported a standardized mortality ratio of 5.05 (95% CI, 0.13–28.20; $n = 1$) and no incident cases. No deaths from cancer of the skin were observed by Koh et al. (2011) in manufacturing workers.

For non-melanoma cancer of the skin, Sorahan et al. (2005) reported one observed death (SMR, 0.55; 95% CI, 0.01–3.05). Stenehjem et al. (2017) reported an adjusted odds ratio of 3.51 (95% CI, 0.45–27.00; n = 6) for squamous cell carcinoma of the forearm and hand after adjustment for age, sunburn frequency, and education.

For malignant melanoma, Sorahan et al. (2005) reported a standardized mortality ratio of 0.81 (95% CI, 0.22–2.06; n = 4) and a standardized incidence ratio of 1.21 (95% CI, 0.64–2.07; n = 13). Among women potentially exposed to benzene, Bulbulyan et al. (1999) reported an elevated risk (SMR, 8.7; 95% CI, 1.1–31.3; n = 2) among press operators and no melanoma deaths among bookbinders. In offshore petroleum workers, Stenehjem et al. (2017) reported an odds ratio for benzene exposure of 2.43 (95% CI, 0.30–20.00; n = 5) for melanomas of the forearm and hand after adjustment for age, sunburn frequency, and education. [The Working Group noted that adjustment for sunburn was not a good proxy for adjusting for occupational exposure to ultraviolet radiation. There may also have been potential confounding as a result of other co-exposures.]

(ix) Cancers of the brain and central nervous system

Wong (1987a) reported an increased mortality from cancer of the CNS (ICD-8, code 191–912) for workers continuously exposed to benzene (SMR, 1.54; 95% CI, 0.56–3.35; n = 6), with no linear trend by cumulative exposure (Wong, 1987b) or overall elevation for the three exposure categories.

An odds ratio of 2.11 (95% CI, 0.66–6.73; n = 16) was reported for transformer assembly workers directly exposed to benzene compared with those indirectly exposed or unexposed (Greenland et al., 1994). These results include both malignant and unspecified tumours of the brain.

An exposure–response analysis for the Chinese cohort (Hayes et al., 1996) saw no trend (P for trend, 0.48), but did find an elevation in the highest exposure category (RR, 2.3; five deaths) with deficits in two intermediate exposure categories. In an update of the study, Linet et al. (2015) reported a relative risk of 0.8 (95% CI, 0.4–1.6; 18 exposed deaths) for benign and malignant tumours of the brain for any exposure to benzene.

For cancers of the brain and spine, Koh et al. (2014) reported a standardized mortality ratio of 1.21 (95% CI, 0.15–4.36; n = 2) and a standardized incidence ratio of 2.36 (95% CI, 0.29–8.52; n = 2). Koh et al. (2011) observed no deaths from neurological cancers in manufacturing workers. Collins et al. (2015) reported a standardized mortality ratio of 1.01 (95% CI, 0.48–1.86; 10 deaths) for cancer of the CNS among chemical production workers.

Several older studies provided only summary standardized mortality ratio results for these cancers. Bulbulyan et al. (1999) reported a standardized mortality ratio of 2.6 (95% CI, 0.5–4.6; n = 3) for cancers of the brain and nervous system among women employed as bookbinders. Tsai et al. (1983) included benign neoplasms of the brain and other parts of the nervous system, and neoplasms of an unspecified nature of the eye, brain, and other parts of the nervous system, and reported a standardized mortality ratio of 3.23 (95% CI, 0.04–17.95; n = 1) among men employed as refinery workers. Sorahan et al. (2005) reported non-significant increases in mortality (SMR, 1.05; 95% CI, 0.60–1.70; n = 16) and morbidity (SIR, 1.16; 95% CI, 0.68–1.83; n = 18) for malignant neoplasms of the brain and other parts of the nervous system.

(x) Cancer of the pancreas

A study of male chemical workers observed a standardized mortality ratio below expectation (Wong 1987a) and no exposure–response association between exposure to benzene and cancer

of the pancreas (Wong, 1987b). Greenland et al. (1994) reported an odds ratio of 0.58 (95% CI, 0.18–1.93; *n* = 33) when comparing transformer assembly workers who had been directly exposed to benzene with those indirectly exposed or unexposed. Elevated risks for mortality (SMR, 1.21; 95% CI, 0.85–1.68; *n* = 36) and incidence (SIR, 1.29; 95% CI, 0.90–1.79; *n* = 36) were observed by Sorahan et al. (2005). Bulbulyan et al. (1999) reported a standardized mortality ratio of 1.1 (95% CI, 0.2–3.3; *n* = 3) in female bookbinders and one of 2.0 (95% CI, 0.3–7.4; *n* = 2) among female press operators potentially exposed to benzene. Koh et al. (2014) observed a standardized mortality ratio of 0.57 (95% CI, 0.07–2.07; *n* = 2) and a standardized incidence ratio of 1.41 (95% CI, 0.17–5.09; *n* = 2) in temporary maintenance workers in a refinery/petrochemical complex in the Republic of Korea. For refinery/petrochemical facility manufacturing workers, Koh et al. (2011) reported a standardized mortality ratio of 1.21 (95% CI, 0.25–3.52; *n* = 3) for cancer of the pancreas. Linet et al. (2015) reported a relative risk of 1.7 (95% CI, 1.0–3.1; 45 exposed deaths) for the Chinese benzene worker cohort.

(xi) Additional cancers

Cancers assessed by less than four studies each include the following malignancies: other urinary and genitourinary (Sorahan et al., 2005); other endocrine (Sorahan et al., 2005); small intestine (Sorahan et al., 2005); testis (Sorahan et al., 2005); ovary (Bulbulyan et al., 1999, Sorahan et al., 2005); uterine corpus (Bulbulyan et al., 1999; Sorahan et al., 2005; Linet et al., 2015); uterine cervix (Bulbulyan et al., 1999; Sorahan et al., 2005); thyroid (Sorahan et al., 2005); pleural cancer and mesothelioma (Sorahan et al., 2005); breast (Bulbulyan et al., 1999; Sorahan et al., 2005; Linet et al., 2015); bone (Wong, 1987a, b; Sorahan et al., 2005); lip (Sorahan et al., 2005); and tongue (Sorahan et al., 2005; Koh et al., 2014). Small numbers of cases or deaths were observed for most of these sites. Exceptions included: a standardized mortality ratio of 2.9 (95% CI, 1.5–5.0; *n* = 12) for cancer of the ovary among bookbinders (Bulbulyan et al., 1999); a relative risk of 2.6 (95% CI, 0.9–10.9; *n* = 19) for death from cancer of the uterus; and a relative risk of 1.2 (95% CI, 0.6–2.5; *n* = 32) for death from cancer of the breast among Chinese workers exposed to benzene (Linet et al., 2015).

2.4.2 General-population studies

(a) Cancer of the lung

One cohort study of environmental exposure and three case–control studies examined cancer of the lung in relation to indicators of exposure to benzene (Table 2.7).

Bove et al. (2014) reported on cancer of the lung in the cohort study of United States military personnel exposed to contaminated drinking-water. No quantitative estimate of benzene or other agents was derived. There was an elevated hazard ratio (adjusted for sex, race, rank, and education, but not for smoking) for cancer of the lung among the personnel exposed to drinking-water contaminated with solvents, including benzene (HR, 1.16; 95% CI, 0.96–1.40; 10-year lag time), where the elevation was due entirely to those with higher cumulative exposures. The standardized mortality ratio was 0.92 (95% CI, 0.80–1.04; 237 deaths), and most people in the cohort were younger than 55 years at the end of follow-up.

A case–control study in Montreal reviewed in *IARC Monographs* Volume 100F showed no association between exposure to benzene and overall cancer of the lung (*n* = 857) or for histological subtypes (see Table 2.16, available at: http://publications.iarc.fr/123). Covariates adjusted for in the study included cumulative smoking index, and exposure to arsenic, asbestos, chromium VI, nickel, crystalline silica, beryllium, cadmium, and polycyclic aromatic hydrocarbons (Gérin et al., 1998).

Villeneuve et al. (2014) reported on a case–control study of 445 incident cases of cancer of the lung, trachea, and bronchus, and 948 hospital- and population-based controls in Toronto (1997–2002). Exposure to ambient volatile organic compounds, including benzene, from outdoor air pollution was assessed using land-use regression models and residential history data. The investigators collected information on confounders including tobacco use and exposure to cigarette smoke. An interquartile range increase in estimated time-weighted average benzene exposure across previous residences was associated with cancer of the lung only when using population-based controls (OR, 1.84; 95% CI, 1.26–2.68). Associations were also positive when using exposure 10 years before interview (OR, 1.58; 95% CI, 1.15–2.16) or at the time of interview (OR, 1.51; 95% CI, 1.13–2.01), but smaller in magnitude.

Yuan et al. (2014) reported on a nested case–control study of 82 cases of cancer of the lung and 83 controls among lifelong non-smoking Chinese men in the Shanghai Cohort Study, aged 45–64 years at enrolment. Prospective urine samples were taken and levels of urinary metabolites of polycyclic aromatic hydrocarbons and volatile organic compounds were examined for an association with risk of cancer of the lung. None of the metabolites of volatile organic compounds were associated with overall risk of cancer of the lung. However, elevated urinary *S*-phenylmercapturic acid (SPMA, a metabolite of benzene) was associated with an increased risk of squamous cell carcinoma of the lung (16 cases); odds ratios for the second and third tertiles of SPMA were 1.97 (95% CI, 0.31–12.65) and 5.76 (95% CI, 1.11–28.96), respectively. Overall, there was a monotonic but non-significant trend in odds ratios across quartiles of SPMA (ORs, 1.00, 1.03, 1.10, and 1.57; *P* for trend, 0.31). [The Working Group noted that the study size was small and, although specific to benzene, SPMA was measured at a single point in time and is not a good proxy for occupational benzene exposure (half-life, 9.1 hours). Results also appear to be sensitive to the grouping of the exposure data.]

(b) Cancer of the kidney

In the previous review of the evidence for associations between exposure to benzene and cancer of the kidney, *IARC Monographs* Volume 100F identified two case–control studies in the general population. In the first study, conducted in Germany, an association was found between exposure to benzene and an increased risk for cancer of the kidney (specifically, renal cell carcinoma; Pesch et al., 2000). The study included 935 incident cases and 4298 controls interviewed between 1991 and 1995, with exposure estimated according to occupational history and a JEM. Results indicated that employment durations exceeding the 90th percentile (classified as "very long exposures") in the chemical, rubber, and printing industries were associated with renal cell carcinoma. Substantial exposure to organic solvents was a significant risk factor for both men and women. In the second study in Montreal, Canada, benzene exposure levels were low for most exposed subjects, and there was little evidence of an association between medium and high levels of exposure and risk of cancer of the kidney (OR, 1.3; 95% CI, 0.7–2.4; $n = 12$) (Gérin et al., 1998). The evidence available at the time was judged to be *inadequate*.

Subsequently, Bove et al. (2014) reported on a cohort study of United States marine ($n = 154\,932$) and naval ($n = 154\,969$) personnel who began service during 1975–1985 and were stationed at two United States military bases. Drinking-water systems in Camp Lejeune, North Carolina, were contaminated with solvents, and drinking-water in Camp Pendleton, South Carolina, was uncontaminated. Although the study population was an occupational group, the exposure of interest was environmental; the agents of primary concern were perchloroethylene and trichloroethylene in drinking-water. Benzene was also a contaminant,

with monthly average concentrations above the current United States maximum contaminant levels for 63 months. Personnel in Camp Lejeune had an elevated mortality for cancer of the kidney (HR, 1.35; 95% CI, 0.84–2.16) and, within the cohort, a monotonic categorical cumulative exposure trend was observed for cancer of the kidney and total contaminants. A risk estimate for the association between benzene exposure and mortality from cancer of the kidney was not reported. Less than 6% of the cohort had died, but a risk estimate for the association between benzene exposure and cancer of the kidney was not reported.

(c) Additional cancers

Several studies of associations between various cancers and environmental exposures to benzene have been published recently.

Bove et al. (2014) reported no excess mortality from cancer of the oral cavity or larynx among marine and naval personnel exposed to contaminated drinking-water.

Garcia et al. (2015) reported on a cohort study of 112 378 participants in the California Teachers Study, including 5676 women diagnosed with cancer of the breast. Modelled annual average air concentrations of 24 mammary gland carcinogen pollutants were derived from the NATA database; the mean benzene concentration was 1.40 μg/m^3. There was little evidence for a trend in the hazard of breast cancer overall with estimated benzene concentration (*P* value for trend, 0.38). Analyses restricted to tumours that were both estrogen-receptor and progesterone-receptor negative (704 cases) suggested increased risk of cancer of the breast with exposure to benzene, with a hazard ratio for the highest quintile of benzene concentration of 1.45 (95% CI, 1.15–1.83; *P* for trend, 0.016). Confining the analysis to never smokers did not weaken the association.

Cigarette smoking is the most important environmental factor for cancer of the pancreas. Among smokers, 90% of benzene exposure comes from smoking (National Cancer Institute, 2015). Antwi et al. (2015) reported on a case–control study based at the Mayo Clinic in Minnesota on environmental exposures and risk of cancer of the pancreas; the study included 2092 cases and 2316 hospital-based matched controls from primary care clinics with self-reported exposure in the form of questionnaire responses. Self-reported regular exposure to benzene was associated with cancer of the pancreas, adjusted for age, sex, smoking, diabetes, body mass index, and education (OR, 1.70; 95% CI, 1.23–2.35). [The Working Group noted that significant risks associated with regular exposure to asbestos (OR, 1.54; 95% CI, 1.23–1.92) and chlorinated hydrocarbons (OR, 1.63; 95% CI, 1.32–2.02) were reported. Exposure assessment in the study was limited by self-reported exposure to benzene.]

2.5 Quantitative data

Following the recommendation of an Advisory Group on quantitative risk characterization (IARC, 2013), the Working Group carried out meta-analyses and meta-regression analyses of quantitative associations between occupational benzene exposure and several cancers of the haematopoietic tissues. These analyses update and extend those published earlier by Vlaanderen et al. (2010, 2011, 2012). The association between occupational benzene exposure and the development of AML and CLL was estimated in meta-analyses. Meta-regression analyses investigating the slope and shape of exposure–disease functions were conducted for AML. Studies were selected for inclusion in these analyses using the criteria applied throughout this section; the analytical approach was similar to that previously reported by Vlaanderen et al. (2010, 2011).

2.5.1 Data extraction

Risk ratios, odds ratios, and standardized mortality or incidence ratios were extracted from published studies. The term "relative risk" will be used to refer to these measures of association collectively. Where both mortality and incidence data were reported, the incidence data were used for analysis.

For the meta-regression analyses, only relative risk estimates reported for cumulative exposure to benzene (expressed in ppm-years or ppm-months) were used.

2.5.2 Statistical analysis

(a) Meta-analysis

Random-effects meta-analyses were performed to pool relative risks for AML and CLL. To allow the inclusion of studies without quantitative exposure estimates, only relative risks for "any occupational benzene exposure" versus "background benzene exposure" were used in the meta-analysis. For those studies which included exposure estimates, relative risks for categories of exposure were pooled either by summing observed and expected cases for studies that reported standardized mortality ratios, or by conducting a within-study random-effects meta-analysis of the non-reference exposure groups for studies which reported relative risks or odds ratios.

(b) Meta-regression

The data extracted for use in meta-regression analysis were relative risk estimates for categories of cumulative benzene exposure. Each exposure category relative risk was assigned a specific cumulative exposure value for the purpose of regression analysis, defined as the mid-point of the exposure category. If an open-ended exposure category was reported for the highest exposure group, then the Working Group assigned it an exposure value equal to the reported lower limit for that exposure category plus one half the width of the previous exposure category. The variance of each relative risk was estimated using the reported confidence intervals under the assumption of Wald-type bounds. As risk estimates of a study based on a common internal reference group will be correlated, the covariance between different risk estimates within a study were estimated. For studies that reported standardized mortality ratios, covariance was not estimated.

Meta-regression analyses were performed on the natural logarithm of the reported relative risk estimates, fitting a linear model as well as natural spline models with knots at the 20th, 50th, and 80th percentiles of the benzene exposure distribution. All statistical analyses were performed using the MIXED and IML procedures in SAS 9.4; the meta-analysis was conducted using the "metafor" package in R, version 3.1.2 (Viechtbauer, 2010).

2.5.3 Results

(a) Meta-analysis

Results of the meta-analysis of selected studies on adult AML and CLL are shown in Fig 2.1, stratified by outcome assessment (incidence, mortality, and both combined).

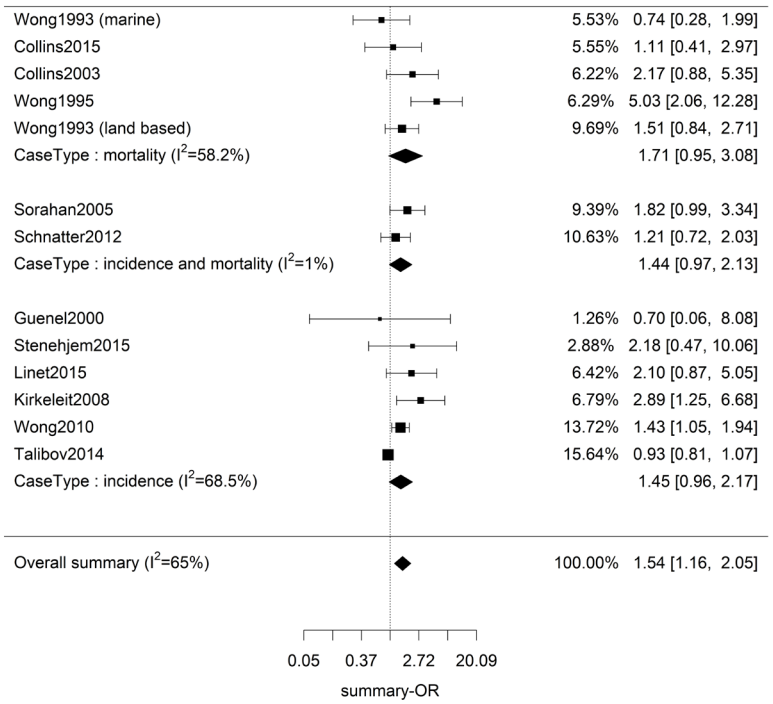
(b) Meta-regression

Visual examination of the natural spline of the cumulative benzene exposure and AML function, including six occupational cohort studies, strongly supported a linear model, as did a statistical comparison of the linear and spline models with respect to goodness of model fit. Subsequent meta-regression analysis focused on results for the linear model, presented in Fig. 2.2.

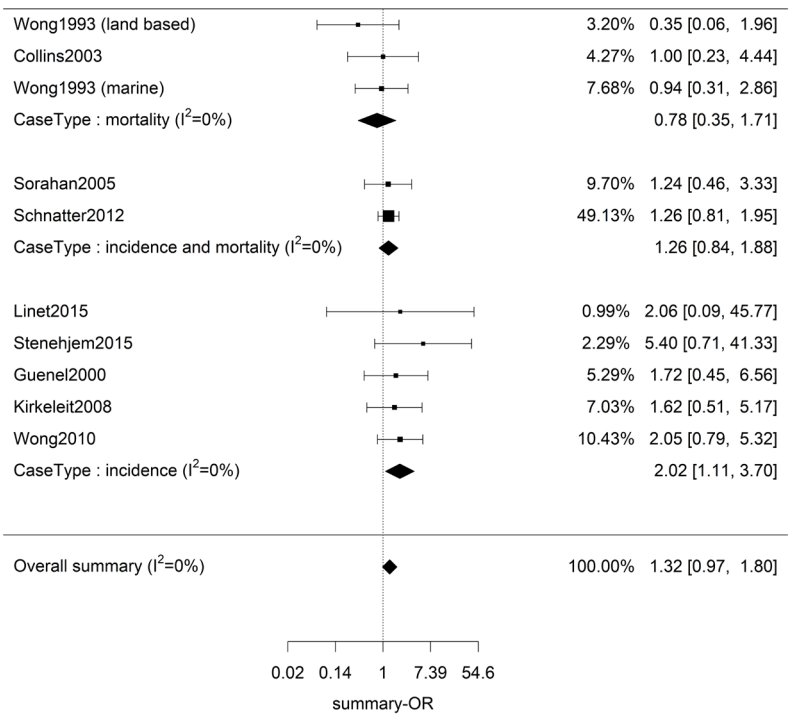
The sensitivity of the exposure–response trend to a single influential study was assessed by refitting the model upon exclusion of one study at a time (Table 2.8). These results indicate that the exposure–response estimate is robust for

Fig. 2.1 Forest plots of (A) AML and (B) CLL stratified by type of outcome (incidence or mortality)

A

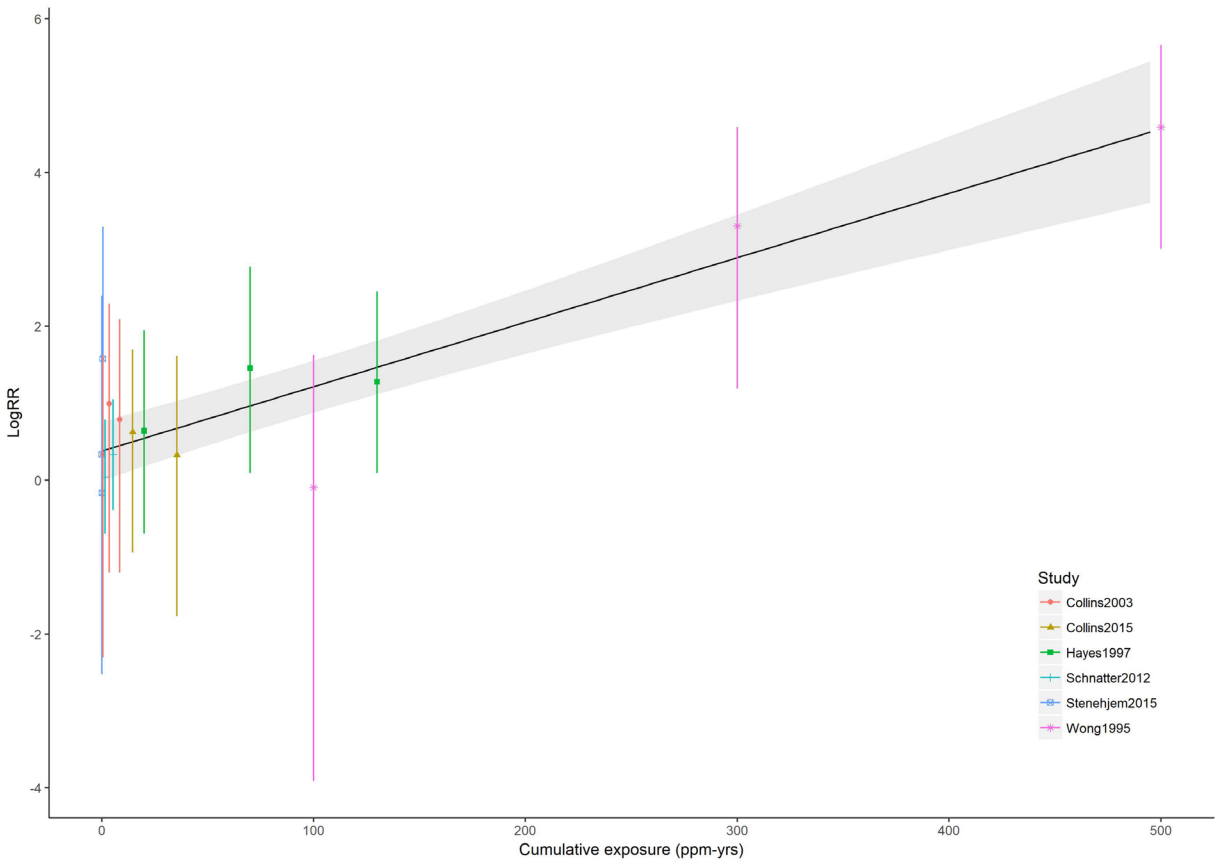


B



Compiled by the Working Group

Fig. 2.2 Meta-regression model of cumulative benzene exposure and AML, including fitted curve and confidence bands



Compiled by the Working Group

Table 2.8 Sensitivity analyses of the linear function of cumulative exposure to benzene (ppm-yr) and AML by sequential exclusion of individual cohort studies

Studies	Intercept	Slope (/100)
All studies	0.38 (0.20)	0.84 (0.11)
Excluding Stenehjem et al. (2015)	0.34 (0.20)	0.85 (0.11)
Excluding Collins et al. (2015)	0.40 (0.22)	0.84 (0.11)
Excluding Schnatter et al. (2012)	0.50 (0.25)	0.81 (0.11)
Excluding Hayes et al. (1997)	0.36 (0.21)	0.86 (0.11)
Excluding Collins et al. (2003)	0.28 (0.23)	0.86 (0.11)
Excluding Wong (1995)	0.45 (0.21)	0.59 (0.40)

AML, acute myeloid leukaemia; ppm, parts per million; yr, year(s)

the exclusion of all individual studies, with the exception of Wong (1995) (reanalysis of Pliofilm manufacturing plants in Ohio), which leads to an overall lower estimated association (0.59 vs 0.85). The Stenehjem et al. (2015) study, which seems to indicate higher risks at low levels of exposure, and the Wong (1995) study were particularly influential on the exposure–response function due to the high exposure estimates. The observed instability in the derivation of the meta-exposure–response association underscores some uncertainty in these results.

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3. CANCER IN EXPERIMENTAL ANIMALS

Benzene has been evaluated by four *IARC Monographs* Working Groups (IARC, 1974, 1982, 1987, 2012). The Working Group in 2012 concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of benzene (IARC, 2012).

3.1 Mouse

3.1.1 Inhalation

See Table 3.1

Numerous studies have been conducted in mice exposed to benzene by inhalation. Even though some of these studies focused on the effects of benzene in transgenic mice, data from these studies reporting the effects of benzene in wildtype mice are included in this section.

C57BL/6 female mice (age, 7–9 weeks) were exposed to clean air (116 mice) or benzene at 300 ppm [purity not reported] (118 mice) for 6 hours per day, 5 days per week, for 16 weeks in whole-body inhalation chambers. The 116 clean-air controls and 118 mice exposed to benzene were reduced to 88 and 90 mice, respectively, by interim killing for assays for haematopoietic stem cells. Preliminary observations were reported 64 weeks after beginning exposure (Cronkite et al., 1984). During this period there was increased mortality in the mice exposed to benzene: one of the clean-air control mice died and 10 mice exposed to benzene died. The dead control mouse did not have lymphoma or leukaemia. Of the 10 mice exposed to benzene that died, 6 had lymphoma of the thymus gland, 2 had unspecified lymphomas, 1 did not have lymphoma or leukaemia, and 1 carcass was lost due to cannibalism and autolysis of tissues. The incidence of lymphoma or leukaemia 64 weeks after beginning exposure was 8/89 (9.0%) in mice exposed to benzene compared with 0/88 in control mice. [These tumour incidences indicated a significant increase in the incidence of lymphoma or leukaemia [$P = 0.007$, Fisher exact test]; however, because 87 of 88 clean-air control mice and 80 of 89 mice exposed to benzene were not comprehensively examined, the actual tumour incidences in the control groups and groups exposed to benzene were unknown. The study was considered inadequate for the evaluation.]

C57BL/6 female mice (age, 8–12 weeks) were exposed to clean air (88 mice) or benzene at a dose of 300 ppm [purity not reported] (89 mice) for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers, and then observed for their lifetimes. The observations reported were made 692 days after beginning exposure (580 days after the end of the exposure period) (Cronkite et al., 1985). During this period, there was increased mortality in the mice exposed to benzene: 23 deaths in the control group and 48 deaths in the group exposed to benzene were observed. There was a significantly higher incidence of tumours in the mice exposed to benzene that had died by day 692 of the experiment: leukaemia (all

Table 3.1 Studies of carcinogenicity in mice exposed to benzene by inhalation

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (F) 7–9 wk 64 wk Cronkite et al. (1984)	Inhalation (whole-body exposure) Benzene, reagent grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 88, 90 87, 80	*Haematopoietic and lymphoid tissues*: lymphoma/leukaemia 0/88, 8/89	See comments	Principal limitations: preliminary data obtained from dead or moribund animals; animals alive at 64 wk were not assessed Surviving mice were not examined, so the tumour incidence in the control and benzene-exposed populations is unknown; the study was considered inadequate
Full carcinogenicity Mouse, C57BL/6 (F) 8–12 wk 692 d Cronkite et al. (1985)	Inhalation (whole-body exposure) Benzene, probably reagent grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 88, 89 65, 41	*Haematopoietic and lymphoid tissues* Thymic lymphoma 1/88, 10/89 Non-thymic lymphoma 2/88, 6/89 Myelogenous leukaemia 3/88, 0/89 Unspecified leukaemia 2/88, 4/89 Leukaemia (all types) 8/88, 20/89 *Zymbal gland*: benign and malignant epidermoid tumours and lymphoepithelioma 1/88, 16/89 *Ovary*: unspecified solid tumours 0/88, 8/89 *Liver*: hepatoma (not specified) 1/88, 1/89 *Other neoplasms*: unspecified solid tumours 2/88, 4/89	See comments See comments See comments See comments See comments See comments See comments See comments See comments	Principal limitations: data obtained from dead or moribund animals; animals alive at 692 d after the commencement of the study were not assessed; the data presented in Table 1 appear to be the continuing observations of the study reported in Cronkite et al. (1984), although this is not specifically stated Surviving mice were not examined, so tumour incidence in the control and in the benzene-exposed populations was therefore unknown; the study was considered inadequate

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, CBA/Ca BNL (M) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 60, 57 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 7/60, 1/57 Myelogenous neoplasms 0/60, 11/57* *Liver*: hepatoma (not specified) 16/60, 6/57 *Other neoplasms*: unspecified solid tumours 13/60, 30/57*	 NS *$P < 0.001$ NS *$P < 0.001$	Principal strengths: lifetime study; studies in male and female mice
Full carcinogenicity Mouse, CBA/Ca BNL (F) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 60, 54 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 5/60, 4/54 Myelogenous neoplasms 1/60, 6/54* *Liver*: hepatoma (not specified) 8/60, 0/54 *Other neoplasms*: unspecified solid tumours 21/60, 43/54*	 NS *$P = 0.040$ (chi-squared test) NS (for an increase) *$P < 0.001$	Principal strengths: lifetime study; studies in male and female mice *P* values for the difference in the incidence of myelogenous neoplasms between control and benzene-exposed females are different when using the one-tail and the two-tail Fisher exact test (as calculated by the Working Group): 0.0420 and 0.0514 (not significant), respectively
Full carcinogenicity Mouse, CBA/Ca BNL (M) 12 wk Lifetime Cronkite et al. (1989)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk, 16 wk 70, 85 NR	*Haematopoietic and lymphoid tissues* Lymphomatous neoplasms 12/70, 7/85 Myelogenous neoplasms 0/70, 2/85 *Liver*: hepatoma (not specified) 27/70, 35/85 *Other neoplasms*: unspecified solid tumours 14/70, 38/85*	 NS NS NS *$P = 0.001$	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, CBA/Ca (M) 10–12 wk 22 mo Farris et al. (1993)	Inhalation (whole-body exposure) Benzene, purity NR but certified HPLC grade Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 16 wk 125, 125 75, 25	*Haematopoietic and lymphoid tissues*: malignant lymphoma 2/119, 14/118* *Preputial gland*: squamous cell carcinoma 0/118, 71/118* *Lung*: adenoma 17/119, 42/118* *Zymbal gland*: carcinoma 1/125, 14/125 *Forestomach*: squamous cell carcinoma 0/125, 9/125 *Harderian gland*: adenoma 6/125, 7/125	*$P < 0.01$ (table), $P < 0.002$ (text) *$P < 0.01$ *$P < 0.01$ [$P < 0.01$]; see comments [$P < 0.01$]; see comments NS; see comments	Principal strengths: covers most of the lifespan Benzene decreased survival ($P < 0.01$); the incidence of Zymbal gland carcinoma and of forestomach squamous cell carcinoma was significantly increased by Fisher's exact test: $P < 0.01$ and $P < 0.01$, respectively; Zymbal gland, forestomach, and Harderian gland were examined microscopically only when gross lesions were evident
Full carcinogenicity Mouse, AKR (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk 50, 50 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/50, 0/50	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described
Full carcinogenicity Mouse, AKR (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 80, 80 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/80, 0/80	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57Bl (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/40, 0/40	NS	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described
Full carcinogenicity Mouse, CD-1 (NR) NR Lifetime Goldstein et al. (1982)	Inhalation Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues*: myelogenous leukaemia 0/40, 2/40	NS; see comments	Principal strengths: lifetime study Principal limitations: only myeloproliferative disorders were assessed; methods were poorly described Spontaneous cases of myelogenous leukaemia are rare in CD-1 mice; the authors argue that the two occurrences of myelogenous leukaemia are likely due to benzene inhalation
Full carcinogenicity Mouse, C57BL/6 (wildtype) (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 33, 100, 300 ppm (in air) 6 h/d, 5 d/wk, 26 wk 20, 19, 19, 18 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/20, 0/19, 2/19, 5/18* Non-thymic lymphoma 2/20, 4/19, 1/19, 5/18 Myeloid leukaemia 0/20, 0/19, 0/19, 0/18 Neoplasms of haematopoietic and lymphoid tissues (combined) 2/20, 4/19, 3/19, 10/18* *Other neoplasms*: solid tumours (not specified) excluding lymphomas 3/20, 3/19, 8/19, 2/18	 *$P < 0.05$ [$P = 0.004$] (Cochran–Armitage trend test) NS NS *$P < 0.05$ NS	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C3H/He (wildtype) (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 100, 300 ppm (in air) 6 h/d, 5 d/wk, 26 wk 23, 24, 23 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/23, 4/24, 0/23 Non-thymic lymphoma 2/23, 2/24, 5/23 Myeloid leukaemia 0/23, 0/24, 2/23 Neoplasms of haematopoietic and lymphoid tissues (combined) 2/23, 6/24, 7/23 *Other neoplasms*: solid tumours (not specified) excluding lymphomas 11/23, 5/24, 8/23	 [$P = 0.0031$] (Cochran–Armitage trend test) NS NS NS NS	Principal strengths: lifetime study
Full carcinogenicity Mouse, C57BL/6 (wildtype) (NR) NR Lifetime Li et al. (2006)	Inhalation (whole-body exposure) Benzene, purity NR (purchased from Wako Fine Chemicals, Japan) Air 0, 300 ppm 6 h/d, 5 d/wk, 26 wk 8, 10 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/8, 3/10 Non-thymic lymphoma 6/8, 5/10	 [NS] [NS]; see comments	Principal strengths: lifetime study Principal limitations: small numbers of animals were used The time to non-thymic lymphoma was shorter in benzene-exposed mice compared with controls
Full carcinogenicity Mouse, AKR/J (M) 8 wk Lifetime Snyder et al. (1980)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 100 ppm (in air) 6 h/d, 5 d/wk 50, 50 NR	*Haematopoietic and lymphoid tissues*: malignant lymphoma 24/50, 29/49	 NS	Principal strengths: lifetime study

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1980)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk 40, 40 NR	*Haematopoietic and lymphoid tissues* Haematopoietic neoplasms (combined) 2/40, 8/40* Bone marrow hyperplasia in animals without haematopoietic neoplasm 0/38, 13/32*	 *$P < 0.005$; see comments *$P < 0.001$	Principal strengths: lifetime study Principal limitations: poor data presentation Using the two-tail Fisher exact test to compare the incidence of haematopoietic neoplasms in clean air and benzene-exposed C57BL/6J mice, the Working Group determined that the *P* value was NS (0.0872). On the other hand, using the log-rank (χ^2) test, which compares events and time to event, the difference in malignant lymphoma incidence between control and benzene-exposed mice was found to be significant by the authors ($P < 0.005$). The significance found by the authors therefore depends on tumour induction time
Full carcinogenicity Mouse, CD-1 (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 1200 ppm (in air) 6 h/d, 5 d/wk, 10 wk 80, 80 71 (at risk), 71 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 11/71, 11/71 *Zymbal gland*: carcinoma 0/71, 4/71* *Lung*: adenoma 17/71, 33/71* *All sites* Malignant tumours 22/71, 24/71* Benign tumours 21/71, 35/71* Benign and malignant tumours 36/71, 45/71*	 NS *$P < 0.05$ [NS]; see comments *$P < 0.001$ *$P < 0.05$ [NS]; see comments *$P < 0.001$ *$P < 0.001$ [NS]; see comments	Principal strengths: lifetime study Principal limitations: short duration of exposure; use of the log-rank (χ^2) test is questionable Using the two-tail Fisher exact test, the Working Group determined that only the incidence of lung adenoma and of benign tumours was significantly increased in benzene-exposed mice ($P = 0.0081$ and 0.0252, respectively)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 1200 ppm (in air) 6 h/d, 5 d/wk, 10 wk 80, 80 67 (at risk), 68 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 15/67, 11/68 *Zymbal gland*: carcinoma 0/67, 4/68 *Lung*: adenoma 11/67, 15/68 *All sites* Malignant tumours 19/67, 15/68 Benign tumours 13/67, 16/68 Benign and malignant tumours 28/67, 30/68	 NS NS NS NS NS NS	Principal strengths: lifetime study Principal limitation: short duration of exposure
Full carcinogenicity Mouse, CD-1 (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 1 wk then 2 wk unexposed, for life 60, 60 46 (at risk), 54 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 1/46, 7/54* *Zymbal gland*: carcinoma 0/46, 2/54 *Lung*: adenoma 3/46, 14/54* *All sites* Malignant tumours 1/46, 12/54* Benign tumours 3/46, 15/54* Benign and malignant tumours 4/46, 25/54*	 *[$P < 0.05$] (one-tail Fisher exact test) NS *$P < 0.005$ *$P < 0.005$ *$P < 0.001$ *$P < 0.001$	Principal strengths: lifetime study In the group of exposed mice, the Working Group determined that increases in the incidence of lung adenoma, malignant tumours, benign tumours, and of total tumours were also significant by the two-tail Fisher exact test, with *P* values of 0.015, 0.003, 0.008, and < 0.0001, respectively

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6J (M) 8 wk Lifetime Snyder et al. (1988)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300 ppm (in air) 6 h/d, 5 d/wk, 1 wk then 2 wk unexposed, for life 60, 60 46 (at risk), 54 (at risk)	*Haematopoietic and lymphoid tissues*: leukaemia/lymphoma 1/46, 3/54 *Zymbal gland*: carcinoma 0/46, 19/54* *Lung*: adenoma 5/46, 3/54 *All sites* Malignant tumours 2/46, 24/54* Benign tumours 6/46, 5/54 Benign and malignant tumours 8/46, 25/54*	 NS *$P < 0.001$ NS *$P < 0.001$ NS *$P < 0.001$	Principal strengths: lifetime study In the group of exposed mice, the Working Group determined that increases in the incidence of Zymbal gland carcinoma, malignant tumours, and of total tumours were also significant by the two-tail Fisher exact test, with P values of < 0.0001, < 0.0001, and 0.003, respectively

d, day(s); F, female; h, hour(s); HPLC, high-performance liquid chromatography; M, male; mo, month(s); NR, not reported; NS, not significant; ppm, parts per million; wk, week(s)

types): control, 8/88 (9.1%) and benzene, 20/89 (22.5%) [$P = 0.0223$, Fisher]; tumours of the Zymbal gland: control, 1/88 (1.1%) and benzene, 16/89 (18.0%) [$P = 0.001$, Fisher]; tumours of the ovary: control, 0/88 and benzene, 8/89 (9.0%) [$P = 0.0066$, Fisher]; hepatoma [not further specified]: control, 1/88 (1.1%) and benzene, 1/89 (1.1%); and other tumours: control, 2/88 (2.3%) and benzene, 4/89 (4.5%). [These incidences indicated a significant increase in carcinogenicity in mice exposed to benzene; however, the animals that were still alive at experimental day 692 (65 control and 41 exposed to benzene) were not comprehensively examined. The actual tumour incidences in the control group and the group exposed to benzene were therefore unknown. The similarities between the mice described in Table 1 of the 1985 article and Table 1 of the 1984 article authored by Cronkite et al. suggested that these were the same groups of experimental animals; this is not specifically stated in the 1985 article, however (Cronkite et al., 1984, 1985). The study was considered inadequate for the evaluation.]

Six groups of CBA/Ca BNL male or female mice (age, 12 weeks) were exposed to clean air or benzene at a dose of 100 or 300 ppm [purity not reported] for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers and then observed for their lifetimes (Cronkite et al., 1989). Groups 1 and 2 consisted of 60 males exposed to clean air and 57 males exposed to benzene at 300 ppm; groups 3 and 4 consisted of 60 females exposed to clean air and 54 females exposed to benzene at 300 ppm; and groups 5 and 6 consisted of 70 males exposed to clean air and 85 males exposed to benzene at 100 ppm. Mice exposed to benzene at 300 pm, but not 100 ppm, had shorter lifespans than controls. Median lifespans of males and females were 1030 and 1100 days (clean air) and 510 and 580 days (300 ppm benzene), respectively, while the median lifespans of males exposed to clean air and 100 ppm benzene were 1020 and 1000 days, respectively. Neither lymphomatous neoplasms nor hepatoma were increased in male or female mice exposed to benzene at 300 ppm; however, the mice exposed to benzene at 300 ppm had increased incidences of myelogenous neoplasms (controls, 0/60 (males) and 1/60 (females); benzene, 11/57 (males) ($P < 0.001$) and 6/54 (females) ($P = 0.040$) [$P = 0.0420$, one-tail Fisher exact test; $P = 0.0514$ (not significant), two-tail Fisher exact test]) and solid tumours other than hepatoma or of the haematopoietic or lymphoid tissues (controls, 13/60 (males) and 21/60 (females); benzene, 30/57 (males) ($P < 0.001$) and 43/54 (females) ($P < 0.001$)). Male mice exposed to benzene at 100 ppm also had an increased incidence of solid tumours other than hepatoma or of the haematopoietic or lymphoid tissues (controls, 14/70; benzene, 38/85 ($P = 0.001$)).

Groups of 125 CBA/Ca male mice (age, 10–12 weeks) were exposed to clean air or benzene [high-performance liquid chromatography grade] at a dose of 300 ppm for 6 hours per day, 5 days per week, for 16 weeks in inhalation chambers, and then observed for up to 18 months (Farris et al., 1993). There was a significant decrease in survival in the group exposed to benzene ($P < 0.01$). There was a significant increase in the incidences of malignant lymphoma (control, 2/119; benzene, 14/118; $P < 0.01$), squamous cell carcinoma of the preputial gland (control, 0/118; benzene, 71/118; $P < 0.01$), adenoma of the lung (control, 17/119; benzene, 42/118; $P < 0.01$), carcinoma of the Zymbal gland (control, 1/125; benzene, 14/125 [$P < 0.01$, Fisher]), and squamous cell carcinoma of the forestomach (control, 0/125; benzene, 9/125 [$P < 0.01$, Fisher]).

Groups of 50–80 AKR mice, 40 C57Bl mice, and 40 CD-1 mice [sex and age not reported] were exposed to clean air or benzene at a dose of 100 or 300 ppm [purity not reported] in inhalation chambers for 6 hours per day, 5 days per week, for life (Goldstein et al., 1982). Of the CD-1 mice exposed to benzene at 300 ppm, one developed chronic myelogenous leukaemia

and another developed acute myelogenous leukaemia. [The Working Group noted that only myeloproliferative disorders were assessed and that, while there was no statistical increase in the incidence of myelogenous leukaemia in mice exposed to benzene compared with controls, spontaneous cases of myelogenous leukaemia are rare in CD-1 mice. The authors argued that "the absence of a background incidence of acute and chronic myelogenous leukaemia in CD-1 mice … suggests that the present observations are due to a direct effect of benzene inhalation."] No leukaemias were observed in AKR or C57Bl mice. [The Working Group also noted the very low incidence of neoplasms of the haematopoietic and lymphoid tissues in all three strains of mice tested.]

In lifetime studies, groups of male C57BL/6 wildtype (18–20 animals per group), heterozygous *Trp53*-deficient or homozygous Trp53-deficient mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 33, 100, or 300 ppm [purity not reported, chemical grade] for 6 hours per day, 5 days per week, for 26 weeks in inhalation chambers, and groups of 18–24 male C3H/He wildtype (23–24 animals per group), heterozygous *Trp53*-deficient or homozygous Trp53-deficient mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 100 or 300 ppm for 6 hours per day, 5 days per week, for 26 weeks in inhalation chambers (Kawasaki et al., 2009; see also Section 3.3.1). Wildtype mice of both strains exposed to benzene at 300 ppm had decreased survival rates ($P < 10^{-5}$). Wildtype C57BL/6 mice exposed to benzene at 300 ppm had significant increases in the incidences of lymphoma of the thymus gland (control, 0/20; benzene, 5/18; $P < 0.05$) and total neoplasms of the haematopoietic and lymphoid tissues (control, 2/20; benzene, 10/18; $P < 0.05$). Wildtype C3H/He mice exposed to benzene at 300 ppm had a non-significant increase in the incidence of total neoplasms of the haematopoietic and lymphoid tissues (control, 2/23; benzene, 7/23; not significant), and two of these mice developed myeloid leukaemia (compared with none from the control group). There was also a significant positive trend in the incidence of lymphoma of the thymus gland for both strains of mice. There was no increase in the incidence of solid tumours in any of the groups of wildtype mice exposed to benzene.

In a study using C57BL/6 h-Trx-Tg transgenic mice, Li et al. (2006) exposed 8 wildtype C57BL/6 mice to clean air and 10 wildtype C57BL/6 mice [sex not reported] to benzene at 300 ppm [purity not reported, chemical grade] in inhalation chambers for 6 hours per day, 5 days per week, for 26 weeks, and monitored the mice for their lifetimes. Until the mice reached an age of approximately 2 years, the proportion surviving in wildtype mice exposed to benzene was lower than in wildtype mice exposed to clean air. The cumulative incidence of thymic lymphoma in clean-air controls and wildtype mice exposed to benzene was 0/8 and 3/10 [no significant increase], respectively, and the cumulative incidence of non-thymic lymphoma was 6/8 and 5/10, respectively; however, the time to non-thymic lymphoma was shorter in mice exposed to benzene compared with controls.

Groups of 50 male AKR/J mice and 40 male C57BL/6J mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 100 ppm [purity not reported] (AKR mice) or 300 ppm (C57BL/6J mice) in inhalation chambers for 6 hours per day, 5 days per week, for life (Snyder et al., 1980). In the AKR mice, there was no difference in weight gain or median survival between those exposed to clean air and those exposed to benzene at 100 ppm. In contrast, C57BL/6J mice exposed to benzene at 300 ppm had a decreased weight gain and median survival: median survival of mice exposed to benzene was 41 weeks compared with 75 weeks for the controls. Inhalation of benzene at 100 ppm did not increase the incidence of malignant lymphoma in AKR mice: malignant lymphoma was found in 24/50 mice exposed to clean air and 29/49 mice exposed to benzene.

Haematopoietic neoplasms were found in 2/40 C57BL/6J mice exposed to clean air and 8/40 C57BL/6J mice exposed to benzene at 300 ppm ($P < 0.005$, log-rank (χ^2) test [$P = 0.0872$, two-tail Fisher exact test]), and hyperplasia of the bone marrow without neoplasia was found in 0/38 control mice and 13/32 mice exposed to benzene ($P < 0.001$, log-rank (χ^2) test [$P < 0.001$, Fisher exact test]). [Using the two-tail Fisher exact test, the incidence of haematopoietic neoplasms was not statistically significantly increased in C57BL/6J mice exposed to benzene at 300 ppm. On the other hand, using the log-rank test, which compares events and times to event, the difference in haematopoietic neoplasm incidence between control groups and mice exposed to benzene was found to be significant. The significance found by Snyder et al. (1980) therefore depends on tumour induction time. In support of the authors' conclusion that C57BL/6J mice exposed to benzene at 300 ppm had a significant increase in the incidence of haematopoietic neoplasms, hyperplasia of the bone marrow without neoplasia was significantly increased in mice exposed to benzene.]

Male CD-1 and C57BL/6J mice (age, 8 weeks) were exposed to clean air or benzene at a dose of 300 or 1200 ppm [purity not reported] in inhalation chambers (Snyder et al., 1988). In a first protocol, groups of 80 CD-1 and 80 C57BL/6 mice were exposed to clean air or to benzene at 1200 ppm for 6 hours per day, 5 days per week, for 10 weeks, and then observed for their lifetimes. In a second protocol, groups of 60 CD-1 and 60 C57BL/6 mice were exposed to clean air or to benzene at 300 ppm for 6 hours per day, 5 days per week, for 1 week, followed by non-exposure for 2 weeks; this regimen was repeated for life. Exposure to benzene did not affect the mortality rate of either CD-1 or C57BL/6 mice; however, for clean-air controls, the 50% mortality rate occurred earlier in mice exposed according to the first protocol than in mice exposed according to the second protocol (approximately 460 days vs 600 days for CD-1 mice, and 740 days vs 840 days for C57BL/6 mice). Tumour incidences in CD-1 mice exposed to clean air or benzene at 1200 ppm were: malignant tumours, 22/71 or 24/71; benign tumours, 21/71 or 35/71; total tumours, 36/71 or 45/71; adenoma of the lung, 17/71 or 33/71; leukaemia/lymphoma, 11/71 or 11/71; and carcinoma of the Zymbal gland, 0/71 or 4/71, respectively. [Using the log-rank (χ^2) test, Snyder et al. (1988) reported that the increases in the incidence of malignant tumours, benign tumours, total tumours, adenoma of the lung, and carcinoma of the Zymbal gland were significant; however, using the two-tail Fisher exact test, the Working Group determined that only the incidence of benign tumours and of adenoma of the lung was significantly increased in the exposed mice.] Tumour incidence was not increased in C57BL/6 mice exposed to benzene at 1200 ppm. Tumour incidence in CD-1 mice intermittently exposed to clean air or benzene at 300 ppm was: malignant tumours, 1/46 or 12/54; benign tumours, 3/46 or 15/54; total tumours, 4/46 or 25/54; adenoma of the lung, 3/46 or 14/54; leukaemia/lymphoma, 1/46 or 7/54 [$P < 0.05$, one-tail Fisher exact test]; and carcinoma of the Zymbal gland, 0/46 or 2/54. [Using the two-tail Fisher exact test, the Working Group confirmed the conclusions of Snyder et al. that the incidence of total tumours, malignant tumours, benign tumours, and of adenoma of the lung was significantly increased in mice exposed to benzene.] Tumour incidence in C57BL/6 mice intermittently exposed to clean air or benzene at 300 ppm was: malignant tumours, 2/46 or 24/54; benign tumours, 6/46 or 5/54; total tumours, 8/46 or 25/54; adenoma of the lung, 5/46 or 3/54; leukaemia/lymphoma, 1/46 or 3/54; and carcinoma of the Zymbal gland, 0/46 or 19/54. [Using the two-tail Fisher exact test, the Working Group confirmed the conclusions of Snyder et al. that the incidence of total tumours, malignant tumours, and of carcinoma of the Zymbal gland was significantly increased in mice exposed to benzene. The Working Group noted the short duration of exposure in the first protocol.]

3.1.2 Oral administration

See Table 3.2

Good laboratory practice (GLP) studies of carcinogenicity with benzene (purity, > 99.7%) were conducted in groups of 50 B6C3F$_1$ mice of each sex (age, 6.5–8.5 weeks); four groups were given benzene at a dose of 0 (control), 25, 50, or 100 mg/kg body weight (bw) in corn oil by gavage 5 days per week, for 103 weeks (NTP, 1986; Huff et al., 1989). At the age of 2 years, mean body weights of male and female mice given the high dose were significantly decreased. Survival of male and female mice given the high dose was also significantly decreased (males, 28/50 (control), 23/50, 18/50, 7/50; females, 30/50 (control), 26/50, 24/50, 18/50). Most mice exposed to benzene that died before week 103 had neoplasia. Compound-related non-neoplastic and neoplastic effects were found for the adrenal gland, forestomach, Harderian gland, haematopoietic and lymphoid tissues, liver, lung, mammary gland, ovary, preputial gland, and Zymbal gland.

In males and females, benzene caused a significant increase in the incidence of the following lesions, with a significant positive trend: epithelial hyperplasia and carcinoma of the Zymbal gland; hyperplasia of the bone marrow (haematopoietic system), and lymphoma or leukaemia (combined); alveolar epithelial hyperplasia, bronchioloalveolar adenoma and carcinoma, and bronchioloalveolar adenoma or carcinoma (combined); hyperplasia and adenoma or carcinoma (combined) of the Harderian gland; epithelial hyperplasia, hyperkeratosis, and squamous cell papilloma of the forestomach; and hyperplasia of the adrenal gland. In males only, benzene caused a significant increase in the incidence of adenoma of the Harderian gland and pheochromocytoma of the adrenal gland. In females only, benzene caused a significant increase in the incidence of carcinoma of the Harderian gland, with a significant positive trend.

In male mice, benzene caused significant increases in the incidence, and a significant positive trend in the incidence, of hyperplasia of the preputial gland, squamous cell carcinoma of the preputial gland, carcinoma (not otherwise specified) of the preputial gland, and of carcinoma (all types) of the preputial gland. There was also a small but significant increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined).

In female mice, benzene caused significant increases in the incidence, and a significant positive trend in the incidence, of tubular adenoma of the ovary, granulosa cell tumours [benign] of the ovary, granulosa cell tumours or carcinoma (combined) of the ovary, mixed tumours (benign) of the ovary, carcinoma of the mammary gland, and of carcinosarcoma of the mammary gland, and a significant increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) (NTP, 1986; Huff et al., 1989). [The Working Group noted that this was a well-conducted GLP study with multiple doses, using both males and females, covering most of the lifespan, and with complete histopathology. Mice treated with benzene had lower body weights and survival.]

A group of 40 male and 40 female Swiss mice (age, 7 weeks) was given benzene (purity, > 99.93%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by stomach tube once per day, 4–5 days per week, for 78 weeks. Mice were observed for life. The authors stated that body weights were lower in treated mice, and particularly in males. Survival was comparable among groups. Necropsies were performed on all animals, with histopathological examinations on 29 tissues and organs (several with multiple sections, e.g. gastrointestinal tract), and all lesions. There were significant increases in the incidence of primary tumours (benign and malignant) (male, 15/40 vs 24/40; female, 16/40 vs 32/40), malignant tumours (female, 11/40 vs 28/40), total tumours [mainly adenomas] of the lung (male, 3/40 vs 17/40; female, 4/40 vs 15/40)

Table 3.2 Studies of carcinogenicity in mice exposed to benzene by gavage or intraperitoneal injection

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6.5–8.5 wk 103 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 5 days/wk, 103 wk 50, 50, 50, 50 28, 23, 18, 7	*Zymbal gland*: squamous cell carcinoma 0/43*, 1/34, 4/40**, 21/39***	*P < 0.001 (trend), **P = 0.012, ***P < 0.001 (life-table test)	Principal strengths: 2 yr bioassay; control and three dose groups; complete histopathology; well-conducted GLP study; studies in both male and female mice Principal limitations: much of the lowered body weights and slightly reduced survival could be attributed to tumour-bearing animals; the survival of high-dose groups of male and female mice was significantly lower than respective vehicle control groups; mean body weights of high-dose groups of male and female mice were lower than vehicle controls The incidence of preneoplastic hyperplasia was increased in tumours of Zymbal gland, preputial gland, Harderian gland, lung, haematopoietic system (bone marrow), forestomach, and adrenal gland caused by benzene
		Haematopoietic and lymphoid tissues Lymphoma 4/49*, 9/48, 9/50**, 15/49***	*P < 0.001 (trend), **P = 0.030, ***P < 0.001 (life-table test)	
		Lymphoma or leukaemia (combined) 4/49*, 10/48**, 10/50***, 15/49****	*P < 0.001 (trend), **P = 0.048, ***P = 0.018, ****P < 0.001 (life-table test) Leukaemia in one low-dose and one intermediate-dose group mouse	
		Lung Alveolar/bronchiolar adenoma 6/49*, 6/48, 8/50, 12/49**	*P < 0.001 (trend), **P = 0.005 (life-table test)	
		Alveolar/bronchiolar carcinoma 5/49*, 11/48, 12/50**, 14/49***	*P < 0.001 (trend), **P = 0.017, ***P < 0.001 (life-table test)	
		Alveolar/bronchiolar adenoma or carcinoma (combined) 10/49*, 16/48, 19/50**, 21/49***	*P < 0.001 (trend), **P = 0.007, ***P < 0.001 (life-table test)	
		Adrenal gland: pheochromocytoma 1/47, 1/48, 7/49*, 1/46	*P = 0.010 (life-table test)	
		Preputial gland Squamous cell carcinoma 0/21*, 3/28, 18/29**, 28/35**	*P < 0.001 (trend), **P < 0.001 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6.5–8.5 wk 103 wk NTP (1986) (cont.)		Carcinoma, NOS 0/21*, 2/28, 1/29, 3/35**	*P < 0.019 (trend), **P < 0.043 (life-table test)	
		Carcinoma (all types) 0/21*, 5/28, 19/29**, 31/35**	*P < 0.001 (trend), **P < 0.001 (life-table test)	
		Harderian gland Adenoma 0/49*, 9/46**, 13/49***, 11/48***	*P < 0.001 (trend), **P = 0.001, ***P < 0.001 (life-table test)	
		Adenoma or carcinoma (combined) 1/49*, 10/46**, 13/49***, 14/48***	*P < 0.001 (trend), **P = 0.002, ***P < 0.001 (life-table test)	
		Forestomach Squamous cell papilloma 2/45*, 1/42, 2/44, 5/38**	*P = 0.003 (trend), **P = 0.014 (life-table test)	
		Squamous cell papilloma or carcinoma (combined) 2/45*, 2/42, 3/44, 5/38**	*P = 0.004 (trend), **P = 0.014 (life-table test) One carcinoma in each benzene-treated group; one mouse in the high-dose group had papilloma and carcinoma	
		Liver Hepatocellular carcinoma 9/49, 8/48, 17/50*, 8/47	*P = 0.028 (life-table test)	
		Hepatocellular adenoma or carcinoma (combined) 15/49, 17/48, 22/50*, 11/47	*P = 0.029 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6.5–8.5 wk 103 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 5 d/wk, 103 wk 50, 50, 50, 50 30, 26, 24, 18	*Zymbal gland*: squamous cell carcinoma 0/43*, 0/32, 1/37, 3/31**	*P = 0.007 (trend), **P = 0.045 (life-table test)	Principal strengths: 2 yr bioassay; control and three dose groups; complete histopathology; well-conducted GLP study; studies in both male and female mice Principal limitations: see principal limitations for NTP (1986) male mice study The incidence of preneoplastic hyperplasia was increased in tumours of the Zymbal gland, ovary, Harderian gland, lung, haematopoietic system (bone marrow), forestomach, and adrenal gland Historical incidence of lymphoma at laboratory (mean ± SD): 22/99 (22.2%); historical incidence in NTP studies: 237/1187 (20.0 ± 8.7%). Historical incidence of ovarian tumours at laboratory: 0/100; historical incidence in NTP studies: no more than two ovarian tumours were present in any single control group. Historical incidence of forestomach squamous cell papilloma at laboratory (mean): 0/99; historical incidence in NTP studies: 7/1077 (0.6%)
		Haematopoietic and lymphoid tissues Lymphoma 15/49*, 24/45**, 24/50***, 20/49****	*P = 0.031 (trend), **P = 0.021, ***P = 0.025, ****P = 0.037 (life-table test)	
		Lymphoma or leukaemia (combined) 15/49*, 25/45**, 26/50***, 22/49****	*P = 0.014 (trend), **P = 0.014, ***P = 0.012, ****P = 0.017 (life-table test) Leukaemia in 1 low-dose, 2 intermediate-dose, and 2 high-dose mice	
		Lung Alveolar/bronchiolar adenoma 4/49*, 2/42, 5/50, 9/49**	*P = 0.003 (trend), **P = 0.011 (life-table test)	
		Alveolar/bronchiolar carcinoma 0/49*, 3/42, 6/50**, 6/49***	*P = 0.002 (trend), **P = 0.010, ***P = 0.004 (life-table test)	
		Alveolar/bronchiolar adenoma or carcinoma (combined) 4/49*, 5/42, 10/50**, 13/49***	*P < 0.001 (trend), **P = 0.039, ***P < 0.001 (life-table test)	
		Ovary Tubular adenoma 0/47*, 0/44, 3/49, 3/48**	*P = 0.008 (trend), **P = 0.047 (life-table test)	
		Granulosa cell tumour [benign] 1/47*, 1/44, 6/49**, 7/48***	*P < 0.001 (trend), **P = 0.040, ***P = 0.008 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6.5–8.5 wk 103 wk NTP (1986) (cont.)		Granulosa cell tumour or carcinoma (combined) 1/47*, 1/44, 6/49**, 8/48*** Mixed tumour, benign 0/47*, 1/44, 12/49**, 7/48*** *Mammary gland* Carcinoma 0/49*, 2/45, 5/50**, 10/49*** Carcinosarcoma 0/49*, 0/45, 1/50, 4/49** *Harderian gland* Carcinoma 0/48*, 0/44, 0/50, 4/47** Adenoma or carcinoma (combined) 5/48*, 6/44, 10/50, 10/47** *Forestomach*: squamous cell papilloma 1/42*, 3/40, 6/45**, 5/42*** *Liver* Hepatocellular adenoma 1/49, 8/44*, 5/50, 4/49 Hepatocellular adenoma or carcinoma (combined) 4/49, 12/44*, 13/50**, 7/49	*P < 0.001 (trend), **P = 0.040, ***P = 0.004 (life-table test) One high-dose group mouse had carcinoma *P < 0.001 (trend), **P < 0.001, ***P = 0.001 (life-table test) *P < 0.001 (trend), **P = 0.026, ***P < 0.001 (life-table test) *P < 0.001 (trend), **P = 0.017 (life-table test) *P < 0.001 (trend), **P = 0.020 (life-table test) *P = 0.009 (trend), **P = 0.017 (life-table test) *P = 0.022 (trend), **P = 0.038, ***P = 0.040 (life-table test) *P = 0.008 (life-table test) *P = 0.014, **P = 0.008 (life-table test)	

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) 7 wk Lifetime Maltoni et al. (1988)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 78 wk 40, 40 NR	*Lung*: all tumours [mainly adenomas] 3/40, 17/40* *All sites* "Benign and malignant tumours" 15/40, 24/40* "Malignant tumours" 9/40, 14/40	*[$P < 0.001$][a] *[$P < 0.04$][b] [NS]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 908; also reported in Maltoni et al. (1989)
Full carcinogenicity Mouse, Swiss (F) 7 wk Lifetime Maltoni et al. (1988)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 78 wk 40, 40 NR	*Mammary gland*: carcinoma 2/40, 19/40* *Lung*: all tumours [adenomas] 4/40, 15/40* *All sites* "Benign and malignant tumours" 16/40, 32/40* "Malignant tumours" 11/40, 28/40*	*[$P < 0.0001$][a] *[$P < 0.01$][a] *[$P \leq 0.0005$][a] *[$P \leq 0.0003$][a]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 908; also reported in Maltoni et al. (1989)
Full carcinogenicity Mouse, RF/J (M) 6 wk Lifetime Maltoni et al. (1989)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 52 wk 45, 45 NR	*Lung*: all tumours [adenomas] 5/45, 23/45* *Haematopoietic and lymphoid tissues*: leukaemia 17/45, 26/45* *All sites* "Benign and malignant tumours" 18/45, 33/45* "Malignant tumours" 19/45, 26/45	*[$P < 0.0001$][a] *[$P < 0.05$][b] *[$P < 0.003$][a] [NS]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 909

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, RF/J (F) 6 wk Lifetime Maltoni et al. (1989)	Gavage Benzene, 99.93% Olive oil 0, 500 mg/kg bw 4–5 d/wk, 52 wk 40, 40 NR	*Mammary gland*: carcinoma 1/40, 9/40* *Haematopoietic and lymphoid tissues*: leukaemia 14/40, 24/40* *Lung*: all tumours [mainly adenomas] 3/40, 18/40* *All sites* "Benign and malignant tumours" 20/40, 34/40* "Malignant tumours" 3/40, 18/40*	*[$P < 0.02$][a] *[$P < 0.05$][a] *[$P < 0.0002$][a] *[$P < 0.002$][a] *[$P \leq 0.0002$][a]	Principal strengths: lifetime study; studies in male and female mice Experiment BT 909
Full carcinogenicity Mouse, A/J (M) 6–8 wk 24 wk Stoner et al. (1986)	Gavage Benzene, purity NR (reagent grade) Tricaprylin 0, 100 mg/kg bw 3×/wk for 8 wk 16, 16 15, 16	*Lung*: adenoma 3/15, 8/16 Tumour multiplicity: 0.27 ± 0.59, 0.63 ± 0.72*	 NR [NS] *$P < 0.05$	Principal limitations: incomplete histopathology reporting; only one dose group Cumulative dose was 2400 mg/kg bw, or 100 mg/kg bw/dose, as animals were gavaged 3×/wk for 8 wk
Full carcinogenicity Mouse, A/J (F) 6–8 wk 24 wk Stoner et al. (1986)	Gavage Benzene, purity NR (reagent grade) Tricaprylin 0, 100 mg/kg bw 3×/wk for 8 wk 16, 16 14, 15	*Lung*: adenoma 2/14, 5/15 Tumour multiplicity: 0.14 ± 0.36, 0.53 ± 0.92	 NR [NS] NS	Principal limitations: incomplete histopathology reporting; only one dose group Cumulative dose was 2400 mg/kg bw or 100 mg/kg bw/dose, as animals were gavaged 3×/wk for 8 wk

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, A/J (M) 6–8 wk 24 wk Stoner et al. (1986)	i.p. Benzene, purity NR (reagent grade) Tricaprylin 0, 20, 50, 100 mg/kg bw 3×/wk for 8 wk 16, 16, 16, 16 16, 15, 16, 16	*Lung*: adenoma 3/16, 5/15, 8/16, 10/16* Tumour multiplicity: 0.25 ± 0.58, 0.53 ± 0.92, 0.63 ± 0.72*, 0.69 ± 0.60*	*[$P < 0.03$] *$P < 0.05$	Principal limitations: incomplete histopathology reporting Cumulative doses were 480, 1200, and 2400 mg/kg bw, or 20, 50, and 100 mg/kg bw, as animals were i.p. injected 3×/wk for 8 wk
Full carcinogenicity Mouse, A/J (F) 6–8 wk 24 wk Stoner et al. (1986)	i.p. Benzene, purity NR (reagent grade) Tricaprylin 0, 20, 50, 100 mg/kg bw 3×/wk for 8 wk 16, 16, 16, 16 16, 16, 16, 15	*Lung*: adenoma 4/16, 4/16, 4/16, 6/15 Tumour multiplicity: 0.31 ± 0.60, 0.44 ± 0.89, 0.25 ± 0.45, 0.47 ± 0.64	NR [NS] NS	Principal limitations: incomplete histopathology reporting Cumulative doses were 480, 1200, and 2400 mg/kg bw, or 20, 50, and 100 mg/kg bw, as animals were i.p. injected 3×/wk for 8 wk
Full carcinogenicity Mouse, CD-1 (M) In utero 12 mo Badham et al. (2010)	i.p. Benzene, purity NR Corn oil 0, 200, 400 mg/kg bw to pregnant dams on gestation days 8, 10, 12, and 14 25, 25, 25 NR	*All sites*: total tumours 6/22, 14/22*, 8/23 *Liver tumours* [primarily adenomas] 3/22, 10/22*, 4/23	*$P = 0.0329$[a] *$P = 0.0452$[a]	Transplacental carcinogenesis The authors also reported an experiment in male C57BP/6N mice with a similar study design, which gave negative results

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) In utero 12 mo Badham et al. (2010)	i.p. Benzene, purity NR Corn oil 0, 200, 400 mg/kg bw to pregnant dams on gestation days 8, 10, 12, and 14 25, 25, 25 NR	*All sites*: total tumours 1/25, 10/24*, 5/22 Lesions of the haematopoietic and lymphoid tissues: hyperplasias, myeloproliferative disorders, and myeloid/lymphoid neoplasias 1/25, 9/24*, 5/22	*P = 0.0019[a] *P = 0.0232[a]	Transplacental carcinogenesis The authors also reported an experiment in female C57BP/6N mice with a similar study design, which gave negative results

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; i.p., intraperitoneal; M, male; mo, month(s); NOS, not otherwise specified; NR, not reported; NS, not significant; NTP, National Toxicology Program; SD, standard deviation; wk, week(s); yr, year(s)

[a] Fisher exact test

[b] One-tail Fisher exact test

in males and/or females, and of carcinoma of the mammary gland in females (2/40 vs 19/40). Carcinoma of the Zymbal gland occurred in four males and one female and dysplasia of the Zymbal gland in three males and four females, compared with none in controls (Maltoni et al., 1988, 1989). [The Working Group noted that this lifetime study did not report on numerical body weight data, statistical analyses, or incidence of benign tumours of the mammary gland.]

A group of 45 male and 40 female RF/J mice (age, 6 weeks) was given benzene (purity, > 99.93%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by stomach tube once per day, 4–5 days per week, for 52 weeks. Mice were observed for life. Necropsies were performed on all animals, with histopathological examinations on 29 tissues and organs (several with multiple sections, e.g. gastrointestinal tract), and all lesions. There were significant increases in the incidence of primary tumours (benign and malignant) (male, 18/45 vs 33/45; female, 20/40 vs 34/40), malignant tumours (female, 3/40 vs 18/40), total tumours [mainly adenomas] of the lung (male, 5/45 vs 23/45; female, 3/40 vs 18/40), and leukaemia (male, 17/45 vs 26/45; female, 14/40 vs 24/40) in males and/or females, and of carcinoma of the mammary gland in females (1/40 vs 9/40) (Maltoni et al., 1989). [The Working Group noted that this lifetime study did not report on body weights, statistical analyses, or incidence of benign tumours of the mammary gland.]

Groups of 16 male and 16 female A/J mice (age, 6–8 weeks) were given benzene (reagent grade) [purity not reported] at a dose of 0 (control) or 100 mg/kg bw [reported as cumulative dose of 2400 mg/kg bw] in 0.1 mL of tricaprylin per mouse, by gavage 3 days per week, for 24 weeks. Of the male mice, 15/16 (control) versus 16/16 (exposed to benzene) survived; for female mice, 14/16 (control) versus 15/16 (exposed to benzene) survived. At necropsy, tumours of the lung (pearly white nodules on the surface of the lungs) were counted under a dissecting microscope. Random samples of lung nodules were taken from control groups and groups treated with benzene for histopathological evaluation and confirmation of adenoma of the lung. The liver, kidneys, spleen, intestines, stomach, thymus, and salivary and endocrine glands were also examined grossly. The incidence of adenoma of the lung was numerically increased, but not significantly (males, 3/15 vs 8/16; females, 2/14 vs 5/15). Lung adenoma multiplicity was increased in exposed males (0.27 ± 0.59 vs 0.63 ± 0.72, $P < 0.05$) but not in exposed females (0.14 ± 0.36 vs 0.53 ± 0.92) (Stoner et al., 1986). [The Working Group noted that benzene purity was not reported, the histopathological report was incomplete, and only one dose was tested.]

3.1.3 *Intraperitoneal injection*

See Table 3.2

Groups of 16 male and 16 female A/J mice (age, 6–8 weeks) were given benzene (reagent grade) [purity not reported] at a dose of 0 (control), 20, 50, and 100 mg/kg bw [reported as cumulative doses of 480, 1200, and 2400 mg/kg bw] in 0.1 mL tricaprylin per mouse, by intraperitoneal injection, 3 days per week, for 24 weeks. One male from the group given the lowest dose and one female from the group given the highest dose died early. At necropsy, tumours of the lung (pearly white nodules on the surface of the lungs) were counted under a dissecting microscope. Random samples of lung nodules were taken from control groups and groups treated for benzene for histopathological evaluation and confirmation of adenoma of the lung. Liver, kidneys, spleen, intestines, stomach, thymus, and salivary and endocrine glands were also examined grossly. The incidence of adenoma of the lung was significantly increased in males given the highest dose (3/16 vs 5/15, 8/16, and 10/16 [$P < 0.03$]), as was multiplicity of adenoma of the lung in males given medium and high doses (0.25 ± 0.58 vs 0.53 ± 0.92, 0.63 ± 0.72 ($P < 0.05$), and 0.69 ± 0.60 ($P < 0.05$)). Neither

lung tumour incidence (4/16 vs 4/16, 4/16, and 6/15) nor multiplicity (0.31 ± 0.60 vs 0.44 ± 0.89, 0.25 ± 0.45, and 0.47 ± 0.64) were significantly increased in treated females (Stoner et al., 1986). [The Working Group noted the incomplete histopathological report.]

CD-1 and C57Bl/6N male and female mice (age, 7–9 weeks) were acclimated for 1 week before use, and given access to rodent chow and tap water. A maximum of 3 females were housed with 1 male overnight, and vaginal plugs the next morning designated day 1 of gestation. Pregnant mice were given corn oil (vehicle) or benzene at a dose of 200 or 400 mg/kg bw [purity not reported] by intraperitoneal injection on days 8, 10, 12, and 14 of gestation (Badham et al., 2010). At an age of 1 year, offspring mice were killed, necropsied, and tissues or organs collected (i.e. heart, intestines, kidneys, liver, lung, spine, spleen, stomach, thymus, and any abnormal tissues). Blinded histopathology was performed under light microscopy.

All tumours observed originated from the lung, liver, or haematopoietic and lymphoid tissues. CD-1 mice exposed in utero to benzene at 200 mg/kg bw had significant increases in the incidence of total tumours (combined), while the group exposed to benzene at 400 mg/kg bw had non-significant numerical increases only: males, 6/22 (27.3%) vs 14/22 (63.6%; $P = 0.0329$, Fisher exact test) and 8/23 (34.8%); females, 1/25 (4.0%) vs 10/24 (41.7%; $P = 0.0019$, Fisher exact test) and 5/22 (22.7%). In C57Bl/6N mice, there was a low incidence of total tumours (combined) only in groups treated with benzene: males, 0/21 vs 1/22 (4.5%) and 1/25 (4.0%); females, 0/19 vs 3/20 (15.0%) and 2/22 (9.1%). CD-1 male mice given the low dose had significant increases in the incidence of tumours of the liver (primarily adenomas, with some focal nodular hyperplasias and carcinomas): 3/22 (13.6%) vs 10/22 (45.5%, $P = 0.0452$) and 4/23 (17.4%). CD-1 female mice given the low dose had significant increases in the incidence of tumours [lesions] of the haematopoietic and lymphoid tissues (hyperplasias, myeloproliferative disorders, or myeloid/lymphoid neoplasias, combined): 1/25 (4.0%) vs 9/24 (37.5%, $P = 0.0232$) and 5/22 (22.7%). In C57Bl/6N mice, there was a low incidence of tumours [lesions] of the haematopoietic and lymphoid tissues only in the groups treated with benzene: males, 0/21 vs 0/22 and 1/25 (4.0%); females, 0/19 vs 3/20 (15.0%) and 2/22 (9.1%) (Badham et al., 2010). [The Working Group noted that dams were not carried to the end of the study for possible tumour occurrence, and that the study did not report benzene purity, injection volumes, beginning and ending body weights, and numbers of surviving mice. The Working Group also noted the short study duration of 12 months, that not all tissues or organs were taken or examined microscopically, and that tumours of the haematopoietic and lymphoid tissues were lesions grouped as hyperplasias, myeloproliferative disorders, and myeloid/lymphoid neoplasias.]

3.2 Rat

See Table 3.3

3.2.1 Oral administration

Groups of 30–35 male and 30–35 female Sprague-Dawley rats (age, 13 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control), 50, or 250 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 52 weeks (Maltoni & Scarnato, 1979; Maltoni et al., 1983; see also Maltoni & Scarnato, 1977; Maltoni et al., 1985, 1989; Mehlman, 2002) [Experiment BT901]. The rats were kept under observation for their lifespan. Mortality, correlated with the direct toxic effects and the higher incidence of malignant tumours, was higher in male and female rats treated with benzene. In treated males, a significant increase in the incidence of tumours of the haematopoietic and lymphoid

Table 3.3 Studies of carcinogenicity in rats exposed to benzene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Maltoni & Scarnato (1979), Maltoni et al. (1983)	Gavage Benzene, > 99.9% Olive oil 0, 50, 250 mg/kg bw 1×/d, 4–5 d/wk, 52 wk 30, 30, 35 NR	*Zymbal gland*: carcinoma 0/30, 0/30, 0/35 *Oral cavity*: carcinoma 0/30, 0/30, 0/35 *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia (lymphoma, leukaemia, or histiocytic sarcoma, combined) 0/30*, 0/30, 4/35 (11.4%) *Mammary gland*: carcinoma 0/30, 0/30, 0/35 *All sites*: malignant tumours 2/30 (6.7%)*, 1/30 (3.3%), 8/35 (22.9%)	[NS] [NS] *[$P = 0.033$] (Cochran–Armitage trend test) [NS] *[$P = 0.025$] (Cochran–Armitage trend test)	Principal strengths: lifetime study; studies in male and female mice; multiple-dose study; complete histopathology Principal limitations: mortality was higher in benzene-treated rats Experiment BT901
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Maltoni & Scarnato (1979), Maltoni et al. (1983)	Gavage Benzene, > 99.9% Olive oil 0, 50, 250 mg/kg bw 1×/d, 4–5 d/wk, 52 wk 30, 30, 35 NR	*Zymbal gland*: carcinoma 0/30*, 2/30 (6.7%), 8/35 (22.9%)** *Oral cavity*: carcinoma 0/30, 0/30, 2/35 (5.7%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia (lymphoma, leukaemia, or histiocytic sarcoma, combined) 1/30 (3.3%), 2/30 (6.7%), 1/35 (3%) *Mammary gland*: carcinoma 3/30 (10%), 4/30 (13%), 7/35 (20.0%) *All sites*: malignant tumours 6/30* (20.0%), 10/30 (33.3%), 21/35 (60.0%)	*[$P = 0.005$] (Cochran–Armitage trend test), **[$P = 0.006$] (Fisher exact test) [NS] [NS] [NS]	Principal strengths: lifetime study; studies in male and female mice; multiple-dose study; complete histopathology Principal limitations: mortality was higher in benzene-treated rats Experiment BT901

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 7 wk Lifetime Maltoni et al. (1982a, 1983, 1985)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 50, 40 NR	*Zymbal gland*: carcinoma 1/50 (2.0%), 18/40 (45.0%)*	*[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; studies in male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights Experiment BT902
		Oral cavity: squamous cell carcinoma 0/50, 21/40 (52.5%)*	*[$P < 0.001$] (Fisher exact test)	
		Nasal cavity: carcinoma 0/50, 3/40 (7.5%)	[NS]	
		Forestomach Acanthomas and dysplasias 0/50, 10/40 (25.0%)*	*[$P < 0.001$] (Fisher exact test)	
		Carcinoma in situ 0/50, 0/40	[NS]	
		Liver Hepatoma [hepatocellular carcinoma] 3/50 (6.0%), 3/40 (7.5%)	[NS]	
		Angiosarcoma 0/50, 2/40 (5.0%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 3/50 (6.0%), 1/40 (2.5%)	[NS]	
		Skin: carcinoma 0/50, 9/40 (22.5%)*	*[$P < 0.001$] (Fisher exact test)	
		All sites: malignant tumours 11/50 (22.0%), 36/40 (90.0%)*	*[$P < 0.001$] (Fisher exact test)	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 7 wk Lifetime Maltoni et al. (1982a, 1983, 1985)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 50, 40 NR	*Zymbal gland*: carcinoma 0/50, 16/40 (40.0%)* *Oral cavity*: squamous carcinoma 0/50, 20/40 (50.0%)* *Nasal cavity*: carcinoma 0/50, 1/40 (2.5%) *Forestomach* Acanthomas and dysplasias 0/50, 7/40 (17.5%)* Carcinoma in situ 0/50, 6/40 (15.0%)* *Liver* Hepatoma [hepatocellular carcinoma] 0/50, 1/40 (2.5%) Angiosarcoma 0/50, 3/40 (7.5%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 1/50 (2.0%), 3/40 (7.5%) *Skin*: carcinoma 1/50 (2.0%), 0/40 *All sites*: malignant tumours 10/50 (20.0%), 35/40 (87.5%)*	*[$P < 0.001$] (Fisher exact test) *[$P < 0.001$] (Fisher exact test) [NS] *[$P = 0.002$] (Fisher exact test) *[$P = 0.006$] (Fisher exact test) [NS] [NS] [NS] [NS] *[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; studies in both male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights Experiment BT902

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) 7 wk Lifetime Maltoni et al. (1988, 1989)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 40, 40 NR	*Zymbal gland*: carcinoma 0/40, 7/40 (17.5%)* *Oral cavity*: carcinoma 1/40 (2.5%), 2/40 (5.0%) *Nasal cavity*: carcinoma 0/40, 2/40 (5.0%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 1/40 (2.5%), 2/40 (5.0%) *All sites*: malignant tumours 8/40 (20.0%), 19/40 (47.5%)*	*[$P = 0.012$] (Fisher exact test) [NS] [NS] [NS] *[$P = 0.017$] (Fisher exact test)	Principal strengths: lifetime study; studies in male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Experiment BT907
Full carcinogenicity Rat, Wistar (F) 7 wk Lifetime Maltoni et al. (1988, 1989)	Gavage Benzene, > 99.9% Olive oil 0, 500 mg/kg bw 1×/d, 4–5 d/wk, 104 wk 40, 40 NR	*Zymbal gland*: carcinoma 0/40, 6/40 (15.0%)* *Oral cavity*: carcinoma 0/40, 4/40 (10.0%) *Nasal cavity*: carcinoma 0/40, 1/40 (2.5%) *Haematopoietic and lymphoid tissues*: haemolymphoreticular neoplasia 3/40 (7.5%), 4/40 (10.0%) *All sites*: malignant tumours 10/40 (25.0%), 21/40 (52.5%)*	*[$P = 0.026$] (Fisher exact test) [NS] [NS] [NS] *[$P = 0.021$] (Fisher exact test)	Principal strengths: lifetime study; studies in both male and female mice; complete histopathology Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Experiment BT907

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7–8 wk 104 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 50, 100, 200 mg/kg bw 1×/d, 5 d/wk, 103 wk 50, 50, 50, 50 32, 29, 24, 16	*Zymbal gland*: carcinoma 2/32 (6.3%)*, 6/46 (13.0%), 10/42 (23.8%)**, 17/42 (40.5%)***	*$P < 0.001$[a], $P < 0.001$[c], $P = 0.003$[d] **$P = 0.040$[b], $P = 0.017$[c] ***$P < 0.001$[b], $P < 0.001$[c], $P = 0.024$[d]	Principal strengths: studies in male and female mice; multiple-dose study; covered most of the lifespan; complete histopathology; well-conducted GLP study Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Dose-related lymphocytopenia was observed in benzene-treated rats Statistical tests used: [a] Cochran–Armitage trend test; [b] Fisher exact test; [c] Life-table test; [d] Incidental tumour test
		Oral cavity Squamous cell carcinoma 0/50*, 3/50, 5/50**, 7/50***	*$P = 0.006$[a], $P = 0.001$[c], $P = 0.002$[d] **$P = 0.028$[b], $P = 0.030$[c] ***$P = 0.006$[b], $P = 0.001$[c], $P = 0.001$[d]	
		Squamous cell papilloma 1/50*, 6/50, 11/50**, 13/50***	*$P < 0.001$[a], $P < 0.001$[b], $P < 0.001$[d] **$P = 0.002$[b], $P = 0.001$[c], $P = 0.004$[d] ***$P < 0.001$[b], $P < 0.001$[c], $P = 0.002$[d]	
		Tongue: squamous cell carcinoma 0/50*, 3/50, 4/50, 4/50**	*NS[a], $P = 0.039$[c], $P = 0.049$[d] **$P = 0.028$[c], $P = 0.012$[d]	
		Lip: squamous cell carcinoma 0/50*, 0/50, 0/50, 3/50**	*$P = 0.012$[a], $P = 0.002$[c], $P = 0.008$[d] **$P = 0.035$[c]	
		Skin Squamous cell carcinoma 0/50*, 5/50**, 3/50, 8/50***	*$P = 0.007$[a], $P < 0.001$[c] **$P = 0.028$[b], $P = 0.032$[c] ***$P = 0.003$[b], $P = 0.001$[c], $P = 0.039$[d]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 7–8 wk 104 wk NTP (1986) (cont.)		Squamous cell papilloma 0/50*, 2/50, 1/50, 5/50**	*$P = 0.014$[a], $P = 0.001$[c], $P = 0.002$[d] **$P = 0.028$[b], $P = 0.005$[c], $P = 0.009$[d]	
		Palate Squamous cell papilloma 0/50*, 4/50, 4/50, 9/50**	*$P = 0.002$[a], $P < 0.001$[c], $P = 0.005$[d] **$P = 0.001$[b], $P < 0.001$[c], $P = 0.006$[d]	
		Squamous cell carcinoma 0/50, 0/50, 1/50, 0/50	NS	
Full carcinogenicity Rat, F344/N (F) 7–8 wk 104 wk NTP (1986)	Gavage Benzene, > 99.7% Corn oil 0, 25, 50, 100 mg/kg bw 1×/d, 5 d/wk, 103 wk 50, 50, 50, 50 46, 38, 33, 25	*Zymbal gland*: carcinoma 0/45*, 5/40**, 5/44***, 14/46 (30.4%)****	*$P < 0.001$[a], $P < 0.001$[c], $P < 0.001$[d] **$P = 0.020$[b], $P = 0.022$[c], $P = 0.036$[d] ***$P = 0.026$[b], $P = 0.018$[c] ****$P < 0.001$[b], $P < 0.001$[c], $P < 0.001$[d]	Principal strengths: studies in male and female mice; multiple-dose study; covered most of the lifespan; complete histopathology; well-conducted GLP study Principal limitations: benzene-treated rats had lower body weights; mortality was higher in benzene-treated rats Dose-related lymphocytopenia was observed in benzene-treated rats; historical incidence of endometrial stromal polyps: 22/98 (22.4%) at laboratory; 248/1125 (22.0 ± 7%) (4/49–17/50) overall Statistical tests used: [a] Cochran–Armitage trend test; [b] Fisher exact test; [c] Life-table test; [d] Incidental tumour test
		Oral cavity Squamous cell carcinoma 0/50*, 1/50, 4/50, 5/50**	*$P = 0.011$[a], $P = 0.003$[c] **$P = 0.028$[b], $P = 0.010$[c]	
		Squamous cell papilloma 1/50*, 4/50, 8/50**, 5/50***	*NS[a], $P = 0.017$[c], $P = 0.047$[d] **$P = 0.015$[b], $P = 0.006$[c], $P = 0.022$[d] ***$P = 0.032$[c]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 7–8 wk 104 wk NTP (1986) (cont.)		*Tongue*: squamous cell carcinoma 0/50*, 0/50, 4/50**, 4/50***	*P = 0.014[a], P = 0.004[c] **P = 0.047[c] ***P = 0.024[c]	
		Uterus: endometrial stromal polyp 7/50* (14.0%), 7/50 (14.0%), 7/49 (14.3%), 14/50 (28.0%)**	*P = 0.032[a], P = 0.001[c], P = 0.049[d] **P = 0.003[c], P = 0.049[d]	
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk (breeders) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200–300 ppm 4–7 h/d, 5 d/wk, 104 wk 60, 54 NR	*Zymbal gland*: carcinoma 1/60 (1.7%), 3/54 (5.6%)	[NS]	Principal strengths: lifetime study; complete histopathology Principal limitations: breeders were aged 13 wk at the start of exposure Experiment BT4004, BT4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/60, 2/54 (3.7%)	[NS]	
		Forestomach: carcinoma in situ 0/60, 0/54	[NS]	
		Liver: hepatoma [hepatocellular carcinoma] 0/60, 1/54 (1.9%)	[NS]	
		Mammary gland: malignant tumours 2/60 (3.3%), 6/54 (11.1%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 2/60 (3.3%), 0/54	[NS]	
		All sites: malignant tumours 9/60 (15.0%), 14/54 (25.9%)	[NS]	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) Embryo (gestation day 12) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200, 200–300 ppm 4–7 h/d, 5 d/wk, 15 wk (200 ppm) or 104 wk (0 ppm or 200–300 ppm) 158, 70, 75 NR	*Zymbal gland*: carcinoma 2/158 (1.3%), 4/70 (5.7%), 6/75 (8.0%)*	*[$P = 0.015$] (Fisher exact test)	Principal strengths: lifetime study; multiple-dose study; studies in male and female mice; in utero exposure; complete histopathology Experiment BT4004, 4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/158, 2/70 (2.9%), 1/75 (1.3%)	[NS]	
		Forestomach: carcinoma in situ 0/158, 0/70, 0/75	[NS]	
		Liver: hepatoma [hepatocellular carcinoma] 1/158 (0.6%), 2/70 (2.9%), 2/75 (2.7%)	[NS]	
		Mammary gland: malignant tumours 3/158 (1.9%), 0/70, 0/75	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 12/158 (7.6%), 4/70 (5.7%), 6/75 (8.0%)	[NS]	
		All sites: malignant tumours 28/158 (17.7%), 20/70 (28.6%), 24/75 (32.0%)**	*[$P = 0.018$] (Fisher exact test)	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) Embryo (gestation day 12) Lifetime Maltoni et al. (1983, 1985, 1989)	Inhalation Benzene, > 99.9% Air 0, 200, 200–300 ppm 4–7 h/d, 5 d/wk, 15 wk (200 ppm) or 104 wk (0 ppm or 200–300 ppm) 149, 59, 65 NR	*Zymbal gland*: carcinoma 0/149, 1/59 (1.7%), 8/65 (12.3%)*	*[$P < 0.001$] (Fisher exact test)	Principal strengths: lifetime study; multiple-dose study; studies in male and female mice; in utero exposure; complete histopathology Experiment BT4004, 4006; benzene-treated rats presented lymphocytopenia
		Oral cavity: carcinoma 0/149, 6/59 (10.2%)*, 10/65 (15.4%)**	*[$P < 0.001$] **[$P < 0.001$] (Fisher exact test)	
		Forestomach: carcinoma in situ 0/149, 0/59, 3/65 (4.6%)*	*[$P = 0.027$] (Fisher exact test)	
		Liver: hepatoma [hepatocellular carcinoma] 0/149, 5/59 (8.5%)*, 7/65 (10.8%)**	*[$P = 0.002$] **[$P < 0.001$] (Fisher exact test)	
		Mammary gland: malignant tumours 8/149 (5.4%), 8/59 (13.6%), 9/65 (13.8%)	[NS]	
		Haematopoietic and lymphoid tissues: haemolymphoreticular neoplasia 1/149 (0.7%), 4/59 (6.8%)*, 0/65	*[$P = 0.024$] (Fisher exact test)	
		All sites: malignant tumours 26/149 (17.4%), 26/59 (44.1%)*, 38/65 (58.5%)**	*[$P < 0.001$] **[$P < 0.001$] (Fisher exact test)	

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; h, hour(s); M, male; NR, not reported; NS, not significant; ppm, parts per million; wk, week

tissues (lymphoma, leukaemia, or histiocytic sarcoma, combined) was observed: 0/30, 0/30, and 4/35 (11%) [P = 0.033, Cochran–Armitage trend test]. A significant dose-related increase in the incidence of carcinoma of the Zymbal gland was observed in treated female rats: 0/30, 2/30 (7%), and 8/35 (23%) [P = 0.006, Fisher exact test; P = 0.005, Cochran–Armitage trend test]. The overall incidence of malignant tumours in male rats was 2/30 (7%), 1/30 (3%), and 8/35 (23%) [P = 0.025, Cochran–Armitage trend test]. The overall incidence of malignant tumours in female rats was 6/30 (20%), 10/30 (33%), and 21/35 (66%) [P = 0.003, Cochrane–Armitage trend test]. [The Working Group noted the small number of animals and the start of the treatment from age 13 weeks, slightly later than the usual 7–8 weeks. This might partly explain why certain malignant tumours increased in incidence, in particular carcinoma of the oral cavity and carcinoma of the mammary gland, but these increases were not statistically significant. The Working Group noted that the principal strengths of the study were: lifespan was covered; both male and female rats were studied; multiple doses tested; and complete histopathology. The principal limitation was that mortality was higher in rats treated with benzene.]

Groups of 40–50 male and 40–50 female Sprague-Dawley rats (age, 7 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 104 weeks (Maltoni et al., 1982a, 1983, 1985; see also Maltoni et al., 1989) [experiment BT902]. The rats were kept under observation for their lifespan. Male and female rats treated with benzene had lower body weights and showed lymphocytopenia. A significant increase in the incidence of carcinoma of the Zymbal gland was observed in treated male rats: 1/50 (2.0%) versus 18/40 (45.0%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of carcinoma of the Zymbal gland was also observed in treated female rats: 0/50 versus 16/40 (40.0%) [P < 0.001, Fisher exact test]. A significantly increased incidence of squamous cell carcinoma of the oral cavity was observed in treated male rats: 0/50 versus 21/40 (53%) [P < 0.001, Fisher exact test]. The incidence of squamous cell carcinoma of the oral cavity was also significantly increased in treated female rats: 0/50 versus 20/40 (50.0%) [P < 0.001, Fisher exact test]. In treated female rats, a significant increase in the incidence of carcinoma in situ of the forestomach was reported: 0/50 versus 6/40 (15.0%) [P = 0.006, Fisher exact test]. The incidence of precancerous lesions of the forestomach (acanthomas and dysplasias) was significantly increased in treated male and female rats: 0/50 versus 10/40 (25.0%) [P < 0.001, Fisher exact test] and 0/50 versus 7/40 (17.5%) [P = 0.002, Fisher exact test]. In treated male rats, the incidence of carcinoma of the skin was significantly increased: 0/50 versus 9/40 (22.5%) [P < 0.001, Fisher exact test]. The incidence of a rare tumour (angiosarcoma of the liver) was non-significantly increased in both male and female rats: 0/50 versus 2/40 (5.0%) and 0/50 versus 3/40 (7.5%). The overall incidence of malignant tumours in male rats was 11/50 (22.0%) versus 36/40 (90.0%) [P < 0.001, Fisher exact test]. The overall incidence of malignant tumours in female rats was 10/50 (20.0%) versus 35/40 (87.5%) [P < 0.001, Fisher exact test]. [The Working Group noted that the principal strengths of the study included: lifespan covered; both male and female rats studied; and complete histopathology. The principal limitation was that rats treated with benzene had lower body weights.]

Groups of 40 male and 40 female Wistar rats (age, 7 weeks) were given benzene (purity, > 99.9%) at a dose of 0 (control) or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 104 weeks (Maltoni et al., 1988, 1989; see also Maltoni et al., 1985) [Experiment BT907]. Rats were kept under observation for their lifespan. Mortality was higher and body weights lower in male and female rats treated with benzene.

A significantly increased incidence of carcinoma of the Zymbal gland was reported in treated male and female rats: 0/40 versus 7/40 (17.5%) [$P = 0.012$, Fisher exact test] and 0/40 versus 6/40 (15.0%) [$P = 0.026$, Fisher exact test]. The overall incidence of malignant tumours in male and female rats was 8/40 (20.0%) versus 19/40 (47.5%) [$P = 0.017$, Fisher exact test] and 10/40 (25.0%) versus 21/40 (52.5%) [$P = 0.021$, Fischer exact test]. [The Working Group noted the principal strengths: the study covered the lifespan; both male and female rats were studied; and complete histopathology was reported. The principal limitations were the lower body weights and higher mortality in rats treated with benzene.]

In a GLP study, groups of 50 male F344/N rats (age, 7–8 weeks) were given benzene (purity, > 99.7%) at a dose of 0 (control), 50, 100, or 200 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 103 weeks. Groups of 50 female F344/N rats (age, 7 weeks) were given benzene (purity, > 99.7%) at a dose of 0 (control), 25, 50, or 100 mg/kg bw in corn oil by gavage once per day, 5 days per week, for 103 weeks (NTP, 1986; see also Maronpot, 1987; Huff et al., 1989). The rats were kept under observation for 104 weeks and then killed. Higher mortality, lower body weights, and dose-related lymphocytopenia were all observed in male and female rats treated with benzene. A significant increase in the incidence, and positive trend in the incidence, of carcinoma of the Zymbal gland was observed in treated male and female rats. A significant increase in the incidence, and positive trend in the incidence, of squamous cell carcinoma of the oral cavity was observed in treated male and female rats. A significant increase in the incidence, and positive trend in the incidence, of squamous cell papilloma of the oral cavity was observed in treated male rats. A significant increase in the incidence of squamous cell papilloma of the oral cavity was also reported in treated female rats. There was a significant increase in the incidence, and positive trend in the incidence, of squamous cell carcinoma of the lip in treated male rats. In male rats, a significant increase in the incidence of squamous cell carcinoma of the tongue was reported. In female rats, a significant positive trend in the incidence of squamous cell carcinoma of the tongue was reported. There was a significant increase in the incidence, and positive trend in the incidence, of squamous cell papilloma of the palate in treated male rats. In male rats, significant increases in the incidence, and positive trend in the incidence, of squamous cell carcinoma and of squamous cell papilloma of the skin were reported. In female rats, a significant positive trend in the incidence of stromal polyp of the endometrium was reported. [The Working Group noted that the principal strengths included: well-conducted GLP study; multiple-dose study; most of the lifespan covered; both male and female rats studied; and complete histopathology was reported. The principal limitations were the lower body weights and higher mortality in rats treated with benzene.]

3.2.2 Inhalation

One group of pregnant breeders (54 female Sprague-Dawley rats; age, 13 weeks) was exposed to benzene (purity, > 99.9%) at a concentration of 200 ppm by inhalation from day 12 of gestation for 4 hours per day, 5 days per week, for 7 weeks (Maltoni et al., 1983, 1985, 1989; see also Maltoni et al., 1982b, c) [Experiments BT4004, BT4006]. The embryos were exposed transplacentally by inhalation during the prenatal period, and possibly by ingestion (via lactation) during weaning. After weaning, a first group of offspring (70 males and 59 females) was exposed to benzene at 200 ppm by inhalation for 7 hours per day, 5 days per week for 8 weeks (total duration of exposure to benzene, 15 weeks). A second group of offspring (75 males and 65 females) and the breeders were exposed to benzene at 200 ppm by inhalation for 7 hours per day, 5 days per week, for 12 weeks, then exposed to benzene at

300 ppm for 7 hours per day, 5 days per week, for 85 weeks (total duration of exposure to benzene, 104 weeks). All groups of animals were located in inhalation chambers. The breeders control group (60 females) and the offspring control group (158 males and 149 females) were not exposed to benzene (untreated controls exposed to filtered air). The rats were then kept under observation for their lifespan. Mortality was higher in groups of male and female offspring treated with benzene that had lymphocytopenia. No significant increase in tumour incidence was found in the breeders. The overall incidence of malignant tumours in breeders was 9/60 (15.0%, control) versus 14/54 (25.9%). [Breeders were treated starting from age 13 weeks, slightly later than the usual 7–8 weeks. This might partly explain why malignant tumours increased in incidence, particularly carcinoma of the Zymbal gland, carcinoma of the oral cavity, and malignant tumours of the mammary gland, but none of these increases were statistically significant.] In the offspring, a significant increase in the incidence of carcinoma of the Zymbal gland was observed in treated male and female rats: 2/158 (1.3%) versus 4/70 (5.7%) and 6/75 (8.0%) [P = 0.015, Fisher exact test], and 0/149 versus 1/59 (1.7%) and 8/65 (12.3%) [P < 0.001, Fisher exact test], respectively. In female offspring, a significant increase in the incidence of carcinoma of the oral cavity was observed: 0/149 versus 6/59 (10.2%) [P < 0.001, Fisher exact test] and 10/65 (15.4%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of carcinoma in situ of the forestomach was observed in treated female offspring: 0/149 versus 0/59 and 3/65 (4.6%) [P = 0.027, Fisher exact test]. A significant increase in the incidence of liver hepatoma [hepatocellular carcinoma] was observed in treated female offspring: 0/149 versus 5/59 (8.5%) [P = 0.002, Fisher exact test] and 7/65 (10.8%) [P < 0.001, Fisher exact test]. A significant increase in the incidence of tumours of the haematopoietic and lymphoid tissues (haemolymphoreticular neoplasia) was observed in treated female offspring: 1/149 (0.7%) versus 4/59 (6.8%) [P = 0.024, Fisher exact test] and 0/65. The overall incidence of malignant tumours in male and female offspring was: 28/158 (17.7%) versus 20/70 (28.6%) and 24/75 (32.0%) [P = 0.018, Fisher exact test], and 26/149 (17.4%) versus 26/59 (44.1%) [P < 0.001, Fisher exact test] and 38/65 (58.5%) [P < 0.001, Fisher exact test]. [The Working Group noted the principal strengths of the study: multiple-dose study; lifespan was covered; both male and female rats studied; in utero exposure; and complete histopathology. The principal limitation was that breeders were aged 13 weeks at the start of exposure.]

3.3 Genetically modified animals

See Table 3.4

Genetically modified animals have been used for accelerated testing of chemicals for their carcinogenic potential under specific conditions of exposure (Tennant et al., 1995, 2001; French et al., 2001a). These models are focused primarily on heritable mutations in: (1) tumour-suppressor genes with loss of function (e.g. transformation protein 53 or *Trp53*, cyclin-dependent kinase 2a or *Cdnk2a* [splice variants produce either p16Ink4a or p19Arf proteins]) and/or (2) proto-oncogenes (e.g. *Hras* or *vHras*) with gain of function due to acquired mutations associated with cancer in humans and mouse models of human cancer. Susceptibility or predisposition to chemical carcinogenesis is based on acquired or heritable mutations in tumour-suppressor gene and/or proto-oncogene functional pathways, and other modifiers of cancer in genetically modified animal models that are consistent with known hallmarks of human cancer (Hanahan & Weinberg, 2000, 2011). Accelerated tests for carcinogenic potential have shown reliability and potential, but with limitations that must be carefully considered (Pritchard et al., 2003; Eastmond et al., 2013).

Table 3.4 Studies of carcinogenicity in genetically modified animals exposed to benzene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, C57BL/6 or h-Trx-Tg (NR) NR Lifetime Li et al. (2006)	Inhalation (whole-body exposure) Benzene, purity NR Air 0, 300, 0, 300 ppm 6 h/d, 5 d/wk, 26 wk 8, 10, 8, 13 NR	*Thymus*: lymphoma 0/8, 3/10, 0/8, 0/13	NS	Principal strengths: lifetime study Principal limitations: small numbers of animals used The h-Trx-Tg mouse overexpresses human thioredoxin; survival was higher in control mice than in benzene-treated mice
Carcinogenicity with other modifying factor Mouse, B6.CBA-*Trp53* ^{tm1Sia} (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR Clean air 0, 33, 100, 300 ppm 6 h/d, 5 d/wk 24, 27, 25, 26 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 0/24, 1/27, 4/25, 19/26* Non-thymic lymphoma 9/24, 10/27, 5/25, 2/26 Acute myelocytic leukaemia 0/24, 0/27, 0/25, 2/26	*$P < 0.05$ (Fisher exact test), [$P < 0.001$] (Cochran–Armitage trend test) NS (for an increase) NS	Principal strengths: lifetime study Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor; heterozygous wildtype and null allele (*Trp53* haploinsufficient) *Trp53* allelotype was used; moribund mice or mice presenting with masses or significant body weight loss were killed for gross and histopathological examination
Carcinogenicity with other modifying factor Mouse, C3.CBA-*Trp53*^{tm1Sia} (M) 8 wk Lifetime Kawasaki et al. (2009)	Inhalation (whole-body exposure) Benzene, purity NR Clean air 0, 100, 300 ppm 6 h/d, 5 d/wk 24, 24, 24 NR	*Haematopoietic and lymphoid tissues* Thymic lymphoma 1/24, 12/24*, 6/24* Non-thymic lymphoma 3/24, 6/24, 10/24* Acute myelocytic leukaemia 2/24, 2/24, 9/24*	*$P < 0.05$ (Fisher exact test), [$P = 0.001$] (Cochran–Armitage trend test) *$P < 0.05$ (Fisher exact test) *$P < 0.05$ (Fisher exact test), [$P = 0.016$] Cochran–Armitage trend test)	Principal strengths: lifetime study Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor; heterozygous wildtype and null allele (*Trp53* haploinsufficient) *Trp53* allelotype was used; moribund mice or mice presenting with masses or significant body weight loss were killed for gross and histopathological examination

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (M) 7–10 wk 26 wk French & Saulnier (2000), French et al. (2001b)	Gavage Benzene, purity NR Corn oil 0, 200 mg/kg bw, 5×/wk, 26 wk 20, 40 20, 39	*Subcutis*: sarcoma, NOS 0/20, 16/39* *Thymus*: lymphoma 0/20, 3/39 *Pancreas*: acinar cell carcinoma 0/20, 1/39 *Lung*: tumours 0/20, 0/22	*[$P \leq 0.004$] (Fisher exact test) LOH in 13/16 sarcomas tested NS LOH in 3/3 lymphomas tested NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); study terminated 1 d after exposure period
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (M) NR 26 wk Storer et al. (2001)	Gavage Benzene, purity NR Corn oil 0, 100 mg/kg bw 5–7×/wk 15 + 15, 15 + 15 NR	*Thymus* Lymphoma 0/30, 4/30 Atypical hyperplasia [preneoplastic lesion] 0/30, 7/30* *Subcutis*: sarcoma 1/30, 1/30 *Bone*: osteosarcoma 0/30, 1/30	[NS] *[$P \leq 0.01$] (Fisher exact test) NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); data (combined) extracted from use of benzene at a single dose as a positive control in two studies of another agent
Carcinogenicity with other modifying factor Mouse, B6.129-*Trp53*^{tm1Bra} N5 (F) NR 26 wk Storer et al. (2001)	Gavage Benzene, purity NR Corn oil 0, 100 mg/kg bw, 5–7×/wk 15 + 15, 15 + 15 NR	*Thymus* Lymphoma 1/30, 1/30 Atypical hyperplasia [preneoplastic lesion] 0/30, 2/30 *Subcutis*: sarcoma 1/30, 1/30 *Bone*: osteosarcoma 1/30, 0/30	NS NS NS NS	Genetically modified mouse model based on *Trp53* tumour-suppressor gene wildtype and null allele (*Trp53* haploinsufficiency) modifying factor (heterozygous); data (combined) extracted from use of benzene at a single dose as a positive control in two studies of another agent

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/ or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, B6.129-*Cdkn2a*^{tm1Dep} (M) 7–8 wk 27 wk NTP (2007)	Gavage Benzene, > 99% Corn oil 0, 25, 50, 100, 200 mg/kg bw, 5×/wk 15, 15, 15, 15, 15 15, 15, 15, 15, 14	*Multiple organs*: malignant lymphoma 0/15, 0/15, 0/15, 0/15, 5/15*	*P = 0.021 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	Genetically modified mouse model based on *Cdkn2a* tumour-suppressor gene wildtype and null allele (*Cdkn2a* haploinsufficiency) modifying factor (heterozygous); both *p16Ink4a* and *p19Arf* tumour-suppressor gene functions were haploinsufficient
		Bone marrow: atrophy 0/15, 0/15, 0/15, 10/15*, 12/15*	*P ≤ 0.01 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	
		Mesentery, lymph node: atrophy 1/15, 2/15, 2/14, 13/15*, 13/15*	*P ≤ 0.01 (Fisher exact test), [P < 0.001] Cochran–Armitage trend test)	
Carcinogenicity with other modifying factor Mouse, B6.129-*Cdkn2a*^{tm1Dep} (F) 7–8 wk 27 wk NTP (2007)	Gavage Benzene, > 99% Corn oil 0, 25, 50, 100, 200 mg/kg bw, 5×/wk 15, 15, 15, 15, 15 15, 15, 15, 15, 15	*Multiple organs*: malignant lymphoma 0/15, 0/15, 0/15, 0/15, 0/15	NS	Genetically modified mouse model based on *Cdkn2a* umour-suppressor gene wildtype and null allele (*Cdkn2a* haploinsufficiency) modifying factor (heterozygous); both *p16Ink4a* and *p19Arf* tumour-suppressor gene functions were haploinsufficient
		Mesentery, lymph node: atrophy 0/15, 2/15, 3/15, 8/15*, 6/15*	*P ≤ 0.01 (Fisher exact test), [P = 0.005] Cochran–Armitage trend test)	
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) 7 wk 20 wk Spalding et al. (1999)	Skin application Benzene, purity NR Acetone, neat 0, 400, 800, 1600 µL/wk 5, 10, 10, 10 4, 8, 8, 8	*Skin*: squamous cell papilloma 3/5, 7/10, 8/10, 10/10*	*P < 0.05 (life-table test)	FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (two copies or homozygous state); to achieve the weekly dosage regimen, benzene was applied 2 d/wk; skin papilloma incidence in control mice due to fighting
		Tumour multiplicity: 1.4 ± 1.7, 7.0 ± 10.3, 10.6 ± 8.5*, 12.6 ± 10.3*	*P < 0.05 (Mann–Whitney U-test)	
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (M) 8 wk 26 wk Holden et al. (1998)	Skin application Benzene, purity NR Acetone, neat 0, 100, 150 µL, 3×/wk for 20 wk 10, 10, 10 9, 8, 10	*Skin*: squamous cell papilloma 0/10, 0/10, 3/10	NS	FVB/N mouse carrying a v-Ha-*Ras* transgene insert (single-copy or hemizygous state); acetone control group treated daily

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/ or multiplicity of tumours	Significance	Comments
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) 8 wk 26 wk Holden et al. (1998)	Skin application Benzene, purity NR Acetone, neat 0, 100, 150 µL, 3×/wk, 20 wk 10, 10, 10 5, 9, 7	*Skin*: squamous cell papilloma 0/10, 1/10, 1/10	NS	FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (single-copy or hemizygous state); acetone control group treated daily
Carcinogenicity with other modifying factor Mouse, FVB/N-Tg.AC (v-Ha-*Ras*) (F) NR 32 wk French & Saulnier (2000)	Skin application Benzene, purity NR Acetone, neat 0, 450, 800 µL/wk, 20 wk NR	*Bone marrow*: leukaemia, granulocytic 0/19, 4/14*, 11/15*	*$P < 0.05$ (Fisher exact test)	Principal limitations: limited reporting FVB/N-Tg.AC (v-Ha-*Ras*) transgene insert (single copy or hemizygous state); acetone control group treated with 200 µL/d; low-dose group was given 150 µL benzene 3×/wk; high-dose group was given 200 µL benzene 2×/d, 2×/wk

bw, body weight; d, day(s); F, female; h, hour(s); LOH, loss of heterozygosity; M, male; NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s)

3.3.1. Inhalation

Groups of 8 and 10 C57BL/6 wildtype and 8 and 13 h-Trx-Tg mice (overexpressing human thioredoxin) [sex and age at start not reported] were sham exposed (controls) or exposed by whole-body inhalation to benzene at a dose of 300 ppm [purity not reported], 6 hours per day, 5 days per week, for 26 weeks. Mice were maintained for their lifetime (or killed when showing symptoms of advanced haematopoietic neoplasms) and examined histopathologically. Survival of control h-Trx-Tg mice was higher than survival of the three other groups (which was comparable). There was a non-significant numerical increase in the cumulative incidence of lymphoma of the thymus gland (30%, 3/10) in the wildtype group exposed to benzene, while no lymphomas of the thymus gland were observed in the h-Trx-Tg group exposed to benzene (0/13) or in either of the control groups (Li et al., 2006). [The Working Group noted that this was a lifetime study, but the number of animals was small.]

Male B6.CBA-*Trp53*^{tm1Sia} congenic inbred mice (age, 8 weeks) (backcrossed repeatedly to C57BL/6 or B6 to homozygosity) were exposed to benzene at a concentration of 0, 33, 100, or 300 ppm [purity not reported, chemical grade] by whole-body inhalation for 6 hours per day, 5 days per week, for 26 weeks, and observed over their lifetime for tumour development (Kawasaki et al., 2009). Male B6.CBA-*Trp53*^{tm1Sia} mice heterozygous for a null and wildtype *Trp53* allele (haploinsufficient) were observed with a significant increase, with a significant positive trend, in the incidence of lymphoma of the thymus gland. [The Working Group noted that this was a lifetime study.]

Male C3.CBA-*Trp53*^{tm1Sia} congenic mice (age, 8 weeks) (backcrossed repeatedly to C3/He or C3 to homozygosity) were exposed to benzene at a concentration of 0, 100, or 300 ppm [purity not reported, chemical grade] for 6 hours per day, 5 days per week, for 26 weeks, and observed over their lifetime for the development of tumours (Kawasaki et al., 2009). Male C3.CBA-Trp53tm1Sia congenic mice heterozygous for a null and wildtype *Trp53* allele (haploinsufficient) were observed with significant increases in the incidence, with significant positive trends, of thymic lymphoma and of myeloid leukaemia, and a significant increase in the incidence of non-thymic lymphoma. [The Working Group noted that this was a lifetime study.]

3.3.2 Oral administration

Male B6.129-*Trp53*^{tm1Bra} N5 (the fifth C57BL/6 backcross generation, 97% homozygous) congenic mice (age, 7–10 weeks), heterozygous for a null and wildtype *Trp53* allele (Donehower et al., 1992), were given benzene [purity not reported] at a dose of 0 (vehicle only; $n = 20$) or 200 mg/kg bw ($n = 40$) by gavage (vehicle, corn oil) for 5 days per week, for 26 weeks (French & Saulnier, 2000; French et al., 2001b). After 26 weeks of exposure, the *Trp53* haploinsufficient mice were observed with sarcoma (of the subcutis, around the head and neck region or thoracic cavity) (0/20, 16/39 [$P \leq 0.004$, Fisher exact test]), lymphoma of the thymus (0/20, 3/39), and acinar cell carcinoma of the pancreas (0/20, 1/39), but without tumours of the lung (0/20, 0/22). Loss of the residual *Trp53* wildtype allele was observed in tested sarcoma (subcutis) (13/16) and lymphoma of the thymus (3/3).

Benzene [purity not reported] was used as a positive control in the Alternatives to Carcinogenicity Testing project of the International Life Sciences Health and Environmental Sciences Institute in two studies. Male and female B6.129-*Trp53*^{tm1Bra} N5 congenic mice (age at start not reported), heterozygous for *Trp53* wildtype and null allele (Donehower et al., 1992), were given benzene at a dose of 0 or 100 mg/kg bw by gavage, for 5 or 7 days per week, for 26 weeks (Storer et al., 2001). There were 15 males and 15 females per group per study.

Male *Trp53* haploinsufficient mice exposed to benzene showed a non-significant increase in the incidence of lymphoma of the thymus, supported by a significant increase in the incidence of atypical hyperplasia of the thymus [preneoplastic lesion]. The incidence of lymphoma of the thymus was 0/30 and 4/30 (males) and 1/30 and 1/30 (females), and of atypical hyperplasia of the thymus 0/30 and 7/30 [$P \leq 0.01$] (males) and 0/30 and 2/30 (females), for control and exposed groups, respectively. [The Working Group noted the combination of data from two different studies with a similar design.]

The National Toxicology Program tested the B6.129-*Cdkn2a*^{tm1Dep} congenic heterozygous mouse for a null and wildtype *Cdkn2a* allele with reduced expression of both the p16Ink4a and transcript variant p19Arf tumour-suppressor proteins (Serrano et al., 1996). Male and female mice (age, 7–8 weeks) were exposed to benzene (purity, > 99%) at a dose of 0, 25, 50, 100, or 200 mg/kg bw by gavage for 5 days a week, for 27 weeks. Malignant lymphoma was observed in males (0/15, 0/15, 0/15, 0/15, 5/15; $P = 0.021$, Fisher exact test [$P < 0.001$, Cochran–Armitage trend test]), but not in females. In addition, male *Cdkn2a* haploinsufficient mice showed several preneoplastic lesions (including bone marrow, thymus, and lymph node atrophy) associated with dose-related benzene exposure. Significantly increased preneoplastic lesions in female mice were restricted to lymph node (mesenteric) atrophy (NTP, 2007).

3.3.3 Skin application

The application of mutagenic or non-mutagenic chemicals to the shaved dorsal skin of the FVB/N-Tg.AC(v-Ha-*Ras*) mouse (Tg.AC for short) can result in squamous cell papilloma of the skin, which can convert to malignant skin neoplasms due to the abrogation of the first step in the two-step process of initiation–promotion of tumorigenesis (Leder et al., 1990).

A quantity of 200 µL of neat benzene [purity not reported] was applied to the skin of a group of 10–15 female Tg.AC mice twice a week, resulting in the rapid development of papillomas of the skin in 5 weeks. After 20 weeks, 76.9% [10/13] of treated Tg.AC mice had an average of 7.4 papillomas per mouse. A control group of 10–15 mice served as vehicle control [results not reported] (Tennant et al., 1995). [The Working Group noted that no results were given for controls. This study was inadequate for the evaluation of the carcinogenicity of benzene.]

Application of neat benzene [purity not reported] to the shaved dorsal skin of female Tg.AC mice (age, 7 weeks) at a dose of 0 (acetone control group), 400, 800, or 1600 µL per week for 20 weeks resulted in a significant increase in the incidence of squamous cell papilloma of the skin (3/5, 7/10, 8/10, 10/10 ($P < 0.05$)) and a significant increase in tumour multiplicity (1.4 ± 1.7, 7.0 ± 10.3, 10.6 ± 8.5 ($P < 0.05$), 12.6 ± 10.3 ($P < 0.05$)) (Spalding et al., 1999).

Compared with the above skin application studies, at lower benzene [purity not reported] exposure levels of 0 (acetone control group), 100, or 150 µL applied three times a week for 20 weeks in hemizygous Tg.AC mice (age, 8 weeks), the incidences of papilloma of the skin were reduced (males, 0/10, 0/10, 3/10; females, 0/10, 1/10, 1/10) after 26 weeks (Holden et al., 1998).

Blanchard et al. (1998) applied neat benzene [purity not reported] to the shaved dorsal skin of Tg.AC male and female mice (age at start not reported) in both hemizygous (single transgene copy) and homozygous (two copies of transgene) states at a dose of 0 (acetone control group) or 200 µL three times per week, for 20 weeks. A significant difference [$P < 0.05$] in the incidence of papilloma of the skin between hemizygous (males, 3/10; females, 4/10) and homozygous (males, 10/10; females, 9/10) Tg.AC mice was reported. [The Working Group noted that no results were given for controls. This study was inadequate for the evaluation of the carcinogenicity of benzene.]

Benzene [purity not reported] was applied neat to the shaved dorsal skin of homozygous Tg.AC female mice [age at start not reported] at a dose of 0 (acetone control group), 450, or 800 µL once per week, for 20 weeks. After an additional observation period of 12 weeks, the incidences of bone marrow leukaemia (granulocytic) (0/19, 4/14 ($P < 0.05$, Fisher exact test), 15/15 ($P < 0.05$, Fisher exact test)) were significantly increased (French & Saulnier, 2000). [The Working Group noted the limited reporting of the study.]

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4. MECHANISTIC AND OTHER RELEVANT DATA

4.1 Toxicokinetic data

Benzene is extensively metabolized, and the fate of benzene in the body is informed by measurements of various metabolites and of benzene itself. The section on metabolism, metabolic activation, and electrophilicity (Section 4.1.1) therefore precedes the discussion of absorption, distribution, and elimination (Section 4.1.2).

4.1.1 Metabolism, metabolic activation, and electrophilicity

The metabolism of benzene is complex (Ross, 1996; Snyder & Hedli, 1996), as summarized in Fig. 4.1. Qualitatively, the same metabolites are excreted by humans after occupational or environmental exposures and in animals exposed to benzene (Inoue et al., 1988, 1989; Sabourin et al., 1988, 1992; Henderson et al., 1989; Boogaard & van Sittert, 1996). Accordingly, the discussion below integrates findings from humans and from experimental systems in providing a synthetic overview of the metabolism and activation of benzene.

Metabolism of benzene is required for benzene toxicity. Studies in experimental systems using both pharmacological tools and genetically modified animals indicate that benzene requires metabolism to generate reactive electrophilic intermediates and subsequent toxicity (Snyder & Hedli, 1996; Ross, 2000). The first step of benzene metabolism is primarily mediated by cytochrome P4502E1 to form benzene oxide (Johansson & Ingelman-Sundberg, 1988; Koop et al., 1989; Snyder & Hedli, 1996), although other forms of cytochrome P450 may also play a role (Gut et al., 1996a; Powley & Carlson, 2001; Sheets et al., 2004). In CYP2E1-knockout mice, urinary benzene metabolites were reduced by approximately 90% with a concomitant complete lack of benzene-induced genotoxicity and cytotoxicity in bone marrow, blood, and lymphoid tissues (Valentine et al., 1996). Inhibition of CYP2E1-mediated metabolism reduced benzene-induced genotoxicity in mice (Tuo et al., 1996). Co-administration of toluene, a competitive inhibitor of benzene metabolism, reduced both benzene metabolism and benzene toxicity (Andrews et al., 1977). In agreement with rodent data that indicated a critical role for metabolism in toxicity, occupationally exposed individuals were more susceptible to benzene toxicity if they had a phenotype corresponding to rapid CYP2E1 metabolism (Rothman et al., 1997).

The sites of benzene metabolism have also been investigated. Benzene is metabolized in both liver and lung by CYP450 (Chaney & Carlson, 1995; Powley & Carlson, 2000). Partial hepatectomy reduced both benzene metabolism and benzene toxicity in rats (Sammett et al., 1979), indicating that the liver may play a primary role in benzene metabolism. Some metabolites distribute to bone marrow from

hepatic or other sites of generation, and metabolism to electrophiles can also occur in situ in bone marrow (Ross et al., 1996a). In particular, secondary metabolism of phenolic metabolites of benzene via myeloperoxidase (MPO) occurs in bone marrow to generate semiquinone radicals and quinones, providing one potential metabolic mechanism of benzene toxicity (Ross et al., 1996a; Smith, 1999). Metabolism of benzene in situ in rat bone marrow may also occur, and CYP450 has been detected at low levels in bone marrow (Gollmer et al., 1984; Schnier et al., 1989).

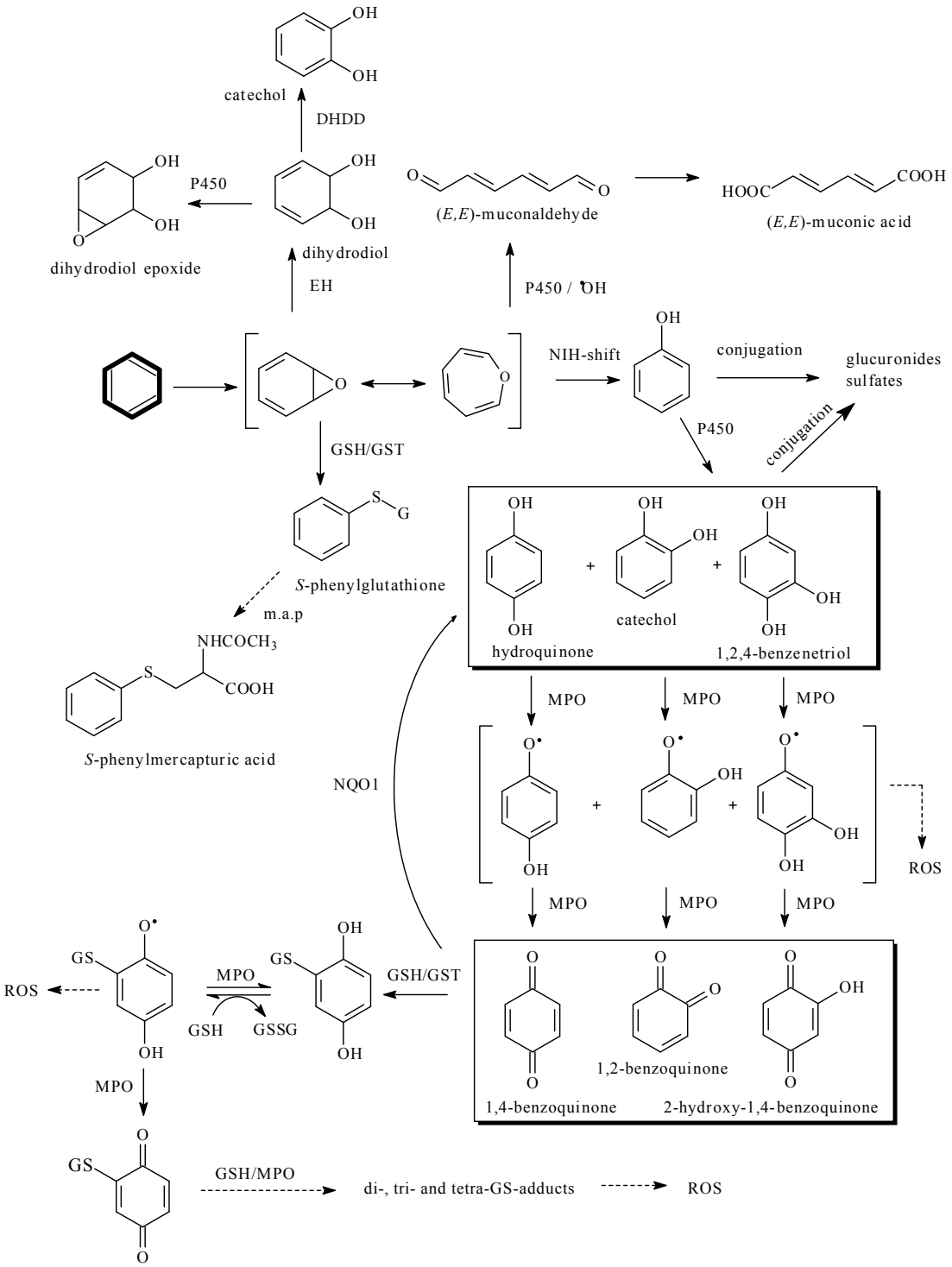
The main urinary metabolites of benzene in humans are phenol, hydroquinone, and catechol (the sum of free plus conjugated), *trans*,*trans*-muconic acid (t,t-MA), and *S*-phenylmercapturic acid (SPMA) (Inoue et al., 1988, 1989; Boogaard & van Sittert, 1996). t,t-MA and SPMA have been commonly used as biomarkers of benzene exposure in occupational and environmental studies in humans (Inoue et al., 1989; Boogaard & van Sittert, 1996). However, several metabolites have only been observed in experimental animals and/or in vitro. Benzene oxide formed by initial metabolic oxidation exists in equilibrium with its tautomer, oxepin. It can rearrange to generate phenol or undergo ring opening mediated by microsomal epoxide hydrolase to form benzene dihydrodiol; benzene dihydrodiol can spontaneously aromatize, releasing a water molecule to give phenol, and can be further oxidized to catechol and/or to a diol epoxide. Phenol can be further oxidized to catechol, hydroquinone, and benzene-1,2,4-triol. Oxidation of these phenols by MPO in bone marrow and bone marrow progenitor cells (Schattenberg et al., 1994) leads to the formation of semiquinone radicals and electrophilic benzoquinones (Gut et al., 1996b; Smith, 1996). Conjugation with glutathione (GSH) also plays an important role in the metabolism of benzene. Reaction of benzene oxide with GSH leads to SPMA.

In parallel, benzene oxide or oxepin and/or benzene dihydrodiol can undergo ring opening reactions to form *trans*,*trans*-muconaldehyde, which is further oxidized to t,t-MA. In studies using both animal and human cells in vitro, quinones generated from polyphenolic metabolites of benzene could be detoxified by NAD(P)H quinone oxidoreductase 1 (NQO1), maintaining them in their hydroquinone forms (Moran et al., 1999). Extensive glucuronidation and sulfation of phenols have been reported in animals and humans (Parke & Williams, 1953a, b; Seaton et al., 1995). GSH is also conjugated with electrophilic quinones (1,2-, 1,4-benzoquinone) leading to the corresponding *S*-(dihydroxyphenyl)glutathione (only one isomer, *S*-(2,5-dihydroxyphenyl) glutathione, is depicted in Fig. 4.1). The multiplicity of electrophilic metabolites formed during benzene metabolism, discussed in the sections that follow, are capable of reaction with GSH, which may occur chemically or be catalysed by glutathione-*S*-transferases (GSTs) (Snyder & Hedli, 1996). Benzene metabolism generates a variety of different reactive electrophiles. Several products of benzene metabolism are electrophilic and can interfere with cellular function.

(a) Epoxides

Benzene oxide is an electrophilic metabolite, identified in vitro when benzene is oxidized by human and mouse microsomes (Lovern et al., 1997) that can spontaneously rearrange to phenol by NIH shift (Jerina & Daly, 1974). It can also form adducts with GSH, cysteine residues in proteins, and DNA (Bechtold et al., 1992a; McDonald et al., 1994; Henderson et al., 2005a; Míčová & Linhart, 2012). The reaction of benzene oxide with soluble thiols such as GSH and N-acetylcysteine, followed by dehydration, leads to the formation of *S*-phenylglutathione and SPMA, respectively, although this reaction is relatively inefficient relative to ring opening in the generation of phenol (Henderson et al., 2005a; Míčová & Linhart, 2012). Benzene oxide is a substrate for GSTs (Zarth et al., 2015) and has sufficient stability in blood (a half-life of

Fig. 4.1 Simplified metabolic scheme of benzene



Compiled by the Working Group from Glatt et al. (1989), Ross (2000), Snyder (2004), Waidyanatha et al. (2005), and Monks et al. (2010).
DHDD, dihydrodiol-dehydrogenase; EH, epoxide hydrolase; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione-*S*-transferase; m.a.p., mercapturic acid pathway; MPO, myeloperoxidase; NQO1, NAD(P)H:quinone oxidoreductase 1; P450, cytochrome P450; ROS, reactive oxygen species.

~ 8 minutes) to reach extrahepatic target sites, as indicated by the production of benzene oxide-protein adducts as biomarkers in animals (McDonald et al., 1994). Benzene oxide-protein adducts have been found in the blood and bone marrow of mice and rats exposed to benzene (McDonald et al., 1994; Rappaport et al., 1996), and benzene oxide-haemoglobin and albumin adducts have been detected in the blood of workers exposed to benzene (Yeowell-O'Connell et al., 1998, 2001; Rappaport et al., 2002; Lin et al., 2007). Benzene oxide can form 7-phenylguanine and other DNA adducts, although its reactivity has been reported to be low relative to its reactions with thiols (Míčová & Linhart, 2012). Recent work failed to detect 7-phenylguanine in DNA from liver, lung, or bone marrow in mice exposed to benzene (Zarth et al., 2014). Benzene dihydrodiol epoxide is another putative electrophilic metabolite. Its half-life at nearly physiological conditions (pH, 7.6) was greater than 5 hours (Waidyanatha & Rappaport, 2005), suggesting it can be distributed to target tissues distal from the initial site of generation.

(b) Muconaldehyde and other ring-opened products

Ring opening of benzene resulting in t,t-MA as a metabolic end-product (Parke & Williams, 1952), commonly used as a biomarker of benzene exposure (Carbonari et al., 2016; Section 1.3.1, occurs in vivo. In vitro or animal studies have identified potential metabolic intermediates in the production of t,t-MA. Latriano et al. (1986) identified muconaldehyde (t,t-MA dialdehyde) in mouse liver microsomes after incubation with benzene. Potential mechanisms of formation include ring opening of benzene oxide or oxepin by cytochrome P450 (CYP) or reactions mediated by oxygen radicals (Zhang et al., 1995; Golding et al., 2010). Muconaldehyde is a bifunctional aldehyde and a reactive electrophilic compound (Latriano et al., 1986; Witz et al., 1996) that reacts with thiols and nucleic acids (Latriano et al., 1989; Bleasdale et al., 1993; Henderson et al., 2005b; Monks et al., 2010; Harris et al., 2011). In studies in vitro, each aldehyde functionality in muconaldehyde can be reduced or oxidized to generate alcohol and/or aldehyde (6-hydroxy-hexa-2,4-dienoic acid) or acid and/or aldehyde (6-oxohexa-2,4-dienoic acid, muconic acid semi-aldehyde) derivatives that retain some of the electrophilicity of the parent dialdehyde but have greater diffusibility (Goon et al., 1992; Witz et al., 1996).

(c) Quinones and semiquinones derived from phenolic metabolites of benzene

The phenolic metabolites, major metabolites of benzene, have been shown to reach the bone marrow of mice and rats in free or conjugated forms (Rickert et al., 1979; Sabourin et al., 1988). In isolated mouse and human bone marrow cells, bi- or triphenolic metabolites could be oxidized to quinones via MPO-mediated reactions to form benzoquinones (Smith et al., 1989; Ross, 1996). Phenol can be metabolized by purified human MPO and horseradish peroxidase to generate 4–4′-diphenoquinone (Eastmond et al., 1986). Quinones are electrophilic compounds that can interact with thiols, proteins, and nucleic acids (Sadler et al., 1988; McDonald et al., 1994; Bodell et al., 1996; Monks et al., 2010). Both 1,2- and 1,4-benzoquinone protein adducts have been found in the blood and bone marrow of mice and rats exposed to benzene (McDonald et al., 1994), and 1,4-benzoquinone protein adducts have been detected in workers exposed to benzene (Rappaport et al., 2002; Lin et al., 2007). The importance of quinones in humans exposed to benzene was suggested by the observation that individuals carrying a homozygous null polymorphism for the quinone-metabolizing enzyme NQO1, and therefore lacking the ability to detoxify electrophilic quinones, were more susceptible to benzene toxicity (Rothman et al., 1997). MPO-catalysed oxidation of bi- or triphenolic metabolites of benzene occurs via

semiquinone radical intermediates, which may disproportionate to generate starting compounds together with their respective benzoquinones, or may react with oxygen to generate reactive oxygen species (ROS) (Sawada et al., 1975; Kalyanaraman et al., 1985, 1988; Sadler et al., 1988; Smith et al., 1989; Smith, 1996; Bolton et al., 2000). Hydroquinone induces chromosomal damage in human lymphocytes in vitro (Eastmond et al., 1994; Stillman et al., 1997). Oxidative damage to DNA in human leukaemia HL-60 cells was induced by hydroquinone, phenol, and benzene triol (Kolachana et al., 1993). Benzene triol was the only phenolic metabolite which resulted in oxidative DNA damage in mice when administered alone; however, combinations of phenol and hydroquinone, phenol and catechol, and hydroquinone and catechol were also effective (Kolachana et al., 1993). In vitro treatment of mouse bone marrow with hydroquinone produced the same DNA adducts as found after treatment of mice with benzene (Bodell et al., 1996).

GSH conjugation of quinones is considered a detoxification reaction, and multiple studies of null polymorphisms in GST-T1 and GST-M1 genes resulting in increased benzene toxicity in exposed human populations suggest that GSTs play an important role in the detoxification of reactive benzene metabolites (Wan et al., 2002). However, GST adducts of 1,4-benzoquinone are haematotoxic and have been demonstrated in the bone marrow of mice after administration of benzene (Bratton et al., 1997). GST conjugation of 1,4-benzoquinone primarily generates 2-(*S*-glutathionyl)hydroquinone, which can undergo sequential oxidation and GST conjugation to produce 2,3,5,6-tetra(*S*-glutathionyl) hydroquinone as the final diphenolic metabolite. This process occurs via the production of both semiquinone and electrophilic quinone derivatives, which can generate oxidative stress and adducts, respectively (Lau et al., 1988, 2010).

Combined exposure to phenol and hydroquinone reproduced the myelotoxicity of benzene in mice (Eastmond et al., 1987; Legathe et al., 1994) and rats (Lau et al., 1988, 2010). Experiments in vitro showed that phenol increased the oxidation of hydroquinone, catalysed by horseradish peroxidase, as well as the binding of radiolabelled hydroquinone to rat liver protein (Eastmond et al., 1987).

In summary, benzene metabolism can generate a multiplicity of metabolites, many of which are electrophilic.

4.1.2 Absorption, distribution, and elimination

(a) Humans

Benzene is well absorbed in humans by inhalation, or by the oral or dermal routes. Inhalation is the major route of human exposure, and is the only route for which extensive human data are available. In experiments on human subjects, values of respiratory uptake (lung retention) of 47–52% were found at exposure levels ranging from 1.6 to 62.0 ppm (Nomiyama & Nomiyama, 1974; Pekari et al., 1992). For absorption of benzene at 32–69 ppm from smoking cigarettes, a higher uptake of 64% was reported (Yu & Weisel, 1996).

Skin absorption of benzene has also been studied experimentally on human subjects. The absorption rate of liquid benzene by the skin (under conditions of complete saturation) was calculated to be approximately 0.4 mg/cm^2 per hour. The absorption rate was determined by the amount of urinary phenol excreted, which was not corrected for urinary phenol not derived from benzene (Hanke et al., 1961). In a series of showering experiments using water contaminated with benzene (367 µg/m^3) it was estimated that the total benzene dose resulting from a 20-minute shower was approximately 281 µg, about 40% of which was a result of inhalation

and the remaining 60% via skin absorption (Lindstrom et al., 1994).

Although experimental studies on oral absorption of benzene in humans are not available, case studies of accidental or intentional poisoning indicate that benzene is also absorbed by the oral route (Thienes & Haley, 1972).

After absorption, benzene is rapidly distributed throughout the human body (Winek et al., 1967; Winek & Collom, 1971; Pekari et al., 1992). Concentrations of benzene at 3.8 mg/L in blood, 13.8 mg/kg in the brain, and 2.6 mg/kg in the liver were reported in a young male worker who died suddenly from a short exposure to very high air concentrations of the chemical (Tauber, 1970).

Unmetabolized benzene is primarily excreted in exhaled air, but small amounts were found also in urine (Nomiyama & Nomiyama, 1974). In contrast, the main portion of the absorbed dose is excreted in the form of water-soluble metabolites in the urine. Human exposure to benzene in air at concentrations of 0.1–10.0 ppm results in urinary metabolite profiles of 70–85% phenol (free + conjugated), 5–10% each of hydroquinone (free + conjugated), catechol (free + conjugated), and t,t-MA, and less than 1% of SPMA (Kim et al., 2006a).

The profile of urinary metabolites may change depending on the level of exposure. When comparing workers occupationally exposed to benzene at more than 25 ppm with those exposed to benzene at less than 25 ppm, Rothman et al. (1998) observed that the ratios of phenol and catechol to total metabolites were significantly higher and the ratios of hydroquinone and t,t-MA to total metabolites were significantly lower in workers exposed to higher concentrations. Workers with less than 50 ng/g creatinine phenol in urine, corresponding to a concentration of benzene in air of approximately 10 ppm, were excluded from this analysis because phenol is not specific to exposure to benzene at lower concentrations. The differences in the ratio of each metabolite to total metabolites between the groups of higher and lower exposure were 0.65 versus 0.61 for phenol, 0.11 versus 0.09 for catechol, 0.13 versus 0.16 for hydroquinone, and 0.11 versus 0.15 for muconic acid. A controlled study of 4 cases exposed to isotopically labelled benzene at 40 ppb resulted in a urinary muconic acid concentration of 3.2–45.0% (Weisel et al., 2003).

Multiple studies have analysed and reanalysed data first reported in Kim et al. (2006a) to determine if, at lower levels of benzene exposure, a nonlinear relationship exists between exposure to benzene and urinary excretion of unmetabolized benzene, phenol, hydroquinone, catechol, muconic acid, and phenylmercapturic acid. The original data were from 389 workers in Tianjin, China, 250 of whom were from factories using benzene and 139 from factories not using benzene (Kim et al., 2006a, b, 2007). In their original analysis, Kim et al. (2006a, b) reported nonlinear relationships between benzene exposure and urinary metabolite concentrations, adjusted for background levels based on controls who had been exposed to benzene at less than 3 ppb. Specifically, the ratio of excreted metabolites to benzene exposure increased markedly at exposures to benzene at less than 1 ppm. The presence or absence of nonlinearity for exposures at less than 1 ppm in these data has been the subject of multiple commentaries and reanalyses (Price et al., 2012; Rappaport et al., 2013a, b; Cox et al., 2017; McNally et al., 2017).

[The Working Group noted that, overall, there are some data suggesting increased metabolism at low exposures, but the data are not definitive.]

(b) Experimental systems

Animal data confirm that benzene is efficiently absorbed by inhalation and by oral and dermal routes. Studies in rats and mice showed that gastrointestinal absorption was greater than 97% in both species when the animals were given benzene by gavage (in corn oil) at doses of 0.5–150 mg/kg body weight (bw) per

day (Sabourin et al., 1987). Low-temperature whole-body autoradiography of ^{14}C-benzene showed that benzene is rapidly distributed in the blood and in well-perfused organs such as the heart muscle, liver, and kidney. A very high level of radioactivity was also observed in the bone marrow, body fat, spinal cord, and white matter of the brain. The radioactivity was rapidly cleared from the central nervous system (Bergman & Appelgren, 1983). The bioavailability of benzene through the oral route was influenced by adsorption on soil. When radiolabelled benzene in soil (clay soil or sandy soil from New Jersey) was administered to rats by gavage, the area under the curve of plasma radioactivity versus time increased compared with that for benzene suspension in water, a difference that was significant with clay soil. The half-life in plasma was not affected by the type of soil used (Turkall et al., 1988). After exposing mice repeatedly to benzene, DNA adducts were detected by ^{32}P-postlabelling in peripheral blood, bone marrow, and liver. The adducts were still detectable in leukocytes 21 days after the last exposure (Li et al., 1996).

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), discussing whether: benzene induces oxidative stress; is genotoxic; alters DNA repair or causes genomic instability; is immunosuppressive; alters cell proliferation, cell death, or nutrient supply; modulates receptor-mediated effects; induces chronic inflammation; and induces epigenetic effects.

In the consideration of mechanistic studies in exposed humans, the Working Group focused on studies in which the following issues were reasonably addressed in their design and/or analysis: definition and comparability of control groups, statistical power, and confounding by relevant covariates or co-exposures. An additional issue was whether bias due to disease (i.e. a diagnosis of benzene poisoning) was avoided.

4.2.1 Oxidative stress

Several potential mechanisms may contribute to benzene-induced oxidative stress. Benzene can produce a multiplicity of electrophilic and pro-oxidant metabolites capable of depleting cellular-reduced GSH (see Section 4.1), a critical defence system against oxygen radicals. Hydroquinones can generally autoxidize in pH-dependent reactions to produce hydrogen peroxide (Song & Buettner, 2010). Polyphenolic metabolites of benzene can generate semiquinone radicals during peroxidase-mediated oxidation (Yamazaki et al., 1960; Yamazaki & Piette, 1963; Kalyanaraman et al., 1991). The primary fate of 1,2- and 1,4-benzosemiquinone radicals is disproportionation to quinone and hydroquinone, although reaction with oxygen can occur in the presence of superoxide dismutase (Sawada et al., 1975; Sadler et al., 1988; Subrahmanyam et al., 1991). The semiquinone derived from 1,2,4-benzenetriol has been shown to react with oxygen (Kalyanaraman et al., 1988), and phenoxy radicals generated during peroxidase-mediated oxidation of phenol can react with GST leading to the generation of thiyl radicals and subsequent production of oxidized GST (Subrahmanyam & O'Brien, 1985; Sadler et al., 1988). Multiglutathione adducts of 1,4-benzoquinone generated by successive cycles of hydroquinone oxidation and glutathione addition retain the capability to generate active oxygen species and oxidative stress (Lau et al., 1988, 2010). Metals such as copper (II) and iron (III) may facilitate the production of reactive and oxidizing species, capable of damaging DNA and inducing lipid peroxidation, from hydroquinone and catechol (Kasai & Nishimura, 1984; Rao & Pandya, 1989; Li et al., 1995).

(a) Humans

Oxidative damage to DNA as indicated by 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels has been commonly used as an indicator of oxidative stress. Examples of studies indicating oxidative damage to DNA in occupationally exposed workers or in environmentally exposed urban populations, where DNA damage was correlated with benzene exposure levels and/or metabolic biomarkers of benzene exposure, are listed in Table 4.1. Four studies are further discussed in Section 4.2.3.

Decreases in GST levels, decreased superoxide dismutase, increased lipid peroxidation, and increased ROS in the blood were detected in 428 gasoline filling station workers compared with 78 unexposed controls (Uzma et al., 2010). The mean benzene exposure over the 12-hour study period in gasoline station attendants was 0.35 ppm (0.12–0.53 ppm). Individual exposure via air sampling was not monitored in controls, but the concentration of benzene in both pre- and post-shift urine and blood in gasoline station attendants was significantly higher than in controls. Both blood and urine benzene concentrations were increased in post-shift samples compared with pre-shift values. Significant correlations were observed between concentrations of benzene in the blood and changes in GST, superoxide dismutase, lipid peroxidation, and ROS (Uzma et al., 2010).

Exposure to benzene in 43 gasoline station attendants significantly increased DNA damage compared with 28 non-exposed individuals, as indicated by comet assay and micronuclei (MN) induction, increased oxidative protein damage, and decreased antioxidant capacity, including decreased GST levels (Moro et al., 2013; see also Table 4.2). Gasoline station attendants were exposed to median benzene values of 76.2 μg/m^3 compared with 42.0 μg/m^3 in controls, and median levels of t,t-MA were increased 4.4-fold in gasoline station attendants compared with the control group (Moro et al., 2013). Personal benzene exposure and urinary muconic acid levels were directly correlated with increases in oxidative protein damage and decreases in antioxidant capacity (Moro et al., 2013).

Two cross-sectional studies evaluated the relationship between benzene exposure and mitochondrial DNA (mtDNA) copy number. Compared with controls without occupational exposure to benzene, one study reported higher mtDNA levels in leukocyte DNA from highly exposed (arithmetic mean, 14 ppm) workers in China (Shen et al., 2008). A second study reported that benzene was associated with increased mtDNA among workers exposed to relatively low levels of benzene in Italy (geometric mean, 21.5 ppb among a group exposed to the highest concentrations) (Carugno et al., 2012).

(b) Human cells in vitro

Benzene metabolites hydroquinone, benzenetriol, and benzoquinone produced ROS in HL-60 cells and enhanced myeloid cell growth (Wiemels & Smith, 1999). A global proteomic analysis in cells after the addition of benzene revealed an enrichment of proteins involved in oxidative stress response (Murugesan et al., 2013). Oxidative DNA damage, indicated by the presence of 8-OHdG, has been detected in several studies of human cells in vitro after treatment with benzene metabolites; these are summarized in Section 4.2.2. Both GST and NQO1, which maintain reactive quinones in their reduced form and can also function as a superoxide reductase (Siegel et al., 2004), are important determinants of hydroquinone-induced toxicity in bone marrow cells (Ross et al., 1996a, b; Trush et al., 1996). The addition of benzene to human myeloid cells led to the production of ROS, an effect suggested to be due to benzene alone (Nishikawa et al., 2011).

Table 4.1 Oxidative damage to DNA in humans exposed to benzene

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
(a) Occupational studies								
8-OHdG Positive Significant correlation $P < 0.01$ between benzene exposure and 8-OHdG (r, 0.34)	65 filling station attendants	Average annual concentration used as a measure of exposure: 0.06–5.85 mg/m^3 (AM ± SD, 0.45 ± 0.96 mg/m^3)	Estimated on the basis of seven repeated personal air samples taken at worksite over 1 yr and questionnaire on personal habits	Not used	Urine	Methyl-benzenes	Increased 8-OHdG with increasing benzene exposure; not related to toluene or xylenes	Lagorio et al. (1994)
8-OHdG Positive $P < 0.05$ in medium- and high-dose groups vs controls; not significant in low-dose group	Three exposed groups and controls: low exposure (35 shoe factory workers); medium exposure (24 paint workers in car factory); high exposure (28 shoe factory workers) Controls: 30 university staff	Mean ± SD: low (n = 35), 2.5 ± 2.4 mg/m^3; medium (n = 24), 103.3 ± 50.3 mg/m^3; high (n = 28), 424.4 ± 181.7 mg/m^3	Personal air sampler	Urinary t,t-MA	Blood lymphocytes	Toluene	8-OHdG significantly correlated with both external and internal measures of benzene; dose–response; higher in women than men exposed to same benzene level; negative correlation of 8-OHdG with toluene levels	Liu et al. (1996)
8-OHdG Positive $P < 0.015$ for coefficient different from 0; adjusted for smoking	Total of 33 men occupationally exposed to gasoline (16 auto mechanics, 14 refinery workers, and 3 gasoline pump repairmen) and 33 male non-occupationally exposed controls	Mean 8-h TWA in workers, 0.13 ppm (range, 0.003–0.6 ppm) Mean exposure of controls 0.002 ppm (range, 0.001–0.003 ppm)	Personal air sampling	Not used	Urine	NR	Late evening/next morning 8-OHdG significant ($P < 0.002$ and $P < 0.02$) relative to pre-shift; no control samples analysed late evening/next morning	Nilsson et al. (1996)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
Urinary markers of nucleic acid oxidation Positive $P < 0.0001$	239 traffic policemen, taxi drivers, and gasoline pump attendants in Parma, Italy		Urine	Urinary t,t-MA, SPMA	Urine	Cotinine	Significant correlation between urinary metabolites of benzene (t,t-MA and SPMA) and DNA and RNA oxidation products	Manini et al. (2010)
8-OHdG Positive $P < 0.001$	31 gasoline service station attendants, 31 petrochemical laboratory workers, and 40 temple workers exposed to incense; controls from an office site with no incense burning in Bangkok, Thailand	Individual benzene exposure (mean ± SE): gasoline service station attendants, 360.9 ± 44.7 μg/m^3; petrochemical laboratory workers, 78.3 ± 18.7 μg/m^3; controls, 4.5 ± 0.5 μg/m^3	Area and personal air sampling	Not used	Leukocytes	1,3-butadiene, PAHs	For occupational exposures, gasoline service station attendants had significantly higher 8-OHdG ($P < 0.001$) than controls; 8-OHdG responding to increasing concentrations of benzene exposure; low to non-detectable 1,3-butadiene and PAHs in gasoline service station attendants and petrochemical laboratory workers	Ruchirawat et al. (2010)
8-OHdG Positive $P < 0.05$	43 gas station attendants, 34 taxi drivers, and 22 controls with no occupational exposure, Rio Grande do Sul, Brazil	NR	NR	Urinary t,t-MA	Urine	Carboxy-haemoglobin	Increased 8-OHdG ($P < 0.05$) in gas station attendants and taxi drivers relative to controls; t,t-MA not measured in taxi drivers	Göethel et al. (2014)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
8-OHdG Negative	18 fuel tanker drivers, 13 filling station attendants, and 20 non-occupationally exposed controls in Bari, Italy	Benzene exposure, mean ± SD: drivers, 279.9 ± 248.6 μg/m³; attendants, 19.9 ± 15.5 μg/m³; controls, 4.7 ± 3.0 μg/m³	Personal air sampling	Urinary t,t-MA, SPMA, benzene	Lymphocytes	NR	No increase in 8-OHdG in benzene-exposed groups relative to controls	Lovreglio et al. (2016)
(b) Exposed urban populations								
8-OHdG Positive (see comments)	40 individuals living and working in Copenhagen	Median (range) benzene exposure, 2.5 (1.9–3.6) μg/m³	Personal air sampling	Urinary t,t-MA, SPMA	Lymphocytes and urine	Toluene, MTBE	Significant correlation between 8-OHdG in lymphocytes (but not urine) and SPMA excretion ($P < 0.04$); no correlation of 8-OHdG with external benzene, toluene or MTBE levels; external benzene was a 5-d cumulative measure while internal markers were measured for only 1 of those days	Sørensen et al. (2003)
8-OHdG Positive (see comments)	Taxi-moto drivers, city residents, and village residents in Cotonou, Benin	Personal benzene exposure, mean ± SD: taxi-moto drivers, 76.0 ± 26.8 μg/m³; village residents, 3.4 ± 3.0 μg/m³	Personal air sampling	Urinary SPMA, benzene	Lymphocytes	Toluene, xylenes	Significantly higher 8-OHdG in taxi-moto drivers than village residents ($P < 0.05$)	Ayi-Fanou et al. (2006)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
8-OHdG Positive (see comments)	109 urban and 62 rural schoolboys in Bangkok, Thailand	Average benzene exposure: 5.50 ± 0.40 ppb in urban vs 2.54 ± 0.23 ppb in rural group	Area and personal air sampling	Urinary t,t-MA, SPMA, benzene	Leukocytes and urine	NR	Level of 8-OHdG in leukocytes was threefold higher in urban vs rural schoolchildren ($P < 0.001$) and was significantly associated with benzene exposure level ($P < 0.05$); urinary 8-OHdG significantly higher in urban vs rural schoolchildren ($P < 0.05$) but no correlation with benzene exposure levels	Buthbumrung et al. (2008)
8-OHdG PAHs were the major contributor to 8-OHdG levels (see comments)	165 city centre and 111 rural schoolchildren in Bangkok, Thailand	Mean ± SE benzene exposure: city, 19.38 ± 1.11 μg/m^3 vs rural, 8.40 ± 0.61 μg/m^3	Area and personal air sampling	Not used	Leukocytes	1,3-butadiene, PAHs	Levels of 8-OHdG were higher in city vs rural schoolchildren ($P < 0.001$); 8-OHdG levels correlated significantly with benzene ($P < 0.001$) and PAH ($P < 0.001$) levels; multivariate analysis identified PAH concentrations as the only factor significantly affecting 8-OHdG levels (r, 0.895; $P < 0.05$)	Ruchirawat et al. (2010)

Table 4.1 (continued)

Oxidized DNA base measured, response, significance	Description of exposed and controls	Benzene exposure levels	Monitoring method	Measure of internal dose	Tissue or cells	Other exposures or biomarkers measured	Comments	Reference
8-OHdG, 8-oxoGuo Positive (see comments)	396 children from central Italy districts with different levels of urbanization and air pollution		Urinary t,t-MA, SPMA, benzene	Urinary t,t-MA, SPMA, benzene	Urine	Cotinine	Multiple linear regression ($P < 0.0001$) indicated that benzene exposure (assessed by urinary SPMA and TTMA) was significantly associated with 8-OHdG and 8-oxoGuo	Andreoli et al. (2012)
8-OHdG, 8-oxoGuo, 8-oxoGua Positive (see comments)	155 children living close (< 15 km) to an oil refinery, 58 children living 70 km from the refinery and not close to an industrial hub		Urinary t,t-MA, SPMA, benzene	Urinary t,t-MA, SPMA, benzene	Urine	Cotinine, MTBE	8-OHdG and 8-oxoGuo significantly correlated with markers of benzene exposure ($P < 0.01$)	Andreoli et al. (2015)

8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxoGua, 8-oxo-guanine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; AM, arithmetic mean; d, day(s); h, hour(s); MTBE, methyl tertiary-butyl ether; NR, not reported; PAHs, polycyclic aromatic hydrocarbons; ppm, parts per million; ppb, parts per billion; SD, standard deviation; SE, standard error; SPMA, *S*-phenylmercapturic acid; t,t-MA, *trans*,*trans*-muconic acid; TWA, time-weighted average; vs, versus; yr, year(s)

Table 4.2 DNA strand breaks, adducts and mutations in humans exposed to benzene

End-point Test system	Tissue, cell type	Description of exposed and controls	Results[a]	Agent Concentration (LEC or HIC)	Comments	Reference
DNA damage/strand breaks DNA elution rate through filters	Blood, peripheral lymphocytes	20 female shoemakers, 12 of which had an additional dermal exposure, 20 matched controls	+ $P < 0.001$	Benzene and toluene 4.16 ± 4.15 µg/m³	No evidence for contribution of skin absorption to the effect	Popp et al. (1992)
DNA damage/strand breaks	Blood, the pellet from centrifuged blood	33 men occupationally exposed to gasoline, 3 unexposed controls (smokers and non-smokers)	± $P = 0.2$	Benzene, VOC from gasoline HIC, 0.6 ppm	No increase overall, but in some benzene exposure subgroups	Nilsson et al. (1996)
Mutation/other NN GPA variant cell frequency	Blood, erythrocytes	44 benzene-exposed workers (23 men and 21 women), 44 matched controls	+ $P < 0.05$ (trend)	Benzene 100 ppm-yr		Rothman et al. (1996)
DNA damage/strand breaks Comet assay, tail moment, heavily damaged cell number	Blood, peripheral lymphocytes	12 gasoline station attendants, unspecified matched controls	+ $P = 0.028$	Benzene, gasoline vapours 0.3 ppm (8h TWA)		Andreoli et al. (1997)
DNA damage/strand breaks Comet assay, percentage of damaged cells	Blood, peripheral lymphocytes	83 exposed workers (29 low exposure, 29 high exposure, 25 benzene poisonings), 29 controls	+	Benzene HIC < 300 mg/m³		Wu et al. (1998)
Mutation/oncogene K-ras mutation	Tumour tissue, exocrine cancer of the pancreas, null	107 patients with exocrine cancer of the pancreas, 83 K-ras mutated and 24 K-ras wildtype; among these, 16 cases were previously exposed to benzene	+ $P < 0.05$	Benzene, possibly other solvents, exposure estimated retrospectively (high, low, none)	Significant for men after the only women exposed to benzene were excluded (OR, 7.07; $P < 0.05$)	Alguacil et al. (2002)
DNA damage/strand breaks Alkaline comet assay, comet tail	Blood, peripheral lymphocytes	133 traffic control policemen exposed to traffic emissions, 59 office policemen	–	Benzene, VOC from traffic HIC 9.5 µg/m³, 7-h TWA		Carere et al. (2002)
DNA damage/strand breaks	Blood, peripheral lymphocytes	158 petrochemical workers, 50 matched controls	+ *P* NR	Benzene 1.75 ± 3.6 ppm (inhalation)	NQO1 deficiency correlated with twofold higher SSBs	Garte et al. (2005)

Table 4.2 (continued)

End-point Test system	Tissue, cell type	Description of exposed and controls	Results[a]	Agent Concentration (LEC or HIC)	Comments	Reference
DNA damage/strand breaks Comet tail moment	Blood, peripheral lymphocytes	22 clothes vendors, 21 grilled meat vendors, 29 gasoline station attendants, 23 factory workers, 27 controls Schoolchildren: 41 from Bangkok and 30 provincial	+ $P < 0.05$	Benzene, VOCs 5.5 ± 0.4 ppb	DNA repair capacity was also altered in exposed subjects	Navasumrit et al. (2005)
DNA damage/adducts Comet assay, olive tail moment	Blood, peripheral lymphocytes	41 workers from six plants: printing, shoemaking, production of nitrobenzene, benzene, methylene dianiline and carbomer	+ $P = 0.001$	Benzene, different solvents ND		Sul et al. (2005)
DNA damage/adducts ^{32}P-postlabelling, bulky adducts	Blood, peripheral lymphocytes	34 taxi-moto drivers from Cotonou, 6 controls from a nearby village	+ $P < 0.05$	Complex mixture containing benzene 76.0 ± 26.8 μg/m^3	8-oxo-dG and m^{5}dC levels were also elevated in the exposed group	Ayi Fanou et al. (2006)
DNA damage/adducts Comet assay, olive tail moment	Blood, peripheral lymphocytes	115 schoolchildren from heavy traffic area in Bangkok, 69 controls from a rural area	+ $P < 0.001$	Benzene, PAHs 17.55 ± 1.29 μg/m^3		Ruchirawat et al. (2007)
DNA damage/strand breaks SSB by alkaline elution method	Blood, peripheral lymphocytes	158 petrochemical workers, 50 matched controls	+ P NR	Benzene 1.75 ± 3.6 ppm, long-term	NQO1 deficiency correlated with a twofold higher and GSTT1 gene deletion in a 35–40% higher SSBs	Garte et al. (2008)
DNA damage/strand breaks Comet assay, tail intensity	Blood, peripheral lymphocytes	20 petrol station attendants (11 smokers), 20 matched controls (11 smokers)	+ $P < 0.05$	Benzene and other petrol VOCs 0.65 ± 0.47 mg/m^3		Keretetse et al. (2008)
DNA damage/strand breaks Comet tail length, tail moment	Blood, peripheral lymphocytes	33 petrochemical industry operators, 28 service station attendants, 21 gasoline pump maintenance workers, 51 non-exposed controls	+ $P < 0.008$	Benzene, VOCs 40 μg/m^3		Fracasso et al. (2010)
DNA damage/adducts Total DNA adducts	Blood, peripheral lymphocytes	57 healthy inhabitants of Cotonou (high exposure), 20 suburbans (low exposure), 17 villagers (control)	+ $P < 0.001$	Benzene, PAHs 76 ± 26.8 μg/m^3	Adducts mainly from exposure to PAHs rather than benzene	Ayi-Fanou et al. (2011)

Table 4.2 (continued)

End-point Test system	Tissue, cell type	Description of exposed and controls	Results[a]	Agent Concentration (LEC or HIC)	Comments	Reference
DNA damage/strand breaks Comet assay, mean tail DNA (%) and tail moment	Blood, peripheral lymphocytes	324 USA Air Force personnel maintenance workers (high), service workers (moderate), others (low exposure)	±	Jet fuel JP-8 ND	Positive correlation between both pre-shift benzene concentrations in breath and the mean tail DNA (%) and tail moment ($P < 0.05$)	Krieg et al. (2012)
DNA damage/strand breaks Comet assay, DNA damage index	Blood peripheral lymphocytes	43 gasoline station attendants with 9.1 ± 1.1 yr of exposure, 28 controls matched by age, all non-smoking	+ $P < 0.001$	Benzene, VOC 76.20 (54.34–1285.48) μg/m^3		Moro et al. (2013)
DNA damage/other Comet assay, tail intensity	Blood, peripheral lymphocytes	18 fuel tanker drivers, 13 filling station attendants, 20 controls with no occupational exposure to benzene	–	Benzene, VOCs, fuel HIC, 280 ± 249 μg/m^3	Smokers and non-smokers in all groups	Lovreglio et al. (2014)

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study)

8-oxo-dG, 8-oxo-2′-deoxyguanosine; HIC, highest ineffective concentration; LEC, lowest effective concentration; m^5 dC, methylated deoxycytosine; ND, not determined; NQO1, NAD(P)H:quinone oxidoreductase 1; NR, not reported; OR, odds ratio; PAH, polycyclic aromatic hydrocarbons; ppb, parts per billion; ppm, parts per million; SSB, single-strand break; TWA, time-weighted average; VOC, volatile organic compounds; yr, year(s)

(c) Experimental systems

In rodents, benzene increased oxygen radical generation and lipid peroxidation (Verma & Rana, 2004). The exposure of mice to benzene via inhalation increased lipid peroxidation and DNA damage in bone marrow (Yu et al., 2014). The activation of bone marrow phagocytes after the administration of benzene to mice led to increased oxidative stress, nitric oxide generation, and protein-bound 3-nitrotyrosine in bone marrow (Laskin et al., 1989; Chen et al., 2005; Melikian et al., 2008). Oxidative stress has also been implicated in several effects induced by benzene metabolites in animal cells, including homologous recombination (Winn, 2003), DNA damage and recombination (Tung et al., 2012), and altered c-Myb transcriptional activity (Wan & Winn, 2007). Attenuation of oxidative stress in mice by transgenic overexpression of thioredoxin reductase decreased clastogenic effects and completely suppressed lymphoma of the thymus gland induced by benzene inhalation (Li et al., 2006).

4.2.2 Genetic and related effects

(a) Oxidative damage to DNA

(i) Humans

Representative studies examining oxidative damage to DNA in either occupationally exposed workers or in environmentally exposed urban populations, as measured by the production of oxidized DNA bases, are described in Table 4.1. Oxidative damage to DNA was detected primarily using the oxidation of guanine residues, and correlated with benzene exposure levels and/or metabolic biomarkers of benzene exposure.

In several occupational studies in Table 4.1 that were the focus of the Working Group's review, discussed in more detail below, significant effects of benzene exposure on oxidative damage to DNA, as indicated by increased 8-OHdG levels or other oxidized DNA bases, were observed. Dose–response relationships between exposure to benzene and 8-OHdG levels were suggested by Lagorio et al. (1994), Liu et al. (1996), and Ruchirawat et al. (2010).

Lagorio et al. (1994) examined urinary 8-OHdG in a random sample of 65 filling station attendants, and both benzene and methylbenzene exposure levels were calculated from seven personal air samples taken over 1 year. No control group was used in this study. A significant correlation was found between urinary 8-OHdG levels and exposure to benzene ($r = 0.34$; $P < 0.01$).

Liu et al. (1996) demonstrated a dose–response increase in oxidative damage to DNA associated with both external and internal measures of benzene exposure. Lymphocyte 8-OHdG was assessed in blood samples of 87 benzene-exposed workers from shoemaking and car-painting factories and 30 controls from a university staff. Workers from different factories were exposed to different concentrations of benzene; median levels in environments considered to have low, medium, and high concentrations of benzene in air were measured as 2.5, 103.3, and 424.4 mg/m^3, respectively.

Manini et al. (2010) measured urinary nucleic acid oxidation in 239 traffic policemen, taxi drivers, and gasoline pump attendants in Parma, Italy. A separate control group was not used. Urinary t,t-MA and SPMA were used as internal markers of benzene exposure. Multiple linear regression analyses showed that benzene exposure was associated with oxidative damage to DNA, particularly RNA as indicated by the production of 8-oxo,7,8-dihydroguanosine (8-oxoGuo). The modulating effects of NQO1 and GST polymorphisms on DNA damage and SPMA excretion, respectively, were reported.

Ruchirawat et al. (2010) performed a study of oxidative damage to DNA in multiple populations in Thailand, either primarily exposed to benzene (31 petrochemical laboratory workers and 31 gasoline service station attendants) or other pollutants (165 city centre and 111 rural

schoolchildren, and 40 temple workers exposed to incense). Individuals from an office building with no incense burning were used as a control population. The group exposed to the highest concentrations of benzene (gas station attendants; mean benzene exposure, 360.9 μg/m^3) had higher leukocyte 8-OHdG than control subjects, and both petrochemical laboratory workers (benzene exposure levels, 78.3 μg/m^3) and gas station attendants had significantly higher single-strand break levels and DNA repair capacity.

(ii) Human cells in vitro

Several studies have demonstrated that phenolic metabolites of benzene can induce oxidative damage to DNA, as indicated by 8-OHdG formation in cellular systems in vitro. Zhang et al. (1993) demonstrated that 1,2,4-benzenetriol induced 8-OHdG and MN formation in the human leukaemia HL-60 cell line. This and other benzene metabolites (phenol, hydroquinone, catechol) were shown to induce 8-OHdG formation in HL-60 cells (Kolachana et al., 1993). Catechol was shown to induce 8-OHdG in HL-60 cells (Oikawa et al., 2001). Hydroquinone induced DNA strand breaks, DNA–protein cross-links, and 8-OHdG formation in human hepatoma HepG2 cells (Luo et al., 2008), and single-strand breaks, chromosomal aberrations (CAs), and 8-OHdG formation in A549 human lung cancer cells (Peng et al., 2013).

(iii) Experimental systems

Increased 8-OHdG formation was also observed in mouse bone marrow 1 hour after administration of benzene or 1,2,4-benzenetriol (Kolachana et al., 1993). Although phenol, catechol, and hydroquinone were without effect when administered separately, binary combinations of phenol, catechol, and hydroquinone induced 8-OHdG formation in mouse bone marrow when administered together (Kolachana et al., 1993).

(b) DNA binding, DNA strand breaks, and gene mutations

In several occupational studies of DNA binding, strand breaks, and mutations that were the focus of the Working Group's review, discussed in more detail below, the observed effects could be reasonably attributed to benzene (Popp et al., 1992; Rothman et al., 1995, 1996; Wu et al., 1998; Table 4.2). The study of Rothman et al. (1995, 1996) showed an apparent dose–response relationship. In this study, glycophorin A gene (*GPA*) loss and subsequent duplication of its NN allele in MN-heterozygous was demonstrated in subjects with long-term occupational exposure to benzene; cumulative exposures ranged from 8 to 3488 ppm-years. The NN *GPA* variant cell frequency was 13.9 ± 1.7 per million cells in workers exposed to benzene versus 7.4 ± 1.1 per million cells in control individuals. In contrast, no significant difference existed between the two groups for the null allele frequency. In this study, benzene produced gene-duplicating mutations, but did not produce gene-inactivating mutations at the *GPA* locus in bone marrow cells of humans.

There are no reports on benzene-specific DNA adducts in exposed humans or in human cells treated with benzene in vitro. However, DNA adducts were detected in human promyelocytic HL-60 cells treated with a mixture of benzene metabolites, hydroquinone and 1,4-benzoquinone, by ^{32}P-postlabelling (Levay et al., 1991). The adduct formed after treatment of the HL-60 cells with either benzoquinone or hydroquinone was later identified as N^2-(4-hydroxyphenyl)-2′-deoxyguanosine-3′-phosphate (Pongracz & Bodell, 1996). Furthermore, two depurinating adducts, namely 7-(3,4-dihydroxyphenyl)-2′-deoxyguanosine and 3-(3,4-dihydroxyphenyl) adenine, were identified in cultured human blood mononuclear cells (Chakravarti et al., 2006).

In human lymphocytes treated in vitro, benzene and its metabolites hydroquinone, catechol, 1,2,4-benzotriol, and 1,4-benzoquinone

(but not t,t-MA) significantly increased DNA damage (single-strand breaks) in the comet assay (Anderson et al., 1995). In HeLa cells treated with hydroquinone, the alkaline comet assay also showed significant DNA damage compared with untreated cells (Galván et al., 2008). In vitro studies on human cells are also listed in Table 4.3.

(c) *Chromosomal damage and cytogenetic effects*

(i) *Humans*

Chromosomal damage

Chromosomal end-points, including sister-chromatid exchanges (SCEs), MN, and CAs, have typically been examined in peripheral blood lymphocytes (PBLs), although some of the benzene studies examined buccal cells, sperm, or other blood cells, such as granulocytes. Two of these end-points, CAs and MN, have been found to be associated with increased cancer risk in humans in large, prospective studies (Liou et al., 1999; Hagmar et al., 2004; Boffetta et al., 2007; Bonassi et al., 2007). The third end-point, SCEs, is an indicator of effects on DNA; however, the relevance of SCEs to cancer risk is uncertain (Norppa et al., 2006), and the data for benzene in exposed humans are inconclusive (see Table 4.4).

Studies that examined structural CAs in humans occupationally exposed to benzene are summarized in Table 4.4. Of this range of occupations, most show increases in CAs associated with jobs involving exposure to benzene, including some large studies (> 100 exposed workers) with significant positive exposure–response relationships (e.g. Kim et al., 2004a; Rekhadevi et al., 2011).

Similarly, although fewer in number, the majority of studies of MN in humans exposed to benzene show increases in MN frequencies associated with jobs involving benzene exposure (see Table 4.4). In addition, several large studies have reported significant positive exposure–response relationships (e.g. Kim et al., 2008; Rekhadevi et al., 2011 in buccal cells; Zhang et al., 2014).

Aneuploidy was reported in other studies of humans occupationally exposed to benzene that examined specific chromosomes, generally in lymphocytes or sperm (e.g. Kim et al., 2004b; Xing et al., 2010 and in studies of human cells in vitro; see Table 4.4, Table 4.5 and Table 4.6). Ji et al. (2012) compared aneuploidy results between lymphocytes and sperm in their study population and across other studies, reporting the induction of aneuploidy in different chromosomes in different cell types.

Over 20 studies of human cells in vitro are available, primarily using PBLs although some have used other lymphohaematopoietic cells or cell lines (see Table 4.5). In vitro studies using benzene without metabolic activation have been inconsistent for chromosomal end-points; however, the few that used S-9 to activate benzene metabolism were uniformly positive. In vitro studies directly assessing the benzene metabolites phenol, hydroquinone, benzoquinone, catechol, and benzenetriol have been consistently positive for the various chromosomal end-points examined, including SCEs (e.g. Morimoto & Wolff, 1980; Yager et al., 1990). In the study of Erexson et al. (1985), catechol exhibited greater potency in inducing SCEs than benzoquinone, hydroquinone, and benzenetriol, which were in turn more potent than phenol, which was in turn more potent than benzene.

Specific cytogenetic effects

Some studies have examined specific cytogenetic changes in humans exposed to benzene to investigate the mechanisms of benzene carcinogenesis (see Table 4.6). The cytogenetic changes include alteration of the number of specific chromosomes, loss of particular regions of certain chromosomes, and acquisition of specific translocations.

Among the variety of cytogenetic changes, significant exposure-related trends for –5, –7,

Table 4.3 DNA damage in human cells in vitro

End-point Test system	Tissue, cell line	Results[a]		Agent	Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation				
DNA adducts ^{32}P-postlabelling	Myeloid leukaemia (HL-60)	+	NT	Benzene metabolites (HQ, 1,4-BQ)	HQ, 50 µM per 8 h; 1,4-BQ, 25 µM per 2 h		Levay et al. (1991)
DNA strand breaks Comet assay	Lymphocytes	+	–	Benzene	12 mM/h		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	+	+	1,2,4-BT	100 µM per 30 min, $P < 0.05$		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	+ NT	– +	HQ CAT	200 µM/h 1 mM per 2 h		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	– NT	– +	1,4-BQ 1,4-BQ	200 µM/h 0.5 mM per 4 h		Anderson et al. (1995)
DNA strand breaks Comet assay	Lymphocytes	–	–	t,t-MA	800 µM/h		Anderson et al. (1995)
DNA strand breaks Comet assay, tail moment	Non-proliferating peripheral lymphocytes	+		Benzene metabolites (HQ, BQ, BT)	LEC: HQ, 0.5 µg/mL; BQ, 0.3 µg/mL; BT, 5.0 µg/mL		Andreoli et al. (1997)
DNA adducts Tandem mass spectrometry	Blood mononuclear cells	+	NT	Benzene metabolite (1,2-BQ)	75 µM		Chakravarti et al. (2006)
DNA strand breaks Comet assay, tail moment	HeLa cells	+		HQ	150 µM per 12 h	WRN depletion increased DNA damage (SSBs by comet assay)	Galván et al. (2008)

[a] +, positive; –, negative

BQ, benzoquinone; BT, benzenetriol; h, hour(s); HIC, highest ineffective concentration; HL, human leukaemia; HQ, hydroquinone; LEC, lowest effective concentration; NT, not tested; SSB, single-strand break; t,t-MA, *trans*,*trans*-muconic acid

Table 4.4 Chromosomal damage in humans exposed to benzene

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
20 (of 38) workers using benzene as solvent, 5 industrial controls from other areas of plant	1–20	NR (mean, ~25–150 in high-exposure area; Tough et al., 1970)	+	NT	NT	Blood samples 2–3 yr after benzene substituted with toluene	Tough & Brown (1965)
Factory workers (solvent) and onsite controls G1: 20 exposed, 5 controls (from Tough & Brown, 1965) G2: 12 exposed, 6 controls G3: 20 exposed, 5 controls Controls listed above from other areas of plant; 8 general-population controls	G2: 6–25 G3: 2–26	G1: ~25–150 in high-exposure area G2: similar to G1 G3: ~12	G1: see above G2: +/– G3: –	NT	NT	G2: blood samples almost 4 yr after benzene; G3: exposure up to time of study G2: + vs general population; – vs onsite controls; onsite controls also had some exposure, although earlier in time	Tough et al. (1970)
52 workers exposed to benzene, 44 pre-employment control group	0.1–26	2.1 (TWA)	+	NT	NT	+ for chromosome breaks (deletions) and other CAs (rings, dicentrics, translocations, exchanges); – for chromatid breaks	Picciano (1979)
22 benzene production workers, 22 controls from metallurgical factory	11.4 (mean)	0.2–12.4 (8-h TWA)	+	–	NT	+ for chromosome-type CAs; (+) for total CAs; – for chromatid-type	Sarto et al. (1984)
16 non-smoking female benzene-exposed worker, 7 controls	NR	3–50	NT	–	NT		Watanabe & Endo (1984)
66 ethylbenzene production workers, 20 general-population controls of same social position, etc. as exposed	3–18	0.47–11.7 (8-h TWA)	(+)	+	NT		Jablonická et al. (1987)
66 refinery workers, 33 controls SCE: 28 refinery workers, 23 controls	NR	< 1–10 (TWA)	NT	(+)	NT		Yardley-Jones et al. (1988)
33 workers exposed to benzene, 15 general-population controls	10–23	< 31.3	+	NT	NT	+ for CAs	Sasiadek et al. (1989)
36 non-smoking female shoemakers, 11 factory worker controls	5.5 (mean)	3–210 (GM, 54) (one 8-h shift)	NT	–	NT		Seiji et al. (1990)
66 refinery workers, 33 controls CAs: 48 refinery workers, 29 controls	> 5	1–10 (TWA)	+	NT	NT		Yardley-Jones et al. (1990)

Table 4.4 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
20 female shoemakers, 20 general-population controls	3–NR (mean, 18)	0.25–5.0 (mean, 1.3) (one 8-h shift)	NT	+	NT		Popp et al. (1992)
56 workers in plants with other exposures, 20 controls	10–20 (not clearly reported) (average, 6 h/d as solvent)	< 10	+	NT	NT		Sasiadek (1992)
42 benzene distillers at oil refinery, 42 controls	2–NR	0.3–15 (mean, 2.3)	+	+	NT		Major et al. (1994)
49 oil refinery workers, 91 historical controls, 122 industrial controls	0–2 (n = 10) 2–10 (n = 22) > 10 (n = 17)	0.9–21.5 in 1990 (start of study) 0.3–5.8 by 1992	+	+	NT	+ in both chromatid- and chromosome-type CAs and achromatic lesions (gaps); followed from 1990 to 1992, during which exposures decreased along with CAs (excluding gaps) and gaps only, but not SCEs	Tompa et al. (1994)
38 high-exposure and 45 low-exposure female shoemakers, 35 worker controls	High exposure, 2–31 (mean, 13.4) Low exposure, 1–33 (mean, 17.7)	High exposure, 2–15 (mean, 8) Low exposure, 2–13 (mean, 5)	+	+	NT		Karacić et al. (1995)
35 low-benzene (high-toluene) shoeworkers, 24 medium-benzene car painters, 28 high-benzene (low-toluene) shoeworkers, 30 university staff controls	NR	Low, 0.77 (mean) Medium, 32 (mean) High, 133 (mean) (8-h TWA)	NT	NT	+		Liu et al. (1996)
58 shoemakers, 20 general-population controls	5–50	NR	+	NT	NT		Tunca & Egeli (1996)
437 factory workers, 150 controls		0.02–9.2 (mean, 1.4)	NT	NT	+		Zhang (1996)
49 exposed female shoemakers, 27 controls employed in confectionary industry	1–33 (mean, 17)	1.9–14.8 (median, 5.9) (at time of study)	(+)	(+)	NT		Bogadi-Sare et al. (1997)

Table 4.4 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
Lymphocytes: 38 petrochemical workers, benzene plant or coke oven workers, 13 controls (included office workers) Buccal: 18 petrochemical workers, benzene plant or coke oven workers, 15 controls (included office workers)	NR	Benzene, 0.8–1.1 Coke, 0.04–0.30 (8-h TWA)	NT	NT	–	Chromosome 9; buccal cells and lymphocytes	Surrallés et al. (1997)
23 painters, 22 factory controls	NR	0.17–3.06 (TWA, 0.71)	NT	–	NT		Xu et al. (1998)
12 benzene factory workers, 5 coke oven workers, 8 rural village population controls	Benzene, 0.7–19 (GM, 4.1) Coke, 0.5–30.6 (GM, 4.8)	Benzene, 0.03–9.0 (GM, 0.41) Coke, 0. 16–0.53 (GM, 0.31)	+	NT	NT	Chromosomes 1 and 9; blood smear granulocytes and lymphocytes, and stimulated (cultured) lymphocytes; + for breakages in both chromosomes in benzene factory workers in cultured lymphocytes; (+) for chromosome 1 (9 not reported) in benzene factory worker smear cells	Marcon et al. (1999)
44 Chinese workers exposed to benzene, 44 controls 12 Estonian benzene production, 5 coke oven workers, and 8 controls	Estonian benzene production, 6.6 (mean) Estonian coke workers, 11.4 (mean)	Chinese, 31 (median) Estonian benzene production, 4.1 (mean) Estonian coke workers, 1.1 (mean)	–	NT	NT		Eastmond et al. (2001)
178 petroleum refinery workers, 36 office worker controls	10.6 (mean)	0.004–4.52	+	NT	NT		Kim et al. (2004a)
82 coke oven workers, 76 controls	0.75–19.67 (mean, 8)	0.014–0.743 (GM, 0.557) (8-h TWA)	+	NT	NT	Chromosomes 8, 21 were evaluated; + for t(8,21) translocations	Kim et al. (2004b)
39 ethylbenzene production workers, 55 controls	NR	0.13–4.7	+	NT	NT	CAs in high-CA subset decreased 10 mo after improved work conditions	Sram et al. (2004)
10 oil refinery workers, 87 industrial controls, 26 matched controls	12–28 (mean, 22.8)	13.7 (mean) in 1994, reduced to 0.56 (mean) in 1995	+	+	NT	Followed annually from 1990 to 2003; decreased over time as exposures decreased	Tompa et al. (2005)
44 factory workers, 44 factory controls	0.7–16 (mean, 6.3) (Smith et al., 1998)	31 (median) 8-h TWA, based on geometric mean of five 8-h measures	+	NT	NT	Chromosomes 2, 4, 6, 11, 12, 14, 18; same study as Smith et al. (1998) and other Zhang et al. studies with different chromosomes evaluated	Zhang et al. (2007)

Table 4.4 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic effects[b]			Comments	Reference
			CA	SCE	MN		
108 petroleum refinery workers, 33 controls	10.5 (mean)	0.004–4.52 (shift TWA, 0.51)	+	NT	+		Kim et al. (2008)
30 petroleum refinery workers, 10 office worker controls		0.51 (mean)	NT	NT	+		Kim et al. (2010)
200 filling station workers, 200 general-population controls	10.7 (mean)	0.34–0.47	+	NT	+		Rekhadevi et al. (2011)
30 workers in China who used benzene-containing glues, 11 factory controls	> 1–NR	< LOD–23.6 (GM, 2.8)	+	NT	NT	Chromosome 1 in sperm; + for "structural aberrations", including duplications, deletions, and breaks; (+) for "numerical aberrations" (disomy, nullisomy)	Marchetti et al. (2012)
459 unspecified benzene-exposed workers, 88 controls			NT	NT	+		Zhang et al. (2012b)
385 shoemakers, 197 controls (102 indoor local controls, 95 teachers from Shanghai as external controls)	> 1	0.8–17.8 (median, 2.0)	NT	NT	+		Zhang et al. (2014)
317 shoemakers, 102 office worker controls	> 1	0.80–12.09 (median, 1.60)	NT	NT	+		Zhang et al. (2016)

[a] Benzene exposure level conversion: 1 ppm = 3.19 mg/m^3 = 3190 µg/m^3

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

CA, chromosomal aberrations; d, day(s); G1, group 1; G2, group 2; GM, geometric mean; h, hour(s); LOD, limit of detection; MN, micronuclei; mo, month(s); NR, not reported; NT, not tested; ppm, parts per million; SCE, sister-chromatid exchanges; TWA, time-weighted average; vs, versus; yr, year(s)

Table 4.5 Chromosomal end-points in human cells in vitro

Cells	End-point	Benzene and metabolite(s)	Results[a]	Reference
Leukocytes	Combined effects of benzene and radiation on CAs	Benzene	+ for CAs	Morimoto (1976)
Lymphocytes	QM staining	Benzene	– for SCE, structural CAs	Gerner-Smidt & Friedrich (1978)
Lymphocytes	SCEs	Benzene	– for SCE	Morimoto & Wolff (1980)
		Phenol, CAT, HQ	+ for SCE	
Lymphocytes	SCEs	Benzene	+ for SCE with S-9	Morimoto (1983)
		CAT, HQ	+ for SCE	
Lymphocytes	SCEs	Benzene, CAT, HQ, phenol	+ for SCE with S-9	Morimoto et al. (1983)
T-lymphocytes	Stained using a modified fluorescence-plus-Giemsa technique for SCE	Benzene, phenol, CAT, BT, HQ, BQ	+ for SCE	Erexson et al. (1985)
		t,t-MA, 4,4′-biphenol, 4,4′-dipheno-quinone, 2,2′-biphenol	(+)/–	
Lymphocytes	Modified MN assay with anti-kinetochore antibody	HQ, phenol, CAT	+ for MN and K+	Yager et al. (1990)
		BQ	+ for MN	
Lymphocytes and HL-60 cells	MN with anti-kinetochore antibody	BT	+ for MN and K+	Zhang et al. (1993)
Lymphocytes	FISH (chromosomes 1, 7, and 9)	HQ	+ for hyperploidy	Eastmond et al. (1994)
Human lymphoblast cell line (GM09948)	FISH with specific probes for chromosomes 5, 7, and 8	HQ	+ for loss of one hybridization signal for chromosomes 5, 7, and 8	Stillman et al. (1997)
Lymphocytes	FISH with centromeric probes for chromosomes 1, 5, 7 and specific probes for 5q31 and 7q36-qter	HQ, BT	+ for HQ and BT for monosomy 5 and 7 and –5q and –7q	Zhang et al. (1998b)
Human lymphoblast cell line (GM09948)	FISH with specific probes for chromosomes 5, 7, and 8	CAT	– for CAT for loss of one hybridization signal for chromosomes 5, 7, and 8	Stillman et al. (1999)
		HQ	+ CAT/HQ synergy; – for CAT/HQ hyperploidy [(+) for 8]	
Lymphocytes	MN	Benzene	– for MN	Zarani et al. (1999)
CD34+ and CD34– cells from cord blood	FISH with probes for chromosomes 7 and 8	HQ	+ for monosomy and trisomy 7 and 8 in CD34+; + for monosomy 7 only in CD34–; – for tetrasomy 7 and 8 in both cell types	Smith et al. (2000)

Table 4.5 (continued)

Cells	End-point	Benzene and metabolite(s)	Results[a]	Reference
Human CD34+CD19– bone marrow cells	FISH with 5q31, 5p15.2, and centromeric probes specific for human chromosomes 7 and 8	HQ	+ for monosomy 7; + for –5q31; – for monosomy 5; no loss or gain of 8	Stillman et al. (2000)
Lymphocytes	FISH (chromosomes 5, 7, 8, and 21)	BT, HQ, t,t-MA	+ for aneuploidy	Chung & Kim (2002)
Lymphocytes	CBMN, FISH (chromosomes 7 and 8)	BT	+ for MN, aneuploidy	Chung et al. (2002)
Metabolites incubated with human topoisomerase IIα and then DNA	Measuring DNA cleavage	BT HQ BQ CAT, 4,4'-biphenol, 2,2'-biphenol	– (+) + for targeting topoisomerase IIα –	Lindsey et al. (2005)
Whole blood	FISH with probes for chromosomes 1, 5, 6, 7, 8, 9, 11, 12, and 21; six chromosomes (1, 5, 7, 8, 9, and 21) analysed for BT and high-dose HQ	HQ BT	(+) for HQ for monosomy for all chromosomes except 21; ++ for 5, 7, 9, and 11; highest IRRs for 5, 6, and 12 + for trisomy for 7, 8, 9, and 21; highest IRRs for 7, 8, 12, and 21; + for BT for monosomy for 5, 7, 8, and 9 but not 1 and 21; highest IRRs for 5 and 7; (+) for BT for trisomy for all chromosomes; no selection for trisomy 8	Zhang et al. (2005)
Lymphocytes (interphase and metaphase)	FISH with probes for chromosome 1	Benzene	(+) for MN in interphase cells, aneuploidy in metaphase	Holeckova et al. (2008)
HL-60 cells	Gamma-H2AX	HQ, BQ	+	Ishihama et al. (2008)
TK-6 cells (lympho-blastoid cell line)	Stained with DAPI, chromosomes 11 and 21	HQ	+ for structural CAs; + for translocations of chromosome 21; – for translocations of chromosome 11	Ji et al. (2009)
Peripheral blood mononuclear cells	CBMN	BQ	+ for MN with PMA act of MPO; – for MN without PMA	Westphal et al. (2009)
Lymphocytes	umuC test, CBMN assay	Benzene	– for umuC; (+)/– for MN	Bonnefoy et al. (2012)
Lymphocytes	MN	Benzene with S-9, HQ	+ for MN	Peng et al. (2012)

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

BQ, benzoquinone; BT, benzenetriol; CA, chromosomal aberration; CAT, catechol; CBMN, cytokinesis-blocked micronucleus; DAPI, 4',6-diamidino-2-phenylindole; FISH, fluorescence in situ hybridization; H2AX, histone H2AX; HL, human leukaemia; HQ, hydroquinone; IRR, incidence rate ratio; K+, kinetochore positive; MN, micronuclei; MPO, myeloperoxidase; PMA, phenylmercapturic acid; QM, QM protein (transcription cofactor inhibiting the activity of AP-1 transcription factors and is also a ribosomal protein participating in protein synthesis); SCE, sister-chromatid exchanges; t,t-MA, *trans*,*trans*-muconic acid

Table 4.6 Cytogenetic changes in humans exposed to benzene

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
Woman (age, 38 yr) with benzene-induced leukaemia (case study)			+ for extra chromosomes, mostly in C group (group C trisomy)	All chromosomes	Forni & Moreo (1967)
Woman (age, 37 yr) with benzene-induced acute erythroleukaemia (case study)			+ for cytogenetic changes	All chromosomes	Forni & Moreo (1969)
5 women with benzene haemopathy, diagnosed 5 yr previously, 1 control	NR	NR	+	% aneuploid lymphocytes (40%) decreased from time of diagnosis (70%)	Pollini et al. (1969)
4 women with benzene myelopathy diagnosed 10 yr previously, 1 control	NR	NR	Same subjects as Pollini et al. (1969)	All had lower % aneuploid cells than control	Pollini et al. (1976)
4 women with benzene myelopathy diagnosed 12 yr previously, 1 control	NR	NR	Same subjects as Pollini et al. (1969)	All had lower % aneuploid cells than at previous follow-ups, but closer to control	Pollini & Biscaldi (1977)
33 workers exposed to benzene, 15 general-population controls; all smokers	10–23	< 31.3	+ for structural CAs In exposed workers, chromosomes 2, 4, and 9 almost twice as susceptible to breaks; 1 and 2 almost twice as susceptible to gaps; chromosome 18 underrepresented for CAs In unexposed controls, more random distribution of the breakpoints	All chromosomes	Sasiadek et al. (1989)
56 workers in plants, 20 controls	10–20 (not clearly defined)	< 10	+ for structural CAs (mainly breaks and gaps) and non-random distribution of breakpoints, which accumulated mainly on chromosomes 2, 4, and 7	All chromosomes	Sasiadek (1992)
58 shoemakers, 20 general-population controls	5–50	NR	(+) for polyploidy	All chromosomes	Tunca & Egeli (1996)
18 petrochemical workers (benzene plant or coke oven workers), 15 controls (including some office workers)	NR	Benzene plant, 1.1 (mean) Coke oven, 0.04 (mean) (8-h TWA)	– for chromosome 9 numerical abnormalities	Chromosome 9; buccal cells	Surrallés et al. (1997)

Table 4.6 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
43 Chinese factory workers, 44 other factory controls	0.7–16 (mean, 6.3)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for hypo- and hyperdiploidy of chromosome 8; hyperdiploidy of chromosome 21; t(8,21), t(8;?), t(21,?), and chromosome 8 breaks but not deletions	Chromosomes 8 and 21; same study population as some Zhang et al. studies (2007 and others), different chromosomes evaluated	Smith et al. (1998)
43 Chinese factory workers, 44 factory controls	0.7–16.0 (mean, 6.3)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for monosomy 5 and 7 but not 1; trisomy 1, 5, and 7; tetrasomy 1, 5, and 7; –5q; –7q; total structural CAs in 5 and 7 (+) for chromosome 1 breaks at the centromere	Chromosomes 1, 5, and 7; same study population as for some Zhang et al. studies (2007 and others), different chromosomes evaluated	Zhang et al. (1998a)
12 benzene factory workers, and 5 cokery workers; 17/8 rural village population controls	0.7–19 (GM, 4.1)	0.0–9.0 (GM, 0.41)	(+) for hyperploidy in both chromosomes 1 and 9 in cultured lymphocytes; (+) for chromosome 1 hyperploidy and breakages (9 not reported) in smear cells; + for breakages in chromosomes 1 and 9 in cultured lymphocytes	Chromosomes 1 and 9; blood smear granulocytes and lymphocytes, and stimulated (cultured) lymphocytes	Marcon et al. (1999)
5 cokery workers, 8 rural village population controls	0.5–30.6 (GM, 4.8)	0.16–0.53 (GM, 0.31)	(+) for hyperploidy in both chromosomes 1 and 9 in cultured lymphocytes		
43 Chinese factory workers, 44 factory controls	0.7–16 (mean, 6.3)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for monosomy (one hybridization signal) 7 and 8 in metaphase but not interphase + dr for trisomy (3 hybridization signals) 7 and 8 in metaphase and interphase, but more pronounced in metaphase	Chromosomes 7 and 8; same study population as for other Zhang et al. studies (e.g. 2007), different chromosomes evaluated; compared sensitivity of metaphase and interphase FISH, metaphase more sensitive	Zhang et al. (1999)
44 Chinese workers, 44 controls	NR	1.6–328.5 (median, 31)	(+) dr for hyperploidy in Ch chromosome 1	Chromosomes 1 and 9	Eastmond et al. (2001)
12 Estonian benzene production workers, 5 coke oven workers, 8 controls	Benzene workers, 6.6 (mean) Coke workers, 11.4 (mean)	Benzene workers, 4.1 (mean) Coke workers, 1.1 (mean)			

Table 4.6 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
82 coke oven workers, 76 controls	0.75–19.67 (mean, 8)	0.014–0.743 (GM, 0.557) (8-h TWA)	+ for both monosomy and trisomy of both 8 and 21; + for t(8,21) translocations	Chromosomes 8 and 21	Kim et al. (2004b)
43 factory workers, 44 factory controls	0.7–16 (mean, 6.3) (from Smith et al., 1998)	1–328 (median, 31) (8-h TWA, based on geometric mean of five 8-h measures)	+ dr for monosomy and trisomy of all 7 chromosomes; + for exposed vs non-exposed for tetrasomy of all 7 chromosomes; some selectivity at lower exposures: only monosomy 6 and trisomy 4, 6, and 11 were + in the < 31 ppm group; + for –6q and t(14;18); – for t(4,11) and t(6,11)	Chromosomes 2, 4, 6, 11, 12, 14, and 18; same study population as for Smith et al. (1998) and other Zhang et al. studies, different chromosomes evaluated	Zhang et al. (2007)
57 Chinese factory workers exposed to benzene (20 low-dose and 37 high-dose), 31 unexposed factory workers (not clearly reported) Subset: 37 benzene-exposed workers, 20 unexposed factory workers (not clearly reported)	NR	Low, 1.8 (mean) High, 21.9 (mean) Subset, 22.6 (mean)	t(14,18) signif ↓d no t(8,21) observed 2 t(15,17), but 1 in unexposed, 1 in exposed	t(15;17) and t(8,21) t(14,18) in subset	McHale et al. (2008)
649 MDS cases, 80 with benzene exposure (13.2%), 29 highly exposed > 21 ppm	High, > 0.5 (mean, 12)	> 21	– *	–5/5q–, –7/7q–, +8, del(20q) and 11q23/*MLL* * – for benzene-exposed vs non-exposed MDS cases	Irons et al. (2010)
30 petroleum refinery workers, 10 office worker controls		0.51 (mean)	+ for chromosomes 7, 9 for aneuploidy	Chromosomes 7 and 9	Kim et al. (2010)
33 Chinese male factory workers using benzene-containing glues, 33 factory worker controls	> 1	< 0.2–23.6 (median, 2.9) (8-h TWA)	+ for disomy X and disomy Y; – for disomy 21	Chromosomes 21, X, and Y in sperm; same population as Ji et al. (2012) (PBL results)	Xing et al. (2010)
47 shoemakers (22 exposed to benzene at < 10 ppm, 25 at ≥ 10 ppm), 27 clothing factory controls	NR	Low, 4.95 (mean) High, 28.33 (mean) (Based on multiple samples over 3 mo)	+ selectivity; + dr for monosomy 5, 6, 7, 10, 16, and 19; + dr for trisomy 5, 6, 7, 8, 10, 14, 16, 21, and 22	All chromosomes; OctoChrome FISH: chromosome-wide aneuploidy study (CWAS)	Zhang et al. (2011)

Table 4.6 (continued)

Description of exposed and controls	Exposure duration (years)	Exposure in air (ppm)[a]	Cytogenetic changes[b]	Comments	Reference
33 Chinese male factory workers, 33 control factory workers	> 1	< 0.2–23.6 (median, 2.9) (8-h TWA)	(+) for trisomy 21; – for gain X and gain Y	Chromosomes 21, X, and Y; same population as Xing et al. (2010) (sperm results)	Ji et al. (2012)
30 workers in China who used benzene-containing glues, 11 factory worker controls	> 1–NR	< LOD–23.6 (GM, 2.8)	(+) for disomy 1	Chromosome 1 in sperm	Marchetti et al. (2012)
Man (age, 43 yr) with MDS and AML (case study)	16 (HQ)		+ CAs in chromosomes 5 and 7		Regev et al. (2012)
28 shoemakers (18 exposed to benzene at < 10 ppm, 10 at ≥ 10 ppm), 14 clothing factory controls	NR	Low, 2.64 (mean) High, 24.19 (mean) (Based on multiple samples over 3 mo)	+ dr for monosomy 7 and 8; no trisomy effects	Chromosomes 7 and 8; in interphase CFU-GM cells	Zhang et al. (2012a)
722 AML cases, 78 with benzene exposure (10.8%), 38 > 0.31 ppm			– *	–5/5q–, –7/7q–, +8, del(20q) and 11q23/*MLL*; t(8,21), t(15,17) * – for benzene-exposed vs non-exposed AML cases	Irons et al. (2013)

[a] Benzene exposure level conversion: 1 ppm = 3.19 mg/m^3 = 3190 ug/m^3

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

AML, acute myeloid leukaemia; CA, chromosomal aberration; CFU-GM, colony-forming-unit granulocyte-macrophage; CWAS, chromosome-wide aneuploidy study; dr, dose–response relationship; FISH, fluorescence in situ hybridization; GM, geometric mean; h, hour(s); HQ, hydroquinone; LOD, limit of detection; MDS, myelodysplastic syndrome; *MLL*, mixed lineage leukaemia gene; mo, month(s); NR, not reported; PBL, peripheral blood lymphocyte; ppm, parts per million; TWA, time-weighted average; vs, versus

–5q, and –7q, as well as for +8 in PBLs, have been observed (Zhang et al., 1998a, 1999, 2011). Zhang et al. (2012a) also observed a significant exposure-related trend for –7, but not +7 or +8, in circulating interphase colony-forming-unit (CFU) granulocyte-macrophage (GM) cells, myeloid progenitor cells that are probable targets for the induction of myeloid leukaemia. In independent studies with PBLs, the benzene-associated aneuploidy of chromosome 7 (Kim et al., 2010) and +8 (Kim et al., 2004b) were confirmed. Zhang et al. (2007) also observed a significant exposure-related trend for trisomy 12.

In studies on several translocations implicated in some cancers of the lymphoid and haematopoietic tissues, Smith et al. (1998) observed a significant exposure-related trend using fluorescence in situ hybridization testing for t(8,21). This association was independently confirmed by Kim et al. (2004b), but not replicated using polymerase chain reaction analysis (McHale et al., 2008; IARC, 2012). Similarly, using fluorescence in situ hybridization Zhang et al. (2007) observed a significant exposure-related increase in t(14,18) in the group exposed to the highest concentration of benzene, but this was not replicated in the polymerase chain reaction analyses of McHale et al. (2008). In addition, Zhang et al. (2007) did not observe significant trends for some common translocations involving the *MLL* (mixed lineage leukaemia) gene on chromosome 11q23; however, a significant exposure-related trend was observed for –6q. McHale et al. (2008) did not observe an exposure-related effect on t(15,17).

In vitro studies similarly report certain cytogenetic changes with exposure to benzene or its metabolites. For example, Chung & Kim (2002) reported significant concentration-related trends for –5 and –7, as well as inductions of +8 without significant trends, in human lymphocytes treated with the benzene metabolites hydroquinone, benzenetriol, or t,t-MA, although no t(8,21) translocations were observed. Similarly, Zhang et al. (1998b) reported significant increases in –5 and –7, as well as in –5q and –7q, in human lymphocytes treated with hydroquinone or benzenetriol. Smith et al. (2000) reported that hydroquinone also induced significant positive dose–response relationships in +8 in CD34+ cells and in –7 in both CD34+ and CD34– cells from human cord blood, CD34+ cells being haematopoietic progenitor cells. Stillman et al. (1997, 1999) observed increased –5 and –7 in a human lymphoblastoid cell line from exposure to hydroquinone, but not to catechol. Stillman et al. (1999) further found that catechol acted synergistically with hydroquinone to induce significant positive dose–response relationships in –5 and –7, as well as –5q, which was not observed for hydroquinone alone. A concentration-related trend for +8 was also observed, although it was reportedly not statistically significant. In addition, Stillman et al. (2000) treated human CD34+CD19– bone marrow cells with hydroquinone and reported significant concentration-related trends for –7 and –5q but not –5 or +8; further, a greater susceptibility to hydroquinone- induced –5q and –7 was seen in the bone marrow cells than in the lymphoblastoid cell line. Zhang et al. (2005) exposed lymphocytes in whole blood to hydroquinone or benzenetriol, and reported that chromosomes 5 and 7 were selectively more susceptible to loss induced by those benzene metabolites than several other chromosomes that were examined; further, chromosome 8 was one of a few chromosomes that were more susceptible to gain. Also of note, similar to the findings of Zhang et al. (2007) in workers exposed to benzene, no increases in translocations involving 11q23 were observed in a lymphoblastoid cell line (TK6) treated with hydroquinone (Ji et al., 2009).

In addition to the investigation of chromosomal end-points in healthy people exposed to benzene, some studies have examined cytogenetic changes in cases of acute myeloid leukaemia (AML) or myelodysplastic syndromes

(MDS) in people who have likely been exposed to benzene. Zhang et al. (2002) reviewed 18 cases of AML attributed to benzene exposure from case reports with cytogenetic analyses. Only 1 of the 18 cases had a normal karyotype (Zhang et al., 2002), in contrast to some cytogenetic studies which reported over 40% of de novo cases of AML with a normal karyotype (Schoch et al., 2004; Sanderson et al., 2006). Zhang et al. (2002) also reviewed over 30 abnormal karyotypes from leukaemia patients with likely prior benzene exposure from several large-scale leukaemia studies, and noted that several cases exhibited the same translocation (e.g. t(8,21) for AML and t(9,22) for chronic myeloid leukaemia). Overall, Zhang et al. (2002) found that there were insufficient data from which to discern a specific pattern of clonal chromosomal changes in patients with leukaemia associated with benzene, indicating that benzene produces a variety of cytogenetic changes that may induce or contribute to leukaemogenesis.

Irons et al. (2013) investigated 722 AML cases identified in Shanghai, China and determined that 78 cases had likely benzene exposure. Irons et al. (2013) compared the cytogenetic findings in the 78 cases exposed to benzene first with those from the 644 unexposed cases and then with those from several studies of therapy-related AML. In a subsequent study of 710 of these AML cases, 75 of which were determined to have likely been exposed to benzene, Kerzic & Irons (2017) assessed chromosome breakpoints across 441 identifiable regions. Likewise, Irons et al. (2010) studied 649 MDS cases in Shanghai, China, and determined that 80 cases had likely been exposed to benzene, 29 of which had likely been exposed to high concentrations (> 21 ppm) of benzene. Irons et al. (2010) first compared the cytogenetic findings in the cases exposed to benzene with those from all of the MDS cases. A case–case analysis was then conducted, in which each of the 29 highly exposed cases was matched by age and sex to two cases with no suspected benzene exposure, and levels of various abnormalities characteristic of t-MDS in the highly exposed cases were compared with levels in the unexposed cases. [The Working Group noted that the implications of the reported comparisons between cases exposed to benzene and unexposed cases are uncertain, given that only a portion of the MDS cases in those exposed to benzene were actually attributable to benzene, which can have a diluting effect.]

(ii) Experimental systems

Benzene induced CAs, MN, and SCEs in bone marrow cells of mice, CAs in bone marrow cells of rats, rabbits, and Chinese hamsters, and sperm-head anomalies in mice treated in vivo. Most of the induced aberrations were breaks or deletions. Chromosome-type aberrations also occurred however, particularly after prolonged exposure when toxicity, manifested by a drop in the peripheral blood leukocyte count, appeared. Benzene did not induce SCE in rodent cells in vitro, but it did induce aneuploidy and CAs in cultured Syrian hamster embryo cells. Benzene induced mutation and DNA damage in some studies in rodent cells in vitro. In *Drosophila*, benzene was reported to be weakly positive in assays for somatic mutation and for crossing-over in spermatogonia; in single studies, it did not induce sex-linked recessive lethal mutations or translocations. It induced aneuploidy, mutation, and gene conversion in fungi. Benzene was not mutagenic to bacteria (IARC, 1982, 1987, 2012). In agreement with a possible role of combinations of multiple metabolites of benzene in genotoxicity, Barale et al. (1990) demonstrated that combinations of phenol and hydroquinone were highly genotoxic to mouse bone marrow as indicated by the formation of MN.

In utero exposure to benzene increased the frequency of MN and SCEs in haematopoietic tissue of fetal and postnatal mice (Ning et al., 1991; Xing et al., 1992). French et al. (2015) observed a dose-dependent increase in benzene-induced

chromosomal damage and estimated a benchmark concentration limit of 0.205 ppm benzene using Diversity Outbred mice. This estimate is an order of magnitude below the value estimated using B6C3F1 mice.

After exposure of mice to benzene, DNA adducts were detected by ^{32}P-postlabelling in both the bone marrow and leukocytes (Bodell et al., 1996; Lévay et al., 1996). Mild but statistically significant mutagenic responses were found in transgenic mice carrying the *lacI* reporter gene exposed to benzene (Mullin et al., 1995; Provost et al., 1996). The clastogenic potential of benzene is partly due to its metabolites. Specifically, benzene oxide, benzoquinones, muconaldehydes, and benzene dihydrodiol epoxides are electrophiles that readily react with peptides, proteins, and DNA (Bechtold et al., 1992b; McDonald et al., 1993; Bodell et al., 1996; Gaskell et al., 2005; Henderson et al., 2005a; Waidyanatha & Rappaport, 2005), and can thereby interfere with cellular function (Smith, 1996).

The importance of CYP2E1 (see Section 4.1) in inducing benzene toxicity was shown in studies of *Cyp2e1*$^{-/-}$ mice, in which no benzene-induced cytotoxicity or genotoxicity were observed (Valentine et al., 1996). Similar studies showed the importance of NQO1, which detoxifies benzoquinones, proposed toxic metabolites of benzene. Compared with *NQO1*$^{+/+}$ mice, *NQO1*$^{-/-}$ mice exhibited more severe benzene-induced haematotoxicity and were more sensitive to benzene-induced MN formation in peripheral blood cells. These results indicate that NQO1 deficiency results in substantially greater benzene-induced toxicity. However, the specific patterns of toxicity differed between the male and female mice (Bauer et al., 2003). In fact, male mice were more sensitive than females to the induction of MN by benzene administered either orally or intraperitoneally (Meyne & Legator, 1980; Siou et al., 1981). This may be due, at least in part, to a function of greater oxidative metabolism in male mice (Kenyon et al., 1996). Castration of males reduces their sensitivity to that of females (Siou et al., 1981).

4.2.3 Altered DNA repair or genomic instability

Several DNA reactive metabolites are formed during benzene metabolism, and the type and the frequency of lesions, the respective DNA repair systems involved in their removal, and the repair capacity of the target organ are influenced by the different metabolites (Winn, 2003; Pandey et al., 2009; Au et al., 2010; Hartwig, 2010). Table 4.7 reports examples of in vivo and in vitro studies indicating altered DNA repair or epigenetic alterations related to benzene exposure or its metabolites.

Benzene exposure at occupational and environmental concentrations influences DNA repair systems in human studies in vivo, as reviewed by Ravegnini et al. (2015). In subjects who worked at a spray-painting plant, the exposure to benzene had significantly altered mRNA expression of some critical cell regulatory and DNA repair genes such as *Xpc*, *Xpa*, and *Apel* (Wang et al., 2012). Exposure to a time-weighted average concentration of benzene in a workplace of up to 1.8 mg/m^3 may cause chromosomal damage in workers; in particular, the *XRCC1* rs25487 and rs1799782 polymorphisms may be associated with an increase in MN frequency (Huang et al., 2016). Frequencies of MN and CAs in 108 petroleum refinery workers exposed to 0.51 ppm of benzene (full-shift time-weighted average) were higher than in 33 office workers, and the frequencies were influenced by the polymorphism of the *XRCC1* gene (Kim et al., 2008).

In human cell systems and in exposed mice, chemically reactive benzene metabolites, particularly 1,4-benzoquinone and hydroquinone, directly inhibited isolated topoisomerase II (Frantz et al., 1996; Hutt & Kalf, 1996; Eastmond et al., 2001, 2005; Ji et al., 2009). Possible mechanisms of this inhibition include covalent binding

Table 4.7 Studies of benzene or metabolites indicating altered DNA repair, genomic instability, or epigenetic alterations

Description of exposed and controls Study type Tissue	Benzene exposure (range or median) or its metabolites	Comments	Reference
DNA repair or genomic instability			
In vitro Isolated topoisomerase IIα	BQ, 10 μM; HQ 10 mM	Metabolites catalytically inhibited topoisomerase II	Baker et al. (2001)
In vitro Isolated topoisomerase IIα; human CEM leukaemia	BQ, 0–100 μM BQ, 10 μM	BQ strongly inhibited topoisomerase II BQ underwent covalent binding with topoisomerase IIα	Lindsey et al. (2004)
108 exposed petroleum refinery workers, 33 controls Occupational exposure	0.004–4.52 ppm	Both the CA and MN frequencies were significantly higher in exposed compared with unexposed workers and influenced by polymorphism of XCCR1 gene	Kim et al. (2008)
Spray painters: A, 46 direct exposed; B, 26 indirect exposed; C, 29 controls Occupational exposure	A, 0.21 ± 0.19 mg/m^3; B, 0.06 ± 0.12 mg/m^3; C, ND	The mRNA expression levels of *Rad51*, *Bcl-2*, *Bax*, *Apel*, *Xpa*, and *Xpc* in groups A and B were downregulated significantly compared with group C	Wang et al. (2012)
Haematopoietic stem and progenitor cells, human CD34+ cells In vitro	0.5–1 mg/mL	DNA breakage	Thys et al. (2015)
CD-1 mouse fetal liver cells In vitro	BQ, 5, 15, and 25 μM	Benzoquinone exposure significantly decreased the transcript levels of 8-oxo-guanine glycosylase	Philbrook & Winn (2016)
Epigenetic alterations			
DNA methylation			
78 gas station attendants, 77 urban traffic officers, and 57 controls Occupational exposure	0.040–0.132, 0.09–0.031, and < 0.006–0.014 mg/m^3	Airborne benzene was associated with hypomethylation of *Line-1* and *AluI*	Bollati et al. (2007)
In vitro Human lymphoblastoid TK6 cells	Benzene: 1, 10, and 100 μM; HQ, 0.005, 0.05, and 0.5 μM	Benzene and its metabolite HQ exposure induced global DNA hypomethylation in TK6 cells	Tabish et al. (2012)
In vitro Human hepatic L02 cells	Benzene, HQ, and BQ: 5, 10, 25, and 50 μM	HQ and 1,4-BQ, but not benzene, induced global DNA hypomethylation	Hu et al. (2014)
Histone modifications			
In vitro Human myeloid leukaemia HL-60 cells	HQ Single treatment: 1, 5, 15, and 25 μM Repeated treatment: 1, 5, and 15 μM four times every 48 h Long-term treatment with 1μM: five times a week for 5 wk	Epigenetic modifications (instauration in *LINE-1* sequences) after in vitro treatment with HQ were transitory and reversible	Mancini et al. (2017)

BQ, benzoquinone; CA, chromosomal aberration; CEM, human acute lymphoblastic leukaemia cells; h, hour(s); HQ, hydroquinone; *LINE-1*, long interspersed nuclear element-1; MN, micronuclei; ND, not detectable; ppm, parts per million; wk, week(s)

or catalytic action (Baker et al., 2001; Lindsey et al., 2004, 2005; Chen et al., 2016a).

Benzoquinone exposure significantly decreased the transcript levels of the critical base excision repair gene, 8-oxo-guanine glycosylase, in CD-1 mouse fetal liver cells in vitro (Philbrook & Winn, 2016); it was also able to rapidly increase ROS production, followed by a statistically significant increase in both c-H2A.X foci and DNA recombination in fetal haematopoietic cells (Tung et al., 2012).

4.2.4 Immunosuppression

This section focuses on the studies that directly or indirectly inform immune response outcomes, and is divided into haematotoxicity (inclusive of all such data in Section 4), genes related to immune function, and immunoproteins.

(a) Humans

(i) Haematotoxicity

Acute exposure to benzene has been associated with diseases and symptoms in the blood-forming system such as aplastic anaemia, specific cytopenias, and pancytopenia (Aksoy et al., 1971; Yin et al., 1987; IARC, 2012). These diseases are associated with a functional reduction in immune competence by virtue of the reduced number of immunocompetent cells resulting from impaired haematopoiesis (IARC, 2012; McHale et al., 2012). In addition, several studies have found that various levels of severity of benzene-associated haematotoxicity have been associated with a future risk of developing a haematological malignancy or related disorder (Aksoy & Erdem, 1978; Yin et al., 1987; Rothman et al., 1997).

Many studies investigating the association between benzene exposure and altered blood cell counts reported haematological changes in exposed humans, especially at relatively high levels of exposure (e.g. > 10 ppm) (Rothman et al., 1996; Ward et al., 1996; Qu et al., 2002; Lan et al., 2004); some studies have demonstrated that haematological alterations can also occur at lower levels of exposure (< 10 ppm) (Ward et al., 1996; Zhang, 1996; Qu et al., 2002; Lan et al., 2004; Miao & Fu, 2004; Uzma et al., 2008; Robert Schnatter et al., 2010; Chen et al., 2012; Wang et al., 2012; Zhang et al., 2016). In particular, leukocyte counts were consistently reduced in an exposure-related manner (Rothman et al., 1996; Ward et al., 1996; Qu et al., 2002; Lan et al., 2004; Robert Schnatter et al., 2010). Reductions in leukocyte counts were observed with median benzene air concentrations of 1.2 ppm in Lan et al. (2004) and 3.8 ppm (4-week average) in Qu et al. (2002), with lowered counts in subgroups of workers exposed to less than 1 ppm. Decreased neutrophil counts were associated with benzene exposure down to a level of about 7.8–8.2 ppm (Robert Schnatter et al., 2010). However, numbers of band neutrophils, which are precursors and later mature into granulocytes, were increased as well as mean corpuscular volume (Bogadi-Sare et al., 2003).

A few studies reported no statistically significant differences in blood cell counts (Hancock et al., 1984; Kipen et al., 1989; Biró et al., 2002). Additionally, several studies reported no or minimal changes in haematological parameters in workers with occupational exposures of less than 5 ppm, in particular, less than 1 ppm. Several of these studies used historical haematological data collected as part of routine surveillance (Collins et al., 1991, 1997; Tsai et al., 2004; Swaen et al., 2010). [The Working Group noted that the timing of collection of blood samples relative to the most recent benzene exposure was not reported.]

Total lymphocyte counts were reduced in humans exposed to benzene (Rothman et al., 1996). Numbers of circulating CD19+ B-lymphocytes were consistently reduced in several studies (Rothman et al., 1996; Bogadi-Sare et al., 2000, 2003; Lan et al., 2004). CD4+ T-lymphocytes were consistently decreased in

multiple studies (Luan, 1992; Lan et al., 2004; Kirkeleit et al., 2006; Uzma et al., 2008; Chen et al., 2007, 2012; Wang et al., 2012); however, CD8+ T-lymphocytes populations were increased (Chen et al., 2012). In a study of paint factory workers exposed to benzene, a continual increase in the percentage of CD8+ T-cells measured every 4 months for a year was observed (Chen et al., 2012). No significant change in absolute number of CD8+ cells was observed in other studies (e.g., Chen et al., 2007). The decreased CD4+ and increased CD8+ T-cells resulted in a lowering of the CD4+/CD8+ ratio (Luan, 1992; Lan et al., 2004; Chen et al., 2007, 2012; Wang et al., 2012). Increased CD3+ lymphocytes were additionally noted (Chen et al., 2012).

Benzene exposure also reduced T-cell receptor excision circles (TRECs), a marker of T-cell maturity. Decreased TRECs in peripheral blood mononuclear cells (PBMCs) of patients with benzene poisoning were found in two separate studies, suggesting impaired T-cell immune function (Li et al., 2005, 2009a). Decreased TRECs were also found in the peripheral blood mononuclear cells in 62 workers exposed to benzene at a concentration in air of 1.72–37.8 mg/m^3 compared with 11 healthy controls (Han et al., 2004). However, Lan et al. (2005a) reported no significant difference in TREC levels in shoe factory workers exposed to benzene at a mean concentration in air of 15.8 ppm.

Relatively low levels of benzene (i.e. < 5 ppm) could result in haematological suppression after continuous exposure with no observed threshold for a response (Ward et al., 1996). Most types of blood cells, with the exception of leukocytes, from complete blood count levels were decreased in workers exposed to benzene, correlated with length of employment (Khuder et al., 1999).

Overall, decreased red blood cell counts (Rothman et al., 1996; Khuder et al., 1999; Qu et al., 2002; Miao & Fu, 2004; Koh et al., 2015), platelets (Rothman et al., 1996; Qu et al., 2002; Uzma et al., 2008; Ye et al., 2008; Chen et al., 2012; Wang et al., 2012), and haemoglobin content (Bogadi-Sare et al., 2003; Wang et al., 2012; D'Andrea & Reddy, 2016) were consistently reported. Benzene exposure increased haemoglobulin content and platelets. Haemoglobin content and red blood cell counts in workers who had been exposed to benzene for longer periods were significantly increased compared with controls (Uzma et al., 2008). Chemical and rubber factory workers who had been exposed to benzene at 0.07–872.0 mg/m^3 (median level, 7.4 mg/m^3) had reduced red blood cell count and mean platelet volume, the most affected haematological peripheral blood parameters (Robert Schnatter et al., 2010).

Finally, several studies examined circulating haematopoietic stem and/or progenitor cells, which may also be affected in individuals exposed to benzene. In a cross-sectional study of 17 petroleum refinery workers exposed to very low levels of benzene (0.28–0.41 ppm), increased burst-forming-unit erythroid and CFU-GM colonies without any growth stimulation were observed compared with 20 unexposed controls; this effect was not observed after the addition of growth factors, either erythropoietin (EPO) or granulocyte colony-stimulating factor (Quitt et al., 2004). However, another study of 10 subjects with occupational exposure to more than 10 ppm benzene (mean, 24.2 ppm), 19 subjects with exposure to less than 10 ppm benzene (mean, 2.6 ppm), and 24 controls with no occupational exposure to benzene reported an inverse monotonic exposure–response relationship with haematopoietic progenitor cell colony formation in cultured peripheral blood, including: CFU granulocyte, erythroid, macrophage, and megakaryocyte (with EPO stimulation); CFU-GM (with and without EPO stimulation); and burst-forming-unit erythroid cells (with EPO stimulation) (Lan et al., 2004).

(ii) Genes related to immune function

Several studies have investigated human susceptibility to benzene exposure and its relationship with single-nucleotide polymorphisms in genes that encode immune-related proteins. For instance, the tumor necrosis factor alpha (TNF-α) single-nucleotide polymorphism was associated specifically with an increased risk of persistent benzene-induced dysplasia in workers (Lv et al., 2007), and a significantly higher frequency of TNF-α was observed in benzene-poisoned patients (Lv et al., 2005).

Corresponding to altered leukocyte counts in benzene-exposed shoe factory workers, changes were reported in the expression of various genes, including the vascular cell adhesion molecule *VCAM1*, interleukin (*IL*)*-1A*, *IL-4*, *IL-10*, *IL-12A*, *CSF3*, *MPO*, and *CRP* (Lan et al., 2005b; Shen et al., 2011). Gene expression related to T-cells was also altered. The distributions of the T-cell receptor variable (TCRV) family TCRVα, TCRV*β*, and TCRVγ gene repertoires in individuals exposed to benzene were significantly lower compared with the reference group (Chen et al., 2006; Li et al., 2007, 2008, 2009b). PBMC gene expression levels of *CD3δ*, *CD3ε*, and *CD3ζ* were increased in workers exposed to benzene versus controls; in workers diagnosed with benzene poisoning, however, some regions were decreased in severe cases and other regions were unchanged (i.e. *CD3γ* and *CD3ε*) in mild cases (Li et al., 2012). *CXCL16*, a gene responsible for encoding a chemokine that activates T-cells and natural killer cells, was found to be consistently upregulated in workers exposed to benzene (Forrest et al., 2005; McHale et al., 2009).

(iii) Immunoproteins

Regarding B-cell effects, immunoglobulin (Ig) G production was positively correlated with air benzene levels (Bogadi-Sare et al., 2000). Increased IgG was also reportedly correlated with benzene urinary metabolite t,t-MA measured in petrochemical workers exposed to benzene (Dimitrova et al., 2005). IgM and IgA were reduced in cargo tank workers exposed to benzene (Kirkeleit et al., 2006). Furthermore, one study reported that plasma concentrations of soluble CD27 and CD30, two immune markers indicative of B-cell activation, were decreased by 17% for sCD27 but non-significantly reduced for sCD30 in the group exposed to the highest concentration of benzene (≥ 10 ppm) compared with control workers, after adjusting for age and sex (Bassig et al., 2016). [The Working Group noted that several prospective cohorts, although not specifically related to benzene, found that higher levels of sCD27 were associated with increased risk of non-Hodgkin lymphoma (Purdue et al., 2011; De Roos et al., 2012; Bassig et al., 2015a; Hosnijeh et al., 2016; Späth et al., 2017).]

Similar to effects in cytokine gene expression, cytokine serum concentrations were also modified. TNF production was significantly reduced in paint factory workers exposed to benzene vapours (Haro-García et al., 2012). IL-10 serum concentrations were positively correlated with the number of working years in those exposed to benzene (Spatari et al., 2015).

(b) Human cells in vitro

Alterations in cytokine production were also observed in studies of human cells in vitro. Both IL-1α and IL-1β were decreased in human blood monocytes after exposure to hydroquinone (Carbonnelle et al., 1995). Catechol, hydroquinone, 1,2,4-benzenetriol, and *p*-benzoquinone were also found to stimulate the production of T-helper cell (Th2) cytokines IL-4 and IL-5 (Gillis et al., 2007).

Supporting the haematotoxicity observed in humans, phenol, hydroquinone, and 1,2,4-benzenetriol decreased haemoglobin synthesis in K562 cells in a concentration-dependent manner (Wu et al., 2011). CD34+ haematopoietic progenitor cells treated with hydroquinone inhibited erythroid differentiation

in an exposure-related response, and miRNA-451a and miRNA-486–5p were upregulated during erythroid differentiation (Liang et al., 2017).

(c) Experimental systems

(i) Mouse

Several murine studies demonstrated consistent immunosuppressive effects on assays for humoral and cell-mediated immune function after oral and inhalation exposure. The only animal study to evaluate the effect of benzene exposure on the ability of T-cells to respond to a tumour challenge was conducted by Rosenthal & Snyder (1987), who exposed C57Bl/6 male mice to three concentrations (10, 30, and 100 ppm) of benzene for 100 days before tumour challenge. Inhalational exposure to 100 ppm increased lethal tumour incidence, which suggests reduced tumour surveillance (Rosenthal & Snyder, 1987). Further examination demonstrated that the same benzene concentration reduced T-cell cytolytic activity after 20 days of exposure, and reduced proliferative responses in the spleen in the mixed lymphocyte reaction (Rosenthal & Snyder, 1987). These reductions in cytotoxic and proliferative activity all occurred without any corresponding changes in the total number of T-cell or lymphocyte subpopulations in the spleen (Rosenthal & Snyder, 1987). In addition, Rosenthal & Snyder (1985) also demonstrated that 9 days of continuous inhalation exposure to benzene reduced cell-mediated immunity to bacterial infection. Exposure to benzene at several concentrations (30–300 ppm) increased bacterial load after a 4-day infection by the intracellular pathogen *Listeria monocytogenes*. Accompanying this effect, total lymphocytes and T- and B-cell populations were all reduced in the spleen for up to 7 days post-infection under the same benzene exposure concentrations (Rosenthal & Snyder, 1985).

Changes in assays for humoral immune function were first observed in BALB/c male mice after inhalation exposure to benzene at concentrations of 50 or 200 ppm for 14 days (Aoyama, 1986). Seven days after immunization with sheep red blood cells (SRBC), both benzene concentrations reduced IgM SRBC-specific plaque-forming cells (PFC) by up to 87% relative to controls, and IgG PFCs were reduced by approximately 94% (Aoyama, 1986). IgG PFCs remained suppressed 10 days after immunization, and IgM PFCs were not significantly different from controls. Reductions in total lymphocytes and B- and T-cells in the blood were also observed at the same concentration.

The oral exposure database of assays for immune function is less robust than that for inhalation; only one study has reported reduced humoral immunity. Male CD-1 mice were exposed to benzene in drinking-water at a concentration of 166 mg/L (the only tested dose) for 28 days, resulting in reduced IgM SRBC-specific PFCs as well as anti-SRBC antibody titres (Hsieh et al., 1990). Reductions were also observed in the mixed lymphocyte response and T-cell proliferation in response to concanavalin A and phytohaemagglutinin stimulation, and in the B-cell proliferative responses to pokeweed mitogen and lipopolysaccharide stimulation. In addition, benzene exposure increased serum corticosterone, which is known to suppress immune function (Hsieh et al., 1991). Different results were observed in a study of BALB/c male mice exposed to benzene by oral gavage at a concentration of 150 mg/kg bw per day (8 hours per day/5 days a week, for 2 weeks); no changes in total serum antibody titres were reported, but reduced counts of leukocytes, total lymphocytes, monocytes, and neutrophils in the blood were observed (Wen et al., 2016). In C57BL/6 mice exposed orally to benzene at 27 mg/kg bw per day for 28 days, briefly increased splenic natural killer cell activity was observed by day 21 and

splenic production of IL-2 was reduced by day 28 (Fan, 1992).

The haematotoxic effects of benzene exposure are well established in experimental animals (Cronkite et al., 1985; Farris et al., 1997a). Rats and mice of both sexes exhibited leukocytopenia and anaemia after subchronic inhalation exposure, but only mice demonstrated evidence of severe femoral hypoplasia (Ward et al., 1985). Nucleated bone marrow cells were significantly reduced in $B6C3F_1$ mice after inhalation exposure at a concentration of 100 ppm benzene for 8 weeks (Farris et al., 1997a). From analysis of the differentiation and maturation of haematopoietic precursor cells, exposure to benzene at 200 ppm for 8 weeks resulted in a sustained reduction of the primitive precursor CFU high proliferative progenitor cells, downstream progenitor CFU-GM cells, bone marrow granulocytes, and leukocytes in the blood (Farris et al., 1997a). In C57BL/6 mice exposed intraperitoneally to hydroquinone at 50 mg/kg bw per day or to benzene at 600 mg/kg bw per day for 2 days, or in DBA/2J mice exposed to benzene at 10 ppm via inhalation for 5 days, CFU-GM proliferation was not significantly affected but differentiation was significantly increased (Dempster & Snyder, 1991; Hazel et al., 1996). When Swiss Webster mice of both sexes were exposed to benzene in utero at 10 ppm and re-exposed to benzene at 10 ppm at age 10 weeks, greater reductions in splenic CFU-GM were observed compared with mice that were not exposed to benzene in utero (Keller & Snyder, 1986). Intraperitoneal exposure to benzene increased the production of nitric oxide in bone marrow cells, which may contribute to the reduced proliferation (Punjabi et al., 1994). Benzene exposure was also found to suppress the progenitor cell cycle of CFU-GM in the bone marrow of C57BL/6 mice by overexpressing the cyclin-dependent kinase inhibitor p21 (Yoon et al., 2001).

(ii) Rat

Two rat studies examined the effect of benzene exposure on assays for immune function: one oral and one inhalation. In male Wistar rats exposed to a single dose of benzene in drinking-water at 0.6 mL/kg of drinking water per day for 90 days, reductions in the total number of SRBC-specific antibody-forming cells in the spleen by 40%, and in the total anti-SRBC serum titres by 64% after immunization with SRBC (immunization protocol not specified), were observed (Karaulov et al., 2017). Cell-mediated immunity, assessed by the delayed-type hypersensitivity response, was also reduced by 52% compared with controls. Karaulov et al. (2017) additionally examined the effect of benzene exposure after 45, 90, and 135 days by stimulating splenocytes ex vivo with the concanavalin A; increased cytokine production of IL-4 and IL-6 and a reduced number of CD4+ T-cells were reported for all time periods.

In male Sprague-Dawley rats exposed to benzene via inhalation at a range of concentrations (30–400 ppm) for 2 or 4 weeks, no changes in anti-SRBC serum antibodies were induced (Robinson et al., 1997). However, the highest tested concentration reduced the numbers of splenic B-cells after 2 and 4 weeks of exposure and of CD4+/CD5+ T-helper cells after 4 weeks of exposure.

4.2.5 Altered cell proliferation, cell death, and nutrient supply

(a) Humans

Representative studies were included if the biological end-point was considered relevant for this key characteristic (studies of peripheral blood cell counts, benzene poisoning, cultured haematological progenitor cells, and genetic susceptibility to these events are presented in Section 4.2.4(a)). The Working Group focused on studies in which presence of benzene in the study population was documented, the presence

of co-exposures was evaluated and addressed, the control group was comparable to the exposed study population, and the study had adequate statistical power.

A case series report in China of 23 subjects with a history of benzene poisoning, with quantitative data for 17 subjects indicating very high exposure to benzene before diagnosis (i.e. concentration in air at 50–300 ppm), described a distinct pattern of bone marrow dysplasia including marked dyserythropoiesis, eosinophilic dysplasia, and abnormal cytoplasmic granulation of neutrophilic precursors. In addition, clonal and oligoclonal proliferation in bone marrow T-lymphocytes, including clonal rearrangements in T-cell receptor gene segments, was present in 14 out of 23 cases (Irons et al., 2005).

Several cross-sectional studies of workers with occupational exposure to benzene and unexposed controls measured miRNA in peripheral leukocytes or plasma and mRNA in leukocytes, and found altered levels of these end-points for genes that play a role in apoptosis; these studies provided indirect evidence of the possible influence of benzene on apoptosis in healthy subjects (Forrest et al., 2005; Sun et al., 2009; McHale et al., 2011; Wang et al., 2012; Li et al., 2014; Chen et al., 2016b, 2017; Hu et al., 2016; Liu et al., 2016).

In studies in vitro, benzene or its metabolites induced apoptosis in CD34+ human bone marrow progenitor cells, PBLs, PBMCs, bone marrow mesenchymal stem cells, and HL-60 human promyelocytic leukaemia cells (Moran et al., 1996; Ross et al., 1996a; Wiemels & Smith, 1999; Bratton et al., 2000; Nishikawa et al., 2011; Hu et al., 2012; Lee et al., 2012; Peng et al., 2012; Zolghadr et al., 2012). Inhibition of nuclear-factor kappa-light-chain-enhancer of activated B-cells (NF-κB) by hydroquinone sensitizes human bone marrow progenitor cells to TNF-α-induced apoptosis (Kerzic et al., 2003). Inhibition of DNA-dependent protein kinase, catalytic subunit, potentiated the apoptotic and growth inhibitory effects of hydroquinone in proerythroid leukaemia K562 cells (You et al., 2013). Apoptosis was prevented when NQO1 was induced by hydroquinone in KG-1a human promyeloblastic leukaemia cells. Induction of NQO1 by hydroquinone in human bone marrow cells depends on its genotype (Moran et al., 1999); in cells with a T/T genotype, NQO1 activity and protein were not detected (Ross et al., 1996b; Traver et al., 1997). This finding is consistent with the observation that the NQO1 null genotype increases the risk of benzene poisoning (i.e. haematotoxicity) (Rothman et al., 1997).

The benzene metabolite orthoquinone stimulated hyperproliferation of human mononuclear cells cultured with T- and B-cell mitogens (Chakravarti et al., 2006).

(b) Experimental systems

Benzene is reported as a bone marrow depressant as it decreases cell counts in circulating blood, bone marrow, and haematopoietic progenitor cells of animals treated with benzene (IARC, 1982). The cycling fraction of bone marrow or progenitor cells is also suppressed during exposure to benzene, although this suppression is rapidly reversed when exposure to benzene ceases (Moeschlin & Speck, 1967; Irons et al., 1979; Cronkite et al., 1982; Lee & Garner, 1991; Farris et al., 1997a). Suppression of the number of progenitor cells as well as of their cycling fraction is induced by a p53-mediated checkpoint for damaged cells (Kastan et al., 1991; el-Deiry et al., 1994), as evidenced by the lack of suppression of either parameter in the Trp53 knockout mouse (Yoon et al., 2001). On and off regulation of Trp53 therefore results not only in the direct suppression of haemopoiesis but also in a dynamic recovery proliferation after suppression of haemopoiesis during and after benzene exposure in wildtype mice. These dynamic changes may be responsible for the oscillatory proliferation of bone marrow cells to counter any additional epigenetic haematopoietic

neoplastic impacts (Yoon et al., 2001). Indeed, the studies of Snyder and co-workers (Snyder et al., 1981; Dempster & Snyder, 1990) demonstrated that exposing mice to benzene by inhalation for varying periods of time resulted in a growth advantage for granulopoietic cells and proliferation of myeloblasts and/or promyelocytes.

Benzene has been shown to induce apoptosis in murine haematopoietic cells in vitro (Martínez-Velázquez et al., 2006; Gao et al., 2011), as well as spleen cells, femoral B-lymphocytes, and thymic T-lymphocytes in vivo (Farris et al., 1997b; Wen et al., 2016). In mice exposed to benzene by inhalation at 100 ppm for 6 hours per day, 5 days per week for 2 weeks, no change in the level of apoptosis in bone marrow as measured by flow cytometric analysis using Annexin V staining (Faiola et al., 2004) was observed. However, genes involved in apoptosis (Trp53-mediated *caspase 11*, *bax*, and *ccng*) were upregulated in the bone marrow cells of mice exposed to benzene by inhalation at 300 ppm for 6 hours per day, 5 days per week for 2 weeks (Yoon et al., 2003). In mice exposed to benzene by inhalation at 300 ppm for 6 hours per day, 5 days per week, a higher ratio of apoptosis (i.e. Annexin V staining) in bone marrow cells was observed on day 60 after the start of the experiment when compared with control mice (Das et al., 2012). Trp53-mediated gene expression alterations were also observed in the bone marrow cells of mice exposed to benzene by inhalation at 100 ppm for 6 hours per day, 5 days per week for 15 weeks (Boley et al., 2002). Simultaneously, in mice exposed to benzene a reduction of immune function (phagocytic capacity and cytotoxic efficacy) of cells derived from bone marrow, a reduced generation of adherent stromal cells, and a decreased expression of the adhesion molecule (CXCR4) in bone marrow cells were observed, which might be responsible for inducing myelodysplasia (Das et al., 2012).

Inhalation of benzene at 300 ppm for 23 hours per day for 7 consecutive days induced apoptotic changes in the parenchymal components of the lung of Sprague-Dawley rats. An assay for terminal deoxynucleotidyl transferase dUTP nick end-labelling (TUNEL) and electrophoretic analysis of internucleosomal DNA fragmentation of benzene-exposed lung tissue exhibited 180–200 base pairs of laddering subunits, indicative of genomic DNA degradation (Weaver et al., 2007).

4.2.6 Receptor-mediated effects

Although no data on aryl hydrocarbon receptor (AhR) were available in exposed humans or in human cells, several experimental studies in vitro and in vivo have examined the potential role of AhR in benzene carcinogenicity. This transcription factor appears to be involved in the regulation of immature haematopoietic stem or progenitor cell populations, and AhR dysregulation may result in changes to the bone marrow microenvironment that can lead to excessive or unnecessary proliferation (Singh et al., 2009, 2014). Yoon et al. (2002) reported that AhR-knockout ($AhR^{-/-}$) mice do not show any haematotoxicity after exposure to benzene (Yoon et al., 2002). Follow-up studies reported that mice in which the bone was ablated by irradiation and repopulated with marrow cells from AhR-knockout mice did not display any sign of benzene-induced haematotoxicity (Hirabayashi et al., 2008; Hirabayashi & Inoue, 2010). Benzene and its metabolites hydroquinone and benzoquinone did not activate AhR in mouse hepatoma cells in vitro, suggesting that direct interaction with AhR is not involved in these haematotoxic effects (Badham & Winn, 2007).

4.2.7 Chronic inflammation

(a) Humans

TNF-α (an important mediator of inflammation), IL-6 (a pro-inflammatory cytokine), and IL-8 (a chemokine) were studied in 196

rural Indian women who used benzene-contaminated biomass to cook, and compared with 149 age-matched women who cooked with the cleaner fuel of liquefied petroleum gas (Dutta et al., 2013). This study analysed sputum samples and revealed markedly elevated levels of TNF-α (6.9-fold) as well as significantly higher IL-6 and IL-8 levels in the exposed women, suggesting airway inflammation and trafficking of inflammatory cells from circulation to the airways, compared with control women who cooked with the cleaner fuel (Dutta et al., 2013). [The Working Group noted that the women using biomass were not only exposed to benzene, but also to particulate matter of diameter less than 10 μm (PM_{10}) and other toxic chemicals such as formaldehyde, which may also induce inflammation.]

Several studies in human cells in vitro indicate that several benzene metabolites (t,t-MA, hydroquinone, catechol, benzoquinone, and 1,2,4-benzenetriol) may play important roles in the mechanisms of benzene toxicity and inflammation. Hydroquinone (1–10 M) inhibited TNF-α-induced activation of NF-κB in primary human CD4+ T-lymphocytes and in primary human CD19+ B-lymphocytes (Pyatt et al., 1998, 2000). Gillis et al. (2007) showed that benzene metabolites (catechol, hydroquinone, 1,2,4-benzenetriol, and benzoquinone) increased production of pro-inflammatory cytokines (TNF-α and IL-6) in PBMCs. TNF-α production was increased in a dose-dependent manner. Concurrently, suppression of anti-inflammatory cytokine IL-10 expression was also observed in the activated PBMCs treated with higher concentrations of hydroquinone and catechol (Gillis et al., 2007).

(b) Experimental systems

(i) Mouse

Exposure to benzene for 14 days has been demonstrated to affect inflammation in mouse models in two studies. Aoyama (1986) reported that exposure to benzene by inhalation at 200 ppm for 14 days increased ear swelling in BALB/c mice immunized with the contact sensitizer picryl chloride. In mice given benzene by oral gavage at 150 mg/kg bw per day (8 hours per day/5 days a week, for 2 weeks), slight, but not significant, paw swelling was observed in the delayed-type hypersensitivity test (Wen et al., 2016).

Benzene metabolites were also shown to directly induce inflammatory responses in mice. In C57BL/6 female mice given a single subcutaneous injection (100 nmol/mouse), benzoquinone and (to a lesser extent) hydroquinone, but not benzene itself, was observed to increase popliteal lymph node cell count indices 6 days later as determined by popliteal lymph-node assay (Ewens et al., 1999). Bando et al. (2017) also demonstrated the direct inflammatory capacity of hydroquinone. The ears of BALB/c and C57BL/6 mice were observed to swell within 24 hours of dermal application of hydroquinone at concentrations of as low as 1% and 8%, respectively. Further examination revealed accumulation of Th2 cytokines such as IL-4, decreased Th1 cytokines, and increased accumulation of T-, B-, and natural killer cells, total serum IgE, hydroquinone-specific IgE, macrophages, neutrophils, and eosinophils (Bando et al., 2017). In female BALB/c mice, hydroquinone induced IL-4 and IgE and increased total and keyhole limpet haemocyanin-specific IgE (Lee et al., 2002).

(ii) Rat

In a single rat study that examined the effect of 1 hour of dermal exposure to benzene of hairless male rats, occlusive and unocclusive dermal exposure increased erythema at the site of application. Blood IL-1 and skin concentrations of TNF-α increased by 2.4-fold and 3.7-fold, respectively (Chatterjee et al., 2005).

4.2.8 Epigenetic alterations

Epigenetic alterations related to benzene exposure were observed in studies in vivo and in vitro, as reviewed by Zhang et al. (2010), Chappell et al. (2016), and Salemi et al. (2017).

Epigenetic alterations, including DNA methylation and non-coding RNA, were correlated with benzene exposure (Fenga et al., 2016). Occupational or environmental exposure to benzene can produce epigenomic changes. More recently, the effect of benzene exposure on miRNA expression has been reported in occupationally exposed workers (Liu et al., 2016). Downregulation of miR-133a was observed in 50 workers exposed to benzene at 3.50 ± 1.6 mg/m^3 compared with 50 controls exposed to benzene at 0.06 ± 0.01 mg/m^3 (Chen et al., 2016b). Overexpression of miR-221 was observed in PBLs of 97 petrol station attendants exposed to benzene at 0.073 ± 0.02 mg/m^3 compared with 103 controls exposed to benzene at 0.008 ± 0.001 mg/m^3 (Hu et al., 2016).

The results of in vitro studies of benzene-induced changes in DNA methylation are influenced by cell line type and substance used for the specific experiment, that is, benzene or its metabolite. A global DNA hypomethylation was observed in human lymphoblastoid TK6 cells after exposure to benzene at concentrations of 1, 10, and 100 μM (Tabish et al., 2012), and after exposure to hydroquinone at concentrations of 2.5, 5, 10, 15, and 20 μM in a dose-dependent manner (Ji et al., 2010). In human normal hepatic L02 cells a global DNA methylation change was observed only after exposure to hydroquinone and 1,4-benzoquinone, but not to benzene itself or other metabolites (Hu et al., 2014). A reversible poised state of chromatin, identified by the simultaneous presence of histone modifications associated with both gene activation and repression in long interspersed nuclear element-1 (*LINE-1*) sequences, was observed after an in vitro long-term treatment of human myeloid leukaemia HL-60 cell line with a low-concentration dose (1μM (correspond to 110 ng/mL)) of hydroquinone (Mancini et al., 2017). In human leukaemia U937 cells exposed to 1,4-benzoquinone, Chen et al. (2016b) observed dose-dependent alterations in miR-133a expression.

4.2.9 Other mechanisms

Other effects of benzene primarily concern telomere length and transformation. Bassig et al. (2014) reported that workers who had been exposed to high concentrations of benzene (> 31 ppm) had a mean telomere length that was increased by about 10% compared with matched unexposed workers. A study of human lung cells in vitro reported that exposure to benzene (0.01 and 1 μM) increased telomerase activity in the fibroblast-like human lung LL24 cell line, but not in the human adenocarcinoma A549 cell line at higher concentrations (10 and 1000 μM) (Giuliano et al., 2009).

Two studies in vitro examined indicators of transformation. Tsutsui et al. (1997) reported increases in transformed colonies of Syrian hamster embryo cells after treatment with benzene and its metabolites phenol, catechol, or hydroquinone (1–100 μM), and Ibuki & Goto (2004) described anchorage-independent growth in soft agar after treatment of NIH3T3 cells with benzoquinone and hydroquinone.

4.3 Data relevant to comparisons across agents and end-points

This section analyses the responses and/or activity of benzene, its metabolites (Fig. 4.1), and its agents, as evaluated by IARC in a diverse set of in vitro assays performed as part of the United States Environmental Protection Agency Toxicity Forecaster (ToxCast) (Kavlock et al., 2012) and Toxicology in the 21st Century (Tox21) (Tice et al., 2013) initiatives. The inclusion of analyses from high-throughput in vitro assays in the

evaluation of the carcinogenicity of agents has been identified as a priority by IARC (Straif et al., 2014). Consequently, analyses involving these assays have been part of recent *Monographs* that have evaluated the carcinogenicity of 2,4-dichlorophenoxyacetic acid (2,4-D) and 4,4′-dichlorodiphenyltrichloroethane (DDT) (Loomis et al., 2015).

Benzene has not been tested as part of ToxCast and Tox21. The benzene metabolites that have been evaluated are phenol (IARC Group 3), hydroquinone (IARC Group 3), catechol (IARC Group 2B), and 1,4-benzoquinone (IARC Group 3).

Exposure to agents could potentially lead to human cancer through a diverse set of mechanisms. Each individual agent has a specific pathway or a set of pathways leading to a particular kind of cancer. Despite this heterogeneity in the possible mechanisms, Smith et al. (2016) have identified 10 common characteristics of carcinogens by examining all agents classified as Group 1 carcinogens by IARC. The *IARC Monographs* Volume 113 Working Group (Loomis et al., 2015; IARC, 2017) systemically evaluated the assays performed as part of ToxCast and Tox21 and assigned an estimation of activity in each assay for an agent as an indication of 1 of the 10 key characteristics of the carcinogens. In this *Monograph*, we use these same assignments of activities (i.e. mapping of assays) to the key characteristics. Assays were assigned to 6 out of the 10 key characteristics of carcinogens, namely: is electrophilic or can undergo metabolic activation (31 end-points); induces epigenetic alterations (11 end-points); induces oxidative stress (18 end-points); induces chronic inflammation (45 end-points); modulates receptor-mediated effects (92 end-points); and alters cell proliferation, cell death, or nutrient supply (68 end-points).

The 10 key characteristics are listed in full as follows.

(1) *Is electrophilic or can be metabolically activated*: 31 assay end-points consisting of CYP biochemical activity assays and aromatase, which regulates conversion of androgens to estrogens. [The Working Group noted that these assays largely indicate inhibition of CYP activity, and do not directly measure metabolic activation or electrophilicity.]

(2) *Is genotoxic*: 0 assay end-points.

(3) Alters DNA repair or causes genomic instability: 0 assay end-points.

(4) *Induces epigenetic alterations*: 11 assay end-points including 4 DNA-binding assays in HepG2 liver cell lines, biochemical assays targeting histone deacetylases, and other enzymes modifying chromatin, as well as cellular transcription factor assays involved in epigenetic regulation. [The Working Group noted these end-points have not been extensively validated with reference compounds for epigenetic alterations.]

(5) *Induces oxidative stress*: 18 assay end-points, all cellular assays, targeting nuclear erythroid-related factor-2, antioxidant response element, and other stress-related transcription factors, as well as protein upregulation in response to ROS.

(6) *Induces chronic inflammation*: 45 assay end-points, mostly using primary human cells, measuring protein expression levels indicative of inflammatory responses, including cytokines, cell adhesion molecules, and NF-κB. [The Working Group noted these in vitro end-points are short-term assays and therefore not directly indicative of chronic inflammation.]

(7) *Is immunosuppressive*: 0 assay end-points.

(8) *Modulates receptor-mediated effects*: 92 assay end-points targeting nuclear receptors (e.g. AhR, androgen receptor (AR), estrogen receptor (ER), farnesoid X receptor, peroxisome proliferator-activated receptor

(PPAR), pregnane X receptor (PXR), and retinoic acid receptor, among others) in cellular assays for transactivation, receptor dimerization, and nuclear translocation, as well as biochemical radioligand binding assays and coregulatory recruitment assays.

(9) *Causes immortalization*: 0 assay end-points.

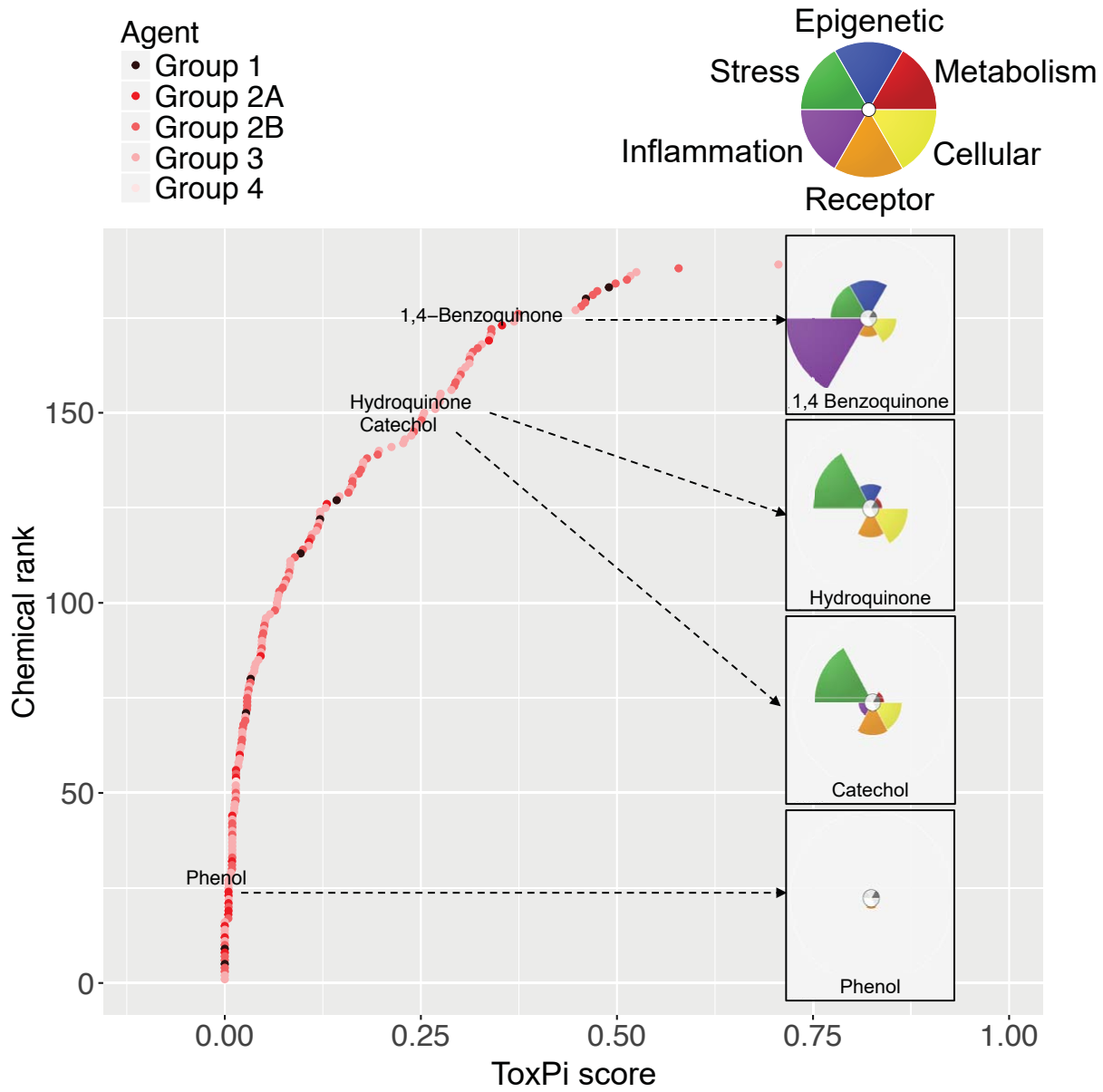
(10) *Alters cell proliferation, cell death, or nutrient supply*: 68 assay end-points measuring cell cycle markers, proliferation, cytotoxicity, and mitochondrial toxicity by a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

The activity of an agent on a given assay is determined by the statistical significance of the association between all tested concentrations of the agent with assay response (Sipes et al., 2013). The activity of each agent in each assay and across groups of assays was summarized using the Toxicological Prioritization Index (ToxPi) approach (Reif et al., 2010). In the Working Group's analysis, each agent–assay pair was summarized as "active" (1) or "inactive" (0). Within each key characteristic slice for a given agent, the distance from the origin represents the relative agent-elicited activity of the component assays (i.e. slices extending further from the origin were associated with "active" calls on more assays). The overall score of an agent is the aggregation of all slice-wise scores (Fig. 4.2, inset) and provides an activity ranking relative to the 189 agents screened in ToxCast/Tox21 that have been evaluated in the *IARC Monographs* (Fig. 4.3, rank chart).

A tabular summary of the results is given in the supplementary information (Annex 1), and a summary for each relevant compound follows. Note that the activity calls across these assays represent exposure to each of the four individual metabolites, and may not necessarily be indicative of exposure to benzene or its other metabolites.

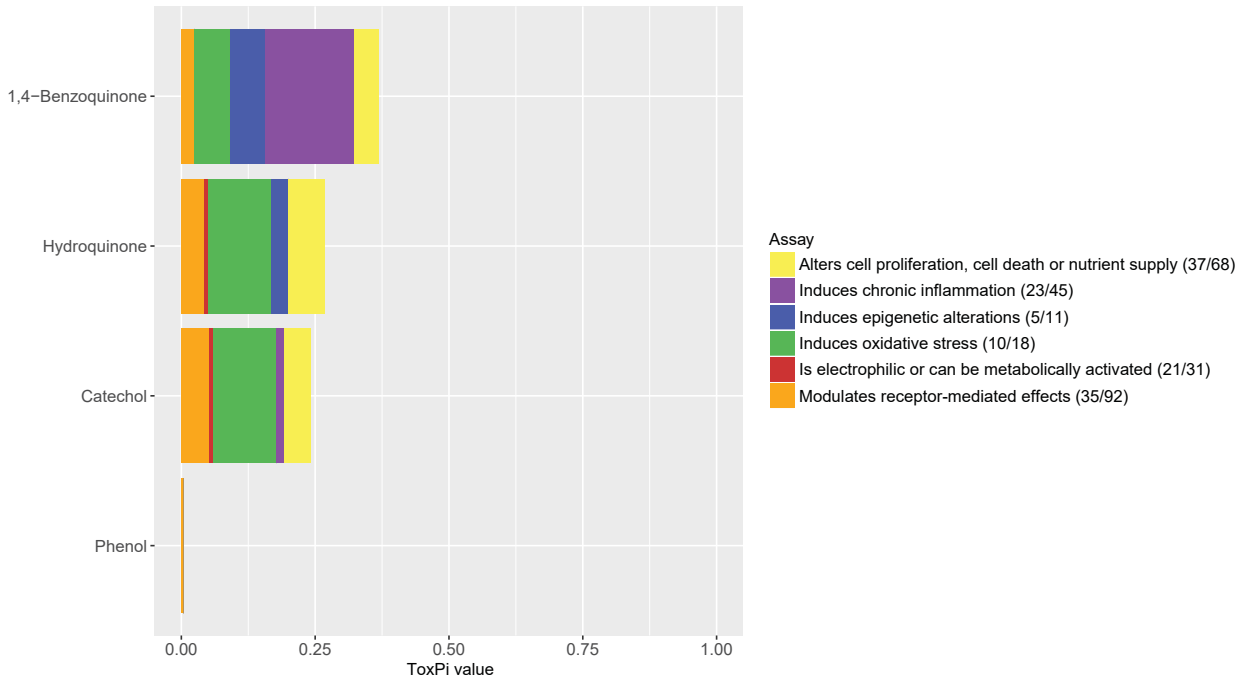
1,4-Benzoquinone (Chemical Abstracts Service, CAS, Registration No. 106-51-4) has the highest ToxPi value among the benzene metabolites evaluated, and has the 16th highest value among the 189 agents (16/189) evaluated by IARC (see Fig. 4.2). The largest contribution to this ToxPi value is from active hits to assays mapped to the "*Induces chronic inflammation*" category (Fig. 4.2). This represents the maximum number of hits to this category among the 189 evaluated IARC agents. Exposure results in upregulation of cell adhesion proteins E-selectin, P-selectin, and vascular cell adhesion molecule 1 (VCAM1), and in the upregulation of chemokines and cytokines such as CXCL9, CXC10, CCL2, IL-1a, IL-8, TNFα, CD38, CD40, and CD69 in multiple human cell cultures and co-cell cultures. NF-κb is also upregulated in the HepG2 cell line. The second-largest contributor to the ToxPi value derives from active hits mapped to the "*Induces epigenetic alterations*" category, with two hits out of four assays mapping to DNA binding (the seven assays associated with measuring chromatin alterations were not performed). Assays indicating upregulation of matrix metalloproteinase 1 in two cells cultures are linked to the "*Induces oxidative stress*" category, and the tissue inhibitor of metalloproteinases 2 is also upregulated in a co-culture involving one of the former lines. Assays suggesting upregulation of hypoxia -inducible factor-a and metal regulatory transcription factor-1 in HepG2 liver cell lines are also linked to 1,4-benzoquinone exposure. Hits associated with upregulation of six genes, including *TGFβ1*, *MYC*, and vascular endothelial growth factor *VEGFRII*, which are markers of cell-cycle across multiple platforms, downregulation of cellular proliferation across multiple cell-cultures as assayed by the sulforhodamine B colorimetric assay, and upregulation of two markers of cell proliferation were mapped to the "*Alters cell proliferation, cell death, or nutrient supply*" category. Upregulation of the gene expression of six receptors, including AhR, AR,

Fig. 4.2 ToxPi ranking for benzene metabolites phenol, catechol, hydroquinone, and 1,4-benzoquinone and all agents evaluated by IARC with available data using ToxCast and Tox21 assay end-points mapped to six key characteristics of carcinogens



Inset are the ToxPi diagrams for these metabolites. ToxPi diagram colour coding is provided in the legend. Each agent in the rank chart is plotted in a colour according to its IARC classification

Fig. 4.3 Stacked bar plots of ToxPi values by assay category for benzene metabolites phenol, catechol, hydroquinone, and 1,4-benzoquinone using ToxCast and Tox21 assay end-points mapped to six key characteristics of carcinogens



The numbers in the parentheses next to each category represent the ratio of the maximum number of hits to assays assigned to category over 189 IARC agents to the total number of assays assigned to the category

glucocorticoid receptor, and retinoid X receptor, all on the Attagene platform performed on HepG2 liver cell line, were linked to the "*Modulates receptor-mediated effects*" component of the ToxPi value.

Hydroquinone (CAS 123-31-9) has the second-highest ToxPi value of the benzene metabolites and the 39th highest ToxPi value among the 189 agents (39/189) evaluated by IARC (see Fig. 4.2). The largest contributor to this value came from active hits linked to the "*Induces oxidative stress*" category. This represents the ninth-highest number of hits to this category among the 189 evaluated IARC agents. This category contains assays mapped to regulation of matrix metalloproteinase 1 in one of the three cell cultures, regulation of oxidative stress and stress kinase after 72 hours of exposure, and four (hypoxia-inducible factor-a, metal regulatory transcription factor-1, nuclear erythroid-related factor-2, and antioxidant response element) out of six markers of oxidative stress measured in three separate assay platforms (Apredica, Attagene, and Tox21). The second-largest contributor came from hits linked to the "*Alters cell proliferation, cell death, or nutrient supply*" category, with indications of upregulation of cell cycle, downregulation of proliferation, and upregulation of mitochondrial toxicity. For assays assigned to "*Modulates receptor-mediated effects*" there are active hits for AhR, AR, glucocorticoid receptor, PPAR-response element, PXR-vitamin D response element, ER, and PPARγ. One out of the four assays associated with DNA binding (from the "*Induces epigenetic*

alterations" category) is active for hydroquinone exposure.

Catechol (CAS 120-80-9) has the third-highest ToxPi value of the benzene metabolites and the 45th highest ToxPi value among the 189 agents (45/189) evaluated by IARC (see Fig. 4.2). The largest contributor to this value came from the exact same active hits as for hydroquinone in assays linked to the "*Induces oxidative stress*" category. For assays assigned to "*Modulates receptor-mediated effects*" there are active hits for AhR, AR, ER, and PPAR. The assays linked to "*Alters cell proliferation, cell death, or nutrient supply*" category suggested upregulation of cell cycle and downregulation of proliferation across multiple cell cultures.

Phenol (CAS 108-95-2) was only active on a biochemical assay for ER, with no activity hits on any other assays assigned to the six categories of carcinogenicity.

In conclusion, 1,4-benzoquinone is the benzene metabolite most strongly associated with assays mapped to the six key characteristics of carcinogens, and with the "*Induces chronic inflammation*" category in particular. Hydroquinone and catechol showed a moderate number of hits. Phenol showed activity on only one assay. These four benzene metabolites were tested in different phases of the ToxCast or Tox21 programmes, resulting in different percentages of missing data (i.e. "not-tested" in a given assay). Relative to the full assay set, 1,4-benzoquinone was tested in 46% of all assays and catechol, hydroquinone, and phenol were tested in 88% of all assays. [The Working Group noted that a reanalysis of data, in which only assays with data for most of the full list of IARC chemicals tested were considered, resulted in similar ToxPi scores.]

4.4 Observed exposure–response relationships in mechanistic studies

Based on the *Report of the IARC Advisory Group to Recommend on Quantitative Risk Characterization* (IARC, 2013), a more detailed review of the availability of exposure–response information for mechanistic and other data from studies in exposed humans was performed. The purpose of this review was to explore the observed exposure–response relationships by summarizing information across studies on the magnitudes of response (e.g. relative percentage change) and the corresponding levels of exposure.

First, the representative studies of human benzene exposure that were the focus of Sections 4.1 and Section 4.2 relating to the key characteristics of carcinogens were further reviewed for availability of information relating level of exposure to degree of response. Only studies relating to key characteristics of carcinogens for which there was strong evidence in exposed humans were considered (see Section 5.4). Additional considerations for selecting studies included the availability of multiple exposure categories with associated measurements of benzene concentrations in air, adequate sample size, consideration of potentially confounding co-exposures, and/or completeness of reporting. Candidate studies were evaluated for their adequacy in terms of exposure assessment.

Based on these considerations, it was determined that exposure–response information was available for the key characteristics of carcinogens of "is genotoxic" and "is immunosuppressive", the second of which includes measures of haematotoxicity. Specifically, exposure–response information was available for the end-points for which there was strong evidence in exposed humans (see Section 5.4): (i) genotoxicity (oxidative DNA damage, indicated by 8-OHdG, and chromosomal effects, indicated by MN and

CAs); and (ii) immunosuppression and haematotoxicity (peripheral pluripotent stem cell and leukocyte counts).

Representative studies, independent of the presence or direction of a statistically significant effect, were then selected (see Section 4.2), and numbers in the exposed and reference groups, duration of exposure, and level of exposure among those exposed were examined. Further, for each end-point the measure of response, the evidence of an exposure–response gradient, the central tendency and measure of variance of end-point in the reference group, and the measure of exposure were all considered. Finally, considerations of each exposure category included: the central tendency and measure of variance of exposure; the central tendency of difference in response from the reference group as a percentage change (i.e. (mean of category – mean of the reference group)/mean of the reference group); and a test for significance of difference from the reference group (e.g. *t*-test) and *P* value.

[The Working Group noted that, because representative studies were selected, the existence of additional mechanistic studies with exposure–response information cannot be excluded. The possibility of publication bias on mechanistic end-points also cannot be excluded.]

4.4.1 Genotoxicity

Two studies with exposure–response information for oxidative DNA damage (8-OHdG) were selected (Lagorio et al., 1994; Liu et al., 1996). Both had statistically significant exposure–response trends, but the population in Lagorio et al. (1994) were exposed to benzene at much lower concentrations (mean, 0.45 mg/m^3 or 0.14 ppm) compared with those in Liu et al. (1996) (mean, 166.1 mg/m^3 or 51 ppm). Of the two studies, only the study conducted by Liu et al. (1996) divided exposed populations into categories; the group exposed to low concentrations (mean, 2.46 mg/m^3 or 0.76 ppm) was not statistically different, with an effect size of 25%, and the group exposed to medium concentrations (mean, 103.3 mg/m^3 or 31 ppm) was statistically significantly increased, with an effect size of 600%.

Four studies with exposure–response information for MN were selected (Liu et al., 1996; Rekhadevi et al., 2011; Zhang et al., 2014, 2016). [The Working Group noted that the study by Rekhadevi et al. (2011) was not informative for exposure–response because it had a narrow range of benzene exposures (1.1–1.5 mg/m^3 or 0.34–0.46 ppm) that was further divided into three categories.] All studies except for that of Rekhadevi et al. (2011) tested for exposure–response trends, all of which were statistically significant. Exposures in the Rekhadevi et al. (2011) (mean, 1.32 mg/m^3 or 0.41 ppm), Zhang et al. (2016) (median, 1.6 ppm), and Zhang et al. (2014) (median, 6.4 mg/m^3 or 2 ppm) studies were lower than those of the Liu et al. (1996) study (mean, 166.1 mg/m^3 or 51 ppm). Three of these studies divided exposed populations into categories that were compared with the reference group and, in each case, the group exposed to the lowest concentrations demonstrated a statistically significant increase in MN compared with the reference group, with effect sizes of 45–55% (Liu et al., 1996; Zhang et al., 2014, 2016).

Numerous publications with exposure–response information for CAs were selected (Bogadi-Sare et al., 1997; Zhang et al., 1998b, 2007, 2011, 2012b; Kim et al., 2004b; Xing et al., 2010; Rekhadevi et al., 2011; Marchetti et al., 2012), although many were of the same study population. All had statistically significant exposure–response trends with the exception of Rekhadevi et al. (2011) (see Working Group comment, above) and Bogadi-Sare et al. (1997). Exposure concentrations considered by Rekhadevi et al. (2011) (mean, 1.322 mg/m^3 or 0.41 ppm), Kim et al. (2004b) (geometric mean, 0.56 ppm), Marchetti et al. (2012) (mean, 2.8 ppm), Xing et al. (2010) (median, 2.9 ppm), and Bogadi-Sare et al. (1997)

(median, 5.9 ppm) were lower than those in the Zhang et al. studies (median, 10–31 ppm). Among the studies of exposure to lower concentrations, two divided exposed populations into categories that were compared with the reference group: Xing et al. (2010) and Marchetti et al. (2012). The response of the group exposed to the lowest concentration (median, 1.2 ppm) in the study by Marchetti et al. (2012) was statistically significantly different from the reference group, with an effect size of 33%. In the case of Xing et al. (2010), the group exposed to the lowest concentration (median, 1.0 ppm) was not statistically different with an effect size of 50%; the group exposed to the higher concentration (median, 7.7 ppm) was statistically significantly increased, with an effect size of 70%.

[The Working Group noted that, in the majority of studies examined, an exposure–response gradient between benzene exposure and both MN and CAs was reported.]

4.4.2 Immunosuppression and haematotoxicity

Numerous studies with exposure–response information for leukocyte counts were selected (Liu et al., 1996; Rothman et al., 1996; Ward et al., 1996; Qu et al., 2002; Lan et al., 2004; Robert Schnatter et al., 2010; Swaen et al., 2010; Zhang et al., 2016). All had statistically significant exposure–response trends with the exception of the study by Swaen et al. (2010), which was not statistically significant, and by Liu et al. (1996), in which no trend test was performed. Exposures in the studies by Swaen et al. (2010) (mean, 0.22 ppm), Zhang et al. (2016) (median, 1.6 ppm), Robert Schnatter et al. (2010) (median, 2.3 ppm), Qu et al. (2002) (mean, 3.8 ppm), and Lan et al. (2004) (mean, 5.1 ppm) were lower than in the studies by Rothman et al. (1996) (median, 31 ppm) and Liu et al. (1996) (mean, 166.1 mg/m^3 or 51 ppm). Ward et al. (1996) only reported the maximum exposure level (34 ppm).

Six studies divided exposed populations into categories (Liu et al., 1996; Rothman et al., 1996; Qu et al., 2002; Lan et al., 2004; Swaen et al., 2010; Zhang et al., 2016). The groups exposed to the lowest concentrations in these studies had effect sizes ranging from a 0.8% increase to a 14.5% decrease in leukocytes, with the effects observed in Lan et al. (2004) (14.5% decrease at a mean exposure of 0.57 ppm) and Qu et al. (2002) (4.3% decrease at a mean exposure of 3.07 ppm) being statistically significant. In the case of Swaen et al. (2010), none of the groups (< 0.5 ppm, 0.5–1.0 ppm, > 1.0 ppm) demonstrated statistically significant changes (effects ranging from 1% increase to 1% decrease). In Zhang et al. (2016), the groups exposed to the two lowest concentrations (3.55 ppm-yr and 6.51 ppm-yr) had non-significant decreases of 5.3%, whereas the third exposure group (10.72 ppm-yr) had a significant decrease of 11.2%. In Liu et al. (1996), the lowest exposure category (mean, 2.46 mg/m^3 or 0.76 ppm) demonstrated a 0% change, but the middle exposure category (mean, 103.3 mg/m^3 or 31 ppm) showed a 17% decrease (no statistical tests were performed). In Rothman et al. (1996), the group exposed to the lower concentration (median, 13.6 ppm) demonstrated a non-significant decrease of 5.8%, whereas the group exposed to the higher concentration (median, 91.9 ppm) had a significant decrease of 17.6%.

[The Working Group noted that, in the majority of studies examined, an exposure–response gradient between exposure to benzene and leukocyte count was reported. Some other studies discussed in Section 4.2.4(a), which evaluated populations exposed to relatively low levels of benzene, reported no effects on leukocytes; these studies are not included here, however, because they were not informative for exposure–response analyses (e.g. they only compared all levels of exposure with the reference group).]

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5. SUMMARY OF DATA REPORTED

5.1 Exposure data

Benzene is the simplest aromatic hydrocarbon. It is a volatile and ubiquitous air pollutant, mainly arising from anthropogenic sources such as combustion processes. It is found in crude oil and hence in petroleum products. Historically, benzene was used as a solvent, for example in glues and paints, and also in the rubber and chemical industries. At the present time, benzene is a high production volume chemical, despite being banned in consumer products in many countries. Its primary use is in the synthesis of ethylbenzene, used in plastics manufacturing.

Benzene exposure may occur in several industries and occupations, including the petroleum, chemical, and manufacturing industries, and coke making. In low- and middle-income countries, exposure may still occur in several industrial sectors, including shoemaking, painting, printing, and rubber product manufacturing. It is a component of gasoline, vehicular exhaust, industrial emissions, and tobacco smoke, all sources of environmental exposure.

Exposure to benzene mainly occurs via inhalation, but skin absorption is also possible. Benzene can be measured in both workplace and community settings using air monitoring and personal monitoring as well as biomonitoring, capturing all routes of exposure. Urinary *S*-phenylmercapturic acid (SPMA) and unmetabolized benzene in blood and urine are specific biomarkers, whereas *trans*,*trans*-muconic acid is not. The current preferred method for biomonitoring is to measure urinary benzene and SPMA; these measurements are non-invasive and reflect daily benzene exposure.

Occupational exposure to benzene is regulated in many countries, and there are also some environmental guidelines for benzene in air and drinking-water.

Full-shift occupational exposures in high-income countries are usually less than 1 ppm (3.19 mg/m^3) for most industries and occupational groups, including the upstream and downstream petroleum industry and automobile repair, and for diverse workers exposed to vehicle exhausts. However, workers in most of these industries may conduct short-term tasks that possibly result in exposure to high concentrations of benzene, such as maintenance activities where pipelines are open, tank cleaning, or top filling of road tankers with gasoline. Data from low-and middle-income countries are sparse; however, exposures considerably higher than those described above have been reported from China.

Environmental air levels, as determined at fixed monitoring sites, are generally orders of magnitude lower than occupational exposures. There is evidence that outdoor air levels have declined significantly over time in both Europe and the USA, where annual average concentrations are currently less than 5 μg/m^3; however, higher concentrations are measured in some cities in other regions of the world.

For occupational cancer epidemiology, high-quality exposure assessments use benzene measurements to derive individual exposure estimates. In studies of occupational exposures, these estimates represent long-term exposure in the workplace. Participants should have complete and detailed job histories for which the measured data are applied. For environmental (air pollution) epidemiology, exposure assessment typically relies on measurements of benzene in outdoor air collected from routine monitoring stations, or from modelled ambient concentrations for geographically defined gridded areas. These spatially referenced data for temporally relevant critical windows are linked to geocoded residences of study participants and used to generate individual-level estimates of benzene exposure.

5.2 Human carcinogenicity data

5.2.1 Acute myeloid leukaemia

The classification of benzene as a Group 1 carcinogen in previous *IARC Monographs* was based on sufficient evidence of an association between benzene exposure and risk of acute myeloid leukaemia (AML) and/or acute non-lymphocytic leukaemia (ANLL). This conclusion was supported by several occupational cohort studies that collected quantitative exposure data, revealing exposure–response trends between benzene exposure and AML and/or ANLL. According to the recent WHO classification of AML, related neoplasms are included in this category as AML not otherwise specified (e.g. pure erythroid leukaemia, acute megakaryoblastic leukaemia, and acute monocytic leukaemia). The following discussion referring to AML therefore includes ANLL.

Occupational and general-population studies published since the previous *IARC Monographs* on benzene, including two studies in occupational cohorts with careful assessment of benzene exposure, confirm the association between AML and exposure to benzene, and also demonstrate an exposure–response trend with quantitative exposure metrics.

5.2.2 Chronic myeloid leukaemia

Several cohort studies in the petroleum industry and other settings demonstrated increased risks for chronic myeloid leukaemia (CML). Other studies showed no evidence of an association, including two studies that were previously included in *IARC Monographs* volume 100F with quantitative estimates of exposure to benzene but did not report any exposure–response relationship.

An elevated risk of CML was reported in two new publications of occupational cohort studies with extended follow-ups, and a significant exposure–response trend was seen in the study that evaluated exposure–response. Among the four studies judged to be the most informative by the Working Group, the point estimates were above the null for all; however, only three studies included 6 or more exposed cases. The Working Group further noted a lack of clear evidence of an exposure–response gradient in the four available studies. Other co-exposures were present, but the potential for confounding could not be assessed.

5.2.3 Non-Hodgkin lymphoma

The broad category of non-Hodgkin lymphoma (NHL) includes chronic lymphocytic leukaemia (CLL), multiple myeloma (MM), and acute lymphocytic leukaemia (ALL), as well as follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma (DLBCL), and hairy cell leukaemia. In considering the data available at the time, the Working Group of *IARC Monographs* Volume 100F concluded that there was *limited evidence* in humans that benzene causes NHL. The current Working Group

examined all of the pertinent studies published before and after Volume 100F. In doing so, the Working Group assessed the quality of the old and new studies and noted that several high-quality cohort studies provided data for NHL in occupational settings and in the general population. These studies showed elevated relative risks for NHL as categorized in the studies, which were statistically significant in two studies. Two of these studies were conducted in China, and with relatively high levels of exposure to benzene in one occupational cohort. The studies that reported on NHL used different classifications of lymphoma, which varied over time and between studies. The Working Group therefore noted that associations were observed between benzene exposure and a heterogeneous classification of NHL.

CLL is currently included as a subgroup of NHL, but in the past it was generally considered as a separate entity (not always reported as such in papers). As noted in *IARC Monographs* Volume 100F, CLL can be an indolent disease of the elderly; this raises questions about cohorts that are not followed up until the study population is relatively old, and about studies that use mortality instead of incidence data. The diagnostic accuracy of CLL has also improved over time. Because of these concerns, the Working Group accorded the greatest weight to recent studies and those that reported incidence data; these included four studies (two occupational cohorts, and case–control studies in Italy and China) that used current classifications for lymphomas, including CLL. Three of these studies (three occupational cohorts) found positive associations between CLL and exposure to benzene, but the 95% confidence intervals included the null. Confounding from other occupational exposures was judged to be unlikely in these studies.

MM was considered separately in one case–control study and in nine occupational cohort studies. The numbers of exposed cases were generally small. Elevated relative risks were observed in four studies. The remaining studies did not find robust positive associations, but some showed elevated risks in the exposure category of highest concentration.

In *IARC Monographs* Volume 100F, the evidence of an association between benzene and ALL in adults was regarded as limited, based on a few occupational cohorts that included very small numbers of exposed cases and reported increased risks that were not statistically significant. Data for adult ALL and benzene exposure remain sparse: only one occupational cohort study has reported on ALL after the publication of the previous review. That study reported a non-significantly elevated risk based on a few incident cases, and did not provide exposure–response results. Among all of the included studies of adult ALL, the magnitude of the risk ratio estimates ranged from 0.8 to 4.5, and all confidence intervals included the null.

Other specific subtypes of NHL were reported in a few studies, including outcomes such as DLBCL, follicular lymphoma, and mantle B-cell lymphoma, but results were inconsistent.

5.2.4 Childhood cancer

Information about a possible association between environmental benzene exposure and childhood cancer derives mainly from case–control studies. Seven studies investigated leukaemia, two studies reported on tumours of the central nervous system, and there were single studies published for each of lymphoma, Wilms tumour, retinoblastoma, and neuroblastoma. The seven studies reporting on leukaemia were inconsistent in the definition of leukaemia (including a variation over all leukaemias, acute leukaemia, ALL, and AML) and reported heterogeneous results for association with benzene exposure. The Working Group noted that all four studies reporting separate results for ALL and AML showed associations between benzene

and AML in children (although most associations were not statistically significant), but no or weaker associations with ALL. Parental exposures to benzene before or during pregnancy were also considered in several studies of both cohort and case–control format. The Working Group noted a consistent association between exposure to benzene and AML for children, and coherence with findings for adult AML and benzene exposure, but could not rule out chance, bias, and confounding as alternative explanations.

5.2.5 Cancer of the lung

Several epidemiological studies of workers exposed to benzene have examined cancer of the lung. The most informative studies, which include those with larger cohort sizes, longer follow-up times, and either larger numbers of workers exposed to high concentrations or better-quality exposure assessments, have all reported statistically significant excesses of cancer of the lung among workers exposed to benzene. Positive trends between cumulative exposure to benzene and cancer of the lung were reported in two of these studies. However, none of these studies controlled for potential confounding by smoking or by occupational exposure to other lung carcinogens. The Working Group noted that smoking is a strong risk factor for cancer of the lung, and an important potential confounder of this association; in addition, the workers in these cohorts were potentially exposed to other occupational lung carcinogens.

5.2.6 Other cancers

Occupational cohort studies also reported data for several other cancer types and tumour sites, including cancer of the: nasal cavity, pharynx, larynx, and related sites; oesophagus; stomach; colon, rectum, and anus; pancreas; kidney; liver and biliary tract; prostate; bladder, brain, and central nervous system; and skin. Each of these cancers was addressed in a small number of studies. For each cancer site, results were inconsistent across studies, exposure–response data were generally lacking, and potential confounding from other occupational exposures and behavioural factors was typically not controlled.

5.2.7 Quantitative data

Meta-regression analysis of data from six occupational cohort studies strongly supported a linear exposure–response relationship for AML and cumulative exposure to benzene.

5.3 Animal carcinogenicity data

There were 17 studies that reported on the effects of benzene inhalation in male and female mice. Several studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in mice exposed to benzene.

In one study in male and female mice, benzene caused significant increases in the incidence of myelogenous neoplasms (myeloid leukaemia) and solid tumours (other than of the liver or lymphomas) in males and females. In a second study in male mice, there was a significant increase in the incidence of solid tumours (other than of the liver or lymphomas). In a third study in male mice, there was a significant increase in the incidence of malignant lymphoma, squamous cell carcinoma of the preputial gland, carcinoma of the Zymbal gland, squamous cell carcinoma of the forestomach, and adenoma of the lung. In a fourth study in male mice, there was a significant increase in the incidence of lymphoma of the thymus gland (with a significant positive trend), and neoplasms of the haematopoietic and lymphoid tissues. In a fifth study in male mice, there was a significant positive trend in the incidence of lymphoma of the thymus gland. In a sixth study in male mice, there was a significant

increase in the incidence of neoplasms of the haematopoietic tissues. In a seventh study in male mice, with a short duration of exposure, there was a significant increase in the incidence of adenoma of the lung. In an eighth study in male mice, there was a significant increase in the incidence of leukaemia or lymphoma (combined) and of adenoma of the lung. In a ninth study in male mice, there was a significant increase in the incidence of carcinoma of the Zymbal gland. Seven other studies were negative. One study was considered inadequate for the evaluation.

There were four oral administration (gavage) and two intraperitoneal studies of benzene in male and female mice. Some studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in mice exposed to benzene.

In a first study in which benzene was administered by gavage, benzene caused a significant increase in the incidence of the following lesions in males and females: bronchioloalveolar adenoma and carcinoma, hepatocellular adenoma or carcinoma (combined), squamous cell carcinoma of the Zymbal gland, adenoma or carcinoma (combined) of the Harderian gland, lymphoma or leukaemia (combined), and squamous cell papilloma of the forestomach. In males only, benzene caused a significant increase in the incidence of hepatocellular carcinoma, adenoma of the Harderian gland, carcinoma of the preputial gland, squamous cell papilloma or carcinoma (combined) of the forestomach, and pheochromocytoma of the adrenal gland. In females only, benzene induced hepatocellular adenoma, carcinoma of the Harderian gland, tubular adenoma of the ovary, mixed tumours (benign) of the ovary, tumour (benign or malignant) of the granulosa cell, and carcinoma and carcinosarcoma of the mammary gland.

In a second gavage study, benzene caused a significant increase in the incidence of tumours of the lung in males and females, and of carcinoma of the mammary gland in females. In a third gavage study, there was a significant increase in the incidence of tumours of the lung and leukaemia in males and females, and of carcinoma of the mammary gland in females. In a fourth gavage study in male and female mice, strain A/J, benzene induced a significant increase in the multiplicity of adenoma of the lung in males.

In a first study in which benzene was administered by intraperitoneal injection, there was a significant increase in the incidence and multiplicity of adenoma of the lung in male A/J mice but not in females. In a second (transplacental) study there was a significant increase in the incidence of tumours of the liver in the male offspring, and of lesions of the haematopoietic and lymphoid tissues (hyperplasia, myeloproliferative disorders, and myeloid/lymphoid neoplasia, combined) in the female offspring of pregnant mice given benzene intraperitoneally.

There were five studies of the carcinogenicity of benzene in rats: four oral administration studies (by gavage of males and females of different strains, i.e. Sprague-Dawley, Wistar, and F344) and one inhalation study in Sprague-Dawley rats (in pregnant females and their male and female offspring). All studies reported an increase in the incidence of one or more types of neoplasms (including tumours of the haematopoietic and lymphoid tissues) in rats exposed to benzene.

Benzene significantly increased the incidence of carcinoma of the Zymbal gland in male and/or female rats in four gavage studies, and in male and female offspring in a study of transplacental exposure followed by inhalation. It also significantly increased the incidence of squamous cell carcinoma of the oral cavity (including lip and tongue) in males and females in two gavage studies, and in the female offspring in the study of transplacental exposure followed by inhalation. Exposure to benzene significantly increased the incidence of carcinoma in situ of the forestomach

in females and of acanthoma or squamous cell dysplasia (combined) of the forestomach in males and females in one gavage study, and carcinoma in situ of the forestomach in the female offspring in the study of transplacental exposure followed by inhalation. A significantly increased incidence of hepatocellular carcinoma was observed in the female offspring in the study of transplacental exposure followed by inhalation. Benzene caused a significant positive trend in the incidence of tumours of the haematopoietic and lymphoid tissues in males in one of the gavage studies, and a significant increased incidence of those same tumours in female offspring in the study of transplacental exposure followed by inhalation. There were also significant increases in the incidence of carcinoma of the skin in males in two gavage studies and of stromal polyps of the endometrium in females in one gavage study.

There were 12 studies that reported on neoplasms and preneoplastic effects induced by benzene (three whole-body inhalation, three oral administration (gavage), and six skin application studies) in one or both sexes of four different genetically modified mouse models of different genetic backgrounds. It was demonstrated that benzene induced cancer in different tissues (including tumours of the haematopoietic and lymphoid tissues) of genetically modified mice, depending upon the route of exposure.

In inhalation studies, B6.CBA-*Trp53*^{tm1Sia} haploinsufficient congenic inbred mice showed significant exposure-related increases in the incidence of lymphoma of the thymus gland in one study; C3.CBA-*Trp53*^{tm1Sia} congenic mice demonstrated a significant exposure-related increase in the incidence of lymphoma of the thymus gland, non-thymic lymphoma, and myeloid leukaemia in another study. One inhalation study in C57BL/6 h-Trx-Tg mice was negative.

In studies of B6.129-*Trp53*^{tm1Bra} N5 haploinsufficient mice exposed to benzene by gavage, increases in the incidence of sarcomas of the subcutis were observed in one study and atypical hyperplasia of the thymus gland in another. In another model of a haploinsufficient mouse with tumour-suppressor gene (the B6.129-*Cdkn2a*^{tm1Dep} congenic), oral exposure to benzene by gavage was associated with a significant dose-related increase in malignant lymphoma in males, but not in females.

Benzene application to the skin of female v-Ha-*Ras* mice resulted in a significant and rapid development of exposure-related squamous cell papillomas of the skin in one study, and of a significant increase in the incidence of granulocytic leukaemia in another; all other skin application studies were negative or inadequate for the evaluation.

5.4 Mechanistic and other relevant data

Benzene is well absorbed via inhalation as well as by oral and dermal exposure in all species studied, including humans and rodents. Benzene is widely distributed in the body by blood circulation; unchanged benzene is largely excreted by exhaled breath, with small amounts appearing in urine. The initial step of metabolism is oxidation to benzene oxide by cytochrome P450. Subsequent metabolism is complex, and includes the creation of a multiplicity of reactive electrophiles via multiple metabolic pathways in multiple tissues, including bone marrow. Major urinary metabolites detected in exposed humans include phenol, hydroquinone, catechol, (*E,E*)-muconic acid, and SPMA. There are some data suggesting increased metabolism at exposure to low concentrations, but these data are not definitive. Electrophiles are generated during benzene metabolism, as indicated by metabolite profiles and the production of epoxide- and benzoquinone-protein adducts in individuals exposed to benzene. There is *strong* evidence, including in exposed humans, that benzene is metabolically activated to electrophilic metabolites. There is

strong evidence, including in exposed humans, that benzene induces oxidative stress and associated oxidative DNA damage. Several studies in exposed humans reported that exposure to benzene is associated with markers of oxidative stress, such as decreased serum glutathione levels, increased lipid peroxidation, increased reactive oxygen species, oxidative protein damage, and/or decreased antioxidant capacity. In addition, multiple studies in exposed humans reported oxidative DNA damage in the form of 8-hydroxy-2′-deoxyguanosine. Benzene or its metabolites induced oxidative stress in human and other mammalian cells in vitro, and in various tissues, including bone marrow, in mice.

There is *strong* evidence, including in exposed humans, that benzene is genotoxic, inducing DNA damage and chromosomal changes. Benzene induces DNA strand breaks and gene mutations in occupationally exposed humans, and DNA damage in human cells in vitro. In experimental animals exposed in vivo, benzene induced DNA adducts in bone marrow and leukocytes. Benzene metabolites induced benzene-derived DNA adducts in several studies in human haematopoietic cells. The multitude of studies of chromosomal end-points in humans exposed to benzene is largely consistent with respect to the induction of chromosomal aberrations and micronuclei. Specific cytogenetic changes have also been observed in exposed humans, including aneuploidy, translocations, and various other structural chromosome changes. Furthermore, in human cells in vitro, benzene with metabolic activation and benzene metabolites consistently induce chromosomal alterations.

The evidence is *strong* that benzene alters DNA repair or causes genomic instability, inhibiting topoisomerase II, which is involved in DNA replication. No data on topoisomerase II were available in exposed humans. Benzene metabolites, particularly 1,4-benzoquinone and hydroquinone, directly inhibited topoisomerase II in human cell systems and in exposed mice.

The evidence is *strong* that benzene is immunosuppressive, including in exposed humans. Although no studies in humans were available that directly examined changes in immune function, many studies in exposed humans have demonstrated haematotoxicity, from decreased leukocyte counts at lower exposures to aplastic anaemia and pancytopenia at higher exposures. Specifically, reduced numbers and/or maturity of B-lymphocytes and CD4+ T-lymphocytes have been reported in multiple studies in exposed humans. Multiple experimental animal studies have demonstrated consistent immunosuppressive effects on assays for humoral and cell-mediated immune function, in addition to haematotoxicity, consistent with studies in exposed humans. In addition, several studies have found that haematotoxicity induced by benzene, at various levels of severity, has been associated with a future risk of developing a haematological malignancy or related disorder.

Haematotoxicity observed in exposed humans and experimental animals provides indirect evidence that benzene exposure leads to alterations of cell proliferation and cell death. In human cells in vitro, benzene or its metabolites induced apoptosis consistently across multiple haematopoietic cell types, which could be prevented by induction of the detoxifying enzyme NAD(P)H quinone oxidoreductase 1. In addition, in mice, benzene depressed the cycling fraction of bone marrow cells/progenitor cells mediated by Trp53, and induced apoptosis in various mouse haematopoietic cells in vivo and in vitro. After cessation of benzene exposure, dynamic recovery proliferation of bone marrow cells/progenitor cells was observed. Overall, the evidence is *strong* that benzene alters cell proliferation, cell death, or nutrient supply, specifically with respect to induction of apoptosis.

The evidence is *strong* that benzene modulates receptor-mediated effects, specifically with respect to aryl hydrocarbon receptor (AhR). No data on AhR were available in exposed humans or

in human cells. Benzene does not induce haematotoxicity in AhR-knockout ($AhR^{-/-}$) mice, or in wildtype mice whose marrow cells were repopulated with cells from $AhR^{-/-}$ mice after irradiation. Benzene and its metabolites hydroquinone and *p*-benzoquinone did not directly activate AhR in vitro in mouse hepatoma cells.

There are few data on the remainder of the 10 key characteristics of carcinogens (induces chronic inflammation, induces epigenetic alterations, or causes immortalization).

In the ToxCast/Tox21 high-throughput testing programmes of the United States government, four metabolites of benzene (phenol, catechol, hydroquinone, and 1,4-benzoquinone) were individually tested in several assays in vitro that have been mapped to the key characteristics of carcinogens. Few of these assays demonstrated metabolic capacity. Phenol was largely inactive, while the activity of the other three metabolites for oxidative stress and AhR corroborated other mechanistic data on these key characteristics. 1,4-Benzoquinone was also active in many assays mapped to inflammation.

Studies in exposed humans examining exposure–response gradients were available for the end-points of micronucleus formation, chromosomal aberrations, and leukocyte counts. In the majority of studies examined, an exposure–response gradient was reported.

6. EVALUATION AND RATIONALE

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of benzene. Benzene causes acute myeloid leukaemia in adults.

Positive associations have been observed for non-Hodgkin lymphoma, chronic lymphoid leukaemia, multiple myeloma, chronic myeloid leukaemia, acute myeloid leukaemia in children, and cancer of the lung.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Benzene is *carcinogenic to humans (Group 1)*.

6.4 Rationale

Support for Group 1 from mechanistic data

A Group 1 evaluation was supported by mechanistic data demonstrating that benzene exhibits many of the key characteristics of carcinogens. In particular, there is *strong* evidence, including in exposed humans, that benzene: is metabolically activated to electrophilic metabolites; induces oxidative stress and associated oxidative DNA damage; is genotoxic, inducing DNA damage and chromosomal changes; is immunosuppressive; and causes haematotoxicity.

LIST OF ABBREVIATIONS

8-OHdG	8-hydroxy-2′-deoxyguanosine
AhR	aryl hydrocarbon receptor
ALL	acute lymphocytic leukaemia
AML	acute myeloid leukaemia
ANLL	acute non-lymphocytic leukaemia
AR	androgen receptor
ASPEN	EPA Assessment System for Population Exposure Nationwide
bw	body weight
CA	chromosomal aberration
CALINE	California Line Source Dispersion model
CAS	Chemical Abstracts Service
CFU	colony-forming unit
CI	confidence interval
CLL	chronic lymphocytic leukaemia
CML	chronic myeloid leukaemia
CNS	central nervous system
DLBCL	diffuse large B-cell lymphoma
EPA	United States Environmental Protection Agency
EPO	erythropoietin
ER	estrogen receptor
GLP	good laboratory practice
GM	granulocyte-macrophage
GSD	geometric standard deviation
GSH	glutathione
GST	glutathione-*S*-transferase
HCL	hairy cell leukaemia
HL	Hodgkin lymphoma
HR	hazard ratio
IARC	International Agency for Research on Cancer
ICD	International Statistical Classification of Diseases and Related Health Problems
Ig	immunoglobulin
IL	interleukin
IQR	interquartile range

JEM	job-exposure matrix
MDS	myelodysplastic syndrome
MLL	mixed lineage leukaemia
MM	multiple myeloma
MN	micronuclei
MPD	myeloproliferative disorder
MPO	myeloperoxidase
mtDNA	mitochondrial DNA
NATA	National-Scale Air Toxics Assessment
NCI-CAPM	National Cancer Institute-Chinese Academy of Preventive Medicine
NF-κB	nuclear-factor kappa-light-chain-enhancer of activated B-cells
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NQO1	NAD(P)H quinone oxidoreductase 1
OEL	occupational exposure limit
OR	odds ratio
PBL	peripheral blood lymphocyte
PBMC	peripheral blood mononuclear cell
PFC	plaque-forming cell
PM_{10}	particulate matter of diameter < 10 μm
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion
ppm	parts per million
PXR	pregnane X receptor
ROS	reactive oxygen species
RR	relative risk
SCE	sister-chromatid exchange
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SPMA	*S*-phenylmercapturic acid
SRBC	sheep red blood cell
TCRV	T-cell receptor variable
Tg.AC	FVB/N-Tg.AC(v-Ha-*Ras*)
Th	T-helper cell
TNF-α	tumour necrosis factor alpha
Tox21	Toxicity in the 21st Century
ToxCast	Toxicity Forecaster
ToxPi	toxicological prioritization index
TREC	T-cell receptor excision circle
t,t-MA	*trans*,*trans*-muconic acid
UOGD	unconventional oil and gas development
vs	versus
WHO	World Health Organization

IARC MONOGRAPHS

This volume presents an evaluation of the carcinogenicity of benzene, updating with new data the most recent evaluation provided in Volume 100F of the *IARC Monographs*. Benzene, a simple aromatic hydrocarbon, occurs naturally and as a result of human activity, notably as a result of combustion, and it is a high-volume chemical now used mostly as a chemical intermediate. Human exposure to benzene is widespread through the air, in consumer products, and in industry.

An *IARC Monographs* Working Group reviewed epidemiological studies, animal cancer bioassays, and mechanistic data to assess the carcinogenicity of benzene and conducted quantitative analyses of data on genotoxicity and human cancer risks.