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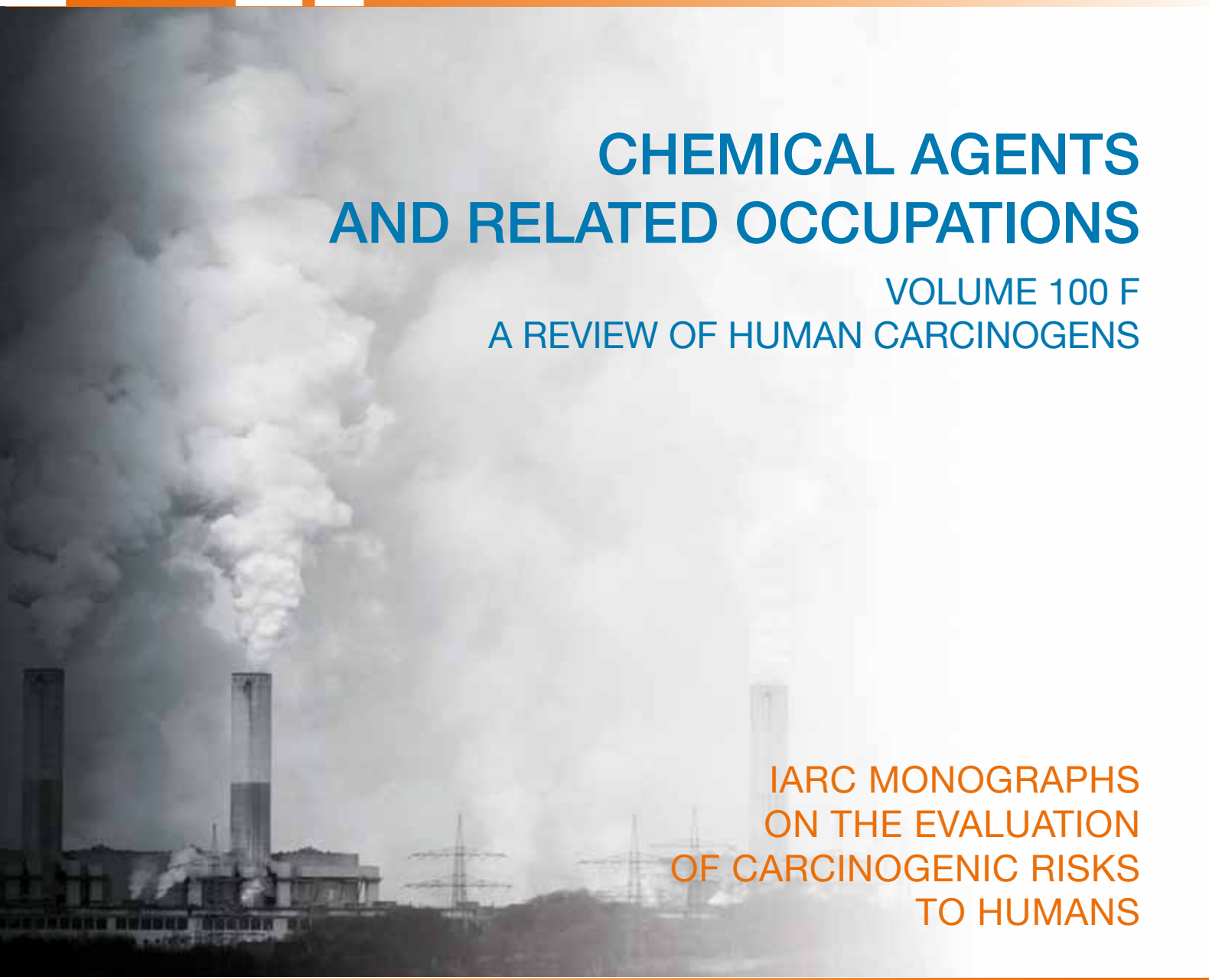


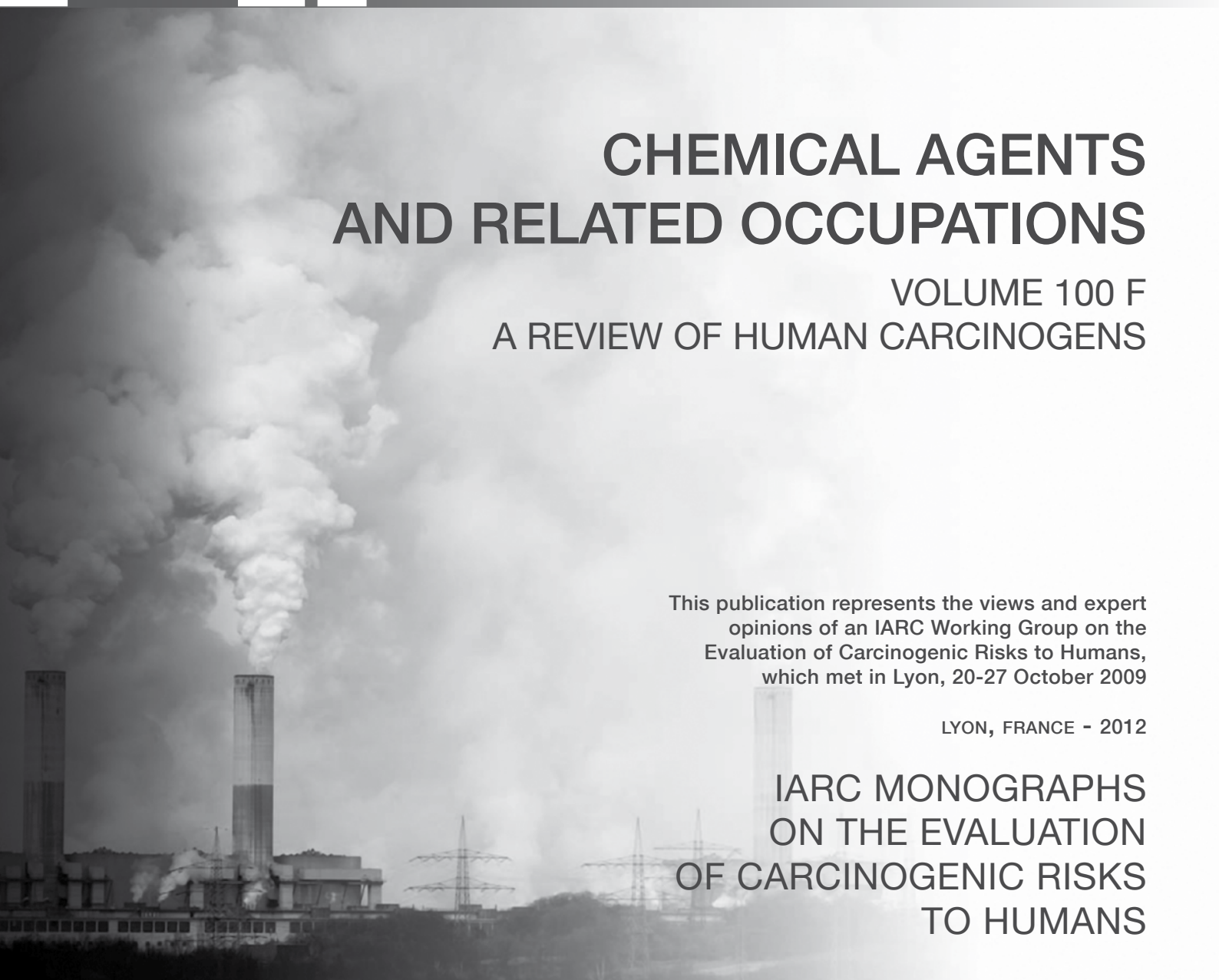
CHEMICAL AGENTS AND RELATED OCCUPATIONS

VOLUME 100 F
A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS



CHEMICAL AGENTS AND RELATED OCCUPATIONS

VOLUME 100 F
A REVIEW OF HUMAN CARCINOGENS

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, 20-27 October 2009

LYON, FRANCE - 2012

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 chemical 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 Health, Safety and Hygiene at Work Unit of the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities, and 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 U.S. National Cancer Institute, the U.S. National Institute of Environmental Health Sciences, the U.S. Department of Health and Human Services, or the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities.

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Lorenzo Tomatis (1929-2007)
Founder of the *IARC Monographs* Programme

Lorenzo Tomatis, MD, with other colleagues knowledgeable in primary prevention and environmental carcinogenesis, perceived in the 1960s the growing need to objectively evaluate carcinogenic risks by international groups of experts in chemical carcinogenesis. His vision and determination to provide a reliable source of knowledge and information on environmental and occupational causes of cancer led to his creating the *IARC Monographs* Programme for evaluating cancer risks to humans from exposures to chemicals. The first meeting, held in Geneva in December 1971, resulted in Volume 1 of the IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man [1972], a series known affectionately since as the "orange books". As a champion of chemical carcinogenesis bioassays, Tomatis defined and promoted the applicability and utility of experimental animal findings for identifying carcinogens and for preventing cancers in humans, especially in workers and children, and to eliminate inequalities in judging cancer risks between industrialized and developing countries. Tomatis' foresight, guidance, leadership, and staunch belief in primary prevention continued to influence the *IARC Monographs* as they expanded to encompass personal habits, as well as physical and biological agents. Lorenzo Tomatis had a distinguished career at the Agency, arriving in 1967 and heading the Unit of Chemical Carcinogenesis, before being Director from 1982 to 1993.

Volume 100 of the *IARC Monographs* Series is respectfully dedicated to him.

(photo: Roland Dray)

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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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Each participant was asked to disclose pertinent research, employment, and financial interests. Current financial interests and research and employment interests during the past 3 years or anticipated in the future are identified here. Minor pertinent interests are not listed and include stock valued at no more than US$10 000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are listed as significant pertinent interests.

[2] Dr Bond reviewed a draft report (on a chemical that is not being reviewed at this meeting) for the American Petroleum Institute, a trade association that represents corporations that produce and distribute some of the chemicals under review.

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[3] Dr Goldstein serves as an expert witness in court cases involving benzene. Support for one position in a dispute generally poses a conflicting interest, however, Dr Goldstein lists similar numbers of cases on behalf of plaintiffs and corporate defendants, and for this reason IARC exceptionally regards this activity as not warranting a limitation on participation. Dr Goldstein also consulted with an insurance company to estimate the number of worker disability cases that will result from the World Trade Center attack.

[4] Dr Groopman served on an advisory board for VICAM, which makes test kits for aflatoxins.

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Laurent Galichet's colleagues gratefully acknowledge his years of service in the *IARC Monographs* Programme, in particular his unrelenting efforts to modernize the production process of the *Monographs*. The new style and layout of the Volume 100 series are the fruits of these efforts. Laurent left the IARC in October 2011.

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.

For most chemicals and some complex mixtures, the major collection of data and the preparation of working papers for the sections on chemical and physical properties, on analysis, on production and use, and on occurrence are carried out under a separate contract funded by the US National Cancer Institute. 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

Part F of Volume 100 of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* contains updated assessments of several chemical agents, complex mixtures, and related occupations that were classified as *carcinogenic to humans (Group 1)* in Volumes 1–99.

Volume 100 – General information

About half of the agents classified in Group 1 were last reviewed more than 20 years ago, before mechanistic studies became prominent in evaluations of carcinogenicity. In addition, more recent epidemiological studies and animal cancer bioassays have demonstrated that many cancer hazards reported in earlier studies were later observed in other organs or through different exposure scenarios. Much can be learned from updating the assessments of agents that are known to cause cancer in humans. Accordingly, IARC has selected A Review of Human Carcinogens to be the topic for Volume 100 of the *IARC Monographs*. It is hoped that this volume, by compiling the knowledge accumulated through several decades of cancer research, will stimulate cancer prevention activities worldwide, and will be a valued resource for future research to identify other agents suspected of causing cancer in humans.

Volume 100 was developed by six separate Working Groups:

Pharmaceuticals
Biological agents
Arsenic, metals, fibres, and dusts
Radiation
Personal habits and indoor combustions
Chemical agents and related occupations

Because the scope of Volume 100 is so broad, its *Monographs* are focused on key information. Each *Monograph* presents a description of a carcinogenic agent and how people are exposed, critical overviews of the epidemiological studies and animal cancer bioassays, and a concise review of the toxicokinetic properties of the agent, plausible mechanisms of carcinogenesis, and potentially susceptible populations and life-stages. Details of the design and results of individual epidemiological studies and animal cancer bioassays are summarized in tables. Short tables that highlight key results appear in the printed version of Volume 100, and more extensive tables that include all studies appear on the website of the *IARC Monographs* programme (http://monographs.iarc.fr). For a few well-established associations (for example, tobacco smoke and human lung cancer), it was impractical to

include all studies, even in the website tables. In those instances, the rationale for inclusion or exclusion of sets of studies is given.

Each section of Volume 100 was reviewed by a subgroup of the Working Group with appropriate subject expertise; then all sections of each *Monograph* were discussed together in a plenary session of the full Working Group. As a result, the evaluation statements and other conclusions reflect the views of the Working Group as a whole.

Volume 100 compiles information on tumour sites and mechanisms of carcinogenesis. This information will be used in two scientific publications that may be considered as annexes to this volume. One publication, Tumour-Site Concordance between Humans and Experimental Animals, will analyse the correspondence of tumour sites among humans and different animal species. It will discuss the predictive value of different animal tumours for cancer in humans, and perhaps identify human tumour sites for which there are no good animal models. Another publication, Mechanisms Involved in Human Carcinogenesis, will describe mechanisms known to or likely to cause cancer in humans. Joint consideration of multiple agents that act through similar mechanisms should facilitate the development of a more comprehensive discussion of these mechanisms. Because susceptibility often has its basis in a mechanism, this could also facilitate a more confident and precise description of populations that may be susceptible to agents acting through each mechanism. This publication will also suggest biomarkers that could render future research more informative. In this way, IARC hopes that Volume 100 will serve to improve the design of future cancer studies.

Introduction to Volume 100F

This sixth and final part of Volume 100 of the *IARC Monographs* contains updated assessments of several chemical agents, complex mixtures, and related occupations that were classified as *carcinogenic to humans (Group 1)* in Volumes 1–99. In the early 1970s, the *IARC Monographs* began as a programme to evaluate chemical substances; among the agents considered in the present volume, 4-aminobiphenyl, auramine production, and benzidine were first reviewed in Volume 1 (IARC, 1972), benzo[*a*]pyrene in Volume 3 (IARC, 1973), and 2-naphthylamine, bis(chloromethyl)ether, and chloromethyl methyl ether in Volume 4 (IARC, 1974). For most of these chemicals, the cancer hazard was recognized already when they were first reviewed, although the classification in Group 1 was formalized later, in Working-Group meetings for Supplements 1, 4, and 7 (IARC, 1979, 1982, 1987).

Some workplace conditions entail complex exposures to varying chemical mixtures. This makes it difficult to attribute an excess cancer risk to specific causal agents, particularly in the absence of long-term follow-up studies showing a reduction of risk following the removal of specific agents from the workplace. Accordingly, this Volume 100F also contains updated assessments of chemical-related occupations and industries that were classified in Volumes 1–99 as *carcinogenic to humans (Group 1)*.

The agents that are assessed in the present volume were last reviewed during *IARC Monograph* meetings dating from 1987 till 2008 (see Table).

It should be noted that, in the present volume, some of these agents are named slightly differently, compared with previous evaluations.

Agents reviewed in this volume

Agent	Volume Number	Year of meeting
4-Aminobiphenyl	Volume 99	2008
Benzidine and dyes metabolized to benzidine	Volume 99	2008
Methylenebis(chloroaniline) (MOCA)	Volume 99	2008
2-Naphthylamine	Volume 99	2008
ortho-Toluidine	Volume 99	2008
Auramine production	Volume 99	2008
Magenta production	Volume 99	2008
Benzo[*a*]pyrene	Volume 92	2005
Coal gasification, occupational exposures during	Volume 92	2005
Coal-tar distillation, occupational exposures during	Volume 92	2005
Coal-tar pitch (paving and roofing with), occupational exposures during	Volume 92	2005
Coke production, occupational exposures during	Volume 92	2005
Mineral oils (untreated and mildly treated)	Supplement 7	1987
Shale oils	Supplement 7	1987
Chimney sweep, occupational exposure as a	Volume 92	2005
Aluminium production	Volume 92	2005
Aflatoxins (naturally occurring mixtures of)	Volume 82	2002
Benzene	Supplement 7	1987
Bis(chloromethyl)ether and chloromethyl methyl ether (technical grade)	Supplement 7	1987
1,3-Butadiene	Volume 97	2007
2,3,7,8-Tetrachlorodibenzo-*para*-dioxin	Volume 69	1997
Ethylene oxide	Volume 97	2007
Formaldehyde	Volume 88	2006
Sulfur mustard	Supplement 7	1987
Vinyl chloride	Volume 97	2007
Isopropyl alcohol manufacture (strong-acid process)	Supplement 7	1987
Strong-inorganic-acid mists containing sulfuric acid (occupational exposure)	Volume 54	1991
Iron and steel founding	Supplement 7	1987
Painter (occupational exposure as a)	Volume 98	2007
Rubber-manufacturing industry	Supplement 7	1987

Specific remarks about some of the agents reviewed in this volume

Four aromatic amines (4-aminobiphenyl, benzidine, 2-naphthylamine, *ortho*-toluidine) and two related industrial processes (auramine production, magenta production) were re-affirmed as Group-1 carcinogens based on *sufficient evidence* that they cause cancer of the urinary bladder in humans. The Group-1 classification of dyes metabolised to benzidine was based on *sufficient evidence* of carcinogenicity for some of these dyes in experimental animals and strong mechanistic evidence indicating that the metabolism of these dyes leads to the release of free benzidine – a re-affirmed Group-1 carcinogen – and to the subsequent induction of chromosomal aberrations in all experimental animal species studied, and in humans exposed to these dyes. Likewise, the Group-1 classification of 4,4′-methylenebis(2-chloroaniline) was based on *sufficient evidence* of carcinogenicity in experimental animals and strong mechanistic evidence, indicating that the toxicological profile of this genotoxic amine is similar to that of *ortho*-toluidine (a re-affirmed Group-1 carcinogen), that it forms DNA adducts in human urothelial cells *in vitro* and haemoglobin adducts in the blood of exposed workers, and that it causes cytogenetic alterations in urothelial cells and lymphocytes of exposed workers.

Exposure to polycyclic aromatic hydrocarbons (PAHs) causes cancers of the skin and lung in humans. Although there are no epidemiological studies of benzo[*a*]pyrene as a single exposure, it is carcinogenic in numerous animal species. There is mechanistic evidence indicating that benzo[*a*]pyrene is metabolized to highly reactive diolepoxides that form covalent DNA adducts, which have been shown to induce mutations in the K-*RAS* oncogene and the *TP53* tumour-suppressor gene in human lung tumours, and in corresponding genes in lung tumours in mice. Exposures to benzo[*a*]pyrene and benzo[*a*]pyrene-containing complex mixtures also induce cytogenetic alterations, DNA breakage, oxidative DNA lesions, and specific mutations in oncogenes and tumour-suppressor genes, all of which can contribute to the carcinogenic effects of benzo[*a*]pyrene and benzo[*a*]pyrene-containing complex mixtures in exposed humans. This consistent and coherent mechanistic evidence from experimental and human studies provides biological plausibility to support the overall classification of benzo[*a*]pyrene as a Group-1 carcinogen.

Two PAH-containing mixtures (chimney soot, coal-tar pitch), and occupational exposures in four PAH-related industries (coal-tar distillation, coal gasification, coke production, aluminium production) were confirmed as Group-1 carcinogens.

Workers in the rubber-manufacturing industry have an increased risk for leukaemia, lymphoma, and cancers of the urinary bladder, lung, and stomach. Due to the diversity and complexity of the exposures during rubber-manufacturing, the Working Group – like the previous one three decades ago (*IARC Monograph* Volume 28, 1982) – could not identify specific causative agents. However, there continues to be strong evidence of genotoxic and cytogenetic effects in workers in this industry.

There is consistent evidence that untreated or mildly treated mineral oils cause cancer of the skin, specifically of the scrotum, in humans. The association is highly unlikely to be due to chance, bias, or confounding, given the large case series, supportive epidemiological evidence, the rarity of scrotal cancer, and the intensity of exposure during the period of interest. Despite the fact that a significant proportion of workers exposed occupationally to mineral oils and shale oils are women, epidemiological studies established a statistically significant risk only for skin cancer in the scrotum, because of the extreme rarity of this type of cancer at this site. This observation does not imply that the skin-cancer hazard is restricted to males.

Evidence from three cohort studies indicated that exposure of humans during the manufacture of isopropyl alcohol by the strong-acid process causes cancer of the paranasal sinuses, an extremely rare cancer. The risk for laryngeal cancer may also have been elevated in these workers. This evaluation re-affirms the conclusion of a previous Working Group (IARC, 1987). It was noted that since that time none of these cohorts nor any other isopropanol-manufacturing unit has been evaluated for cancer mortality or incidence without the inclusion in the study population of workers in other production units. An increased incidence of other cancers in the upper respiratory tract was observed in these studies, but it was not possible to attribute these cancers to exposures during isopropanol production. Therefore, the possible association of these tumours with this specific exposure could not be evaluated.

The Working Group reviewed more than 100 epidemiological studies of benzene and confirmed its carcinogenicity, with *sufficient evidence* for acute non-lymphocytic leukaemia and *limited evidence* for acute and chronic lymphocytic leukaemia, multiple myeloma, and non-Hodgkin lymphoma. The Working Group noted the extraordinary expansion in the epidemiological literature on benzene-related cancers since the previous evaluation, the diversity of industrial and environmental exposure scenarios where benzene has been studied, and the evolution in the histological classification of leukaemias and lymphoid neoplasms over the past decades, and suggested that a re-evaluation of the cancer hazards from exposure to benzene be conducted in due time.

Dioxin (2,3,7,8-tetrachlorodibenzo-*para*-dioxin, TCDD) was previously classified in Group 1, based on *limited evidence* of carcinogenicity in humans, *sufficient evidence* in rodents, and strong evidence in humans and animals for a mechanism via initial binding to the aryl-hydrocarbon receptor (AhR), which leads to changes in gene expression, cell replication, and apoptosis. There is now *sufficient evidence* from epidemiological studies for all cancers combined, making TCDD the first agent classified initially in Group 1 based on *sufficient evidence* of carcinogenicity in experimental animals and strong mechanistic data, to be later confirmed by increased cancer incidence in humans. This highlights the ability of mechanistic information to provide robust evidence of carcinogenicity. Like TCDD, 2,3,4,7,8-pentachlorodibenzofuran and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) are complete carcinogens in experimental animals, and there is ample evidence that they act through the same AhR-mediated mechanism. The Working Group classified these two chemicals in Group 1. The Working Group discussed the possibility of evaluating an additional 26 polychlorinated dibenzodioxins, dibenzofurans and biphenyls for carcinogenicity, based upon data showing similarity to the mechanism outlined for TCDD. According to the *IARC Monographs* Preamble (Part B, Section 6d), an evaluation may be made for a broad group of agents, even including substances for which there is no direct information on cancer in humans or experimental animals, if it is warranted by the overall evidence. Recognizing the complexity of the mechanistic evaluation, the Working Group decided this time to make evaluations for two indicator chemicals, 2,3,4,7,8-pentachlorodibenzofuran and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), for which there are recent positive NTP bioassays, supported by data on a wide range of mechanistic events. The Working Group suggested that a future generic evaluation be focused on the entire class of dioxin-like compounds, taking into account the available mechanistic data.

The Working Group unanimously reaffirmed the classification of formaldehyde in Group 1, based on *sufficient evidence* in humans of nasopharyngeal cancer. A possible association with leukaemia was considered strong "but not sufficient" by a previous Working Group, mainly because of the lack of a plausible mechanism. Since that time, the epidemiological evidence has become stronger: a recent

study found that embalming was significantly associated with an increased risk for myeloid leukaemia, with cumulative years of embalming and with increasing peak-exposure to formaldehyde. In addition, a recent study of a small group of workers exposed to formaldehyde showed numerical chromosomal aberrations in myeloid progenitor cells (chromosome-7 monosomy, chromosome-8 trisomy) as also observed in myeloid leukaemia, and haematological changes in peripheral blood that are indicative of effects on the bone marrow. A small majority of the Working Group concluded that, overall, there is *sufficient evidence* of a causal association between exposure to formaldehyde and an increased risk for leukaemia, particularly myeloid leukaemia.

Identification of tumour sites

One of the goals of the review of Group-1 carcinogens in Volume 100 of the *IARC Monographs* is the identification of tumour sites with *sufficient evidence* in humans. The tumour-site identification in this volume was – naturally – limited by the research that has been published to date, and it should be noted that many plausible tumour sites identified in rodents have not been considered in humans. For example, several aromatic amines induce mammary gland tumours in rats, and there is mechanistic evidence that supports a potential for aromatic amines to cause this cancer, but the epidemiological studies on cancer associated with exposure to these substances have not considered breast cancer, mainly because the industrial cohorts were generally small and did not include women. In addition, no case–control studies are available on breast cancer associated with exposure to aromatic amines. Similarly, mammary gland tumours are the only tumour induced by 1,3-butadiene in both rats and mice, but epidemiological studies – while demonstrating an increased risk for leukaemia – have just started to explore the possibility of this chemical being associated with breast cancer.

Changes in occupational exposures over time

Changes in occupational hygiene or industrial processes can alter the profile of workplace exposures over time. Moreover, regulations and working conditions may differ greatly from one country to another. For these reasons, it is useful to identify the specific agents responsible for excess cancer risks whenever possible (see *IARC Monographs* Preamble Part B, Section 6a). It is important to recognize that new studies of modern and improved workplaces would not reflect risks that might still exist in areas where adequate regulations or process improvements are not fully implemented. Consequently, previous evaluations of occupations that were classified as *carcinogenic to humans* stand as a historical record of hazards that are known to cause cancer. In future monographs, as historical exposures described as occupations are reduced and risk is abated, previous findings should be noted as indicative of potential carcinogenic risk in this occupation should historical exposure levels return or continue to exist elsewhere. Also, as industrial processes change, carcinogenicity profiles in the workplace will change and evaluations need to take these changing processes into account.

Incorporation of new mechanistic data in future evaluations

The ever-increasing understanding of the molecular mechanisms underpinning the classical concepts of initiation, promotion and progression in cancer provides a challenge for its integration into cancer-hazard identification. In most instances, genotoxic pathways have been considered central to the carcinogenic process and mechanistic studies characterizing these outcomes have provided important supporting information for the evaluation. The panel of genotoxic endpoints has been extended over the years, from classical mutagenicity in bacteria to the current demonstration of mutation induction in oncogenes and tumour-suppressor genes.

Mechanisms of non-genotoxic carcinogenesis are being identified for an increasing number of chemicals evaluated by the IARC *Monographs*. In the future, this area will undoubtedly expand into processes mediated by epigenetic events. Collectively, new types of information are being used to assess these mechanisms, such as epigenomics, proteomics, metabolomics and systems-biology approaches. These strategies have identified changes in specific genes, proteins, signalling pathways, networks of pathways, cell-cycle genes and transcription factors. Other studies have focused on changes in methylation, and on patterns of microRNA expression and effects of small interfering RNA (si-RNA). Proteomics studies have identified post-translational modifications, and metabolomic studies have revealed how endogenous metabolite patterns change after exposure. These studies are conducted in human and non-human cells, tissues from experimental animals, and in human tissues following occupational and/or environmental exposures. It is recommended that IARC convene an Advisory Group to develop guidelines for the evaluation and incorporation of this type of information to support a specific mechanism and/or to derive an evaluation of carcinogenicity for a specific exposure.

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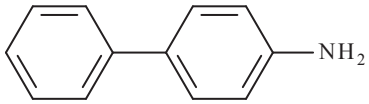
4-AMINOBIPHENYL

4-Aminobiphenyl was considered by previous IARC Working Groups in 1971, 1987, and 2008 (IARC, 1972, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 92-67-1
Chem. Abstr. Serv. Name:
[1,1'-Biphenyl]-4-amine



$C_{12}H_{11}N$
Relative molecular mass: 169.22
Description: Colourless, crystalline solid that turns purple when exposed to air
Solubility: Slightly soluble in cold water; soluble in acetone, chloroform, ethanol, diethyl ether, and hot water

From O'Neil (2006), Lide (2008), and IARC (2010)

1.2 Uses

4-Aminobiphenyl has been used in the past as a rubber antioxidant, as a dye intermediate, and in the detection of sulfates. It is reportedly used as a model carcinogen in mutagenicity studies and in cancer research (NTP, 2005; O'Neil, 2006; HSDB, 2009).

1.3 Human exposure

1.3.1 Occupational exposure

Historically, occupational exposure to 4-aminobiphenyl mainly occurred during its production and its use as a rubber antioxidant and dye intermediate. No exposure measurements are available for these occupational exposure situations (IARC, 2010).

Occupational exposure can also occur when workers are exposed to products contaminated with 4-aminobiphenyl, or in the case of exposure to benzidine and benzidine-based dyes, from which 4-aminobiphenyl can be metabolically released (IARC, 2010). In a study from India on workers exposed to benzidine or benzidine-based dyes and a non-exposed control group, urine samples were analysed for 4-aminobiphenyl and acetylated 4-aminobiphenyl (Ac4ABP). 4-Aminobiphenyl was found in 30 of 33 urine samples from exposed workers and in one sample from the 13 control workers. The workers exposed to benzidine had significantly higher median 4-aminobiphenyl concentrations (57 pmol/mL) than those exposed to benzidine-based dyes (29.3 pmol/mL). Ac4ABP was only detected (79.5 pmol/mL) in the urine sample that was provided by the person who had the highest 4-aminobiphenyl concentration (Beyerbach *et al.*, 2006).

1.3.2 Non-occupational exposure

The main sources of exposure to 4-aminobiphenyl for the general population are cigarette smoking and second-hand tobacco smoke, as 4-aminobiphenyl is formed during tobacco combustion. The following amounts of 4-aminobiphenyl have been reported in unfiltered mainstream, filtered mainstream and side-stream cigarette smoke, respectively: 2.4 to 4.6 ng/cigarette; 0.2 to 23 ng/cigarette; and up to 140 ng/cigarette (Patrianakos & Hoffmann, 1979; Hoffmann *et al.*, 1997).

Other potential sources include hair dyes and food colourant. 4-Aminobiphenyl can occur as a contaminant in 2-aminobiphenyl, which is used in the manufacture of dyes. 4-Aminobiphenyl has been detected in aniline, in the drug and cosmetic colour additive D&C Yellow No. 1, in the food dye FD&C Yellow No. 6, and in hair dyes (Richfield-Fratz *et al.*, 1985; Chiang *et al.*, 1999; Turesky *et al.*, 2003; Akyüz, 2007; Bafana *et al.*, 2007). 4-Aminobiphenyl has also been found as a contaminant in diphenylamine, a fungicide that has been used on apples.

4-Aminobiphenyl has been detected in fume from cooking oils. In a study from Taiwan, China, concentrations of 4-aminobiphenyl were 35.7 μg/m^3 in fumes from cooking with sunflower oil, 26.4 μg/m^3 in vegetable oil fumes and 23.3 μg/m^3 in oil fumes from refined lard (Chiang *et al.*, 1999).

Living near benzidine-contaminated sites may result in exposure to 4-aminobiphenyl, as benzidine in the environment can be degraded to 4-aminobiphenyl by certain bacteria (Bafana *et al.*, 2007).

2. Cancer in Humans

2.1 Descriptive studies

Melick *et al.* (1955) reported a series of 19 cases of cancer of the urinary bladder in 171 male workers (11.1%) engaged in the production of 4-aminobiphenyl. The exposure took place in a chemical plant in the United States of America (USA) between 1935 and 1955. In a follow-up study it was reported that among 315 male workers exposed to 4-aminobiphenyl, 53 had developed bladder tumours (Melick *et al.*, 1971).

2.2 Cohort studies

Following the cessation of industrial production of 4-aminobiphenyl in 1955, a surveillance programme in exposed workers revealed 31 of 285 men with significantly abnormal epithelial cells in urinary sediments, of whom ten were diagnosed with histologically confirmed bladder carcinoma (Melamed *et al.*, 1960). Subsequently, 11 additional cases were found among 18 of the men reported in 1960 to have abnormal cells (Koss *et al.*, 1965). Expanded surveillance programmes identified 35 workers with cancer of the urinary bladder among 503 workers (Koss *et al.*, 1969) and 43 men with confirmed bladder carcinoma among 86 men with suspicious or positive histology (Melamed, 1972).

Cancer mortality was studied among 884 male workers at a chemical plant in West Virginia (USA) that produced a variety of chemicals. A ten-fold increase in mortality from bladder cancer was reported, with all nine cases having started work before 4-aminobiphenyl production ceased in the plant in 1952 (Zack & Gaffey, 1983). An analysis of mortality through 1987 showed 11 deaths from cancer of the urinary bladder among workers in jobs with possible exposure to 4-aminobiphenyl, compared to 0.54 expected (Collins *et al.*, 1993). Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

(TCDD) was also considered, based on reports of chloracne related to an industrial accident with TCDD in 1949, but ten of the 11 workers who died of cancer of the urinary bladder did not have chloracne. Collins *et al.* (1999) conducted another cohort study in the same plant and evaluated the risk for cancer of the urinary bladder associated with exposure to 4-aminobiphenyl and another bladder carcinogen, 2-mercaptobenzothiazole (MBT). Eight workers in jobs with exposure to 4-aminobiphenyl and MBT died of cancer of the urinary bladder, 0.3 deaths were expected (SMR 27.1; 95%CI: 11.7–53.4), while five workers exposed to MBT in jobs associated with little or no exposure to 4-aminobiphenyl died of cancer of the urinary bladder, compared with 1.2 expected.

2.3 Synthesis

Case reports and cohort-surveillance studies indicate a high occurrence of cancer of the urinary bladder in workers occupationally exposed to 4-aminobiphenyl, supported by evaluations of mortality in a chemical plant in the USA. Bladder cancer is strongly associated with occupational exposure to 4-aminobiphenyl.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of 4-aminobiphenyl in the mouse, rat, dog, and rabbit after oral administration or after subcutaneous or intraperitoneal injection have been reviewed in previous *IARC Monographs* (IARC, 1972, 1987, 2010). The results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010).

4-Aminobiphenyl was tested for carcinogenicity by oral administration in three studies in mice, six studies in dogs and one study in rabbits, by subcutaneous injection in one study in mice and one study in rats and by intraperitoneal injection in four studies in mice.

Oral administration of 4-aminobiphenyl caused increased incidences of angiosarcoma (all sites) in male and female mice, bladder carcinoma in male mice (Schieferstein *et al.*, 1985), hepatocellular carcinoma in female mice (Clayson *et al.*, 1967; Schieferstein *et al.*, 1985), and bladder carcinoma in male and female dogs (Walpole *et al.*, 1954; Deichmann *et al.*, 1958, 1965; Block *et al.*, 1978) and in rabbits (sex not specified) (Bonser, 1962). [The Working Group noted that there were limitations in the design and reporting of these studies.] The incidence of hepatocellular adenoma and/or carcinoma was increased in male mice after subcutaneous (Gorrod *et al.*, 1968) or intraperitoneal injection (Dooley *et al.*, 1992; Parsons *et al.*, 2005). [Most of these studies were designed to study tumour formation in the liver and the histopathology is limited to examination of the liver only.]

4. Other Relevant Data

4.1 Aromatic amines: metabolism, genotoxicity, and cancer susceptibility

Biotransformation pathways and genotoxic effects of aromatic amines are described in detail in IARC (2010); highlights are summarized below.

Exposures to aromatic amines, such as 2-naphthylamine, 4-aminobiphenyl and benzidine in the textile dye and rubber tyre industries have long been known to cause cancer of the urinary bladder in humans. These substances also induce neoplasms at multiple organ sites in laboratory animals. Tobacco smoke and hair dyes are major non-occupational sources of exposure to

Table 3.1 Carcinogenicity studies of 4-aminobiphenyl in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse C57 x IF F_1 (M, F) 70 wk Clayson *et al.* (1967)	Oral–Gavage A group of 21 M and 28 F mice were dosed with 0.2 ml of a 25% 4-amino-biphenyl solution in arachis oil, twice/wk for 50 wk, and kept for an additional 20 wk. A group of 19 M and 31 F served as untreated controls	Hepatoma, malignant: [hepatocellular carcinoma] M–0/19, 4/21; F–0/31, 13/28* Bladder carcinoma: M–0/19, 1/21; F–0/31, 0/28	No statistics *[$P < 0.0001$]	Also reported were four "probably malignant" hepatomas in female mice. Purity of 4-aminobiphenyl NR
Mouse BALB/cStCrlfC3Hf/Nctr (M, F) 96 wk Schieferstein *et al.* (1985)	Oral–Drinking-water Groups of 120 M and 120 F mice were given 4-aminobiphenyl as the hydrochloride salt (> 99.5% pure) at doses of 0, 7, 14, 28, 55, 110, and 220 ppm (M) and 0, 7, 19, 38, 75, 150, and 300 ppm (F). Interim sacrifices were at 13, 26, 39, 52, and 96 wk	Angiosarcoma (all sites): M–1/118, 1/117, 1/118, 2/119, 4/115, 5/119, 14/118* F–1/119, 4/120, 4/120, 2/120, 14/120, 26/118, 11/117* Bladder carcinoma: M–0/116, 1/117, 1/118, 0/118, 6/115, 15/118, 23/118* F–0/118, 0/118, 0/119, 1/118, 0/118, 5/117, 1/117 Hepatocellular carcinoma: M–2/118, 1/117, 0/118, 0/117, 0/114, 3/118, 2/117 F–0/117, 0/120, 2/120, 4/119, 10/119, 14/118, 7/117*	*$P < 5x10^{-5}$, positive trend	
Dog Beagle (M) 33 mo Walpole *et al.* (1954)	Oral Two, 7 mo-old male dogs were given 4-aminobiphenyl in a gelatin capsule once daily, 6 × /wk until termination of the study. The dose level was lowered during the course of the experiment and dosing was also interrupted temporarily for 6 mo. The experiment was terminated after 33 mo; total dose for the two dogs was 2.9 and 3.3 g/kg bw, respectively	Bladder carcinomas occurred in both dogs	NR	No concurrent controls. Historical data from this laboratory show that thirty Beagle dogs (age, 3–9 yrs) that died of various causes did not develop bladder tumours. Purity of 4-aminobiphenyl NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Dog Mongrel (F) Lifetime Deichmann *et al.* (1958)	Oral A group of four young adult female mongrel dogs were given 0.3 g of 4-aminobiphenyl admixed into the food on 5 d/wk for 1 yr. The dogs then received an oral dose (capsule) of 0.3 g of this compound 3 × /wk for the rest of the study. The total dose (range) at first appearance of tumours was 87.5–144.0 g per dog, corresponding to 8.2–14.1 g/kg bw	Bladder carcinomas occurred in all four dogs after 21–34 mo	NR	No concurrent controls. Experimental design poorly described. Bladder is only tissue examined.
Dog Beagle (F) Up to 37 mo Deichmann *et al.* (1965)	Oral A group of six 6–12 mo-old female dogs were given an oral dose (capsule) of 4-aminobiphenyl at 1.0 mg/kg bw, 5 × / wk for up to 37 mo. The total dose range was 5.35–7.34 g per dog.	Bladder carcinomas (transitional cell type) were observed in three dogs, bladder papillomas in the three other dogs.	NR	No concurrent controls. Bladder is only tissue examined. Purity of 4-aminobiphenyl NR
Dog Beagle (F) 42 mo Block *et al.* (1978)	Oral A group of 24 female dogs (age, 4 mo) were given 4-aminobiphenyl orally in a corn-oil suspension contained in a capsule on 5 d/wk for 36 mo.	Transitional cell urinary bladder carcinomas 20/24 Grade-2 and -3 tumours 2/24 Grade-1 tumours 2/24 no detectable tumours		Authors indicate that twenty matched littermates served as controls, but no other information was provided for the control animals. Purity of 4-aminobiphenyl NR
Rabbit Strain NR (sex NR) Lifetime Bonser (1962)	Oral A group of 7 rabbits were treated with 4-aminobiphenyl to the limit of tolerance, which was continued until the onset of the final illness. Three animals were sacrificed in the first 2 yr and two each at 3–4 and 5–6 yr after the start of treatment. A group of 12 rabbits served as controls, 5 of which were sacrificed in the first two yr, one at 3–4 yr, and three each at 5–6 and > 7 yr after the start of treatment.	Bladder carcinoma: 0/12, 3/7	NR [$P < 0.036$]	Experimental design very poorly described. It appears bladder is the only tissue examined. Sex NR. Purity of 4-aminobiphenyl NR Dose and dose regimen NR
Mouse Swiss (M, F) 52 wk Gorrod *et al.* (1968)	Subcutaneous injection A group of 52 newborn mice were injected s.c. with 200 µg of 4-amino-biphenyl on each of the first three d of life, separated at weaning into a group of 24 M and 27 F, and kept for up to 52 wk. Groups of 41 M and 41 F newborn mice served as vehicle controls.	Male Hepatomas: M–5/41, 19/20 F–2/41, 4/23	NR [$P < 0.0001$] [NR]	Purity of 4-aminobiphenyl NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat Albino (M, F) Lifetime (animals kept for up to 582 d) Walpole *et al.* (1952)	Subcutaneous injection Groups of 11 M and 12 F rats were each divided in two groups and given 4-aminobiphenyl in arachis oil 5 × /wk for a mean duration of 250–376 dosing d, to a total mean dose per animal of 3.6–5.8 g/kg bw. A control group of 12 M and 11 F rats received arachis oil only.	Intestinal tumours M–0/12, 3/6 (4.4 g/kg bw), 1/5 (5.8 g/kg bw) F–1/11, 1/6 (3.6 g/kg bw), 2/6 (4.2 g/kg bw)	[$P < 0.05$], [NR] [NR], [NR]	Experimental design, especially exposure durations, poorly described. Small numbers of animals make study results difficult to interpret.
Mouse B6C3F$_1$/nctr (M) 12 mo Dooley *et al.* (1992)	Intraperitoneal injection Newborn male mice were given 4-aminobiphenyl (> 98%). The amounts administered were 0, 0.625 and 1.25 µmol dissolved in 35 µl DMSO, injected in portions of 5, 10 and 20 µl on d 1, 8 and 15 after birth, respectively. Surviving pups were weaned on d 21 and designated for necropsy at 8 or 12 mo of age.	At 12 mo: Hepatocellular adenomas: 5/44, 19/19*, 15/15* Hepatocellular carcinomas: 0/44, 5/19*$^{\$}$, 5/15* At 8 mo: Hepatocellular adenomas: 1/44, 22/24*, 8/11* No carcinomas	*$P < 0.001$, Fisher exact test), $^{\$}$[$P < 0.005$]	Initial number of animals NR
Mouse B6C3F$_1$ (M) 12 mo Parsons *et al.* (2005)	Intraperitoneal injection Newborn male mice were given 0.3 µmol 4-aminobiphenyl dissolved in DMSO, by a series of injections: 1/7th of the dose on postnatal Day 1, 2/7th on Day 8, and 4/7th on Day 15. Control mice received DMSO only.	Hepatocellular adenoma: 4/18, 19/24 Hepatocellular carcinoma: 0/18, 2/24	[$P < 0.001$] [NR]	Liver is the only tissue examined. Initial number of animals NR
Mouse CD1 (M) 12 mo Von Tungeln *et al.* (1996)	Intraperitoneal injection Newborn male mice were given a total dose of 625 nmol 4-amino-biphenyl (> 99% pure) in 35 µl dimethyl sulfoxide, given in portions of 5, 10 and 20 µl on Days 1, 8, and 15 after birth, respectively. At weaning, the animals were divided over two groups, which were fed *ad libitum* until the age of 14 wk. Thereafter, one group received 90% of the calories of the *ad libitum* feeding regimen during one wk, followed by 75% of the calories during one wk, and then 60% of the calories in the diet until sacrifice at 12 mo.	*Ad-libitum* group: Hepatocellular carcinoma: 6/22 Hepatocellular adenoma: 12/22 Caloric restriction group: Liver tumours: 0/19	$P < 0.02$ $P < 0.001$ Between *ad libitum* and caloric restriction groups	No tumours in calorie-restricted group No untreated controls Initial number of animals NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse CYP1A2 –/– and CYP1A2+/+ (M, F) 16 mo Kimura *et al.* (1999)	Intraperitoneal injection Groups of male and female mice were injected a total dose 600 or 1200 nmol of 4-aminobiphenyl in DMSO. Control animals were injected with DMSO only.	Hepatocellular carcinoma: -/- M–0/12, 5/27, 8/42 +/+ M–0/12, 6/30, 4/26 -/- F– 0/25, 0/25, 0/27 +/+ F– 0/25, 1/23, 0/33 Hepatocellular adenoma: -/- M–2/12, 13/27**, 29/42* +/+ M–2/12, 21/30*, 18/26* -/- F– 2/25, 2/25, 3/27 +/+ F– 2/25, 4/23, 1/33	*$P < 0.01$ **$P < 0.05$	Liver was the only tissue examined. Initial number of animals NR Mice derived from a mixed background of 129/Sv and C57BL/6 strains

bw, body weight; d, day or days; DMSO, dimethyl sulfoxide; F, female; M, male; mo, month or months; NR, not reported; wk, week or weeks; yr, year or years

arylamines, as is demonstrated by the detection of aminobiphenyl-haemoglobin adducts (Bryant *et al.*, 1988; Ward *et al.*, 1996; Beyerbach *et al.*, 2006), which can be 3- to 10-fold more abundant in tobacco smokers than in nonsmokers (Yu *et al.*, 2002). Other environmental sources of exposure are likely to exist as well, because biomarkers derived from aromatic amines, such as haemoglobin adducts or urinary metabolites have been identified also in non-smokers who are not occupationally exposed to these chemicals.

Multiple metabolic pathways are involved in the activation of aromatic amines to DNA-reactive intermediates. Metabolism is initiated in the liver with either *N*-oxidation (by cytochrome P-450-associated enzymes) or *N*-acetylation (by *N*-acetyltransferase 2, NAT2). *N*-oxidation to *N*-hydroxyarylamine is mainly mediated by CYP1A2, but also CYP1A1 and CYP4B1 iso-enzymes may play a role (Landi *et al.*, 1996; Ketelslegers *et al.*, 2009). NAT2-catalysed *N*-acetylation can provide a detoxification pathway for aromatic amines since it reduces the amount of parent compound that may undergo CYP-mediated *N*-hydroxylation. The *N*-hydroxy metabolite is highly electrophilic: *N*-hydroxyaminobiphenyl, the oxidation product of 4-aminobiphenyl (4-ABP) forms adducts with hepatic DNA at the C8 position of deoxyguanosine and deoxyadenosine in rats (Jones & Sabbioni, 2003). *N*-hydroxyarylamines may be transported in free form to the blood or be conjugated with glucuronide. The acid-labile glucuronidated intermediate is excreted via the kidney and hydrolysed in the bladder lumen where it eventually forms the *N*-hydroxy metabolite again. The acidic pH of urine enhances the hydrolysis reaction and thus represents an additional risk factor for aromatic amine-related bladder cancer. NAT1-mediated *O*-acetylation may represent the final activation step of *N*-hydroxyarylamines; it takes place in the bladder epithelium and forms *N*-acetoxyarylamine. Breakdown of this unstable aromatic acetoxy ester produces the highly reactive aryl nitrenium ion that may serve as electrophilic intermediate leading to DNA adducts and tumour initiation. The highly active NAT1*10 isoform was correlated with higher levels of arylamine-DNA adducts in the human bladder (Kadlubar & Badawi, 1995).

Other activation pathways of aromatic amines to DNA-reactive intermediates include the sulfotransferase-mediated activation of *N*-hydroxyarylamine to an *N*-sulfate ester (Chou *et al.*, 1995), the myeloperoxidase- (Lakshmi *et al.*, 2000) and lactoperoxidase-mediated pathways that catalyse activation in the mammary gland (Gorlewska-Roberts *et al.*, 2004), the peroxidative activation by prostaglandin H synthase (Flammang *et al.*, 1989) – likely predominant in extra-hepatic tissues with low levels of cytochrome P450 isoenzymes –, and non-enzymatic protonation of the *N*-hydroxylamine nitrogen (Beland *et al.*, 1983). Genotoxic aromatic amines may induce tumour formation at different sites depending on substrate specificity and different bio-activation pathways. Inter-individual variability in prostaglandin H synthases in the urinary bladder and in myeloperoxidases in the lung may account for differences in target-site susceptibility to aromatic amines in cigarette smokers (Flammang *et al.*, 1989).

The genotoxic effects of aromatic amines are well established on the basis of mutagenicity and clastogenicity observed in numerous *in vitro* and *in vivo* assays that show the capability of these compounds to form DNA adducts after metabolic activation to electrophilic intermediates. The predominant site for covalent binding of aromatic amines to DNA is the C8 position of guanine, but adducts at other sites, including C8 of adenine and N^2 of guanine, have also been identified (Beland *et al.*, 1983; Kaderlik *et al.*, 1993; Lin *et al.*, 1994; Rothman *et al.*, 1996a). As DNA adducts may lead to somatic point mutations, it is reasonable to assume that activated aromatic amines may lead to bladder-tumour development by inducing mutations in key genes

such as the *TP53* tumour suppressor gene (Sørlie *et al.*, 1998; Feng *et al.*, 2002) and the *H-RAS* gene (Boulalas *et al.*, 2009), both involved in bladder carcinogenesis.

Organ specificity and inter- and intra-species differences in cancer susceptibility to aromatic amines are likely related to polymorphisms in genes that regulate DNA-repair, since deficient DNA-repair capacity is associated with increased bladder cancer risk (Lin *et al.*, 2005), and to polymorphisms in genes that encode enzymes involved in activation or detoxification pathways. The *NAT2* slow-acetylator genotype accounts for a greater risk for cancer of the urinary bladder in individuals exposed to 2-naphthylamine or 4-aminobiphenyl (Yu *et al.*, 2002) and for a lower risk in workers exposed to benzidine (Carreón *et al.*, 2006). Conflicting findings between studies may be a consequence of the interdependence of pathways of arylamine metabolism and of the capability of *N*-acetyltransferases both to detoxify the parent compound and to activate metabolites at different rates in different tissues.

4.2 4-Aminobiphenyl

N-hydroxylation of 4-aminobiphenyl (4-ABP) in human and rat-liver microsomes is primarily catalysed by CYP1A2 (Kimura *et al.*, 1999), an enzyme with a large inter-individual variability (Butler *et al.*, 1989), and by extra-hepatic cytochrome P450s including CYP1A1, CYP1B1, and CYP2A13 (Shimada *et al.*, 1996; Nakajima *et al.*, 2006). In extra-hepatic tissues, the binding of 4-ABP to DNA may be catalysed by peroxidase enzymes, such as prostaglandin H synthase (Flammang *et al.*, 1989).

The major 4-ABP-DNA adduct identified in human bladder and lung is *N*-(deoxyguanosin-8-yl)-4-ABP (Lin *et al.*, 1994): other adducts include *N*-(deoxyadenosin-8-yl)-4-ABP and *N*-(deoxyguanosin-N^2-yl)-4-ABP (Beland *et al.*, 1983). *N*-(deoxyguanosin-8-yl)-4-ABP has also been detected in female breast tissue of both smokers and non-smokers (Lin *et al.*, 1994; Faraglia *et al.*, 2003) indicating that 4-ABP-reactive intermediates are distributed systemically and/or that multiple organs are capable of activating 4-ABP or its metabolites. Experiments in animals show that 4-ABP induces bladder tumours in mice, rabbits, and dogs, liver tumours, mammary gland tumours and angiosarcomas in mice, and intestinal tumours in rats. Increased levels of 4-ABP-haemoglobin adducts are associated with cigarette smoking (Bryant *et al.*, 1988), and occupational exposure to 4-ABP is associated with an increased risk for cancer of the urinary bladder (Beyerbach *et al.*, 2006).

To explore the role of various metabolic intermediates in the mutagenicity of aromatic amines, the DNA-damaging potential of 4-ABP was studied in different bacterial strains. In *S. typhimurium* in the presence of S-9-mediated metabolic activation, 4-ABP was found to induce mutations such as frameshifts and base substitutions in TA98 and TA100 strains, respectively (Chung *et al.*, 2000), and oxidant-induced mutations in TA102, suggesting an oxidative mechanism (Makena & Chung, 2007). 4-ABP-induced DNA damage was mainly due to activation by NAT1 (Oda, 2004) and was increased with higher *O*-acetyltransferase activity (Dang & McQueen, 1999), thus demonstrating the potentially important role of *N*-acetoxy-4-ABP in the mutagenicity of this aromatic amine. In *E. coli*, 4-ABP induced base-pair substitutions predominantly at G sites, including G→T, G→C transversions, and G→A transitions (Verghis *et al.*, 1997). In addition, G→C transversion mutations were triggered by incorporating an oligonucleotide containing the *N*-(deoxyadenosin-8-yl)-4-ABP adduct into the single-stranded DNA of the cloning vector, demonstrating the role of this adduct in 4-ABP-induced mutagenesis.

4-ABP induced mutations at the *HPRT* locus and chromosomal instability in human bladder epithelial cells. In 4-ABP-induced liver tumours in B6C3F1 and CD-1 mice, primarily C→A and A→T

mutations, respectively, were detected at codon 61 of the *H-Ras* gene. 4-ABP also increased the mutation frequency in the bladder, liver, and bone marrow of mice. In human bladder cells treated with *N*-hydroxy-4-ABP, preferential sites of adduct formation in *TP53* were at codons 175, 248, 280, and 285, which are mutational hotspots for cancer of the urinary bladder (Feng *et al.*, 2002).

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of 4-aminobiphenyl. 4-Aminobiphenyl causes cancer of the urinary bladder.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-aminobiphenyl.

There is strong mechanistic evidence indicating that the carcinogenicity of 4-aminobiphenyl in humans operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including *N*-oxidation in the liver, *O*-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.

4-Aminobiphenyl is *carcinogenic to humans (Group 1).*

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BENZIDINE

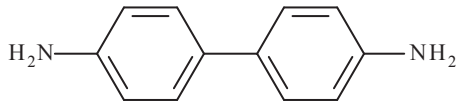
Benzidine was considered by previous IARC Working Groups in 1987 and 2008 (IARC, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

1.1.1 Benzidine

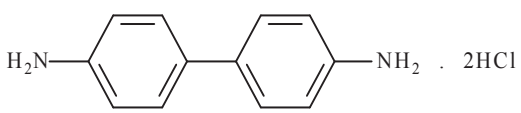
Chem. Abstr. Serv. Reg. No.: 92-87-5
Chem. Abstr. Serv. Name:
[1,1′-Biphenyl]-4,4′-diamine



$C_{12}H_{12}N_2$
Relative molecular mass: 184.24
Description: White to slightly reddish, crystalline powder; darkens on exposure to air and light (O'Neil, 2006).
Solubility: Slightly soluble in water, diethyl ether, and dimethyl sulfoxide (DMSO); soluble in ethanol (Lide, 2008)

1.1.2 Benzidine dihydrochloride

Chem. Abstr. Serv. Reg. No.: 531-85-1
Chem. Abstr. Serv. Name: [1,1′-Biphenyl]-4,4′-diamine, dihydrochloride



$C_{12}H_{10}N_2.2HCl$
Relative molecular mass: 257.18
Description: Crystalline solid (O'Neil, 2006)
Solubility: Soluble in water and alcohol (O'Neil, 2006)

1.2 Uses

Benzidine has been used since the 1850s as the reagent base for the production of a large number of dyes, particularly azo dyes for wool, cotton, and leather (IARC, 2010). In the past, benzidine also has been used in clinical laboratories for detection of blood, as a rubber compounding agent, in the manufacture of plastic films, for detection of hydrogen peroxide in milk, and for quantitative determination of nicotine. Most of these uses have been discontinued because of toxicological concerns. Some dyes used as stains for microscopy and similar laboratory applications may contain benzidine as an impurity (ATSDR, 2001; NTP, 2005).

Manufacture of benzidine is prohibited in several individual countries (e.g. Japan, Republic of Korea, Canada, and Switzerland) and in Europe through European Union (EU) legislation. It has not been manufactured on a large scale for commercial purposes in the United

Table 1.1 Estimated numbers of workers exposed to benzidine in the European Union

Industry, occupational activity	
Manufacture of textiles	160
Education services	3700
Research and scientific institutes	1900
Medical, dental, other health & veterinary services	1300
TOTAL	6900

From: CAREX (1999)

States of America (USA) since 1976, although small quantities remain available for diagnostic testing. It is reportedly produced and/or supplied in research quantities in Germany, Hong Kong Special Administrative Region, India, the People's Republic of China (China), Switzerland, and the USA (IARC, 2010). Production and use of benzidine in dye production has been reported in some developing countries, as has the transfer of benzidine production from other European countries to the former Serbia and Montenegro and to the Republic of Korea (Carreón *et al.*, 2006a).

In 1994, the German Government prohibited the use of certain azo dyes in consumer goods that come in direct, prolonged contact with human skin (e.g. clothing, bedding, footwear, gloves, etc.). The dyes affected are those that, after reduction of one or more azo groups, may release one or more of 20 specific aromatic amines (including benzidine) in detectable concentrations (i.e. > 30 parts per million (ppm)). In 2002, a EU Directive (76/769/EEC) expanded coverage to articles that come in contact with the oral cavity and added two amines to the list (Ahlström *et al.*, 2005; ETAD, 2008). The US Food and Drug Administration limits benzidine content in food colourants to 1 part per billion (ppb). While exposure via ingestion is considered highly unlikely, other impurities in synthetic colouring agents may be metabolized to benzidine after ingestion (ATSDR, 2001).

1.3 Human exposure

1.3.1 Occupational exposure

Estimates of numbers of workers potentially exposed to benzidine have been published by CAREX (CARcinogen EXposure) in Europe. CAREX is an international information system that provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Based on data on occupational exposure to known and suspected carcinogens collected from 1990 to 1993, it is estimated from the CAREX database that 6900 workers were exposed to benzidine in the European Union. Table 1.1 presents the results for benzidine by industry in the EU (CAREX, 1999).

NIOSH (1984) estimated from the US National Occupational Exposure Survey (1981–83) that 1554 workers (including 426 women) were potentially exposed to benzidine and 2987 workers (2464 women) to benzidine dihydrochloride.

Studies that reported airborne or urinary concentrations and results of dermal wipes of benzidine or benzidine derivatives in the benzidine-based dye industry have been reviewed (IARC, 2010).

1.3.2 Non-occupational exposure

Because benzidine may be produced only for captive consumption (i.e. in-house by the producer only, in closed systems with stringent controls), its direct release into the environment is expected to be low.

Benzidine-based dyes can contain various amounts of benzidine as a contaminant. The general population can be exposed to benzidine when in contact with consumer goods containing benzidine or benzidine based-dyes such as leather products (Ahlström *et al.*, 2005), clothes and toys (Garrigós *et al.*, 2002). Some food colourants such as tartrazine and sunset yellow FCF have been

reported to contain trace amounts of benzidine (< 5 to 270 ng/g) (Lancaster & Lawrence, 1999).

2. Cancer in Humans

In a previous *IARC Monograph* (IARC, 2010) it was concluded that there is *sufficient evidence* in humans for the carcinogenicity of benzidine in the human bladder. Numerous case reports from different countries have been published (IARC, 1972, 1982, 1987, 2010). In one extreme instance, all five of a group of workers permanently employed in the manufacture of benzidine for 15 years or more developed bladder cancer (IARC, 1982). Vigliani & Barsotti (1962) reported on 20 workers with tumours of the urinary bladder between 1931 and 1948 among 83 Italian dyestuff workers involved in benzidine production and use. Case *et al.* (1954) found 10 bladder-cancer deaths among dyestuff workers exposed only to benzidine (standardized mortality ratio (SMR) 13.9 [95%CI: 6.7–25.5]). In benzidine-exposed workers in the chemical dye industry in China, the morbidity from bladder cancer increased with increasing duration of exposure (p for trend < 0.01) (Sun & Deng, 1980), while in a cohort of benzidine manufacturers in the USA, risks were significantly elevated for those with ≥2 years exposure to benzidine (SMR 13.0 [95%CI: 4.8–28.4]) (Meigs *et al.*, 1986). In a cohort of dyestuff workers in Torino, Italy, the SMR was 100.8 [95%CI: 60.8–167.2] during exposure and 14.8 [95%CI: 71–31.0] at 20 or more years after exposure ceased (Piolatto *et al.*, 1991). In a study of workers from a chemical manufacturing plant in Shanghai, China, an interaction was found between benzidine exposure and cigarette smoking in the development of bladder cancer (Wu, 1988). Relative to those who did not smoke and had no exposure to benzidine, the risks (RR) for bladder cancer were 6.2 ($P = 0.05$) for smokers who were not exposed to benzidine; 63.4 ($P < 0.05$) for non-smokers exposed to benzidine; and 152.3 ($P < 0.01$) for smokers exposed to benzidine. In another study of Chinese workers in benzidine production and use facilities, the odds ratios (OR) for bladder cancer were 1.0, 2.7 (1.1–6.3) and 4.4 (1.8–10.8) for low, medium and high cumulative exposure to benzidine, respectively, after adjustment for life-time cigarette smoking (Carreón *et al.*, 2006b). In a case–control study in Canada, excesses of renal cell cancer in relation to duration of exposure to benzidine ($P < 0.004$) were noted, but other consistently supporting data were not found (Hu *et al.*, 2002).

Overall, case reports and epidemiological investigations from several countries show strong and consistent associations between benzidine exposure and risk for bladder cancer.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of benzidine in the mouse, rat, hamster, rabbit, dog, and frog by oral, subcutaneous injection, intraperitoneal injection, or inhalation routes of exposure have been reviewed by previous IARC Working Groups (IARC, 1972, 1982, 1987, 2010). There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010). Results of adequately conducted carcinogenicity studies are summarized in Table 3.1.

Benzidine and its dihydrochloride were adequately tested for carcinogenicity by oral administration (feed, drinking-water or gavage) in eight experiments in mice and one experiment in rats; by subcutaneous injection in one experiment in rats and one experiment in frogs; and in rats in one experiment by intraperitoneal injection.

Following oral administration to male and female mice, newborn and adult, of different strains, benzidine significantly increased the

Table 3.1 Carcinogenicity studies of benzidine in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 90 wk Vesselinovitch *et al.* (1975)	Feed Four groups of 50 M mice were fed 150 ppm benzidine as the dihydrochloride (certified ACS grade) in the diet for 45 wk. Groups of 50 mice were sacrificed at 45, 60, 75, and 90 wk of age. A group of 100 male mice that were sacrificed at 90 wk of age served as untreated controls	Hepatocellular carcinomas: 1/98, 2/50, 5/50$^{\$}$, 14/50*, 24/50* Hepatocellular adenomas: 1/98, 6/50$^{\$}$, 15/50*, 17/50*, 11/50*	*[$P < 0.0001$], $^{\$}$[$P < 0.05$]	
Mouse, B6C3F$_1$ (M) 90 wk Vesselinovitch *et al.* (1975)	Feed Three groups of 50 M mice were fed 150 ppm benzidine as the dihydrochloride salt (certified ACS grade) in the diet for 39, 54, or 84 wk. All animals were sacrificed at 90 wk of age. A group of 100 M mice that were sacrificed at 90 wk of age served as untreated controls.	Hepatocellular tumours [benign and malignant]: 1/98, 35/50*, 25/50*, 44/50*	*[$P < 0.0001$]	
Mouse, B6C3F$_1$ (M, F) 90 wk Vesselinovitch *et al.* (1979)	Feed Groups of 43–65 M and F offspring mice were fed and/or exposed to a diet containing 150 ppm benzidine as the dihydrochloride (1) through fed mothers from the 12th d of gestation (prenatal) to delivery; (2) through fed mothers with litters from delivery to weaning; (3) to offspring from weaning to 90 wk of age; (4) during the pre-natal and pre-weaning period; or (5) prenatally, during pre-weaning and in adulthood. Groups 98 M and 100 F mice served as untreated controls.	Hepatocellular tumours [benign and malignant]: Group 1–17/55* (M), 2/62 (F) Group 2–62/65*, 2/43 Group 3–25/44*, 48/50* Group 4–49/49*, 12/48* Group 5–50/50*, 47/50* Controls–1/98, 0/100	*[$P < 0.0001$]	Age NR Purity NR
Mouse, B6C3F$_1$ (M, F) 90 wk Vesselinovitch (1983)	Feed Groups of pregnant F or weanling M + F B6C3F$_1$ mice were fed diets containing 150 ppm of benzidine as the dihydrochloride: group 1–prenatal exposure (12th d of gestation to delivery) group 2–pre-weaning exposure (delivery to weaning) group 3–from weaning to 90 wk Groups of 98 M + 96 F mice were sacrificed at 90 wk and served as controls.	Hepatocellular adenomas and carcinomas: Group 1: 8/36* (M), 2/56 (F) Group 2: 35/52$^{\$}$, 9/43$^{\$}$ Group 3: 22/26$^{\$}$, 16/25$^{\$}$ Controls: 1/98, 0/96 Hepatocellular carcinomas: Group 1: 3/36* (M), 1/56 (F) Group 2: 26/52$^{\$}$, 5/43* Group 3: 17/26$^{\$}$, 16/25$^{\$}$ Controls: 0/98, 0/96	*[$P < 0.05$], $^{\$}$[$P < 0.0001$)	Liver was the only tissue examined. Purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse , F_1 (C57BL/6Jf C3Hf/Nctr females × BALB/cStCrLfC3Hf/Nctr males) and mono-hybrid (F_1 females and F_1 males) up to 80 wk Nelson *et al.* (1982)	Drinking-water Groups of M + F mice (F_1) and MC mice were given drinking-water containing 0, 30, 60, 120, 200, or 400 ppm of benzidine as the dihydrochloride. Groups of mice were sacrificed after 40, 60, or 80 wk of treatment.	Hepatocellular adenomas and carcinomas: F_1 M, 40 wk–0/49, 0/98, 0/72, 0/51, 3/50, 1/28 F_1 F, 40 wk–0/48, 2/98, 1/72, 0/49, 5/50, 13/29 F_1 M, 60 wk–1/48, 0/73, 4/49, 9/48, 9/47, 12/23 F_1 F, 60 wk–1/48, 3/74, 4/52, 24/58, 54/61, 41/41 F_1 M, 80 wk–0/46, 5/44, 6/47, 13/45, 8/21, 16/20 F_1 F, 80 wk–0/47, 9/43, 23/43, 34/37, 9/9, 0/1 MC M, 40 wk–0/50, 1/101, 0/71, 1/48, 0/52, 1/27 MC F, 40 wk–0/48, 0/97, 0/72, 3/51, 6/50, 10/26 MC M, 60 wk–0/48, 3/69, 3/46, 8/50, 8/43, 7/26 MC F, 60 wk–1/48, 7/72, 12/54, 26/56, 47/60, 33/38 MC M, 80 wk–2/45, 2/41, 7/43, 14/44, 7/19, 11/17 MC F, 80 wk–0/48, 12/43, 20/42, 31/32, 7/8, 5/6	No statistics provided [significant for many groups]	Age NR Purity NR Initial number of animals unclear
Mouse, F_1 (C57BL/6Jf C3Hf/Nctr females × BALB/cStCrLfC3Hf/Nctr males) and mono-hybrid (F_1 females and F_1 males) 33 mo (Lifetime) Littlefield *et al.* (1983, 1984)	Drinking-water Groups of 72–120 M + F mice (F_1) and MC mice were given drinking-water containing 0, 30, 40, 60, 80, 120, or 160 ppm (M) and 0, 20, 30, 40, 60, 80, or 120 ppm (F) of benzidine as the dihydrochloride.	Hepatocellular carcinomas: F_1 M: 14/125, 24/119, 30/96, 23/71, 35/71, 51/71, 49/71 F_1 F: 3/124, 51/120, 52/95, 45/72, 55/71, 60/69, 64/72 MC M: 17/123, 20/118, 20/95, 23/72, 24/71, 37/71, 32/71 MC F: 10/125, 54/119, 43/95, 31/71, 37/72, 51/69, 56/72 The authors also noted that a dose effect was observed for Harderian gland adenomas in F_1 and MC males ($P = 0.02$) and females ($P = 0.02$) and for angiomas of the uterus in F_1 and MC females ($P = 0.07$).	Authors indicate that for all four strain/sex combinations, there was a significant dose-related trend for fatal liver tumours, incidental liver tumours, and the pooled estimate using Peto's test, however details on statistics were not provided.	Purity NR
Mouse, $B6C3F_1$ (M, F) 90 wk Vesselinovitch *et al.* (1975)	Gavage Groups of 75 M and 75 F mice were given 0.5 or 1.0 mg benzidine as the dihydrochloride salt twice weekly. All animals were sacrificed at 90 wk of age. Groups of 100 M and 100 F mice served as untreated controls, killed at 90 wk.	Hepatocellular tumours [benign and malignant]: M–1/98, 3/75, 12/75* F–0/100, 4/75$^{\$}$, 17/75*	No statistics provided *[$P < 0.0005$], $^{\$}$[$P < 0.05$]	Purity NR Vehicle NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, ICR (F) 63 wk Miyakawa & Yoshida (1980)	Feed Groups of F mice were implanted with a 45-mg glass bead in the urinary bladder and divided into three groups: 30 were fed basal diet; 60 were fed diet containing 0.2% benzidine; and 60 were fed diet containing a mixture of 0.2% benzidine and 2% DL-tryptophan. Treated groups received diets starting at six wk of age for 20 wk and were then fed the control diet for 40–43 wk.	Hepatomas [adenomas]: 0/30, 34/41*, 24/51 No bladder tumours were found in any of the animals.	*$P < 0.01$, compared with control and tryptophan treated groups	Purity NR
Mouse, Strain NR (M, F) Lifetime Bonser *et al.* (1956)	Subcutaneous Injection Groups of 30 M and 30 F mice received 0.1 ml of a freshly made 3% solution of benzidine (purity NR) in arachis oil by subcutaneous injection, twice/wk for 50 wk, and were then observed for life. Groups of 30 M and 30 F mice served as vehicle controls.	Hepatomas: M–0/30, 4/30; F–0/30, 3/30	No statistics provided	Age NR
Rat, Sprague-Dawley (F) 9 mo Griswold *et al.* (1968)	Gavage Four groups of 10–20 F rats were given benzidine at doses of 12, 25, 35 or 50 mg/rat in sesame oil by stomach tube, daily for 30 d, and sacrificed after a 9-mo observation period. A group of 140 F rats served as vehicle controls.	Mammary carcinomas: 3/132, 5/10*, 7/9*, -, 4/5*	No statistics provided *[$P < 0.0001$]	Small number of animals per group, high mortality in top 2 dose groups. Purity NR
Rat, Sherman (M, F) Lifetime Spitz *et al.* (1950)	Subcutaneous injection Groups of 45 to 155 M and F rats were injected with 15 mg/rat of "technical" benzidine, "pure" benzidine, or benzidine sulfate, dissolved in 1 mL of olive oil once/wk throughout life. A group of 50 rats served as vehicle controls.	Hepatomas: Technical–8/78 (M), 0/155 (F); Pure–5/45 (M), 1/107 (F); Sulfate–not tested (M), 1/153 (F) External auditory canal carcinomas (M+F): Technical –54/233*; Pure –32/152*; Sulfate –16/153* None of the above tumours were reported in the 50 control animals.	No statistics provided *[significant]	Survival at 300 d was 56% for controls and 15% to 3% for treated rats. Poor survival due to heat (no air conditioning in animal rooms). Sex of control animals NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, CD (F) 46 wk Morton *et al.* (1981)	Intraperitoneal injection Three groups of 30 F rats were given injections of 0, 10, or 30 µmol/kg bw benzidine in trioctanoin suspensions, twice/wk for 4 wk.	Mammary gland tumours (all): 3/30, 7/30, 12/29* Mammary gland adenocarcinomas: 1/30, 2/30, 7/29** Zymbal gland tumours (benign and malignant): 1/30, 1/30, 7/29** No tumours of the liver were found.	*$P < 0.01$ **$P < 0.05$	Purity NR
Frog, *Rana temporaria* 38 wk Khudoley (1977)	Subcutaneous injection A group of 37 frogs (1–1.5 yr old) received a subcutaneous injection, once/wk, of 0.2–0.5 mL of a 0.5% solution of benzidine in mineral oil, for up to 38 wk (total dose, 45–114 mg/animal). A group of 67 frogs given subcutaneous injections of 0.2–0.5 mL mineral oil once weekly for 42 wk served as vehicle controls.	Liver tumours: 0/67, 3/14* Tumours of the haematopoietic system: 0/67, 4/14*	*[$P < 0.01$]	Fourteen animals in the treated group were still alive when the first tumour appeared at 16 wk. The high mortality was the result of exceeding the maximum tolerated dose. Sex NR. Purity NR. Histopathology not further specified

bw, body weight; d, day or days; F, female; M, male; MC, monohybrid cross; mo, month or months; NR, not reported; d, day; wk, week or weeks

incidence of hepatocellular tumours (benign and/or malignant) in both sexes (Vesselinovitch *et al.*, 1975, 1979; Miyakawa & Yoshida, 1980; Vesselinovitch, 1983; Nelson *et al.*, 1982; Littlefield *et al.*, 1983, 1984). Oral administration of benzidine caused a markedly increased incidence of mammary carcinomas in female rats (Griswold *et al.*, 1968). Subcutaneous administration of benzidine or its sulfate to male and female rats produced a high incidence of external auditory canal carcinomas (Spitz *et al.*, 1950), while subcutaneous administration of benzidine to frogs caused an increase in tumours of the liver and haematopoietic system (Khudoley, 1977). The intraperitoneal administration of benzidine to female rats resulted in an increase in the incidence of mammary gland adenocarcinomas and combined benign and malignant Zymbal gland tumours (Morton *et al.*, 1981). Other studies were found to be inadequate for evaluation.

4. Other Relevant Data

A general section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this Volume.

Benzidine, *N*-acetylbenzidine, and *N,N′*-diacetylbenzidine have been detected in the urine of workers exposed to benzidine. The predominant DNA adduct identified in exfoliated bladder cells was *N′*-(deoxyguanosin-8-yl)-*N*-acetylbenzidine (Rothman *et al.*, 1996a). Thus, *N*-monoacetylation of benzidine does not interfere with the formation of a DNA-reactive intermediate, and may occur before the cytochrome P450-catalysed formation of *N′*-hydroxy-*N*-acetylbenzidine. In contrast, *N,N′*-diacetylation may be a detoxification pathway. *N*-Acetylbenzidine may be *N*-glucuronidated or *N*-hydroxylated in the liver. In the bladder, the *N′*-hydroxyl-*N*-acetylbenzidine or *N′*-acetoxy-*N*-acetylbenzidine formed by NAT-mediated *O*-acetylation may react with DNA to form covalent adducts. NAT1 is more efficient than NAT2 in catalysing the *N*-acetylation of benzidine or of *N*-acetylbenzidine (Zenser *et al.*, 1996). At low exposure levels, *N*-acetylation of benzidine is favoured over that of *N*-acetylbenzidine. Human neutrophils can also form *N′*-(deoxyguanosin-8-yl)-*N*-acetylbenzidine from *N*-acetylbenzidine by a reaction catalysed by myeloperoxidase (Lakshmi *et al.*, 2000). *N′*-hydroxy-*N*-acetylbenzidine may also be formed through peroxidative activation by prostaglandin H synthase (Zenser *et al.*, 1999a, b). Levels of benzidine-related DNA adducts in exfoliated urothelial cells among exposed workers were not affected by acetylator phenotype (Rothman *et al.*, 1996a), or *GSTM1* genotype (Rothman *et al.*, 1996b).

For several benzidine-based azo dyes, both metabolism and molecular changes identical to those of benzidine have been observed. Metabolic conversion of Direct Black 38, Direct Blue 6, and Direct Brown 95 to benzidine has been observed in the Rhesus monkey (Rinde & Troll, 1975). Azoreductase activity in intestinal bacteria and in the liver catalyses the formation of benzidine from benzidine-based dyes (Cerniglia *et al.*, 1982; Bos *et al.*, 1986).

Benzidine is a multiorgan carcinogen in experimental animals; it induces bladder tumours in dogs, liver tumours in mice and hamsters, and mammary gland tumours in rats. In the presence of a liver-derived metabolic activation system – which in some cases leads to reductive metabolism followed by oxidative metabolism – benzidine and benzidine-based dyes (e.g. Direct Black 38, CI Acid Red 114, CI Direct Blue 15, and CI Pigment Red) were mutagenic in several strains of *S. typhimurium*. Benzidine, *N*-acetylbenzidine, and *N,N′*-diacetylbenzidine have been measured in the urine of workers exposed to Direct Black 38, and benzidine- or 4-ABP-related haemoglobin adducts have been

measured in blood (Dewan *et al.*, 1988; Beyerbach *et al.*, 2006). Significant increases in the incidence of chromosomal aberrations in peripheral lymphocytes have been observed in workers exposed to benzidine or benzidine-based dyes (Mirkova & Lalchev, 1990). In workers exposed to benzidine, the accumulation of mutant p53 protein increased with increasing exposures (Xiang *et al.*, 2007). Similarly, benzidine induced DNA lesions in *TP53* in the bladder, liver, and lung of exposed rats (Wu & Heng, 2006), increased the frequency of micronucleated bone-marrow cells and induced unscheduled DNA synthesis in mice, and increased DNA strand-breaks in the liver of exposed rats.

Based on bio-monitoring studies in workers, animal carcinogenicity data and genotoxicity data, it is reasonable to use the same carcinogenic hazard classification for benzidine-based dyes that are metabolized to benzidine as for benzidine.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of benzidine. Benzidine causes cancer of the urinary bladder.

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

There is strong mechanistic evidence indicating that the carcinogenicity of benzidine in humans operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including *N*-oxidation in the liver, *O*-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.

Benzidine is *carcinogenic to humans (Group 1).*

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DYES METABOLIZED TO BENZIDINE

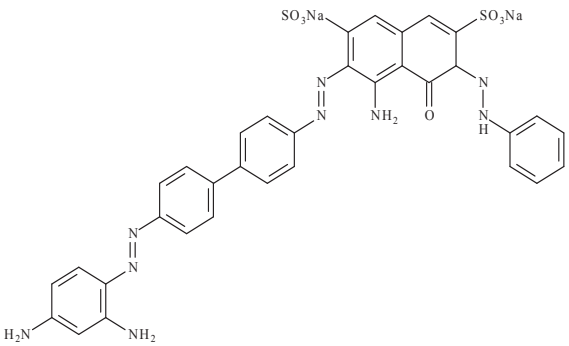
Dyes metabolized to benzidine (benzidine-based dyes) were considered by a previous IARC Working Group in 2008 (IARC, 2010). Three azo dyes metabolized to benzidine, Direct Black 38, Direct Blue 6, and Direct Brown 95 were considered by IARC Working Groups in 1982, 1987, and 2008 (IARC, 1982, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

1.1.1 Direct Black 38

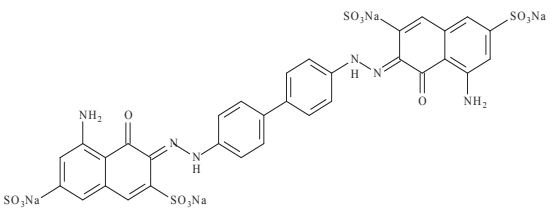
Chem. Abstr. Serv. Reg. No.: 1937-37-7
Chem. Abstr. Serv. Name: 4-amino-3-[2-[4′-[2-(2,4-diaminophenyl)diazenyl][1,1′-biphenyl]-4-yl]diazenyl]-5-hydroxy-6-(2-phenyldiazenyl)-2,7-naphthalenedisulfonic acid, sodium salt (1:2)
Synonyms: C.I. 30235; C.I. Direct Black 38, disodium salt



$C_{34}H_{25}N_9Na_2O_7S_2$
Relative molecular mass: 781.72
Description: Black powder
Solubility: Soluble in water; moderately soluble in ethanol and ethylene glycol mono-ethyl ether; insoluble in other organic solvents (IARC, 2010)

1.1.2 Direct Blue 6

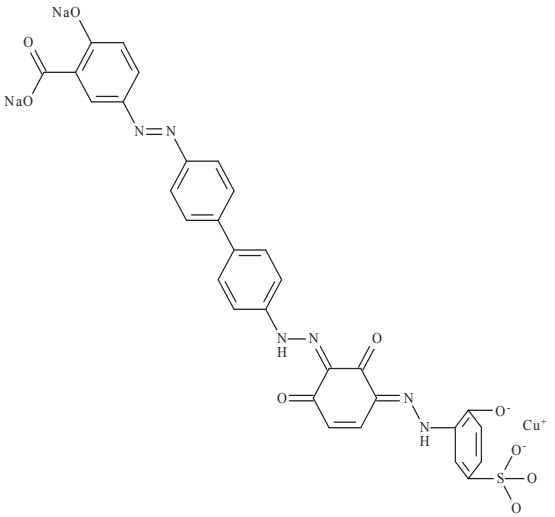
Chem. Abstr. Serv. Reg. No.: 2602-46-2
Chem. Abstr. Serv. Name: 3,3′-[[1,1′-biphenyl]-4,4′-diylbis(2,1-diazenediyl)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid], sodium salt (1:4)
Synonyms: C.I. 22610; C.I. Direct Blue 6; C.I. Direct Blue 6, tetra-sodium salt



$C_{32}H_{20}N_6Na_4O_{14}S_4$
Relative molecular mass: 932.74
Description: Blue powder
Solubility: Soluble in water; moderately soluble in ethanol and ethylene glycol mono-ethyl ether; insoluble in other organic solvents (IARC, 2010)

1.1.3 Direct Brown 95

Chem. Abstr. Serv. Reg. No.: 16071-86-6
Chem. Abstr. Serv. Name: [2-hydroxy-5-[2-[4′-[2-[2-(hydroxy-κO)-6-hydroxy-3-[2-[2-(hydroxy-κO)-5-sulfophenyl]diazenyl-κN1]phenyl]diazenyl][1,1′-biphenyl]-4-yl]diazenyl]benzoato(4-)]-cuprate(2-), sodium (1:2)
Synonyms: C.I. 30145; C.I. Direct Brown 95



$C_{31}H_{18}CuN_6Na_2O_9S$
Relative molecular mass: 760.10
Description: Reddish-brown powder
Solubility: Soluble in water; moderately soluble in ethanol; insoluble in acetone (IARC, 2010)

1.2 Uses

In general, benzidine-based dyes were used primarily to colour textiles, leather, and paper products and also in the petroleum, rubber, plastics, wood, soap, fur, and hair-dye industries. More than 300 benzidine-based dyes are listed in the Colour Index, including 18 that were commercially available in the United States of America (USA). Access to these dyes for home use is no longer permitted in the USA, but some – in particular direct browns, greens, and blacks – were available as consumer products in the 1970s (IARC, 2010). Direct Black 38, Direct Blue 6, and Direct Brown 95 were used on textiles (such as cotton, silk, wool, nylon, and acetate) and on leather. In addition, Direct Black 38 and Direct Blue 6 were used in aqueous printing inks, in hair dyes and as biological stains; Direct Black 38 and Direct Brown 95 were used in plastics; Direct Blue 6 and Direct Brown 95 were used in paper; and, Direct Black 38 was used in wood stains and wood flour (Society of Dyers and Colourists, 1971; NCI, 1978).

By the mid-1970s, most manufacturers in the USA started phasing out the use of benzidine-based dyes and replacing them with other types of dyes (NIOSH, 1980). In 1994, the German Government prohibited the use of certain azo dyes in consumer goods that come in direct prolonged contact with human skin (e.g. clothing, bedding, footwear, gloves, etc.). The dyes affected are those that, after reduction of one or more azo groups, may release one or more of 20 specific aromatic amines (including benzidine) in detectable concentrations (i.e. > 30 ppm). In 2002, the European Union published a Directive (76/769/EEC) that expanded the coverage to compounds that come in contact with the oral cavity, and added two amines to the list (Ahlström *et al.*, 2005; ETAD, 2008).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to benzidine-based dyes can occur during the production and use of these substances. The primary routes of potential exposure to benzidine-based dyes are inhalation and accidental ingestion; dermal absorption can also occur. The potential for exposure has declined since the late 1970s, as benzidine-based dyes were removed from both industrial and consumer markets and replaced with other types of dye. Since 1980, use of mixtures containing benzidine at concentrations of 0.1% or more is

Table 1.1 Estimated numbers of workers exposed to benzidine-based dyes in the European Union

Industry, occupational activity	
Manufacture of textiles	650
Manufacture of leather and products of leather or of its substitutes	220
Manufacture of furniture and fixtures, except primarily of metal	380
Manufacture of plastic products not elsewhere classified	1470
Iron and steel basic industries	10
Manufacture of fabricated metal products, except machinery and equipment	20
Manufacture of machinery except electrical	640
Manufacture of instruments, and of photographic and optical goods	50
Other manufacturing industries	120
Research and scientific institutes	9280
Medical, dental, other health and veterinary services	1090
TOTAL	13920

From CAREX (1999)

permitted only in closed systems; all workers must observe special precautions to reduce exposure, and strict procedures must be followed to transport such materials (IARC, 1982).

Exposure studies in workers involved in manufacture and use of benzidine-based dyes generally were designed to measure benzidine rather than the benzidine-based dyes. Studies that reported airborne and urinary concentrations and results of dermal wipes of benzidine in the benzidine-based dye industry have been reviewed (IARC, 2010).

Estimates of numbers of workers potentially exposed to benzidine-based dyes have been published by CAREX (CARcinogen EXposure) in Europe. CAREX is an international information system that provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Based on data on occupational exposure to known and suspected carcinogens collected from 1990 to 1993, it is estimated that 13920 workers were exposed to benzidine-based dyes in the European Union (CAREX, 1999; Table 1.1).

From the US National Occupational Hazard Survey (1972–74) it was estimated that 79200 workers in 63 occupations (primarily the dye manufacturing, textile dyeing, printing, paper, and leather industries) were potentially exposed to benzidine-based dyes (NIOSH, 1980). A decade later, estimates from another US National Occupational Exposure Survey (1981–83) indicated that about 33900 workers were potentially exposed to one or more of 13 benzidine-based dyes. Although no current estimate of occupational exposure to this type of dye is available, the number of potentially exposed workers is expected to be much lower than in the past (NTP, 2005).

1.3.2 Non-occupational exposure

Benzidine-based dyes can contain various amounts of benzidine as a contaminant. The general population can be exposed to benzidine-based dyes when in contact with consumer goods containing such dyes, e.g. leather products (Ahlström *et al.*, 2005), clothes and toys (Garrigós *et al.*, 2002). Some food colourants such as tartrazine and sunset yellow FCF have been reported to contain trace amounts of benzidine (< 5 to 270 ng/g) (Lancaster & Lawrence, 1999).

Benzidine and benzidine-based dyes have been detected in waste effluents from plants manufacturing or using these dyes (IARC, 2010).

2. Cancer in Humans

Benzidine-based dyes (i.e. dyes metabolized to benzidine) were most recently reviewed in *IARC Monograph* Volume 99 (IARC, 2010).

2.1 Cohort Studies

Three azo dyes metabolized to benzidine, Direct Black 38, Direct Blue 6 and Direct Brown 95, were evaluated in previous *IARC Monographs* (IARC, 1982, 1987, 2010). The most recent evaluation (IARC, 2010) stated that there was *inadequate evidence* in humans for the carcinogenicity of dyes metabolized to benzidine. Stern *et al.* (1987) studied 9365 production workers (7085 men, 2280 women) employed at two leather tanneries where detectable quantities of benzidine were identified in bulk dyes (probably derived from the dyes). Excesses of cancer of the urinary bladder were not observed. The same author (Stern, 2003) conducted a follow-up study with the same result (see Table 2.1, available online at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-03-Table2.1.pdf). Costantini *et al.* (1989) carried out a cohort-mortality study of 2926 male workers employed in tanneries between 1950 and 1981 in Italy. Known exposures in the plants included dyes metabolized to benzidine, dyes metabolized to 3,3′-dimethylbenzidine and dyes metabolized to 3,3′-dimethoxybenzidine. No significant excesses of cancer of the urinary bladder were found (5 deaths, SMR 1.5, 95%CI: 0.5–3.5). You *et al.* (1990) observed only one case of cancer of the urinary bladder in 1210 workers (1060 men, 150 women) from 17 knitting factories, 10 stocking factories, nine silk printing and dyeing factories and seven printing and dyeing factories in China. Fifteen types of benzidine-derived dye had been used in these 43 factories, Direct Black 38 being the most common. Montanaro *et al.* (1997) reported on mortality in a cohort of 1224 workers employed at a chrome tannery in Italy between 1955 and 1988 who were exposed to azo dyes metabolized to benzidine and other chemicals in the tanning process. An excess risk for cancer of the urinary bladder was observed (10 deaths, SMR 2.4, 95%CI: 1.2–4.5).

2.2 Case–control studies

Yoshida *et al.* (1971) reported an excess risk for cancer of the urinary bladder (OR, 6.8, $P = 0.002$) in male workers in the silk-dyeing industry in Japan, in a case–control study of 200 bladder-cancer cases and 148 controls. At least seven of the 17 patients with bladder cancer worked as kimono painters, reportedly using benzidine-based dyes. Myslak *et al.* (1991) found an excess risk for cancer of the urinary bladder among painters in Germany (OR, 2.9, 95%CI: 1.3–6.6). Benzidine-based dyes were manufactured in that country before 1950 and painters were reported to prepare the paints themselves. These studies do not provide data specifically on dyes metabolized to benzidine.

2.3 Synthesis

There is a lack of clear and consistent evidence from epidemiological studies that dyes metabolized to benzidine cause cancer in humans.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of dyes metabolized to benzidine in mice and rats exposed by the oral route or through bladder implantation have been reviewed (IARC, 1987, 2010). There

Table 3.1 Carcinogenicity studies of dyes metabolized to benzidine in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Direct Black 38				
Mouse, ICR (M, F) 60 wk Asada *et al.* (1981)	Drinking-water Groups of 60 mice were given drinking-water containing 3 g/L Direct Black 38. Surviving animals were sacrificed 60 wk after the start of the study. A group of 20 mice served as untreated controls.	Hepatocellular carcinoma: 0/20, 46/59 Mammary gland cancers: 0/20, 20/59	[$P < 0.0001$] [$P < 0.005$]	Gender distribution NR Purity NR
Rat, F344 (M, F) 13 wk NTP (1978), Robens *et al.* (1980)	Feed Groups of 10 male and 10 female rats were fed 0, 190, 375, 750, 1500, or 3000 ppm Direct Black 38 (87% pure, containing ~7% water, ~8% NaCl, no benzidine detected) in the diet for 13 wk and then sacrificed.	Hepatocellular carcinoma or neoplastic nodules: M–0/10, 0/10, 0/10, 0/10, 9/9*(hepatocellular carcinoma, 4/9**), 0/9; F–0/10, 0/10, 0/10, 0/10, 5/10** (only neoplastic nodules), 0/8	*$P < 0.001$ Fisher exact test **$P = 0.01–0.05$ Fisher exact test	Study is of short duration with small number of animals. High mortality in highest dose groups
Direct Blue 6				
Rat, F344 (M, F) 13 wk NTP (1978), Robens *et al.* (1980)	Feed Groups of 10 male and 10 female rats were fed 0, 190, 375, 750, 1500, or 3000 ppm Direct Blue 6 (60% pure, containing ~9% water, ~21% NaCl, no benzidine detected) in the diet for 13 wk and then sacrificed.	Hepatocellular carcinoma or neoplastic nodules: M–0/10, 0/10, 0/10, 0/10, 8/10*(hepatocellular carcinoma, 2/10), 1/9 F–0/10, 0/10, 0/10, 0/10, 0/10, 7/9**(hepatocellular carcinoma, 4/9***)	*$P < 0.001$ Fisher exact test **$P = 0.001–0.009$ Fisher exact test ***$P = 0.01–0.05$	Study is of short duration with small number of animals. High mortality in highest dose groups
Mouse, dd (F) 40 wk Niitsu (1973)	Bladder implantation Two groups of 50 female mice received either a paraffin wax pellet (20 mg) containing 10% Direct Blue BB or a wax pellet alone implanted in the bladder, and were kept for up to 40 wk.	Bladder carcinomas: 1/36 (2.7%), 3/21(14.2%)	[NS, Fisher exact test]	Study duration was short. "Direct Blue BB" is a synonym for Direct Black 38. Purity unspecified. Age not specified (weight, 20 g).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Direct Brown 95				
Rat, F344 (M, F) 13 wk NTP (1978), Robens *et al.* (1980)	Feed Groups of 10 male and 10 female rats were fed 0, 190, 375, 750, 1500, or 3000 ppm Direct Brown 95 (72% pure, containing ~5% water, ~15% NaCl, no benzidine detected) in the diet for 13 wk, and then sacrificed.	Hepatocellular carcinoma or neoplastic nodules: M–0/10, 0/10, 0/10, 0/9, 0/9, 0/9; F–0/10, 0/10, 0/10, 0/10, 5/8*(hepatocellular carcinoma, 1/8), 0/8	*P = 0.001–0.009 Fisher exact test	Study is of short duration with small number of animals. High mortality in highest dose groups.

F, female; M, male; NR, not reported; NS, not significant; wk, week or weeks

have been no additional carcinogenicity studies in animals reported since those reviews. Results of adequately conducted carcinogenicity studies are summarized in Table 3.1.

Three dyes that would be metabolized to benzidine, Direct Black 38, Direct Blue 6 and Direct Brown 95, were tested for carcinogenicity by oral administration (feed or drinking-water) or bladder implantation. Direct Black 38 was adequately tested in one drinking-water study in mice and in one feeding study in mice and rats. Following its oral administration to mice in the drinking-water for 60 weeks, Direct Black 38 significantly increased the incidence of hepatocellular carcinomas and mammary gland cancers (Asada *et al.*, 1981). Oral administration of Direct Black 38 to male and female rats in the diet for only 13 weeks caused a significant increase of hepatocellular carcinomas in males and of liver neoplastic nodules in both sexes (NTP, 1978; Robens *et al.*, 1980). Direct Blue 6 was adequately tested in one feeding study in mice and rats and one bladder-implantation study in mice. Oral administration of Direct Blue 6 to male and female rats in the diet for only 13 weeks caused a significant increase in hepatocellular carcinomas in females and of hepatocellular carcinomas and liver neoplastic nodules combined in both sexes (NTP, 1978; Robens *et al.*, 1980). The bladder-implantation study in mice showed a slight increase in transitional cell carcinoma of the urinary bladder (Niitsu, 1973). Direct Brown 95 was adequately tested in a feeding study in mice and rats. Oral administration of Direct Brown 95 to male and female rats in the diet for only 13 weeks caused a significant increase in hepatocellular carcinomas and liver neoplastic nodules combined in females (NTP, 1978; Robens *et al.*, 1980).

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

For details on benzidine and benzidine-based dyes, see Section 4 of the *Monograph* on Benzidine in this volume.

5. Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of dyes metabolized to benzidine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of Direct Black 38, Direct Blue 6, and Direct Brown 95.

There is *sufficient evidence* in experimental animals for the carcinogenicity of dyes metabolized to benzidine.

There is strong mechanistic evidence indicating that benzidine-based dyes are converted by azoreduction to benzidine in humans and in experimental animals and, consequently, produce DNA adducts and genotoxic effects similar to those of benzidine.

Dyes metabolized to benzidine are *carcinogenic to humans (Group 1).*

In making the overall evaluation, the Working Group considered the following:

- there is *sufficient evidence* in humans and in experimental animals for the carcinogenicity of benzidine.
- the metabolism of benzidine-based dyes results in the release of free benzidine and the induction of chromosomal aberrations in humans, and in all experimental animal species studied.

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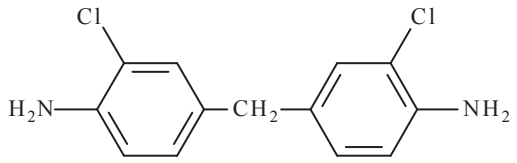
4,4′-METHYLENEBIS(2-CHLOROBENZENAMINE)

4,4′-Methylenebis(2-chlorobenzenamine) (MOCA) was considered by previous IARC Working Groups in 1973, 1992, and 2008 (IARC, 1974, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

From IARC (2010) unless indicated otherwise
Chem. Abstr. Serv. Reg. No.: 101-14-4
Chem. Abstr. Serv. Name: 4,4′-Methylenebis(2-chlorobenzenamine)
Synonym: this compound is commonly known as methylenebis(*ortho*-chloroaniline), MOCA



$C_{13}H_{12}Cl_2N_2$
Relative molecular mass: 267.15
Description: Colourless to yellow or light-brown crystalline solid with a faint amine-like odour
Solubility: Slightly soluble in water; soluble in dilute acids, ether, alcohol (O'Neil, 2006)

1.2 Uses

MOCA is used primarily as a curing agent for polyurethane pre-polymers in the manufacture of castable urethane rubber products (e.g. shock-absorption pads and conveyor belting). In the laboratory, MOCA is used as a model compound for studying carcinogens (NTP, 2005; O'Neil, 2006; IARC, 2010).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to MOCA can occur during its production and use in the polyurethane industry. Workers can be exposed to MOCA when it is processed in the form of a liquid emulsion, as solid pellets with dust, or as solid pellets without dust. In most cases, dermal absorption after contact with contaminated surfaces is the most important occupational exposure route, with inhalation and ingestion representing minor exposure pathways (IARC, 1993).

CAREX (CARcinogen EXposure) is an international information system on occupational

exposure to known and suspected carcinogens, based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for MOCA by industry in the EU (CAREX, 1999).

An estimated 10000 workers were exposed to MOCA in industrialized countries in 1972 (Rappaport & Morales, 1979; Will *et al.*, 1981). In 1979, an estimated 1400 workers in the United States of America (USA) were directly exposed and 7400 indirectly exposed by working in polyurethane-manufacturing processes involving MOCA (Ward *et al.*, 1987). More recently, the Health and Safety Executive estimated that in 2005–06 approximately 300 workers in the United Kingdom were directly exposed to MOCA during polyurethane-elastomer production and over 1000 workers were indirectly exposed (e.g. office staff) (HSE, 2007; Cocker *et al.*, 2009).

(a) Exposure measurements

MOCA levels in air, blood and urine and in surface-wipe samples have been reported for workers employed in the production and use of this chemical in several countries. Different analytical methods have been applied, which complicates comparison of reported MOCA levels (IARC, 2010). Surface wipes have been used mainly to give an indication of potential for dermal exposure to MOCA by anyone working in the area. As measurement of airborne MOCA alone is considered ineffective in the assessment of worker exposure (Robert *et al.*, 1999), post-shift measurement in urine is the most employed method to assess exposure. The concentration of MOCA in urine reflects recent exposure, since the biological half-life of this compound is approximately 23 hours (Osorio *et al.*, 1990). In some studies urinary concentrations of acetyl-MOCA have been determined in addition to those of MOCA, showing that *N*-acetyl-MOCA is a minor urinary metabolite compared with the elimination of the parent amine (Cocker *et al.*, 1988; Shih *et al.*, 2007).

An alternative to measuring MOCA in urine is to determine haemoglobin-MOCA adducts in blood. These adducts are stable for the lifespan of haemoglobin, which in humans is about 120 days (Vaughan & Kenyon, 1996).

(b) MOCA production

Air concentrations of MOCA have been reported from two production plants. In a study from the USA (Linch *et al.*, 1971), the airborne concentration of MOCA was below the detection limit. In a study from Taiwan, China (Chen *et al.*, 2005), the highest concentrations in air (0.41 mg/m^3) were recorded during the purification of MOCA.

Measurements of MOCA concentrations in the urine of production workers from France, Taiwan (China), and the USA were reviewed recently (IARC, 2010). In workers without gloves or protective clothing, concentrations ranged up to several thousand μg/L; the values were much lower when proper protection had been used.

(c) Polyurethane-production workers

Measurements of MOCA concentrations in the urine of polyurethane-production workers from Australia, Canada, France, Germany, Japan, the United Kingdom and the USA were reviewed recently (IARC, 2010). The use of protective equipment and the application of safety procedures reduced the values from several hundred μg/L to as low as 1–10 μg/L.

1.3.2 Non-occupational exposure

The general population can be exposed to MOCA in areas that have been contaminated with MOCA (Keeslar, 1986) or through consumption of certain types of plant (e.g. root crops) grown in contaminated soil (ATSDR, 1994). Immediate family members of workers exposed to MOCA

Table 1.1 Estimated numbers of workers exposed to MOCA in the European Union

Industry, occupational activity	
Manufacture of plastic products not elsewhere classified	1390
Manufacture of rubber products	1360
Manufacture of industrial chemicals	100
Manufacture of miscellaneous products from petroleum and coal	10
Research and scientific institutes	430
TOTAL	3300

From: CAREX (1999)

were reported to have concentrations of up to 15 µg/L in their urine (Keeslar, 1986).

In 1979, extensive environmental contamination with MOCA was discovered on several hundred hectares of land surrounding a MOCA plant in Adrian, MI, USA. Concentrations of up to several mg/kg were found in gardens and community-recreation areas. MOCA was also found in the urine of young children living in the contaminated area (Keeslar, 1986). Of 12 selected children, aged 2 to 16 years, six were found to have detectable concentrations of MOCA in their urine, ranging from 0.3–1.0 ppb (µg/L). These six children were all under the age of six years. Contact with contaminated soil during playing and going barefoot were considered the most likely routes of exposure. The general adult population living in the contaminated area had no detectable MOCA concentrations in urine (IARC, 2010).

2. Cancer in Humans

Bladder-cytology surveys have identified bladder-cancer cases in workers exposed to MOCA in Michigan, USA (Ward *et al.*, 1988, 1990), New Jersey, USA (Mason & Vogler, 1990; Mason *et al.*, 1992), and Taiwan (China) (Chen *et al.*, 2005), but expected numbers were not calculated, so risks for bladder cancer cannot be evaluated. Dost *et al.* (2009) reported on a cohort of 308 male MOCA-production workers in the United Kingdom and found one bladder-cancer death in the period 1979–2007, with 0.18 deaths expected (SMR 5.6; 95%CI: 0.14–31.2), based on the United Kingdom mortality rates.

No adequate epidemiological studies were available to the Working Group to evaluate an association between MOCA and bladder-cancer risk.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of MOCA in mice, rats, and dogs after oral administration, subcutaneous injection or dermal application have been reviewed in previous *IARC Monographs* (IARC, 1974, 1987, 1993, 2010). Results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010).

MOCA was tested for carcinogenicity by oral administration (in the feed or in a gelatin capsule) in one experiment in mice, five experiments in rats and one experiment in dogs; by subcutaneous injection in one experiment in rats; and as an initiator or as a promoter in three experiments in mice, following dermal application.

Following its oral administration (feed) to female mice and male rats, MOCA increased the incidence of hepatomas (Russfield *et al.*, 1975). Oral administration (feed) to male and female

Table 3.1 Carcinogenicity studies of 4,4′-methylenebis(2-chloroaniline) in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, HaM/ICR (M, F) ~24 mo Russfield *et al.* (1975)	Feed Groups of mice were fed diets containing 0, 1000, or 2000 ppm MOCA as the hydrochloride salt for 18 mo. 25/group/sex	Haemangiomas or haemangiosarcomas (combined) M–0/18, 3/13, 8/20 F–1/20, 0/21, 6/14 Hepatomas: M–3/18, 3/13, 4/20 F–0/20, 9/21*, 7/14*	*$P < 0.01$, Fisher exact test	Purity, 97% Authors stated that the incidence of vascular tumours in the high-dose groups was comparable with that in historical controls of the same strain. [The Working Group noted that these vascular tumours were probably not treatment-related]
Rat, Wistar (M, F) Lifetime Grundmann & Steinhoff (1970)	Feed Groups of rats were fed 0 or 1000 ppm MOCA in a protein-deficient diet for 500 d (total dose 27 g/kg bw). 25/group/sex	Hepatomas: M–0/25, 22/25* F–0/25, 18/25* Lung tumours: M–0/25, 8/25** F–0/25, 5/25***	*[$P < 0.001$ Fisher exact test] **[$P = 0.002$] ***[$P = 0.025$]	Purity NR Mean survival times of treated males and females were 565 d and 535 d, respectively, and mean survival of male and female controls was 730 d. Lung tumours were mainly carcinomas.
Rat, Charles River CD1 (M) ~24 mo Russfield *et al.* (1975)	Feed Groups of rats were fed diets containing 0, 500, or 1000 ppm MOCA as the hydrochloride salt for 18 mo 25/group	Hepatomas: 0/22, 1/22, 4/19	[$P < 0.05$, Cochran-Armitage trend test]	Purity, 97%
Rat, Charles River CD (M, F) 2 yr Stula *et al.* (1975)	Feed Groups of rats were fed 0 or 1000 ppm MOCA in a standard diet for up to two yr. 50/group/sex	Lung adenocarcinomas: M–0/44, 21/44* F–0/44, 27/44* Lung adenomatosis: M–1/44, 14/44* F–1/44, 11/44* Hepatocellular carcinomas: M–0/44, 3/44 F–0/44, 3/44 Hepatocellular adenomas: M–0/44, 3/44 F–0/44, 2/44 Pleural mesotheliomas: M–0/44, 4/44 F–0/44, 2/44	*$P < 0.05$, χ^2-test	Purity NR Lung adenomatosis considered as pre-neoplastic or early neoplastic lesion

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Charles River CD (M, F) 16 mo Stula *et al.* (1975)	Feed Groups of rats were fed 0 or 1000 ppm MOCA in a low-protein diet for up to 16 mo. 25/group/sex	Lung adenocarcinomas: M–0/21, 5/21* F–0/21, 6/21* Lung adenomatosis: M–1/21, 8/21* F–1/21, 14/21* Hepatocellular carcinomas: M–0/21, 11/21* F–0/21, 1/21 Hepatocellular adenomas: M–0/21, 5/21* F–0/21, 2/21 Mammary gland adenocarcinomas: F–0/21, 6/21*	*$P < 0.05$, χ^2-test	Purity NR Lung adenomatosis considered as pre-neoplastic or early neoplastic lesion
Rat, Charles River CD (M) 24 mo Kommineni *et al.* (1979)	Feed Groups of rats were fed a 'protein-adequate' diet (Group A) containing 0, 250, 500, or 1000 ppm MOCA or a 'protein-deficient' diet (Group B) containing 0, 125, 250, and 500 ppm MOCA for 18 mo, then kept on these same diets without MOCA. 50, 75 or 100/group	Lung adenocarcinomas: A–0/100, 14/100*, 20/75*, 31/50* B–0/100, 3/100, 7/75**, 8/50* All lung tumours: A–1/100, 23/100*, 28/75*, 35/50* B–0/100, 6/100**, 11/75*, 13/50* Mammary gland adenocarcinomas: A–1/100, 5/100, 8/75**, 14/50* B–0/100, 1/100, 3/75, 3/50*** Zymbal gland carcinomas: A–1/100, 8/100***, 5/75, 11/50* B–0/100, 0/100, 4/75***, 6/50* Hepatocellular carcinomas: A–0/100, 3/100, 3/75, 18/50* B–0/100, 0/100, 0/75, 9/50* Haemangiosarcomas: A–2/100, 4/100, 3/75, 0/50 B–1/100, 2/100, 4/75, 4/50***	* $P < 0.001$ (two-tailed test) **$P < 0.01$ (two-tailed test) ***$P < 0.05$ (two-tailed test)	Industrial grade, purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Dog, Beagle (F) 9 yr Stula *et al.* (1978)	Oral administration A group of one-yr-old dogs was given 100 mg MOCA in gelatin capsules 3 × /wk for 6 wk and then 5 × /wk for up to 9 yr. A group of dogs served as untreated controls. 6/group	Urinary bladder carcinomas: 0/6, 4/5	[$P < 0.05$]	~90% pure One treated dog died prematurely of infection. A transitional-cell carcinoma/adenocarcinoma of the urethra developed in the one treated dog that did not develop a bladder carcinoma
Rat, Wistar (M, F) Lifetime Steinhoff & Grundmann (1971)	Subcutaneous injection Groups of rats were given subcutaneous injections of MOCA (suspension in saline) at doses of 500 or 1000 mg/kg bw, either once a wk or at longer time intervals during 620 d (total dose, 25 g/kg bw). A group of rats served as untreated controls. 17 or 25 (controls)/group/sex	Hepatocellular carcinomas: 0/50, 9/34* Lung cancers: 1/50, 7/34**	*[$P \leq 0.0042$, Fisher exact test] **[$P \leq 0.016$, Fisher exact test]	94% pure Age NR Study inadequately described in a short communication. Lung cancers in treated rats were six adenocarcinomas and one carcinoma.

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NR, not reported; wk, week or weeks; yr, year or years

rats increased the incidence of hepatomas and lung tumours (Grundmann & Steinhoff, 1970). Oral administration (feed) to rats of both sexes caused an increased incidence of lung and mammary gland adenocarcinoma, and hepatocellular carcinoma in two studies (Stula *et al.*, 1975, Kommineni *et al.*, 1979). MOCA also caused haemangiosarcomas and Zymbal gland carcinomas in male rats in one of these studies (Kommineni *et al.*, 1979). Oral administration of MOCA in a gelatin capsule caused carcinomas of the urinary bladder in female dogs (Stula *et al.*, 1978). Subcutaneous injection of MOCA produced an increased incidence in hepatocellular carcinoma and lung cancer in rats (Steinhoff & Grundmann, 1971). After dermal application, MOCA was neither an initiator nor a promoter in the two experiments in mice (Nesnow *et al.*, 1985; Rozinova *et al.*, 1998).

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

Studies on the metabolism, genotoxicity, and animal carcinogenicity of MOCA indicate that this substance acts similarly to other aromatic amines that are known to cause cancer of the urinary bladder in humans.

CYP2A6 or CYP3A4 catalyse *N*-oxidation of MOCA (Butler *et al.*, 1989; Yun *et al.*, 1992) to *N*-hydroxy-MOCA, which can bind to DNA or haemoglobin, or can be further activated to an *N*-sulfate ester by liver sulfotransferases (Chou *et al.*, 1995). Also prostaglandin H synthase (Wiese *et al.*, 2001) and myeloperoxidase (Culp *et al.*, 1997) may catalyse the binding of MOCA to DNA. In exfoliated urothelial cells from MOCA-exposed workers, the predominant DNA adduct was *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol (Kaderlik *et al.*, 1993). The same adduct was found in the liver, lung and kidney of rats exposed to MOCA (Silk *et al.*, 1989). In rat liver the adduct *N*-(deoxyadenosin-8-yl)-4-amino-3-chlorotoluene was also detected. MOCA-DNA adducts were found in the liver and bladder of exposed dogs. Metabolites in urine and blood and haemoglobin adducts have been detected in workers in the polyurethane elastomer industry (Vaughan & Kenyon, 1996). In contrast to the situation with benzidine, very low levels of *N*-acetylated metabolites were observed in urine. Because human liver can also catalyse the *N*-acetylation of MOCA, these low urinary levels may reflect an efficient de-acetylation pathway (Lakshmi *et al.*, 1995).

MOCA is a multiorgan carcinogen in experimental animals: it induces bladder tumours in dogs, liver tumours in rats and mice, and haemangiosarcomas, lung, and mammary gland tumours in rats. Particularly compelling data on the genotoxicity of MOCA include the higher micronucleus frequencies measured in exfoliated bladder epithelial cells and in peripheral lymphocytes of exposed workers (Murray & Edwards, 1999). MOCA and *N*-hydroxy-MOCA are mutagenic in *S. typhimurium* (Bridges *et al.*, 1981; Kuslikis *et al.*, 1991). In addition, MOCA induced mutations at the *HPRT* locus in human lymphoblastoid cells, it stimulated prophage induction in *E. coli*, and caused aneuploidy in *S. cerevisiae*, unscheduled DNA synthesis in cultured mouse hepatocytes, transformation in several mammalian cell cultures, and sister chromatid exchange in lymphocytes of rats treated *in vivo*, and in cultured Chinese hamster ovary cells.

5. Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 4,4′-methylenebis(2-chlorobenzenamine).

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4,4′-methylenebis(2-chlorobenzenamine).

There is strong mechanistic evidence indicating that the carcinogenicity of 4,4′-methylenebis(2-chlorobenzenamine) involves a genotoxic mechanism of action that includes metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects in humans. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including *N*-oxidation in the liver, *O*-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.

4,4′-Methylenebis(2-chlorobenzenamine) is *carcinogenic to humans (Group 1).*

In making the overall evaluation, the Working Group considered that:

The genotoxicity of 4,4′-methylenebis(2-chlorobenzenamine) is well documented and its toxicological profile is similar to that of *ortho*-toluidine, thus indicating a common mode of action. 4,4′-Methylenebis(2-chlorobenzenamine) has been shown to interact with DNA to form adducts in urothelial cells, and with haemoglobin to form adducts in the blood of workers exposed to this compound. It has also been shown to cause the formation of sister chromatid exchange and micronuclei in urothelial cells and lymphocytes of exposed workers.

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2-NAPHTHYLAMINE

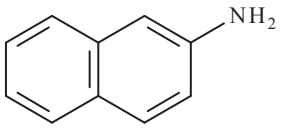
2-Naphthylamine was considered by previous IARC Working Groups in 1973, 1987, and 2008 (IARC, 1974, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

From IARC (2010)

Chem. Abstr. Serv. Reg. No.: 91-59-8
Chem. Abstr. Serv. Name:
2-Naphthalenamine
Synonym: this compound is also known as β-naphthylamine



$C_{10}H_9N$
Relative molecular mass: 143.18
Description: White to reddish crystals (O'Neil, 2006)
Solubility: Soluble in water, diethyl ether, and ethanol (O'Neil, 2006; Lide, 2008)

1.2 Uses

2-Naphthylamine formerly was used commercially as an intermediate in the manufacture of dyes, as an antioxidant in the rubber industry, and to produce 2-chloronaphthalene (IARC, 2010). Because of its carcinogenicity, the manufacture and use of 2-naphthylamine have been prohibited in the European Union (EU) since 1998, in Italy since 1960, in the United Kingdom since 1952, and in Switzerland since 1938. Production and use of dyestuffs containing 2-naphthylamine have been banned in Japan since 1972 (Olfert *et al.*, 2006). In the United States of America (USA), 2-naphthylamine is a carcinogen regulated by the Occupational Safety and Health Administration (OSHA). As such, exposure must be strictly controlled through mandatory use of engineering controls, safe work practices, and personal protective equipment (OSHA, 2011).

Small quantities of 2-naphthylamine are used in laboratory research (IARC, 2010). The substance has been found as a contaminant in other chemicals and industries (Olfert *et al.*, 2006). Phenyl-β-naphthylamine (PBNA) has been used as a substitute; however, it partially metabolizes in the body to 2-naphthylamine (Olfert *et al.*, 2006).

1.3 Human exposure

1.3.1 Occupational exposure

In the past, occupational exposure to 2-naphthylamine mainly occurred during its production and when it was used in the manufacture of azo dyes. Exposure may still occur in laboratories where it is used as a model compound in cancer research, when workers are exposed to pyrolysis fumes containing 2-naphthylamine (e.g. foundry fumes, second-hand tobacco smoke, heated cooking-oils), to 2-nitronaphthalene (e.g. foundry workers), a nitro-PAH that can be metabolized to 2-naphthylamine, or to products containing 2-naphthylamine as a contaminant, such as certain rubber chemicals (IARC, 2010). Countries in which exposure was reported include: Italy, Japan, the Russian Federation, the United Kingdom, and the USA (IARC, 2010).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for 2-naphthylamine by industry in the EU (CAREX, 1999).

From the US National Occupational Exposure Survey (1981–83) it was estimated that 275 workers, including 265 women, were potentially exposed to 2-naphthylamine (NIOSH, 1990). Exposure data were reported for a 2-naphthylamine/benzidine dye factory in Moscow (Bulbulyan *et al.*, 1995). 2-Naphthylamine concentrations in indoor air samples taken in the factory during the period 1939–1948 ranged from < 1 µg/L to > 3 µg/L. Factory-wall wipes sampled in 1948 contained 60.0–115 mg/m^2 wall surface. Dermal wipes sampled in 1940 and 1947 from workers after a shower at work contained between 0.018 and 37.5 mg 2-naphthylamine (data on the surface area sampled were not available).

In a German study of workers primarily exposed to aniline and 4-chloroaniline, urinary 2-naphthylamine concentrations were 0–9.8 µg/L (mean, 3.9 ± 2.2) in 22 smokers and 0–11.6 µg/L (mean, 2.1 ± 2.8) in 21 non-smokers, both significantly higher than the concentrations measured in non-smoking, non-exposed workers (0.0–1.6 µg/L; mean, 0.5 ± 0.7) (Riffelmann *et al.*, 1995).

In a study of two Danish iron foundries, airborne PAH concentrations were measured in relation to 2-naphthylamine (as a possible marker of 2-nitronaphthalene) in the urine of PAH-exposed workers (Hansen *et al.*, 1994). The concentration of 2-naphthylamine in urine was significantly higher in PAH-exposed workers than in controls (matched for smoking habits). Hand moulders, finishing workers and truck drivers tended to have the highest levels. These results may be explained by the presence of 2-nitronaphthalene (which can metabolized to 2-naphthylamine), the presence of aromatic amines, e.g. in moulding sand, or the presence of nitrogen oxides, e.g. in diesel exhaust. It has been estimated that a maximum of 1% of total *N*-phenyl-2-naphthylamine uptake can be transformed into 2-naphthylamine (Weiss *et al.*, 2007).

1.3.2 Non-occupational exposure

The general population can be exposed to 2-naphthylamine through tobacco smoke and other fumes containing 2-naphthylamine, or reportedly when in contact with dyes and hair dyes contaminated with 2-naphthylamine [The Working Group could not find evidence of current contamination of these consumer products]. Exposure to 2-nitronaphthalene, which is formed by incomplete combustion of organic material and generally found in the environment in a mixture with other nitro-PAH and non-nitro-PAH, can also become an indirect source of contact with 2-naphthylamine (IARC, 2010).

Table 1.1 Estimated numbers of workers exposed to 2-naphthylamine in the European Union

Industry, occupational activity	
Education services	1260
Research and scientific institutes	450
Medical, dental, other health and veterinary services	340
TOTAL	2050

From CAREX (1999)

Mainstream cigarette smoke from eight different conventional market cigarettes in the USA contained 2-naphthylamine at concentrations of 1.47 to 14.06 ng/cigarette (Stabbert *et al.*, 2003). A review of similar studies in the IARC Monograph on tobacco indicated that amounts of 2-naphthylamine in mainstream cigarette smoke range from 1–22 ng/cigarette; those in sidestream cigarette smoke range from 113.5–171.6 ng/cigarette (IARC, 2004).

In a German study (Grimmer *et al.*, 2000), 2-naphthylamine was found in urine in comparable levels for non-smokers (n = 14; mean, 120.8 ng/24 hours), smokers (n = 12; mean, 84.5 ng/24 hours) and persons exposed to second-hand tobacco smoke (n = 22; mean, 94.9 ng/24 hours). In a study by Riedel *et al.* (2006), smokers (n = 10) excreted significantly higher amounts of 2-naphthylamine compared with non-smokers (n = 10) (20.8 *vs* 10.7 ng/24 hours).

2-Naphthylamine has been detected in fumes of heated cooking-oils (Chiang *et al.*, 1999) in a study in Taiwan (China) looking at three different commercial cooking oils. Concentrations of 2-naphthylamine in oil fumes were 31.5 μg/m^3 for sunflower oil, 31.9 μg/m^3 for vegetable oil, and 48.3 μg/m^3 for refined lard oil.

2. Cancer in Humans

Studies of cancer in humans exposed to 2-naphthylamine were most recently reviewed by a Working Group in 2008 (IARC, 2010): it was concluded that there was *sufficient evidence* in humans for the carcinogenicity of 2-naphthylamine in the human urinary bladder. Numerous case series reported bladder cancer in workers exposed to 2-naphthylamine; in coal-tar dye workers exposed to 2-naphthylamine, and not to other aromatic amines, the cumulative incidence of bladder cancer was 25% (Goldwater *et al.*, 1965). Eleven cohort studies (four in the USA, two in the United Kingdom, two in Japan, and one each in Poland, the Russian Federation and Italy) are available concerning bladder-cancer risks in workers engaged in the manufacture and use of 2-naphthylamine (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-05-Table2.1.pdf). All these studies indicate markedly elevated bladder cancer risks associated with the manufacture and use of 2-naphthylamine. In most of the studies, it was not possible to quantify the relative contributions of exposures to benzidine and 2-naphthylamine to the overall excess risks. However, Case *et al.* (1954) had reported 26 bladder-cancer deaths, with 0.3 expected [SMR 86.7, 95%CI: 56.6–127.0], in British dyestuff-industry workers exposed to 2-naphthylamine. Also, in a study of bladder-cancer risks in the British rubber industry, Veys (2004) showed excesses of bladder cancer in workers employed between 1946 and 1949 when 2-naphthylamine-contaminated antioxidants were still used (58 cases, SIR 1.71; 95%CI: 1.3–2.21) and no excess in workers employed after this exposure was removed (39 cases, SIR 1.02 95%CI: 0.72–1.39).

3. Cancer in Experimental Animals

Studies on the carcinogenicity of 2-naphthylamine in the mouse, rat, hamster, rabbit, dog, and monkey after oral administration, after subcutaneous or intraperitoneal injection, after intravesicular implantation, or after dermal application have been reviewed in previous *IARC Monographs* (IARC, 1974, 1987, 2010). There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010).

2-Naphthylamine was tested for carcinogenicity by oral administration (in the feed, by gavage or in a gelatin capsule) in five experiments in mice, three experiments in rats, ten experiments in dogs, and one experiment each in hamsters, rabbits and monkeys; by subcutaneous administration in five experiments in mice; by intraperitoneal injection in two experiments in mice and one in rats; and by one intravesicular implantation and one skin-painting study in mice and one bladder instillation study in dogs. Results of adequately conducted carcinogenicity studies are summarized in Table 3.1.

Oral administration of 2-naphthylamine to mice caused a significant increase in the incidence of cholangiomas, hepatomas and liver adenomas (Bonser *et al.*, 1952; Yoshida *et al.*, 1979) and induced 'malignant' hepatomas (Bonser *et al.*, 1952). 2-Naphthylamine caused a significant increase in urinary bladder tumours (including carcinomas) following its oral administration to rats (Hicks *et al.*, 1982; Hicks & Chowaniec, 1977), hamsters (Saffiotti *et al.*, 1967), dogs (Hueper *et al.*, 1938; Bonser *et al.*, 1956; Harrison *et al.*, 1969; Conzelman & Moulton, 1972; Romanenko & Martynenko, 1972; Rigotti *et al.*, 1977; Purchase *et al.*, 1981) and monkeys (Conzelman *et al.*, 1969). Intraperitoneal injection of 2-naphthylamine (Theiss *et al.*, 1981) or its administration by gavage (Stoner *et al.*, 1986) increased the multiplicity of benign lung tumours in strain A mice.

The results of the oral study in rabbits, the intraperitoneal injection study in rats, the subcutaneous injection study, the intravesicular implant study and the skin-painting study in mice, and the bladder-instillation study in dogs were found to be inadequate for the evaluation of the carcinogenicity of 2-naphthylamine.

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

2-Naphthylamine is a constituent of tobacco smoke, and the amounts of 2-naphthylamine-haemoglobin adducts are higher in cigarette smokers than in non-smokers (Bryant *et al.*, 1988). Similarly to other aromatic amines, 2-naphthylamine may undergo *N*-hydroxylation by CYP1A2 (Butler *et al.*, 1989) followed by conjugation of the hydroxyl group with sulfate or glucuronide, or conjugation of the amino group with acetate (*N*-acetylation), sulfate, or glucuronide. In addition, 2-naphthylamine may undergo *N*-oxidation and ring oxidation by peroxidative enzymes such as prostaglandin H synthase in the bladder (Wise *et al.*, 1984; Yamazoe *et al.*, 1985) to form an arene oxide. The *N*-hydroxylated intermediate may re-arrange to form 2-amino-1-naphthol and conjugates with sulfate or glucuronide, or form DNA adducts such as *N*-(deoxyguanosin-8-yl)-2-NA, 1-(deoxyguanosin-N^2-yl)-2-naphthylamine, and 1-(deoxyadenosin-N^6-yl)-2-naphthylamine (Beland *et al.*, 1983). These adducts are also formed by prostaglandin H synthase (Yamazoe *et al.*, 1985), or from the 2-imino-1-naphthoquinone intermediate (e.g. N^4-deoxyguanosin-N^2-yl)-2-amino-1,4-naphthoquinone-imine).

Table 3.1 Carcinogenicity studies of exposure to 2-naphthylamine in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, IF (M, F) up to 72 wk Bonser *et al.* (1952)	Gavage A group of 13 M and 12 F mice received 2-naphthylamine suspended in arachis oil, twice weekly at a dose of 400 mg/kg bw per wk. A group of 6 M and 5 F served as arachis-oil controls.	Liver cholangiomas: M–0/6 (controls), 5/13 F–0/5 (controls), 5/12 M+F–0/11 (controls), 10/25	NR, [NS] [$P < 0.02$]	Age NR Small number of animals studied, especially controls
Mouse, CBA (M, F) up to 89 wk Bonser *et al.* (1952)	Gavage A group of 9 M and 14 F mice received 2-naphthylamine suspended in arachis oil twice/wk at a dose of 240 mg/kg bw per wk. A group of 7 M and 7 F served as arachis-oil controls	Hepatomas: M–0/7 (controls), 6/9 (67%) F–0/7 (controls), 7/14 (50%)	NR [$P < 0.05$] [$P < 0.05$]	Age NR Small number of animals studied, especially controls. Authors reported the incidence of hepatomas in the control breeding mice of the laboratory to be 8%
Mouse, CBA (M, F) up to 89 wk Bonser *et al.* (1952)	Feed Groups of 14–15 M and 12–15 F mice were given four different synthetic diets containing 2-naphthylamine at a dose of 160 mg/kg bw per wk	Hepatomas: M–24/57 F–25/54 Hepatomas (malignant): M+F–16/111	-	Age NR No controls
Mouse, BALB/c (F) 55 wk Yoshida *et al.* (1979)	Feed Groups of 20 F mice were fed a diet containing 0 or 2000 ppm 2-naphthylamine for 40 wk	Liver tumours: Adenoma–0/17, 10/16 Hepatoma–0/17, 3/16 Urinary bladder-epithelium hyperplasia: 0/17, 6/16	NR [$P < 0.0001$] [NS] [$P < 0.01$]	Purity NR
Mouse, A/J (M, F) 24 wk Stoner *et al.* (1986)	Gavage A group of 16 M and 16 F mice received 2-naphthylamine in tricaprylin by gavage 3 × /wk for 8 wk, resulting in a total dose per animal of 600 mg/kg bw. A group of 16 M and 16 F mice served as tricaprylin controls.	Lung tumours: M–3/15 (controls), 8/14 F–2/14 (controls), 4/13 Lung tumour multiplicity: M–0.93 ± 1.00 *vs* 0.27 ± 0.59 (controls)	[NS] [NS] $P < 0.05$	
Rat, Albino (strain NR) (M, F) over 90 wk Bonser *et al.* (1952)	Feed Three groups of 18, 15 and 17 M and F rats were fed low-, mid- and high-protein diets containing 2-naphthylamine at a dose of 310 mg/kg bw per wk for life. Three groups of 15, 17 M and 17 F rats served as controls.	Urinary bladder papillomas: 0/49 (controls), 4/50	NR, [NS]	Age NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (F) up to 104 wk Hicks & Chowaniec (1977)	Gavage A group of 25 F rats were given 2-naphthylamine in arachis oil by gavage, at a dose of 300 mg/kg bw per wk for one yr, and were then left untreated until symptoms of bladder disease were seen. A group of 50 F served as untreated controls.	Urinary bladder tumours: 0/50, 5/17	NR, [$P < 0.001$]	Purity NR Bladder tumours not further specified
Rat, Wistar (F) 100 wk Hicks *et al.* (1982)	Gavage A group of 20 F rats received 2-naphthylamine suspended in arachis oil by gavage at a dose of 300 mg/kg bw per wk for 57 wk. A group of 20 F served as vehicle controls.	Urinary bladder: Carcinomas–0/20, 4/18 Urothelial hyperplasias–0/20, 8/18	NR [$P < 0.05$] [$P < 0.001$]	Purity NR
Hamster, Random-bred Syrian golden (M, F) Lifetime Saffiotti *et al.* (1967)	Feed Groups of 30 M and 30 F hamsters were fed diets containing 0, 0.1 or 1.0% (w/w) 2-naphthylamine.	Urinary bladder carcinomas: M–0/30, 0/30, 10/23* F–0/30, 0/30, 8/16*	NR *[$P < 0.0001$]	Purity NR Hepatoma was found in one high-dose male and one high-dose female.
Dog, Mongrel (F) 89 wk Hueper *et al.* (1938)	Oral and subcutaneous injection A group of 16 F mongrel dogs received subcutaneous injections of 4 mg/d (for dogs ≤ 12 kg) or 5 mg/d (for dogs > 12 kg) 2-naphthylamine, daily for 14 wk followed by doubling of the dose for 12 wk and a tripling for the subsequent 63 wk. During the last 54 wk of the study, dogs started to receive a daily oral dose of 2-naphthylamine by capsule that started at 100 mg/dog and increased to 300 mg/dog by the end of the treatment. A group of four F dogs served as controls.	Urinary bladder tumours (papillomas and carcinomas combined): 0/4, 13/16	NR, [$P < 0.01$]	Purity NR Bladder-tumour incidence based partly on autopsy and partly on cystoscopy and biopsy. Dosing poorly described.
Dog, Mongrel (F) up to 3 yr Bonser *et al.* (1956)	Oral A group of 4 F dogs were given 2-naphthylamine by mouth in a capsule at a dose of 200 mg/d, on six d/wk during 6 mo and then 600 mg/d for up to 2 yr.	Urinary bladder carcinomas: 2/4	-	Age NR, purity NR One dog died after 14.5 mo of treatment. No controls
Dog, Mongrel (F) 79 mo Harrison *et al.* (1969)	Oral A group of 4 F dogs were fed 2-naphthylamine at a dose of 400 mg/d for 2 yr	Urinary bladder carcinomas: 4/4	-	Age NR, purity NR No controls Experimental details poorly described

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Dog, Beagle (M, F) up to 30 mo Conzelman & Moulton (1972)	Oral Groups of 2–7 M and 2–5 F 8–10-mo-old dogs were given 2-naphthylamine by mouth in a capsule at doses of 0, 6.25, 12.5, 25, or 50 mg/kg bw per d on 6 d/wk for 2–26 mo. Total dose/dog ranged from 12 g to 204 g	Urinary bladder carcinomas: M–0/2, 4/6, 2/5, 6/7, 1/2 F–0/2, 0/3, 2/5, 2/3, 3/3 M+F–0/4 (controls), 20/34* (treated)	NR *[$P < 0.05$]	Purity NR
Dog, Breed NR (F) 55 mo Romanenko & Martynenko (1972)	Oral A group of eight F dogs were given 2-naphthylamine at a dose of 5–30 mg/kg bw, 4–6 d/wk, for 7.5 mo and then 30 mg/kg bw, 4–6 d/wk, for 8.5 mo	Urinary bladder carcinomas: 7/8	-	Age NR, purity NR No controls. Route of administration poorly described.
Dog, Breed NR (F) 26 mo Rigotti *et al.* (1977)	Oral A group of 20 F dogs were given 2-naphthylamine in a capsule, daily at a dose of 500–600 mg for 20–26 mo.	Urinary bladder carcinomas: 20/20	-	Age NR, purity NR No controls.
Dog, Beagle (M, F) up to 47 mo Purchase *et al.* (1981)	Oral 3 M and 2 F dogs (age, 9 mo) were given capsules containing 400 mg 2-naphthylamine 5 × /wk for 34 mo. A group of 4 M and 4 F control dogs received capsules with lactose.	Urinary bladder transitional-cell carcinomas: 0/8, 5/5	NR, [$P < 0.001$]	
Monkey, Rhesus *Macaca mulatta* (M, F) up to 60 mo Conzelman *et al.* (1969)	Gavage A group of 8 M and 19 female monkeys (weighing 2–3 kg) were given 2-naphthylamine in a capsule by gavage at a dose of 0 (control, three females) or 6.25–400 mg/kg bw per d, on 6 d/wk for 33–60 mo.	Urinary bladder carcinomas: 0/3 (controls), 9/24	NR	Age NR Some animals received a fixed dose during the entire experiment, others received different doses over the course of five yr. The majority of tumours occurred in animals given high doses.
Mouse, Inbred A/St mice (M, F) 24 wk Theiss *et al.* (1981)	Intraperitoneal injection A group of 10 M and 10 F mice were given 2-naphthylamine in tricaprylin by injection at doses of 0, 62.5, 125 or 250 mg/kg bw, 3 × /wk for 8 wk.	Lung adenomas/mouse: 0.19, 0.40, 0.50, 1.38*	*$P < 0.01$	Purity NR

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; vs, versus; wk, week or weeks; yr, year or years

2-Naphthylamine was mutagenic in *S. typhimurium* strains TA98 and TA100 in the presence of bovine bladder cells (Hix *et al.*, 1983). 2-Naphthylamine-induced DNA damage was triggered by an NAT1-overexpressing *S. typhimurium* strain, but not by an *O*-acetyltransferase-deficient strain, in the presence of human CYP1A2 (*umu* response) (Oda, 2004). In another *umu* gene-expression assay, DNA damage in *S. typhimurium* strain NM2009 exposed to 2-naphthylamine was induced by the CYP4B1 isoenzyme from rat-bladder epithelium (Imaoka *et al.*, 1997). 2-Naphthylamine was mutagenic in Chinese hamster ovary cells in the presence or absence of an exogenous activating system (Gupta & Singh, 1982).

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of 2-naphthylamine. 2-Naphthylamine causes cancer of the urinary bladder.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-naphthylamine.

There is strong mechanistic evidence indicating that the carcinogenicity of 2-naphthylamine operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including *N*-oxidation in the liver, *O*-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.

2-Naphthylamine is *carcinogenic to humans (Group 1)*.

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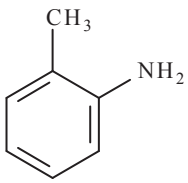
ortho-TOLUIDINE

ortho-Toluidine was considered by previous IARC Working Groups in 1977, 1981, 1987, 2000, and 2008 (IARC, 1978, 1982, 1987, 2000, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

From IARC (2010), unless indicated otherwise
Chem. Abstr. Serv. Reg. No.: 95-53-4
Chem. Abstr. Serv. Name:
2-Methylbenzenamine



C_7H_9N
Relative molecular mass: 107.15
Description: Light yellow liquid becoming reddish brown on exposure to air and light
Boiling-point: 200–202 °C (O'Neil, 2006)
Solubility: Slightly soluble in water; soluble in alcohol, ether, and dilute acids

1.2 Uses

ortho-Toluidine is used as an intermediate in the synthesis of the large-volume herbicides, metolachlor and acetochlor, in the manufacture of more than 90 dyes and pigments (e.g. acid-fast dyestuffs, azo pigment dyes, triarylmethane dyes, sulfur dyes, and indigo compounds), and as an intermediate for synthetic rubber and rubber-vulcanizing chemicals, pharmaceuticals, pesticides, and other chemicals. *ortho*-Toluidine is also used in the clinical laboratory as an ingredient in a reagent for glucose analysis, and for tissue staining (IARC, 2010; NTP, 2004).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to *ortho*-toluidine can occur by inhalation or skin contact during its production, or during the production of dyes, pigments and rubber chemicals manufactured from this chemical. Laboratory and medical personnel may be exposed when using *ortho*-toluidine for staining tissues (IARC, 2010).

From the US National Occupational Exposure Survey (1981–83) it was estimated that 30000 workers, including approximately 15500 women, were potentially exposed to *ortho*-toluidine (NIOSH, 1990). No estimates of the number of exposed workers in the European Union have been reported.

At a chemical plant in the former Soviet Union where *ortho*-toluidine was produced via reduction of *ortho*-nitrotoluene, workers were exposed to concentrations of *ortho*-toluidine in the air that generally exceeded the maximum permissible concentration [of 3 mg/m^3, IARC (1982)] by 2–7-fold. In a total of 215 air samples, the highest exposure levels were observed during distillation and extraction processes (25–28.6 mg/m^3). Dermal exposures also were documented (Khlebnikova *et al.*, 1970). Measurements in the 1940s in a US dye-production plant indicated that the concentration of *ortho*-toluidine was < 0.5 ppm [2 mg/m^3] in the workroom air and in the breathing zone of the workers, and < 0.3–1.7 mg/L in the urine of workers engaged in the production of thioindigo (Ott & Langner, 1983). Exposure to *ortho*-toluidine was also reported to occur in plants involved in dye-production in Italy (Rubino *et al.*, 1982), Germany (Stasik, 1988), and the USA (New Jersey) (Delzell *et al.*, 1989), but no data on exposure levels were provided.

Concentrations of *ortho*-toluidine in indoor air in plants producing rubber antioxidants or vulcanising rubber articles ranged up to several hundred µg/m^3 and *ortho*-toluidine concentrations in post-shift urine samples were around 100 µg/L (Ward *et al.*, 1991; Teass *et al.*, 1993; Ward *et al.*, 1996; Korinth *et al.*, 2006).

Medical and laboratory personnel also are potentially exposed to *ortho*-toluidine, although air concentrations are reportedly low (EPA, 1984; Kauppinen *et al.*, 2003).

1.3.2 Non-occupational exposure

Significant non-occupational exposures to *ortho*-toluidine may result from the use of some hair dyes, the local anaesthetic prilocaine, or tobacco smoke. In a study from Turkey (Akyüz & Ata, 2008), *ortho*-toluidine was found in 34 of the 54 hair dyes tested, at levels up to 1547 µg/g. Prilocaine, a widely used anaesthetic, is metabolized to *ortho*-toluidine. In 25 patients who received local anaesthesia, the average amount of *ortho*-toluidine adducts to haemoglobin (Hb) increased 6–360-fold, from 0.54 ± 0.95 ng/g Hb before treatment to 22 ± 13.2 ng/g Hb at 24 hours after surgery (Gaber *et al.*, 2007). *ortho*-Toluidine has been measured in mainstream cigarette smoke at 9–144 ng per cigarette (Stabbert *et al.*, 2003), and concentrations in urine of smokers are higher than in non-smokers (Riffelmann *et al.*, 1995; Riedel *et al.*, 2006). *ortho*-Toluidine has also been detected in surface waters and industrial effluents (Shackelford & Keith, 1976; Neurath *et al.*, 1977; EPA, 1984; NTP, 2004), in vegetables such as kale, celery and carrots, in the volatile aroma of black tea (Vitzthum *et al.*, 1975; Neurath *et al.*, 1977), and in breast milk (DeBruin *et al.*, 1999), but levels are generally very low.

2. Cancer in Humans

Several cohort studies have been conducted among workers potentially exposed to *ortho*-toluidine (Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-06-Table2.1.pdf). Rubino *et al.* (1982) reported excess bladder-cancer risks in relation to *ortho*-toluidine exposure, however, other exposure to potential bladder carcinogens also occurred in this work environment. Ward *et al.* (1991) reported an excess in bladder cancer in 1749 US workers employed in the production of rubber additives from *ortho*-toluidine and aniline. Risks were greatest for workers with the strongest likelihood of exposure and for those with long-term exposure (> 10 years). Further cases of bladder cancer in this facility were reported by Markowitz & Levin (2004), but rates were not calculated. Exposure to low-level 4-aminobiphenyl was suspected, so a protein-adduct biomarker study was carried out (Ward *et al.*, 1996), which supported the conclusion that

ortho-toluidine was the most likely cause of the bladder-cancer excess, because 4-aminobiphenyl adducts to haemoglobin were unrelated to work in the facility. Using revised exposure categories, Carreón *et al.* (2010) conducted a re-analysis of the data and confirmed that workers in this plant have an increased risk for bladder cancer.

Sorahan *et al.* (2000) and Sorahan (2008) reported an excess in bladder-cancer risk in workers exposed to *ortho*-toluidine in the United Kingdom. Sorahan (2008) found increased risks with longer duration of employment in departments where *ortho*-toluidine was processed ($P < 0.05$), after adjusting for exposure to other bladder carcinogens in the factory.

Overall, the epidemiological studies show consistent associations between exposure to *ortho*-toluidine and bladder cancer. Although exposure to other bladder carcinogens occurred for several of the cohorts, the overall evidence is consistent with an association of exposure to *ortho*-toluidine and bladder cancer.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of *ortho*-toluidine in the mouse, rat and hamster after oral administration or subcutaneous injection were reviewed in previous *IARC Monographs* (IARC, 2000, 2010). There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010).

ortho-Toluidine was tested for carcinogenicity as its hydrochloride salt by oral administration in the feed in two experiments in mice and in three experiments in rats, and as the free base in one limited subcutaneous-injection experiment in hamsters. Results of adequately conducted carcinogenicity studies are summarized in Table 3.1.

Oral administration of *ortho*-toluidine to male and female mice caused an increased incidence of haemangiomas and haemangiosarcomas (combined) in both sexes in one study (Weisburger *et al.*, 1978). The same result was found in male rats in another study, but the separate incidence for haemangiosarcomas was also increased (NTP, 1979). The incidences of hepatocellular carcinomas and of hepatocellular adenomas and carcinomas combined were increased in females in the latter study (NTP, 1979).

Oral administration of *ortho*-toluidine to male rats caused an increased incidence of subcutaneous fibromas and fibrosarcomas (combined) in one study (Weisburger *et al.*, 1978), and of skin and spleen fibromas, mammary gland fibroadenomas and peritoneal sarcomas in another (Hecht *et al.*, 1982). In a third study in male and female rats, *ortho*-toluidine increased the incidence of subcutaneous fibromas and of mesotheliomas of multiple organs or the *tunica vaginalis* in males, and of mammary gland fibroadenomas and urinary bladder transitional-cell carcinomas in females. An increased incidence of fibrosarcomas, angiosarcomas, osteosarcomas or sarcomas (not otherwise specified) (combined) of multiple organs (mainly subcutis and spleen or bone) was also observed in both sexes; and a significant increase in the incidence of fibrosarcomas and sarcomas of multiple organs in males, and of spleen angiosarcomas and osteosarcomas of multiple organs in females (NTP, 1979).

When administered as the free base by subcutaneous injection to male and female Syrian golden hamsters, *ortho*-toluidine produced no increase in tumour incidence compared with controls (Hecht *et al.*, 1983).

Table 3.1 Carcinogenicity studies in experimental animals fed *ortho*-toluidine

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Swiss CD-1 (M, F) 21 mo Weisburger *et al.* (1978)	Groups of 25 M and 25 F mice were fed a diet containing 0, 16000 or 32000 ppm *ortho*-toluidine hydrochloride. After 3 mo, due to toxicity, doses were lowered to 8000 or 16000 ppm for a further 15 mo. Treated animals were then kept without treatment for an additional 3 mo.	Haemangiomas and Haemangiosarcomas (combined): M–0/14 (concurrent control), 5/99 (pooled control), 5/14*, 9/11* F–0/15, 9/102, 5/18**, 9/21*	*$P < 0.025$ (vs all controls **$P < 0.05$ (vs all controls)	Purity, 97–99% Pooled controls: additional controls used for the other compounds tested in the study. Tumour incidence of concurrent and pooled controls were compared statistically (both separately and together) with those of treated groups. Separate incidence for haemangiomas and haemangiosarcomas NR.
Mouse, B6C3F$_1$ (M, F) 103 wk NTP (1979)	Groups of 50 M and 50 F mice, were fed a diet containing 1000 or 3000-ppm *ortho*-toluidine hydrochloride for 103 wk. A group of 20 M and 20 F mice served as untreated controls.	*Males* Haemangiomas and Haemangiosarcomas (combined): 1/19, 2/50, 12/50* Haemangiosarcomas: 1/19, 1/50, 10/50* *Females* Hepatocellular adenomas and carcinomas (combined): 0/20, 4/49, 13/50** Hepatocellular carcinomas: 0/20, 2/49, 7/50***	*$P < 0.005$ (trend test) **$P < 0.007$ (Fisher's exact test), $P < 0.001$ (trend test) ***$P = 0.015$ (trend test)	Purity > 99%
Rat, Sprague-Dawley CD (M) 24 mo Weisburger *et al.* (1978)	Groups of 25 M rats were fed a diet containing 0, 8000 or 16000-ppm *ortho*-toluidine hydrochloride. After 3 mo, due to toxicity, doses were lowered to 4000 or 8000 ppm for a further 15 mo. Treated animals were then kept without treatment for an additional 6 mo.	Subcutaneous fibromas and fibrosarcomas (combined): M–0/16 (concurrent control), 18/111 (pooled control), 18/23*, 21/24* Urinary bladder transitional-cell carcinomas: M–0/16, 5/111, 3/23, 4/24	*$P < 0.025$ (vs all controls)	Purity, 97–99% Pooled controls: additional controls used for the other compounds tested in the study. Tumour incidences of concurrent and pooled controls were compared statistically (both separately and together) with those of treated groups
Rat, Fischer F344 (M) 93 wk Hecht *et al.* (1982)	Groups of 30 M rats were fed a diet containing 0 or 4000 ppm *ortho*-toluidine hydrochloride for 72 wk. Total dose of *ortho*-toluidine hydrochloride ingested was 31.3 g/rat.	Skin fibromas: 1/27, 25/30* Spleen fibromas: 0/27, 10/30* Mammary gland fibroadenomas: 0/27, 11/30* Peritoneal sarcomas: 0/27, 9/30**	*$P < 0.001$ (Fisher's exact test) **$P < 0.01$ (Fisher's exact test)	Purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Fischer F344 (M, F) 104 wk NTP (1979)	Groups of 50 M and 50 F rats, were fed a diet containing 3000 or 6000-ppm *ortho*-toluidine hydrochloride for 101–104 wk. A group of 20 M and 20 F rats served as untreated controls.	*Males* Sarcomas NOS, fibrosarcomas, angiosarcomas or osteosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone): 0/20, 15/50**, 37/49* Sarcomas NOS of multiple organs: 0/20, 3/50, 11/49*** Fibrosarcomas of multiple organs: 0/20, 8/50, 20/49* Subcutaneous integumentary fibromas: 0/20, 28/50*, 27/49* Mesotheliomas of multiple organs or *tunica vaginalis*: 0/20, 17/50*, 9/49*** *Females* Sarcomas NOS, fibrosarcomas, osteosarcomas or angiosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone): 0/20, 3/50, 21/49* Osteosarcomas of multiple organs: 0/20, 0/50, 18/49**** Spleen angiosarcomas: 0/20, 7/49, 9/49*** Urinary bladder transitional-cell carcinomas: 0/20, 9/45***, 22/47* Mammary gland fibroadenomas: 6/20, 20/50, 35/49*****	*$P < 0.001$ **$P = 0.003$ ***$P < 0.05$ ****$P = 0.001$ *****$P = 0.002$	Purity > 99% Mortality of male and female rats was significantly increased by treatment ($P < 0.001$).

F, female; M, male; mo, month or months; NOS, not otherwise specified; NR, not reported; vs, versus; wk, week or weeks

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

ortho-Toluidine is a constituent of tobacco smoke and it is excreted in larger amounts in the urine of smokers than of non-smokers (Riedel *et al.*, 2006). *ortho*-Toluidine induced urinary bladder and mammary gland tumours in rats and liver tumours and haemangiosarcomas in mice. The risk for cancer of the urinary bladder was elevated in workers exposed to *ortho*-toluidine. This substance has been evaluated in a large number of genetic toxicology studies (IARC, 2010); however, there has been much inconsistency in the results reported.

The metabolism of *ortho*-toluidine has not yet been fully characterized, but the available data indicate a preferential ring-oxidation or *N*-acetylation rather than *N*-oxidation (Son *et al.*, 1980). Similarly, cancers of the urinary bladder associated with occupational exposure to *ortho*-toluidine may result from peroxidative activation of the chemical, catalysed by prostaglandin H synthase in the epithelium of the urinary bladder (Zenser *et al.*, 2002). *ortho*-Toluidine-haemoglobin adduct levels were increased in patients treated with the anaesthetic prilocaine (Gaber *et al.*, 2007) and in workers employed in the rubber chemicals manufacturing area of a chemical plant (Ward *et al.*, 1996). Metabolites are excreted primarily as sulfate or glucuronide conjugates, since *ortho*-toluidine is not a substrate for human NAT1-mediated acetylation (Zhang *et al.*, 2006).

ortho-Toluidine induces tumours in rodents and DNA lesions in multiple organs. Most studies reported that *ortho*-toluidine was not mutagenic in *S. typhimurium*, other studies showed positive responses in the same strains. The *N*-oxidized metabolite of *ortho*-toluidine, *N*-hydroxy-*ortho*-toluidine, was mutagenic in *S. typhimurium* strain TA100 (Gupta *et al.*, 1987). *ortho*-Toluidine induced intrachromosomal recombination in *Saccharomyces cerevisiae* in an assay that is responsive to the induction of DNA deletions (Carls & Schiestl, 1994); this response was reduced in the presence of an antioxidant. Other reported effects of *ortho*-toluidine (Danford, 1991) include the induction of sister chromatid exchange, aneuploidy, unscheduled DNA synthesis, DNA strand breaks, and cell transformation *in vitro*, and the induction of micronuclei in peripheral blood of rats treated *in vivo* (Suzuki *et al.*, 2005). The formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine in calf thymus DNA incubated *in vitro* with 4-amino-3-methylphenol, a metabolite of *ortho*-toluidine, suggests a potential role of reactive oxygen species in the DNA-damaging effects of this aromatic amine (Ohkuma *et al.*, 1999). *ortho*-Toluidine induced DNA lesions – measured by means of the comet assay – in multiple organs of exposed rats and mice (Sekihashi *et al.*, 2002): increased DNA migration was observed in the liver, bladder, lung, and brain of mice, and in the liver, bladder, and stomach of rats.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of *ortho*-toluidine. *ortho*-Toluidine causes cancer of the urinary bladder.

There is *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-toluidine.

There is moderate mechanistic evidence indicating that the carcinogenicity of *ortho*-toluidine involves metabolic activation, formation of DNA adducts, and induction of DNA-damaging effects.

ortho-Toluidine is *carcinogenic to humans (Group 1)*.

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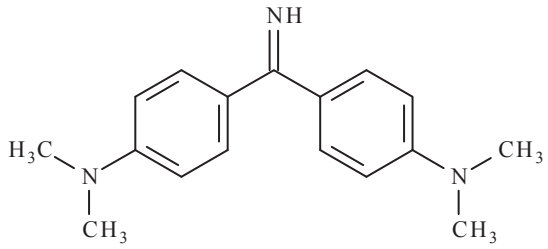
AURAMINE AND AURAMINE PRODUCTION

Auramine and auramine production were considered by previous IARC Working Groups in 1971, 1987, and 2008 (IARC, 1972, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 492-80-8
CAS Name: 4,4′-Carbonimidoylbis[*N*,*N*-dimethylbenzenamine]
Synonyms: C.I. 41000B; C.I. Solvent Yellow 34; 4,4′-dimethylaminobenzophenonimide; 4,4′-(imidocarbonyl)bis(*N*,*N*-dimethylaniline); glauramine; Solvent Yellow 34; yellow pyoctanine



$C_{17}H_{21}N_3$
Relative molecular mass: 267.37
Description: Yellow flakes or powder, decomposes at >70 °C.
Solubility: as base: insoluble in water, soluble in ethanol and diethyl ether; as the hydrochloride: soluble in water, ether, ethanol and chloroform.

1.2 Manufacture and use

Auramine is manufactured industrially from *N*,*N*-dimethylaniline and formaldehyde, which react to form Michler's base (tetramethyldiaminodiphenylmethane). This base is subsequently converted to auramine by heating with sulfur and ammonium chloride in the presence of ammonia. It was reported that a 98% pure auramine contains salts, water and Michler's ketone, an hydrolysis product (Kirsch *et al.*, 1978).

Production of auramine took place first in Europe (Switzerland, Germany, the United Kingdom, and France), and later also in the United States of America (USA). Production in these countries has generally been discontinued. Auramine manufacturing is currently mainly located in India and the People's Republic of China.

Auramine colourants are used for dyeing of leather, jute, tanned cotton, and paints, and as dye components in inking ribbons, ballpoint pastes, oils and waxes, and carbon paper. The most important applications are in paper dyeing and flexographic printing (IARC, 2010). More detailed information on the use of auramine dyes and auramine compounds is provided in the recent *Monograph* (IARC, 2010).

1.3 Human exposure

1.3.1 Occupational exposure

The only well described groups of workers exposed during auramine production include those in the United Kingdom (Case & Pearson, 1954) and Germany (Kirsch *et al.*, 1978; Thiess *et al.*, 1982). Results from exposure measurements in the workplace or from biological samples of workers employed in the production of auramine are not available.

The manufacture of auramine involves potential exposure to its process chemicals (e.g. dimethyl-aniline, formaldehyde, sulfur, ammonium chloride, ammonia, Michler's base), as well as to other chemicals that may be used and produced at the same location (e.g. benzidine, 1-naphthylamine, 2-naphthylamine, magenta, aniline) (Case & Pearson, 1954).

2. Cancer in Humans

Auramine was last reviewed in *IARC Monograph* Volume 99 (IARC, 2010). A 13-fold excess of bladder tumours was observed among men engaged in the manufacture of auramine ($P < 0.005$), compared with mortality rates for the male population in England and Wales (Case & Pearson, 1954; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-07-Table2.1.pdf). Although care had been taken to eliminate from the analysis those workers who were recorded as also having been in contact with 1- or 2-naphthylamine or benzidine, exposure to non-auramine bladder carcinogens could not be entirely excluded. A cohort-mortality study of auramine workers at the 'Badische Anilin und Soda-Fabrik' (BASF) in Germany (Kirsch *et al.*, 1978) identified two bladder cancer deaths, with < 0.4 expected. Case reports of bladder cancer among Swiss auramine-production workers have also been published (Von Müller, 1933).

3. Cancer in Experimental Animals

Studies on the carcinogenicity of auramine in the mouse, rat, and rabbit after oral administration or subcutaneous injection were reviewed in previous *IARC Monographs* (IARC, 1972, 1987, 2010). There have been no additional carcinogenicity studies in experimental animals reported since the most recent evaluation (IARC, 2010).

Auramine was tested for carcinogenicity by oral administration in two experiments in mice, two in rats, one in rabbits, and one in dogs, and by subcutaneous administration in one experiment in rats. Results of adequately conducted carcinogenicity studies are summarized in Table 3.1.

Oral administration of auramine caused a significant increase in the incidence of hepatomas in male and female mice and in male rats (Bonser *et al.*, 1956; Williams & Bonser, 1962; Walpole, 1963), and of lymphomas in female mice (Bonser *et al.*, 1956). The subcutaneous-injection study in rats (in which local sarcomas were observed) and the oral studies in rabbits and dogs were found to be inadequate for the evaluation of the carcinogenic hazards of auramine.

For data on Michler's base and Michler's ketone, see IARC (2010).

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

While no studies were found on the metabolism of auramine in laboratory animals or humans, the finding that auramine-induced intrachromosomal recombination in *Saccharomyces cerevisiae* was reduced in the presence of a free-radical scavenger (*N*-acetylcysteine) suggests that auramine may induce genotoxic effects in yeast by generating

Table 3.1 Carcinogenicity studies of auramine in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Strain NR (M, F) Lifetime Bonser *et al.* (1956)	Feed A group of 15 M and 15 F mice were fed a diet containing 0.1% auramine for 52 wk (total dose, 728 mg/mouse). A group of 30 M and 30 F mice received twice weekly a subcutaneous injection of arachis oil, and served as controls	Hepatomas: M–0/30, 4/15; F–0/30, 3/15 Lymphomas: M–1/30, 3/15; F–4/30, 8/15	[$P < 0.05$, both sexes] [$P < 0.01$, F]	Age NR, purity NR (commercial grade). Small number of animals per group. High mortality during study, especially in females. Inadequate controls.
Mouse, "Stock" albino and CBA (M, F) Lifetime Williams & Bonser (1962), Walpole (1963)	Feed A group of 15 M and 15 F "Stock" mice were fed a diet containing 0.1% auramine for 52 wk (total dose ~1820 mg/mouse). Sixteen M and F mice served as untreated controls. A group of 12 M and 15 F CBA mice were fed a diet containing 0.2% auramine for 52 wk (total dose ~3640 mg/mouse). Ninety M and F mice served as untreated controls	Hepatoma: "Stock" mice M–0/8, 4/7 F–0/8, 3/10 CBA mice M–4/35, 7/12 F–3/55, 11/15	 [$P < 0.05$] [NS] [$P < 0.005$] [$P < 0.0001$]	Purity NR (commercial grade) Animals at risk are the animals surviving ≥ 50 wk.
Rat, Wilmslow Wistar (M) Lifetime Williams & Bonser (1962), Walpole (1963)	Feed Groups of 12 M rats were given a basic diet for life or a diet containing 0.1% auramine for 87 wk (estimated total auramine intake, 10 g/rat)	Hepatoma: 0/12, 11/12	NR, [$P < 0.0001$]	Purity NR (commercial grade) Animals at risk are the animals surviving ≥ 90 wk.
Rat, Sprague-Dawley (M, F) 24 mo Kirsch *et al.* (1978)	Feed Groups of 20 M and F rats were given a diet containing auramine (technical grade) at 0, 50, 100 and 200 ppm	All sites (benign and malignant): M–6/20, 13/20, 8/20, 10/20 F–19/20, 18/20, 15/20, 19/20	NS	Purity, 87% (technical grade) Age NR

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

free radicals (Brennan & Schiestl, 1998). Commercial preparations of auramine were mutagenic in several strains of *S. typhimurium*, when tested with metabolic activation systems. Other *in vitro* effects of auramine include induction of deletions and aneuploidy in *Saccharomyces cereviasiae*, DNA strand breaks in rat hepatocytes, unscheduled DNA synthesis in rat and hamster hepatocytes, mutations in Chinese hamster ovary cells, and micronucleus formation and transformation of Syrian hamster embryo cells. DNA strand breaks were induced in liver, kidney, and urinary bladder cells of exposed rats (Parodi *et al.*, 1982; Martelli *et al.*, 1998) and in liver and bone-marrow cells of exposed mice (Parodi *et al.*, 1982; Sasaki *et al.*, 1997).

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of auramine production. Auramine production causes cancer of the urinary bladder.

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

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

There are insufficient mechanistic data relevant to the carcinogenicity of auramine in humans. Auramine induces DNA strand-breaks in experimental animals.

Auramine production is *carcinogenic to humans (Group 1).*

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

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MAGENTA AND MAGENTA PRODUCTION

Magenta and magenta production were considered by previous IARC Working Groups in 1973, 1986, 1987, and 2008 (IARC, 1974, 1987a, b, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

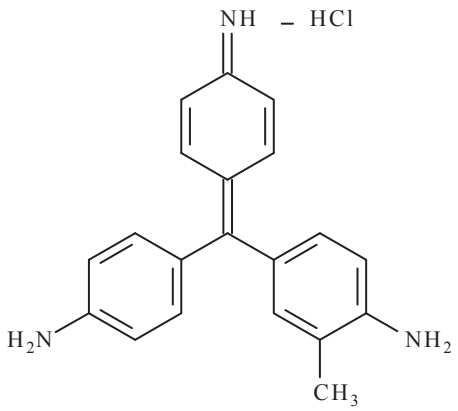
1. Exposure Data

Historically, the dye name Magenta has been used to refer to the mixture Basic Fuchsin, with its four major constituents Basic Red 9 (Magenta 0), Magenta I (Rosanilin), Magenta II, and Magenta III (New Fuchsin). Although samples of Basic Fuchsin can vary considerably in the proportions of these four constituents, today all except Magenta II are available commercially under their own name. Of these, Magenta I and Basic Red 9 are the most common (IARC, 2010).

1.1 Identification of the agents

1.1.1 Magenta I

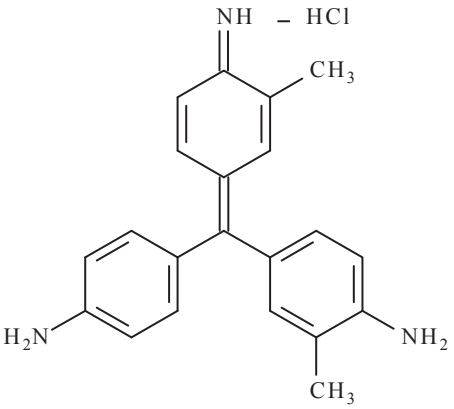
Chem. Abstr. Serv. Reg. No.: 632-99-5
CAS Name: 4-[(4-Aminophenyl)(4-imino-2,5-cyclohexadien-1-ylidene)methyl]-2-methylbenzenamine, hydrochloride (1:1)



$C_{20}H_{19}N_3.HCl$
Relative molecular mass: 337.85
Description: Metallic green, lustrous crystals
Melting-point: Decomposes above 200 °C
Solubility: Slightly soluble in water (4 mg/mL); soluble in ethanol (30 mg/mL) and ethylene glycol methyl ether (30 mg/mL); insoluble in diethyl ether

1.1.2 Magenta II

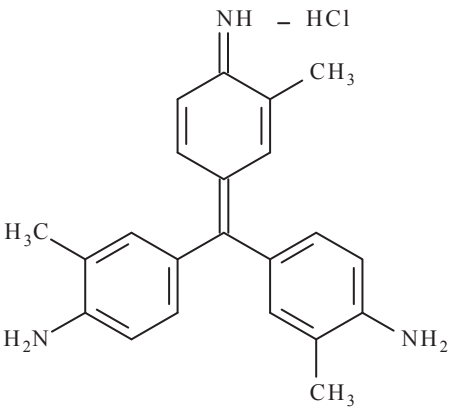
Chem. Abstr. Serv. Reg. No.: 26261-57-4
CAS Name: 4-[(4-Aminophenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene) methyl]-2-methylbenzenamine, hydrochloride (1:1)



$C_{21}H_2N_3.HCl$
Relative molecular mass: 351.87
No information regarding the chemical and physical properties of Magenta II was available to the Working Group.

1.1.3 Magenta III

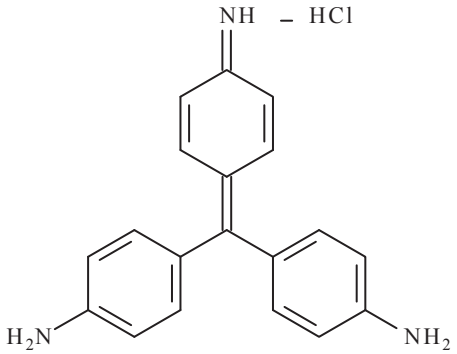
Chem. Abstr. Serv. Reg. No.: *3248-91-7*
CAS Name: 4,4′-[(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methylene]bis[2-methylbenzenamine], hydrochloride (1:1)



$C_{22}H_{23}N_3.HCl$
Relative molecular mass: 365.90
Description: Green crystalline powder
Solubility: Soluble in water (20 mg/mL), ethanol (20 mg/mL), and ethylene glycol methyl ether (20 mg/mL)

1.1.4 Basic Red 9 (Magenta 0)

Chem. Abstr. Serv. Reg. No.: 569-61-9
CAS Name: 4,4′-[(4-imino-2,5-cyclohexadien-1-ylidene)methylene] bis[benzenamine], hydrochloride (1:1)



$C_{19}H_{17}N_3.HCl$
Description: Pale violet powder
Melting-point: 269 °C (decomposes)
Solubility: Slightly soluble in water (3 mg/mL); soluble in ethanol (25 mg/mL) and ethylene glycol methyl ether (70 mg/mL)

From Green (1990), O'Neil (2006), and Lide (2008).

1.2 Manufacturing processes

Magenta was among the first synthetic dyes to be produced in the 1850s. It has been produced commercially in the United States of America (USA) since at least 1921 (IARC, 2010).

In the United Kingdom, the process for manufacturing Magenta has involved condensation of *ortho*-toluidine and formaldehyde in the presence of nitrotoluene, resulting mainly in the production of Magenta III. Magenta I is prepared by the reaction of a mixture of aniline, *ortho*- and

para-toluidine and their hydrochlorides with nitrobenzene or a mixture of nitrobenzene and *ortho*-nitrotoluene in the presence of ferrous chloride, ferrous oxide and zinc chloride. CI Basic Red 9 is prepared by the reaction of aniline with formaldehyde in the presence of hydrogen chloride, forming 4,4′-methylenedianiline, which is then heated with aniline and aniline hydrochloride in the presence of nitrobenzene and ferric chloride (IARC, 2010).

1.3 Human exposure

1.3.1 Occupational exposure

The only well described groups of workers exposed during magenta production include those in a dyestuff-manufacturing plant in Ludwigshafen, Germany (Rehn, 1895), in the manufacture of magenta in the chemical industry in the United Kingdom (1910–52) (Case & Pearson, 1954) and the in manufacture of 'new fuchsin' in an Italian dyestuffs factory (Rubino *et al.*, 1982; Piolatto *et al.*, 1991). Reported exposures were based on duration of exposure, years since first exposure, age at first exposure, job category or years since last exposure. No environmental or biological measurements have been reported for these plants or any other plants historically or currently producing magenta.

Production of magenta may involve exposure to process chemicals (e.g. aniline, *ortho*- and *para*-toluidine, and – historically – arsenic acid). Exposure to other chemicals used and produced at the same location may also occur (e.g. benzidine, 1-naphthylamine, 2-naphthylamine, auramine, aniline) (Case & Pearson, 1954).

2. Cancer in Humans

Magenta production was last reviewed in *IARC Monograph* Volume 99 (IARC, 2010). A 23-fold excess of bladder tumours was found in men engaged in the manufacture of magenta ($P < 0.005$), compared with mortality rates for the male population in England and Wales (Case & Pearson, 1954). Although care had been taken to eliminate from the analysis those workers who were recorded as also having been in contact with auramine, 1- or 2-naphthylamine or benzidine, exposure to non-magenta bladder carcinogens could not be entirely excluded. Rubino *et al.* (1982) reported two deaths from bladder cancer after exposure to magenta in the manufacture of 'new fuchsin' (Magenta III) (see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-08-Table2.1.pdf). Bladder cancer was associated with employment in industries with potential magenta exposure in Torino, Italy (Vineis & Magnani, 1985; see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-08-Table2.2.pdf). These studies indicate that excess bladder cancer risks are caused by the production of magenta, but co-exposures preclude a conclusion for magenta itself.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of magenta in the mouse, rat, and hamster after oral administration, and studies on the carcinogenicity of CI Basic Red 9 in these species after oral administration or subcutaneous injection, have been reviewed in previous *IARC Monographs* (IARC, 1974, 1987b, 1993, 2010). There have been no additional carcinogenicity studies in animals reported since the most recent evaluation (IARC, 2010).

3.1 Magenta

Magenta was tested for carcinogenicity by oral administration in one experiment in mice (Bonser *et al.*, 1956), one experiment in rats

Table 3.1 Carcinogenicity studies of oral administration of Magenta (CI Basic Red 9) in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CI Basic Red 9				
Mouse, B6C3F$_1$ (M, F) 103 wk NTP (1986)	Feed Groups of 50 M and 50 F mice were fed a diet containing 0, 500 or 1000 ppm CI Basic Red 9 for 103 wk.	Hepatocellular carcinomas:		Purity 93–99%
		M–10/50, 20/50, 27/50	$P < 0.001$ (trend)	
		F–3/49, 19/50, 37/49	$P < 0.001$ (trend)	
		Adrenal phaeochromocytomas (benign or malignant):		
		F–1/48, 8/47, 8/45	$P = 0.015$ (trend)	
Rat, F344/N (M, F) 103 wk NTP (1986)	Feed Groups of 50 M and 50 F rats were fed a diet containing 0, 1000 or 2000 ppm CI Basic Red 9 for 103 wk.	Hepatocellular carcinomas:		Purity 93–99%
		M–0/50, 2/50, 8/50	$P = 0.001$ (trend)	
		Thyroid-follicular cell carcinomas:		
		M–0/49, 5/46, 18/44	$P < 0.001$ (trend)	
		F–0/47, 2/48, 2/50	NS	
		Thyroid-follicular cell adenomas or carcinomas:		
		M–0/49, 5/46, 25/44	$P < 0.001$ (trend)	
		F–0/47, 2/48, 6/50	$P = 0.009$ (trend)	
		Zymbal gland carcinomas:		
		M–1/50, 1/50, 13/50	$P < 0.001$ (trend)	
		F–0/50, 2/50, 7/50	$P = 0.003$ (trend)	
		Subcutaneous-fibromas:		
		M–2/50, 20/50, 16/50	$P < 0.001$ (trend)	
		F–0/50, 15/50, 10/50	$P = 0.005$ (trend)	
		Skin-squamous cell carcinomas:		
		M–0/50, 1/50, 10/50	$P < 0.001$ (trend)	
		Skin-trichoepitheliomas:		
		M–0/50, 0/50, 7/50	$P = 0.001$ (trend)	
		Skin-sebaceous adenomas:		
		M–0/50, 0/50, 5/50	$P = 0.006$ (trend)	

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

(Ketkar & Mohr, 1982) and one experiment in hamsters (Green *et al.*, 1979). These studies were found to be inadequate to evaluate the carcinogenicity of magenta in experimental animals.

3.2 CI Basic Red 9

CI Basic Red 9 was tested for carcinogenicity by oral administration in one study in mice, (NTP, 1986) in two studies in rats (Ketkar & Mohr, 1982; NTP, 1986) and one study in hamsters (Green *et al.*, 1979). It has also been tested by subcutaneous injection in one study in rats (Druckrey *et al.*, 1956). Only the study by NTP (1986) was adequate to evaluate the carcinogenicity of CI Basic Red 9 in experimental animals.

After oral administration in the diet, CI Basic Red 9 increased the incidence of hepatocellular carcinomas in male and female mice and in male rats. It increased the incidence of adrenal gland phaeochromocytomas (benign or malignant) in female mice, and of benign (trichoepitheliomas and sebaceous adenomas) and malignant (squamous cell carcinomas) skin tumours in male rats. In rats, it also increased the incidence of subcutaneous fibromas and Zymbal gland carcinomas in males and females, of thyroid follicular-cell carcinomas in males, and of thyroid follicular-cell adenomas and carcinomas combined in females (NTP, 1986; Table 3.1). In an early study, subcutaneous injection of Basic Red 9 (parafuchsin) in rats resulted in a high incidence of local sarcomas (Druckrey *et al.*, 1956). [The Working Group noted that this study lacks detail and has no concurrent controls.]

4. Other Relevant Data

A general Section on "Aromatic amines: metabolism, genotoxicity, and cancer susceptibility" appears as Section 4.1 in the *Monograph* on 4-aminobiphenyl in this volume.

No studies in laboratory animals or in humans were found on the metabolism of magenta. No adequate studies were available on the carcinogenicity of magenta in experimental animals. However, the related compound CI Basic Red 9, which is a component of commercial magenta, induced liver tumours in rats and mice. Excess risk for cancer of the urinary bladder was reported in workers involved in the manufacture of magenta. Magenta was mutagenic in *S. typhimurium* strains TA98, TA100, and TA1535 when tested in the presence of metabolic activation (Mortelmans *et al.*, 1986).

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of magenta production. Magenta production causes cancer of the urinary bladder.

There is *sufficient evidence* in experimental animals for the carcinogenicity of CI Basic Red 9.

There are insufficient mechanistic data relevant to the carcinogenicity of magenta in humans or experimental animals.

Magenta production is *carcinogenic to humans (Group 1).*

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

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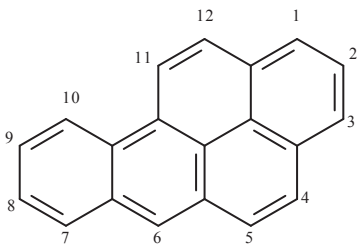
BENZO[*a*]PYRENE

Benzo[*a*]pyrene was considered by previous IARC Working Groups in 1972, 1983, and 2005 (IARC, 1973, 1983, 2010). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Services Reg. No.: 50-32-8
Chem. Abstr. Name: Benzo[*a*]pyrene
IUPAC Systematic Name: Benzo[*a*]pyrene
Synonyms: B*a*P; benzo[*def*]chrysene; 3,4-benzopyrene*; 6,7-benzopyrene*; benz[*a*]pyrene; 3,4-benz[*a*]pyrene*; 3,4-benzpyrene*; 4,5-benzpyrene*
(*alternative numbering conventions)



$C_{20}H_{12}$
Relative molecular mass: 252.31
Description: Yellowish plates, needles from benzene/methanol; crystals may be monoclinic or orthorhombic
Boiling-point: 310–312 °C at 10 mm Hg
Melting-point: 179–179.3 °C; 178.1 °C
Spectroscopy data: Ultraviolet/visual, infrared, fluorescence, mass and nuclear magnetic-resonance spectral data have been reported
Water solubility: 0.00162 mg/L at 25 °C; 0.0038 mg/L at 25 °C
log K_{ow} (octanol–water): 6.35
Henry's Law Constant: 0.034 Pa m^3/mol at 20 °C
From IARC (2010)

1.2 Occurrence and exposure

Benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants formed during incomplete combustion or pyrolysis of organic material. These substances are found in air, water, soils and sediments, generally at trace levels except near their sources. PAHs are present in some foods and in a few pharmaceutical products based on coal tar that are applied to the skin. Tobacco smoke contains high concentrations of PAHs (IARC, 2010).

1.2.1 Exposure of the general population

The general population can be exposed to benzo[*a*]pyrene through tobacco smoke, ambient air, water, soils, food and pharmaceutical products. Concentrations of benzo[*a*]pyrene in

sidestream cigarette smoke have been reported to range from 52 to 95 ng/cigarette — more than three times the concentration in mainstream smoke. Major sources of PAHs in ambient air (both outdoors and indoors) include residential and commercial heating with wood, coal or other biomasses (oil and gas heating produce much lower quantities of PAH), other indoor sources such as cooking and tobacco smoke, and outdoor sources like motor-vehicle exhaust (especially from diesel engines), industrial emissions and forest fires. Average concentrations of individual PAHs in the ambient air in urban areas typically range from 1 to 30 ng/m^3; however, concentrations up to several tens of nanograms per cubic metre have been reported in road tunnels, or in large cities that make extensive use of coal or other biomass as residential heating fuel. Estimates of PAH intake from food vary widely, ranging from a few nanograms to a few micrograms per person per day. Sources of PAHs in the diet include barbecued/grilled/broiled and smoke-cured meats; roasted, baked and fried foods (high-temperature processing); bread, cereals and grains (at least in part from gas/flame-drying of grains); and vegetables grown in contaminated soils, or in areas with surface contamination from atmospheric PAH fall-out (IARC, 2010).

1.2.2 Occupational exposure

Occupational exposure to PAHs occurs primarily through inhalation and via skin contact. Monitoring by means of ambient air-sampling or personal air-sampling at the workplace, to determine individual PAHs, sets of PAHs or surrogates (e.g. coal-tar pitch volatiles) has been used to characterize exposure via inhalation; more recently, biological monitoring methods have been applied to characterize the uptake of certain specific PAHs (e.g. benzo[*a*]pyrene) to be used as biomarkers of total exposure (IARC, 2010).

Industries where occupational exposure to benzo[*a*]pyrene has been measured and reported include: coal liquefaction, coal gasification, coke production and coke ovens, coal-tar distillation, roofing and paving (involving coal-tar pitch), wood impregnation/preservation with creosote, aluminium production (including anode manufacture), carbon-electrode manufacture, chimney sweeping, and power plants. Highest levels of exposure to PAHs are observed in aluminium production (Söderberg process) with values up to 100 µg/m^3. Mid-range levels are observed in roofing and paving (e.g. 10–20 µg/m^3) and the lowest concentrations (i.e. at or below 1µg/m^3) are observed in coal liquefaction, coal-tar distillation, wood impregnation, chimney sweeping and power plants (IARC, 2010).

2. Cancer in Humans

No epidemiological data on benzo[*a*]pyrene alone were available to the Working Group.

3. Cancer in Experimental Animals

Benzo[*a*]pyrene was considered by three previous Working Groups (IARC, 1973, 1983, 2010).

In *IARC Monograph* Volume 3 (IARC, 1973) it was concluded that benzo[*a*]pyrene produced tumours in all species tested (mouse, rat, hamster, guinea-pig, rabbit, duck, newt, monkey) for which data were reported following exposure by many different routes (oral, dermal, inhalation, intratracheal, intrabronchial, subcutaneous, intraperitoneal, intravenous). Benzo[*a*]pyrene had both a local and a systemic carcinogenic effect, was an initiator of skin carcinogenesis in mice, and was carcinogenic in single-dose studies and following prenatal and transplacental exposures.

In *IARC Monograph* Volume 32 (IARC, 1983) no evaluation was made of studies of carcinogenicity in experimental animals published since 1972, but it was concluded that there is *sufficient evidence* for the carcinogenicity of benzo[*a*]pyrene in experimental animals.

Carcinogenicity studies with administration of benzo[*a*]pyrene by multiple route of exposure, reported after the initial evaluations, were subsequently reviewed in *IARC Monograph* Volume 92 (IARC, 2010) and are summarized below (Table 3.1). See Table 3.2 for an overview of malignant tumours induced in different animal species.

3.1 Skin application

In several studies in which benzo[*a*]pyrene was applied to the skin of different strains of mice, benign (squamous cell papillomas and keratoacanthomas) and malignant (mainly squamous-cell carcinomas) skin tumours were observed (Van Duuren *et al.*, 1973; Cavalieri *et al.*, 1977, 1988a; Levin *et al.*, 1977; Habs *et al.*, 1980, 1984; Warshawsky & Barkley, 1987; Albert *et al.*, 1991; Andrews *et al.*, 1991; Warshawsky *et al.*, 1993). No skin-tumour development was seen in $AhR^{-/-}$ mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice developed squamous-cell carcinomas of the skin (Shimizu *et al.*, 2000).

In a large number of initiation–promotion studies in mice, benzo[*a*]pyrene was active as an initiator (mainly of squamous-cell papillomas) when applied to the skin (IARC, 2010).

3.2 Subcutaneous injection

In subcutaneous injection studies of benzo[*a*]pyrene, malignant tumours (mainly fibrosarcomas) were observed at the injection site in mice (Kouri *et al.*, 1980; Rippe & Pott, 1989) and rats (Pott *et al.*, 1973a, b; Rippe & Pott, 1989). No fibrosarcomas were observed in $AhR^{-/-}$ mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice did develop these tumours (Shimizu *et al.*, 2000).

In another study, male and female newborn Swiss mice that were given benzo[*a*]pyrene subcutaneously showed a significant increase in lung-adenoma incidence and multiplicity (Balansky *et al.*, 2007).

A single study in 12 strains of hamsters resulted in sarcomas at the site of injection in both sexes of all 12 strains (Homburger *et al.*, 1972).

3.3 Oral administration

After administration of benzo[*a*]pyrene by gavage or in the diet to mice of different strains (Sparnins *et al.*, 1986; Estensen & Wattenberg, 1993; Weyand *et al.*, 1995; Kroese *et al.*, 1997; Culp *et al.*, 1998; Hakura *et al.*, 1998; Badary *et al.*, 1999; Wijnhoven *et al.*, 2000; Estensen *et al.*, 2004), increased tumour responses were observed in lymphoid and haematopoeitic tissues and in several organs, including the lung, forestomach, liver, oesophagus and tongue.

Oral administration of benzo[*a*]pyrene to $XPA^{-/-}$ mice resulted in a significantly higher increase of lymphomas than that observed in similarly treated $XPA^{+/-}$ and $XPA^{+/+}$ mice (de Vries *et al.*, 1997). Benzo[*a*]pyrene given by gavage to $XPA^{-/-}/p53^{+/-}$ double-transgenic mice induced tumours (mainly splenic lymphomas and forestomach tumours) much earlier and at higher incidences than in similarly treated single transgenic and wild-type counterparts. These cancer-prone $XPA^{-/-}$ or $XPA^{-/-}/p53^{+/-}$ mice also developed a high incidence of tumours (mainly of the forestomach) when fed benzo[*a*]pyrene in the diet (van Oostrom *et al.*, 1999; Hoogervorst *et al.*, 2003).

Oral administration of benzo[*a*]pyrene by gavage to rats resulted in an increased incidence of mammary gland adenocarcinomas (el-Bayoumy *et al.*, 1995).

Table 3.1 Carcinogenicity studies of benzo[*a*]pyrene in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Skin application				
Mouse, Swiss ICR/Ha (F) 52 wk Van Duuren *et al.* (1973)	0 (untreated), 0 (vehicle control), 5 µg/animal, 3 × /wk, 52 wk 50/group	Skin T: 0/50, 0/50, 23/50 (46%; 13 P; 10 C)	+	NR (acetone)
Mouse, Swiss (F) 38–65 wk Cavalieri *et al.* (1977)	0 and 0.396 µmol [0.1 mg] per animal, twice/wk, 30 wk 40/group	Skin T: 0% [0/29], 78.9% [30/38] (7 P, 7 K, 36 C, 1 malignant Schwannoma)	+	99% (acetone)
Mouse, C57BL/6J (F) 60 wk Levin *et al.* (1977)	Experiment 1 and 2: 0 (DMSO/acetone), 0.02 [5.28 µg], 0.1 [26.43 µg], 0.4 [105.75 µg] µmol/animal, once/2 wk, 60 wk (high dose given in two paintings, 30 min apart) Experiment 3: 0 (acetone/NH_4OH), 0.025 [6.6 µg], 0.05 [13.21 µg], 0.1 [26.43 µg] µmol/animal, once/2 wk, 60 wk 30/group	Skin T (mainly SCC): Experiment 1–0%, 0%, 38% (13 T), 100% (44 T) Experiment 2–0%, 4% (1 T), 50% (15 T), 100% (40 T) Experiment 3– 0%, 7% (2 T), 59% (20 T), 91% (24 T)	+	NR (DMSO/acetone (1:3) or acetone/NH_4OH (1 000:1)) Effective number of animals not clearly specified At most, seven animals/group died prematurely without a skin tumour.
Mouse, NMRI (F) 63–109 wk Habs *et al.* (1984)	0, 2, 4 µg/animal, twice/wk 20/group	Skin T: 0/20, 9/20 (45%; 2 P, 7 C), 17/20 (85%; 17 C)	+	> 96% (acetone)
Mouse C3H/HeJ (M) 99 wk Warshawsky & Barkley (1987)	0 (untreated), 0 (vehicle control) or 12.5 µg/animal, twice/wk 50/group	Skin T: 0/50, 0/50, 48/50 (96%; 47 C, 1 P)	+	99.5% (acetone)
Mouse, Swiss (F) 42 wk Cavalieri *et al.* (1988a)	0, 0.1 [26.4 µg], 0.4 [105.7 µg] µmol/animal, twice/wk, 20 wk 30/group	Skin T incidence: 0/30, 26/29 (90%; SGA, 3 P, 23 SCC), 26/30 (90%; 2 P, 26 SCC)	+	Purified [NR] (acetone)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, C3H/fCum (M) Experiment 1: 15 mo Experiment 2: 18 mo Experiment 3: 18 mo Kouri *et al.* (1980)	Experiment 1: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or DMSO, Experiment 2: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or in DMSO, Experiment 3: 0 (trioctanoin/DMSO, 100:1), 0.9 µmol [0.23 mg] in trioctanoin/DMSO (100:1), 1 × 20 or 40/group	FibroS at injection site: *Experiment 1*–0/16, 0/20, 15/18 (83%), 12/19 (63%) *Experiment 2*–0/20, 0/18, 14/18 (78%), 7/19 (37%) *Experiment 3*–0/20, 36/40 (90%)	+	Pure (trioctanoin, DMSO)
Mouse, NR (F) 78 wk Rippe & Pott (1989)	0, 10, 100 µg/animal, 1 × NR/group	S at injection site: 1/30 (3%), 13/30 (43%), 20/30 (67%)	+	NR (tricaprylin)
Mouse, Swiss (newborn) (M, F) 75–200 d Balansky *et al.* (2007)	0 and 1.0 mg/animal, 1 × 12–15 M/group, 12–15 F/group	Lung A: M – 0/15, 9/12; F – 0/15, 11/12	$P < 0.001$	Pure (olive oil)
Rat, Wistar (F) ~530 d Pott *et al.* (1973a)	0, 33, 100, 300, 900, 2 700 µg/animal, 1 × 50/group	T (mainly fibroS) at injection site [incidence derived from dose–response curves]: 2/50 (~4%), 4/50 (~8%), 7/50 (~14%), 23/50 (~46%), 35/50 (~70%), 38/50 (~76%)	+	NR (tricaprylin)
Rat, NR (F) 132 wk Rippe & Pott (1989)	0 and 1 mg, 1 × NR/group	S at injection site: 0/24 (0%), 20/24 (83%)	+	NR (tricaprylin)
Rat, NR (F) 132 wk Rippe & Pott (1989)	0 and 15 mg, 1 × NR/group	S at injection site: 1/24 (4%), 19/24 (79%)	+	NR (DMSO)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian, RB (randomly bred), BIO inbred strains designated as: 1.5, 4.22, 4.24, 7.88, 12.14, 15.16, 45.5, 54.7, 82.73, 86.93, 87.20 (M, F) 53 wk Homburger *et al.* (1972)	500 µg/animal, 1 × 25 M/group, 25 F/group	FibroS at injection site: RB–M, 4/25 (16%); F, 6/23 (26%) 1.5–M, 5/25 (20%); F, 4/23 (17%) 4.22–M, 3/25 (12%); F, 8/25 (32%) 4.24–M, not tested; F, 9/25 (36%) 7.88–M, 13/25 (52%); F, 5/23 (23%) 12.14–M, 3/25 (12%); F, 9/22 (41%) 15.16–M, 9/25 (36%); F, 16/25 (64%) 45.5–M, 12/25 (48%); F, 7/15 (47%) 54.7:–M, 5/25 (20%); F, 5/25 (20%) 82.73–M, 4/21 (19%); F, 4/24 (17%) 86.93–M, 9/25 (36%); F, 8/25 (32%) 87.20–M, 16/25 (64%); F, 11/25 (42%)	+	NR (tricaprylin) No subcutaneous T observed in historical controls
Oral administration				
Mouse, A/J (F) 260 d Weyand *et al.* (1995)	0, 16, 98 ppm (total dose; 0, 11, 67 mg) in the diet 30/group	Lung T: 4/21 (19%; 4 A; 0.19 ± 0.09 A/animal), 9/25 (36%*; 7 A, 2 AdC; 0.48 ± 0.14 T/animal), 14/27 (52%*; 14 A; 0.59 ± 0.12 A/animal) Forestomach T: (0%) 0/21, (5/25) (20%; 3 P, 2 C; 0.24 ± 0.11** T/animal), 27/27 (100%**; 13 P, 14 C; 4.22 ± 0.41**)	*$P < 0.05$ **$P < 0.001$	NR (gel diet)
Mouse B6C3F1 (F) 2 yr Culp *et al.* (1998)	0 (acetone control diet), 5 ppm, 25 ppm, 100 ppm in the diet 48/group	Liver (A): 2/48 (4%), 7/48 (15%), 5/47 (11%), 0/45 (0%) Lung (A and/or C): 5/48 (10%), 0/48 (0%), 4/45 (9%), 0/48 (0%) Forestomach (P and/or C): 1/48 (2%), 3/47 (6%), 36/46 (78%****), 46/47 (98%****) Oesophagus (P and/or C): 0/48 (0%), 0/48 (0%), 2/45 (4%), 27/46 (59%**) Tongue (P and/or C): 0/48 (0%), 0/48 (0%), 2/46 (4%), 23/48 (48%***) Larynx (P and/or C): 0/35 (0%), 0/35 (0%), 3/34 (9%), 5/38 (13%*)	*$P < 0.014$ **$P < 0.0014$ ***$P < 0.0003$ ****$P < 0.00001$	98.5% (acetone)
Mouse, Swiss albino, inbred (F) 27 wk Badary *et al.* (1999)	0 and 1 mg/animal by gavage, twice/wk, 4 wk 10/group	0, 10/10 (100%) (forestomach P; multiplicity, 7.11 ± 1.05)	+	Highest purity grade (corn oil) Drinking-water contained 0.005% ethanol

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, $CSB^{-/-}$ or wild-type ($CSP^{\pm}$ or $CSB^{+/+}$) (M, F) 52 wk Wijnhoven *et al.* (2000)	0 and 13 mg/kg bw by gavage, 3 × / wk, 13 wk 6–18 M/group, 6–13 F/group	Wild-type: 5/27 (14 M, 13 F; 19%; 4 bronchiolo-alveolar A, 2 lymphoma), 17/29* (18 M, 11 F; 59%; 6 bronchiolo-alveolar A, 10 forestomach P, 2 forestomach SCC, 2 histiocytic S, 2 hepatocellular A, 1 intestinal AdC, 1 skin P) $CSB^{-/-}$: 0/13 (6 M, 7 F), 7/12** (6 M, 6 F; 58%; 2 bronchiolo-alveolar A, 2 uterine S, 1 forestomach SCC, 1 intestinal AdC, 1 skin histiocytic S)	*$P = 0.0023$ **$P = 0.0017$	NR (soya oil)
Rat, Crl:CD(SD)BR (F) 49 wk el-Bayoumy *et al.* (1995)	0 and 50 µmol/animal, once/wk, 8 wk by gavage 30/group	Mammary T incidence: 11/30 [37%] [incidence not clearly specified] (8 desmoplastic A, 2 A, 1 AdC), 29/30 (96.7%; 8 fibroA**, 17 desmoplastic A*, 7 A, 22 AdC**) Numbers of mammary T: controls, 14 desmoplastic A, 2 A, 1 AdC; treated animals, 14 fibroA*, 35 desmoplastic A, 11 A, 56 AdC**	*$P < 0.05$ **$P < 0.01$	99% (trioctanoin)
Intraperitoneal injection				
Mouse, B6C3F1; C3A/JF1 (M, F) 90 wk or lifetime Vesselinovitch *et al.* (1975a, b)	0, 75, 150 µg in 10 µL/g bw, 1 × at 1, 15, 42 d of age 30–63/group, 96–100 controls/group	B6C3F1 mice (all ages combined): Liver T (A and hepatocellular C)– M, 1/98 (1%), 69/162 (43%), 81/165 (49%); F, 0/96 (0%), 7/147 (5%), 10/126 (8%) Lung T (A and AdC)– M, 7/98 (7%), 57/162 (35%), 73/165 (44%); F, 2/90 (2%), 53/147 (36%), 50/126 (40%) Forestomach T (P and SCC)– M, 0/98 (0%), 39/162 (24%), 64/165 (39%); F, 0/96 (0%), 22/147 (15%), 40/126 (32%) Lymphoreticular T (mainly reticulum-cell S)– M, 2/98 (2%), 104/314 (33%) (high- and low-dose groups combined); F, 2/96 (2%), 148/281 (53%) (high-and low-dose groups combined)	+	NR (trioctanoin)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Vesselinovitch *et al.* (1975a, b) Contd.		C3A/JF1 mice (all ages combined): Liver T (A and hepatocellular C)– M, 3/97 (3%), 30/148 (20%), 33/137 (24%); F, 0/100 (0%), 1.3% 2/126 (1.3%), 2/153 1.3%) Lung T (A and AdC)– M, 49/97 (49%), 1 438/148 (93%), 125/137 (91%); F, 26/100 (26%), 115/126 (91%), 141/153 (92%) Forestomach T (P and SCC)– M, 0/97 (0%), 18/148 (12%), 42/137 (31%); F, 0/100 (0%), 18/126 (14%), 31/153 (20%) Lymphoreticular T (mainly reticulum-cell S)– M, 0/97 (0%), 26/285 (9%); F, 2/100 (2%), 50/278 (18%) (high- and low-dose groups combined)	+	
Mouse, CD-1 (M, F) 1 yr Wislocki *et al.* (1986)	0 and 560 nmol [148 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 0, 8, 15) 37 M/group, 27 F/group	Liver T: M, 2/28 (7%; 2 A), 18/37* (49%; 11 A, 7 C*); F, no liver T found Lung T: M, 1/28 (4%; 1 A), 13/37** (35%; 13 A); F, 0/31, 13/27** (48%) (13 A) Malignant lymphoma: M, 1/28 (4%), 2/37 (5%); F, 1/31 (3%), 4/27 (15%)	*$P < 0.005$ **$P < 0.05$	> 99% (DMSO)
Mouse, CD-1 (M, F) 52 wk Lavoie *et al.* (1987)	0 and 1.1 µmol [290 µg] (total dose; given as 1/6, 2/6, 4/6 on PND 1, 8, 15) 17 M/group, 14–18 F/group	Liver T: M, 1/17 (6%; 1 H), 13/17* (76%; 9 hepatic A, 4 H); F, 0/18, 0/14 Lung A: M, 0/17, 14/17* (82%); F, 0/18, 9/14** (64%)	*$P < 0.005$ **[$P < 0.0005$]	> 99% (DMSO)
Mouse, Swiss-Webster BLU: Ha(ICR) (M, F) 26 wk Busby *et al.* (1989)	0 and 59.5 µg (total dose; given as 8.5, 17, 34 µg on PND 1, 8, 15) NR/group	Lung T: M, 12/91 (13%; 12 A, 1 AdC; 0.15 ± 0.04 T/mouse), 13/28 (46%; 13 A; 0.71 ± 0.19 A/mouse); F, 7/101 (7%; 7 A; 0.08 ± 0.03 A/mouse), 18/27 (67%; 18 A, 1 AdC; 1.19 ± 0.21 T/mouse)	+	> 99% (DMSO) statistics NR
Mouse, NR, newborn (M, F) 30 wk Rippe & Pott (1989)	0, 10, 100 µg/animal, 1 × NR/group	Lung T: 13% [5/38] (0.13 T/animal), 16% [5/31] (0.23 T/animal), 64% [21/33] (2.52 T/animal)	+	NR (saline solution + 1% gelatine + 0.4% Tween 20) Type of lung tumour NR; statistics NR
Mouse, A/J (F) 260 d Weyand *et al.* (1995)	0 (untreated), 0 (vehicle control), 1.79 mg/animal, 1 × 29–30/group	Lung T: 7/30 (23%; 7 A; 0.27 ± 0.12 A/animal), 11/30 (37%; 11 A; 0.43 ± 0.11 A/animal), 29/29* (100%; 27 A, 2 AdC; 15.8 ± 1.28** T/animal); forestomach T: 0/30 (0%), 0/30 (0%), 24/29** (83%; 15 P, 9 C; 1.83 ± 0.25** T/animal)	*$P < 0.05$ **$P < 0.001$	NR (tricaprylin)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, B6C3F1 infant (M, F) 26 wk, 39 wk, 52 wk Rodriguez *et al.* (1997)	0 (untreated), 0 (vehicle controls), 125, 250, 375 µg/7 g bw, 1 × > 30 M/group, > 30 F/group	Liver T (M): At wk 26: 0/41, 0/58, 0/29, 0/25, 3/34 (9%; multiplicity, 1.0); at wk 39: 0/34, 0/59, 6/26 (23%; multiplicity, 1.0), 13/34 (38%; multiplicity, 1.9), 15/23 (65%; multiplicity, 1.9); at wk 52: 4/64 (6%; multiplicity, 1.0), 3/63 (5%; multiplicity, 1.0), 13/29 (45%; multiplicity, 1.8), 14/27 (52%; multiplicity, 2.2), 19/24 (79%; multiplicity, 2.5) No liver T in F	+	NR (corn oil) No forestomach tumours
Mouse, CD-1 (M) 12 mo Von Tungeln *et al.* (1999)	0, 100, 400 nmol [26, 111 µg]/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15) 24/group	Liver T: 3/20 (15%; 1 A, 2 C; 1.7 T/liver section), 5/21 (24%; 4 A, 1 C; 1.5 T/liver section), 9/20 (45%; 7 A*, 2 C; > 2.3 T/liver section) Lung T: 4/20 (20%; 4 A; 1.0 T/lung section), 1/21 (5%; 1 A; 1.0 T/lung section), 9/20 (45%; 7 A, 2 C; 1.9 T/lung section)	*P = 0.0234	> 99% (DMSO)
Rat, Wistar (F) ~112 wk Roller *et al.* (1992)	0 and 5 mg/animal, 1 × NR/group	Abdominal mesothelioma and S: 3/41 (7.3%), 33/37 (89.2%)	+	NR (3:1 mixture of tricaprylin/ beeswax) Limited reporting
Rat, Wistar (F) ~116 wk Roller *et al.* (1992)	5 mg/animal, 1 × NR/group	Abdominal mesothelioma and S: 19/38 (50%); historical controls, 11/369 (3%)	+	NR (saline solution) No control; limited reporting of tumour data
Inhalation				
Hamster, Syrian golden (M) Lifetime Thyssen *et al.* (1981)	0, 2.2, 9.5, 46.5 mg/m3, 4.5 h/d, 7 d/wk, 10 wk; thereafter 3 h/d, 7 d/wk (total average doses: 0, 29, 127, 383 mg/animal) 24/group (+ animals added during the study)	Respiratory tract T: (polyps, P, SCC)– 0/27, 0/27, 34.6% [9/26; 3 nasal, 8 laryngeal, 1 tracheal], 52% [13/25; 1 nasal, 13 laryngeal, 3 tracheal; no bronchogenic T] Upper digestive tract T: (polyps, P, SCC)– 0/27, 0/27, 26.9% [6/26; 6 pharyngeal, 1 forestomach], 56% [14/25; 14 pharyngeal, 2 oesophageal, 1 forestomach]	+	NR (0.1% saline solution); particle size, > 99% diameter 0.2–0.5 µm, > 80% diameter 0.2–0.3 µm Survival decreased for high dose-exposed animals (59 wk) *vs* other groups (96 wk).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Intrapulmonary injection				
Rat, OM (F) 64 (high-dose group)–133 wk (untreated controls) Deutsch-Wenzel *et al.* (1983)	0 (untreated), 0 (vehicle control), 0.1, 0.3, 1.0 mg/animal, 1 × 35/group	Lung T: [0/35] (0%), [0/35] (0%), [10/35] (28.6%) (4 epidermoid C; 6 pleomorphic S), [23/35] (65.7%) (21 epidermoid C; 2 pleomorphic S), [33/35] (94.3%) (33 epidermoid C)	+	99.1% (1:1 mixture of beeswax and trioctanoin)
Rats, F344/NSlc (M) 104 wk Iwagawa *et al.* (1989)	0, 0.03, 0.1, 0.3, 1.0 mg/animal, 1 × NR/group	Lung T: 0/40, 1/29 (3%; 1 undifferentiated T), 7/30 (23%; 6 SCC, 1 undifferentiated T), 22/29 (76%; 20 SCC, 2 undifferentiated T), 9/13 (69%; 9 SCC)	+	NR (1:1 mixture of beeswax/tricaprylin)
Rat, Osborne-Mendel (F) 134 wk (low-dose group)–140 wk (vehicle controls) Wenzel-Hartung *et al.* (1990)	0 (untreated), 0 (vehicle control), 30, 100, 300 µg/animal, 1 × 35/group	Lung T: [0/35] (0%), [0/35] (0%), [3/35] (8.6%; 3 SCC), [11/35] (31.4%; 11 SCC), [27/35] (77.1%; 27 SCC).	+	99.6% (beeswax/trioctanoin mixture of varying composition) SCC predominantly keratinized
Rat, F344/DuCrj (M) 100 wk Horikawa *et al.* (1991)	0, 50, 100, 200 µg/animal, 1 × 9–10/group	Lung T: 0/19, 0/10, 3/10 (30%; 2 SCC, 1 AdSC), 4/9 (44.4%; 3 SCC, 1 undifferentiated T)	+	NR (1:1 mixture of beeswax/tricaprylin)
Intratracheal administration				
Rat, Wistar-WU/Kisslegg (F) 124–126 wk Pott *et al.* (1987)	0 and 1 mg/animal, once/wk, 20 wk NR/group	Lung T: 0/40, 7/36 (19%; 1 A, 5 SCC, 1 mixed AdC/SCC)	+	NR (0.9% saline solution)
Rat, Sprague-Dawley (M, F) Controls, 131 wk; treated animals, 112 wk Steinhoff *et al.* (1991)	0 and 0 (physiological saline), 7 mg/kg bw/instillation (physiological saline with Tween 60), once/2 wk, 44 wk 20 or 50/group	M: 0/50, 0/50, 19/20 (95%; 19 malignant lung T) F: 0/50, 0/50, 19/20 (95%; 18 malignant, 1 benign lung T)	+	NR (physiological saline solution with or without Tween 60) Limited histology

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 78 wk Feron (1972)	0 (only for M), 1 mg/animal, once/wk, 36 wk 35/group	Respiratory tract T/adenomatoid lesions: M–6/27 (22%; 1 tracheal P, 5 pulmonary adenomatoid lesion), 19/29 (66%; 1 tracheal P, 17 SCC, 26 pulmonary adenomatoid lesion, 5 A, 1 AdC, 1 SCC) F–22/27 (81%; 1 laryngeal SCC, 16 tracheal SCC, 2 bronchial A, 1 AdC, 21 pulmonary adenomatoid lesion, 8 A, 1 AdC)	+	> 99% (0.9% saline solution) No female controls; Statistics NR
Hamster, Syrian golden (M) 78 wk Feron *et al.* (1973)	0, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/animal, once/wk, 52 wk 30/group	Respiratory tract T: 0/29, 3/30 (10%; 3 tracheal P, 1 pulmonary A), 4/30 (13%; 1 tracheal P, 4 pulmonary A), 9/30 (30%; 5 tracheal P, 7 pulmonary A), 25/29 (86%; 2 tracheal polyp, 9 P, 5 SCC, 1 AdSC, 1 fibroS, 2 bronchial polyp, 1 P, 2 SCC, 1 AdSC), 26/28 (93%; 6 tracheal P, 11 SCC, 1 AdSC, 1 bronchial polyp, 2 P, 4 SCC, 2 AdSC, 4 AdC, 1 anaplastic C, 16 pulmonary A, 4 SCC, 3 AdSC, 1 AdC, 2 anaplastic C)	+	NR (0.9% saline solution)
Hamster, Syrian golden (M, F) M, 67–88 wk; F, 60–88 wk Henry *et al.* (1973)	0, 13.3–15.5 mg/animal, once/wk, 8 wk 50/group, 25 controls/group	Respiratory tract T: Controls– 1 tracheal polyp, 6 pulmonary bronchiolar adenomatoid lesions/47 animals Treated animals– 26/65 (40%; 1 nasal polyp; 6 laryngeal polyps, 1 P, 1 A, 1 AdC, 7 tracheal polyps, 1 AdC, 1 SCC, 1 fibroS, 2 bronchial AdC, 13 pulmonary bronchiolar adenomatoid lesion, 3 A, 5 AdC, 1 SCC, 2 anaplastic C, 1 mixed C, 1 myelogenous leukaemia, 1 neurofibroS) T at other sites: Controls– 1 renal A Treated animals– 3 blast-cell leukaemia, 2 adrenocortical A, 1 renal AdC, 1 oesophageal fibroS, 1 haemangioma	+	NR (0.5% gelatine in 0.9% saline solution) Tumour data for M and F combined; statistics NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 60 wk Kobayashi (1975)	0 and 1 mg/animal, once/wk, 30 wk 20–32 M/group, 20–28 F/group	Respiratory tract T: M–0/20, 11/26 (42.3%; 1 laryngeal polyp, 1 tracheal polyp, 1 P, 1 bronchial SCC, 9 lung A, 7 AdC, 3 SCC, 1 anaplastic C, 2 AdSC) F–0/20, 14/26 (53.8%; 1 laryngeal P, 2 tracheal polyps, 1 bronchial SCC, 10 lung A, 3 AdC, 1 SCC)	+	NR (0.9% saline)
Hamster, Syrian golden (M, F) 78 wk Kruysse & Feron (1976)	0 (untreated), 0 (vehicle controls), 1 mg/animal, once/2 wk, 52 wk 17 or 40/group	Respiratory tract T: M–0/40, 0/40, 13/14 (93%; 2 laryngeal P, 1 SCC, 4 tracheal P, 3 SCC, 1 anaplastic C, 1 S, 1 bronchial SCC, 1 AdC, 5 pulmonary A, 1 AdC) F–0/40, 0/40, 7/12 (58%; 2 tracheal P, 3 SCC, 1 bronchial P, 5 pulmonary A)	+	> 99% (saline solution)
Hamster, Syrian golden (M) 100 wk Sellakumar *et al.* (1976)	0 (untreated), 3 mg/animal, once/wk, 10 wk 48/group	Respiratory tract T: 0/48, 7/48 (15%; 2 laryngeal P, 4 tracheal P, 1 lung A) T at other sites: 6/48 (13%; 3 forestomach P, 2 lymphoma, 1 anaplastic C), 26/48 (54%; 21 forestomach P, 1 skin melanoma, 1 liver haemangioma, 1 adrenocorticoA, 3 adrenocorticoC)	+	> 99% (0.9% saline solution)
Hamster, Syrian golden (M, F) Experiment 1: up to 89 wk for M and 70 wk for F Experiment 2: up to 83 wk for M and 68 wk for F Ketkar *et al.* (1977)	Experiment 1: 0, 4, 8, 16 mg in 0.9% saline solution/animal, 1 × 30/group	Respiratory tract T: Experiment 1– M 0/24, 3/30 (10%; 1 laryngeal P, 1 tracheal P, 1 lung S), 5/28 (18%; 1 laryngeal SCC, 1 tracheal P, 4 lung S), 4/27 (15%; 3 tracheal P, 1 lung A, 1 S) F 0/28, 3/29 (10%; 1 tracheal P, 2 lung A), 1/30 (3%; 1 lung A), 3/28 (13%; 1 laryngeal P, 2 lung A)	+	97% (0.9% saline solution or Tris buffer)
	Experiment 2: 0, 4, 8, 16 mg in Tris buffer/animal, 1 × 30/group	Experiment 2– M 0/27, 5/24 (21%; 1 tracheal P, 5 lung A), 13/25 (52%; 1 laryngeal P, 7 tracheal P, 4 lung A, 3 AdC), 8/27 (30%; 2 laryngeal P, 1 SCC, 3 tracheal P, 3 lung A) F 0/27, 3/27 (11%; 2 tracheal P, 1 lung AdC), 2/29 (7%; 2 tracheal P), 8/29 (28%; 1 laryngeal P, 4 tracheal P, 5 lung A)	+	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 81 wk Feron & Kruysse (1978)	0 (untreated), 0 (vehicle controls), 0.35, 0.7 mg/animal, once/wk, 52 wk 15 or 30/group	Respiratory tract T: M–0/30 (untreated and vehicle controls combined), 4/29 (14%; 2 tracheal P, 1 bronchial P, 2 pulmonary A), 19/30 (63%; 1 laryngeal P, 5 tracheal P, 1 SCC, 1 anaplastic C, 1 S, 2 bronchial P, 1 AdC, 11 pulmonary A, 2 AdC, 1 SCC, 1 anaplastic C) F–0/28 (untreated and vehicle controls combined), 3/27 (11%; 1 laryngeal P, 1 bronchial P, 1 pulmonary A), 7/24 (29%; 1 tracheal P, 2 SCC, 1 bronchial AdC, 5 pulmonary A)	+	> 99% (0.9% saline solution) Statistics NR
Hamster, Syrian golden (M, F) Average survival up to 41 wk for M and 35 wk for F Ketkar *et al.* (1978)	0, 0.1, 0.33, 1.0 mg/animal, once/wk 30/group	Respiratory tract T: M–0/29, 5/26 (19%; 5 bronchiogenic A), 7/29 (24%; 5 tracheal P, 2 bronchiogenic A), 6/27 (22%; 5 tracheal P, 2 bronchiogenic A) F–0/30, 12/30 (40%; 1 tracheal P, 1 SCC, 10 bronchiogenic A), 10/28 (36%; 7 tracheal P, 5 bronchiogenic A, 1 SCC), 6/30 (20%; 3 tracheal P, 3 bronchiogenic A, 3 SCC)	+	97% (10% bovine serum albumin) Average survival time much lower in the high-dose group than in the other groups
Hamster, Syrian golden (M, F) Lifetime, up to 90 wk Stenbäck & Rowland (1978)	0, 3 mg large particles, 3 mg small particles/animal, once/wk, 18 wk 48 (M + F)/group	Respiratory tract T (M + F combined): 0/46, 31/47 (66%; 5 laryngeal P, 12 tracheal P, 20 SCC, 2 unspecified T, 2 bronchial P, 9 SCC, 3 A, 2 anaplastic C), 5/46 (11%; 1 laryngeal P, 1 SCC, 4 tracheal P)	+	99.4% (0.9% saline solution); particle size by weight: large-98% < 30 µm, 90% < 20 µm, 36% < 10 µm, 10% < 5 µm; small-98% < 10 µm, 79% < 5 µm, 5% < 1 µm
Hamster, Syrian golden (M) Average survival up to 88 wk Ketkar *et al.* (1979)	0 (untreated), 0 (vehicle controls), 0.125, 0.25, 0.5, 1.0 mg/animal, once/wk 30/group	Respiratory tract T: 0/29, 0/28, 9/29 (31%; 2 laryngeal polyps/P, 1 tracheal P, 1 SCC, 2 lung A, 2 SCC, 5 AdC), 24/29 (83%; 1 nasal SCC, 2 laryngeal polyps/P, 4 tracheal P, 9 SCC, 5 lung A, 5 SCC, 11 AdC), 19/29 (66%; 1 laryngeal P, 2 SCC, 5 tracheal P, 11 SCC, 7 lung SCC, 2 AdC), 9/29 (31%; 1 laryngeal P, 1 SCC, 1 tracheal P, 5 SCC, 1 lung A, 4 SCC)	$P < 0.001$, all treated groups	97% (Tris buffer + 0.9% saline solution); particle size: majority < 10 µm but particles up to 80 µm also present Average survival in two highest-dose groups much lower than that in the other groups due to many early deaths from pulmonary lesions other than tumours

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 105 wk Feron *et al.* (1980)	0 (untreated), 0 (gelatine in 0.9% saline), 0.5, 1.0 mg fine particles, 0.5, 1.0 mg coarse particles, 1.0 mg wide-range particles/animal, once/wk, 52 wk 30–35/group	Respiratory tract T: M–0/29, 2/34 (6%; 2 laryngeal P), 7/34 (21%; 1 laryngeal P, 6 tracheal P, 1 lung A), 6/31 (19%; 2 laryngeal P, 1 tracheal P, 1 S,1 pulmonary A), 13/31 (42%; 2 laryngeal P, 3 tracheal P, 9 pulmonary A), 25/34 (74%; 2 laryngeal P, 9 tracheal P, 4 SCC, 2 S, 1 pulmonary A, 1 AdC), 23/34 (68%; 2 laryngeal P, 1 SCC, 6 tracheal P, 2 SCC, 1 bronchial P, 1 SCC, 13 pulmonary A, 2 AdC, 2 anaplastic C) F–0/28, 2/33 (6%; 1 tracheal P, 1 pulmonary A), 2/34 (6%; 1 bronchial P, 1 A), 5/32 (16%; 1 laryngeal P, 2 tracheal P, 3 pulmonary A), 9/32 (28%; 2 laryngeal P, 5 tracheal P, 6 pulmonary A), 19/32 (31%; 4 tracheal P, 1 SCC, 1 S, 1 bronchial P, 7 pulmonary A, 1 AdC), 11/34 (34%; 1 laryngeal P, 3 tracheal P, 2 bronchial P, 7 pulmonary A, 1 AdC)	+	NR; particles size by weight: fine, 77% < 5.2 µm, 60% < 3.9 µm; coarse, 77% < 42 µm, 3% < 16 µm; wide-range, 72% < 30 µm, 19% < 10 µm (gelatine in 0.9% saline solution) Statistics NR
Hamster, Syrian golden (M) 129 wk Godleski *et al.* (1984)	0 and 5 mg/animal, once/wk, 15 wk 80/group	Malignant T: 4/80 (5%; 1 multicentric undifferentiated lung C, 3 lymphoma), 25/80* (31%; 9 SCC, 2 undifferentiated C of the respiratory tract, 5 lymphoma, 1 SCC, 2 AdC of the gastrointestinal tract, 2 soft-tissue T, 1 hepatoma, 2 mouth SCC, 1 skin C)	*$P < 0.001$	> 99% (0.5% gelatine in 0.9% saline solution)
Intratracheal administration of combinations of benzo[*a*]pyrene and 'particles/fibres'				
Rat, Sprague-Dawley (M, F) Up to 130 wk Steinhoff *et al.* (1991)	0, (untreated), 0 (physiological saline), 10–40 mg/kg bw Bayferrox 130 (96.2% cubic α-Fe_2O_3), 10–40 mg/kg bw Bayferrox 920 (86.1% fibrous α-FeOOH), 7 mg/kg bw, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 130, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 920, ~once/2 wk, 44–~130 wk 20 or 50/group	Lung T: M–0/50, 0/50, 0/50, 0/50, 19 malignant T in 20 animals, 21 malignant and 1 benign T in 20 animals, 17 malignant and 1 benign T in 20 animals F–0/50, 0/50, 0/50, 1 malignant and 1 benign T in 50 animals, 18 malignant and 1 benign T in 20 animals, 16 malignant T in 20 animals, 17 malignant and 2 benign T in 20 animals	+	NR (physiological saline solution with or without Tween 60); Bayferrox 130, Bayferrox 920 Limited histology

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) Lifetime (up to 140 wk) Saffiotti *et al.* (1972)	Experiment 1 0, 50 mg ferric oxide, 5 mg + 45 mg ferric oxide, 12.5 mg + 37.5 mg ferric oxide/ animal, 1 × Experiment 2 (2 groups/dose level) 5 mg + 5 mg ferric oxide, 10 mg + 10 mg ferric oxide, 15 mg + 15 mg ferric oxide, once/wk, 15 wk 23–110 M/group, 18–107 F/group	Experiment 1: Respiratory tract T– M 0/45, 0/101, 3/92 (3%; 1 tracheal polyp, 1 P, 1 bronchial A), 3/27 (11%; 1 bronchial A, 1 bronchogenic SCC, 1 anaplastic C) F 0/44, 0/89, 4/97 (4%; 1 tracheal polyp, 1 P, 1 bronchiolar A, 1 AdC), 6/33 (18%; 1 bronchial P, 1 A, 2 bronchogenic SCC, 1 anaplastic C, 2 bronchiolar A) Forestomach P– M 5/45 (11%; 6 T), 5/101 (5%; 5 T), 15/92 (16%; 35 T), 8/27 (30%; 16 T) F 2/44 (5%; 2 T), 2/89 (2%; 3 T), 5/97 (5%; 5 T), 4/33 (12%; 6 T)	+	NR (0.9% saline solution); ferric oxide
		Experiment 2: Respiratory tract T (M + F combined)– 7/50 (14%; 2 tracheal P, 1 SCC, 1 bronchial P, 1 A, 2 SCC, 1 anaplastic C, 1 pulmonary SCC), 8/58 (14%; 2 tracheal polyps, 1 bronchial polyp, 2 SCC, 2 AdC, 2 pulmonary A, 2 AdC), 17/61 (28%; 2 tracheal polyps, 2 P, 5 SCC, 5 bronchial SCC, 1 pulmonary A, 1 SCC, 1 AdC, 1 anaplastic C), 25/60 (42%; 4 tracheal polyps, 3 P, 3 SCC, 4 anaplastic C, 1 bronchial P, 1 SCC, 2 anaplastic C, 4 AdC, 1 A, 2 pulmonary SCC, 2 anaplastic C, 6 A), 25/39 (64%; 1 tracheal P, 10 SCC, 1 anaplastic C, 3 bronchial P, 7 SCC, 11 anaplastic C, 2 AdC, 2 pulmonary SCC, 2 A), 35/55 (64%; 2 laryngeal SCC, 11 tracheal P, 1 polyp, 12 SCC, 1 carcinoS, 2 fibroS, 16 bronchial SCC, 10 anaplastic C, 6 AdC, 3 A, 2 pulmonary A) Forestomach T– M 8/22 (36%; 13 P, 1 SCC), 6/28 (21%; 9 P), 11/34 (32%; 28 P, 1 SCC), 11/30 (37%; 18 P), 5/22 (23%; 10 P), 1/28 (4%; 1 P) F 9/28 (32%; 14 P), 6/30 (20%; 9 P), 5/27 (19%; 20 P), 8/30 (27%; 10 P, 1 SCC), 5/17 (29%; 11 P), 3/27 (11%; 3 P)	+	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (F) Presumably lifetime Pott *et al.* (1973b)	340 µg in tricaprylin, 340 µg in Tween 60/saline solution, 340 µg in Tween 60/saline solution + 850 µg atmospheric dust/animal, 45 × within a period of 6.5 mo (total dose, ~15 mg; dust, 38 mg) 48/group	Respiratory tract T (benign and malignant T of the larynx, trachea or bronchi): 2/48 (4%), 14/48 (29%), 16/48 (33%)	+	NR (tricaprylin, Tween 60/saline solution); atmospheric dust from Bochum, Germany (particle size < 5 µm)
Hamster, Syrian (M, F) 100 wk Sellakumar *et al.* (1973)	0 (untreated), 3 mg + 3 mg ferric oxide, 3 mg + 6 mg ferric oxide, 3 mg + 9 mg ferric oxide, once/2 wk, 20 wk 36/group, 193 controls/group	Respiratory tract T (M + F combined): 0/193, 26/67 (39%; 3 laryngeal polyp, 3 P, 3 SCC, 7 tracheal polyp, 6 P, 2 SCC, 2 bronchial polyp, 5 SCC, 9 AdC, 1 anaplasic C, 7 lung A, 1 AdC), 28/64 (44%; 1 laryngeal polyp, 3 P, 6 SCC, 3 tracheal polyp, 9 P, 3 SCC, 3 bronchial polyp, 1 P, 4 SCC, 3 AdC, 1 anaplastic C, 7 lung A, 4 AdC), 26/66 (39%; 3 laryngeal polyp, 6 SCC, 6 tracheal polyp, 11 P, 1 SCC, 1 bronchial polyp, 1 P, 4 SCC, 4 AdC, 2 anaplastic C, 6 lung A, 6 AdC) Forestomach T: M–0/193 (M + F), 17/32 (53%; 37 P), 10/31 (32%; 16 P, 1 SCC), 6/35 (17%; 15 P) F–0/193 (M + F), 10/35 (29%; 30 P), 12/33 (36%; 25 P), 15/31 (48%; 33 P)	+	NR (0.9% saline solution); ferric oxide
Hamster, Syrian golden (M, F) Lifetime (up to 120 wk) Stenbäck *et al.* (1975)	0 (untreated), 2 mg + 1 mg magnesium oxide/animal, once/wk, 20 wk, 3 mg + 3 mg ferric oxide/animal, once/wk, 15 wk 48 or 90/group	Respiratory tract tumours (M + F combined): 0/89, 32/45 [71%] (11 laryngeal P, 3 SCC, 1 tracheal polyp, 20 P, 5 SCC, 1 AdC, 1 bronchial P, 3 A, 8 AdC, 9 SCC, 1 AdSC), 31/44 (70%; 10 laryngeal P, 4 SCC, 8 tracheal P, 12 SCC, 2 anaplastic C, 2 bronchial P, 4 A, 2 AdC, 17 SCC, 3 anaplastic C)	+	NR (0.2% saline solution); ferric oxide, magnesium oxide

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) Lifetime (up to 100 wk) Stenbäck & Rowland (1979)	0 (untreated), 0 (saline), 0 (gelatine in saline), 3 mg silicon dioxide in saline, 1.5 mg manganese dioxide in saline, 3 mg in saline, 3 mg in gelatine/saline, 3 mg + 3 mg silicon dioxide in saline, 1.5 mg + 1.5 mg manganese dioxide in saline/animal, once/wk, 20 wk 50/group	All T (M + F combined): 2/100 (2%; 2 lymphoma), 1/48 (2%; 2 forestomach P), 2/45 (4%; 2 lymphoma), 0/48 (0%), 2/48 (4%; 1 forestomach P, 1 lymphoma), 18/46 (39%; 1 laryngeal P, 1 SCC, 4 tracheal P, 15 forestomach P), 11/47 (23%; 2 tracheal P, 1 SCC, 3 bronchial SCC, 1 splenic haemangioma, 1 adrenal cortical A, 1 lymphoma, 2 forestomach SCC), 25/48 (52%; 1 laryngeal SCC, 8 tracheal P, 2 SCC, 3 bronchial SCC, 6 lung A, 3 AdC, 10 forestomach P, 1 thyroid A, 1 uterine fibroma, 1 A, 1 lymphoma), 20/48 (42%; 1 laryngeal P, 3 tracheal P, 1 SCC, 1 bronchial SCC, 24 forestomach P, 1 ovarian fibroma, 1 thyroid A, 2 forestomach SCC, 1 squamous-cell fibroma)	+	> 99% (saline, 0.5% gelatine in saline); manganese dioxide, silicon dioxide
Hamster, Syrian golden (M, F) 82 wk Reynders *et al.* (1985)	0 and 8 mg + 6 mg ferric oxide/animal, once/wk, 6 wk 35/group	Respiratory tract T: M–0/32, 12/24 (50%; 15 T: 3 laryngeal P, 1 tracheal P, 1 SCC, 2 bronchial polyp, 2 SCC, 1AdC, 3 pulmonary SCC, 1 AdSC, 1 AdC) F–0/35, 9/26 (35%; 12 T: 1 laryngeal P, 5 tracheal P, 2 bronchial polyp, 2 pulmonary SCC, 1 AdSC, 1 AdC)	+	NR (0.9% saline solution); ferric oxide
Buccal pouch				
Hamster, Syrian golden (M) Up to 40–44 wk with interim kills after 5, 20, and 24–32 wk Solt *et al.* (1987)	Painting of both buccal pouch with 0, 20 mM solution/animal, twice/wk, 20 wk 28/group, 20 controls/group	Forestomach P: 0/6, 8/10* (after 40–44 wk) Buccal pouch SCC: 0/6, 1/10 (after 40–44 wk)	*[$P < 0.01$]	NR (paraffin oil)
Intramammary or intramamillary administration				
Rat, Sprague-Dawley (F) 20 wk Cavalieri *et al.* (1988a)	0 and 0 (untreated contralateral mammary gland), 4 [1 mg], 16 µmol [4.2 mg] (5th right mammary gland), 1 × 20/group	Mammary gland T: [0/20] (0%), [0/20] (0%), [10/20] (50%; 6 AdC, 4 fibroS), [16/20] (80%; 8 AdC, 2 fibroA, 10 fibroS)	+	> 99% (no vehicle)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Rat, Sprague-Dawley (F) 45 wk Cavalieri *et al.* (1988a, b)	0 and 4 µmol [1 mg]/mammary gland (2nd, 3rd, 4th and 5th mammary gland on both sides injected), 1 × 20/group	Epithelial mammary T: [3/20] (15%; 3 fibroA), [14/20] (70%; 13 AdC, 3 fibroA); multiplicity: controls, 3/3 [1]; treated rats: AdC, 18/13 [1.4]; fibroA, 4/3 [1.3] Mesenchymal (mammary) T: [0/20] (0%), [11/20] (55%; 11 fibroS; multiplicity, 20/11 [1.8]) Skin T: [0/20] (0%), [9/20] (45%; 9 SCC; multiplicity, 11/9 [1.2])	+	> 99% (trioctanoin)
Rat, Sprague-Dawley (F) 24 wk Cavalieri *et al.* (1991)	0, 0.25 [66 µg], 1 µmol [264 µg]/mammary gland (the 2nd, 3rd, 4th and 5th on both sides), 1 × 20/group	Epithelial mammary gland T: 1/18 (6%; 1 fibroA), 1/20 (5%; 1 AdC), 0/20 (0%) Mesenchymal (mammary) T: 0/18 (0%), 6/20 (30%; 6 fibroS; multiplicity, 7/6), 8/20 (40%; 8 fibroS; multiplicity, 10/8) Skin T: 0/18 (0%), 0/20 (0%), 1/20 (5%; 1 SCC)	+	> 99% (trioctanoin) Statistics NR
Intracolonic instillation				
Mouse, Swiss albino (M, F) 120 wk Toth (1980)	0, 200, 2000 µg/g bw (total doses); control and high-dose group, 10 × / wk instillations of 0 and 200 µg, respectively; low-dose group, 1 instillation 50/group/sex	Malignant lymphoma: M–0/50, 6/50* (12%; 1 histiocytic, 4 lymphocytic, 1 mixed), 7/50** (14%; 2 histiocytic, 3 lymphocytic, 2 mixed) F–11/49 (22%; 5 histiocytic, 6 lymphocytic), 21/50*** (42%; 5 histiocytic, 16 lymphocytic), 18/49 (36%; 6 histiocytic, 8 lymphocytic, 4 mixed) Oesophagus T: M–no tumour F–0/49, 0/50, 5/49 (10%) Forestomach T: M–0/50, 2/50 (4%; 2 P), 10/50**** (20%; 9 P, 1 SCC) F–1/49 (2%; 1 SCC), 5/10 (20%; 3 P, 2 SCC), 11/49**** (22%; 9 P, 2 SCC)	*$P < 0.04$ **$P < 0.02$ ***$P < 0.053$ ****$P < 0.006$ *****$P < 0.0001$	98% (olive oil) Anal and skin tumours probably due to release of benzo[*a*]pyrene through the anal orifice

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Toth (1980) Contd.		Anal T : M–0/50, 0/50, 7/50** (14%; 4 P, 3 SCC) F–0/49, 1/50 (2%; 1 P), 6/49* (12%; 1 P, 4 SCC, 1 K) Skin T : M–1/50 (2%; 1 K), 0/50, 13/50***** (26%; 5 P, 7 SCC, 1 K) F–0/49, 2/50 (4%; 2 SCC), 11/49**** (22%; 4 P, 5 SCC, 2 K)		
Mouse, C57Bl/6 (F) 18 mo Anderson *et al.* (1983)	0 (untreated, olive oil or β-naphthoflavone in olive oil), 1 mg/animal (in olive oil), once/wk, 14 wk 45–60/group	Forestomach P: 7/34 (21%; multiplicity, 1.1 ± 0.4), 17/18* (94%; multiplicity, 3.2 ± 2.3*) Peritoneal S: 0/40, 5/32* Lymphoma: 1/40 (2.5%), 9/32* (28%)	*$P < 0.05$	99% (olive oil, enzyme inducer β-naphthoflavone) No colon tumours found
Intravaginal application				
Mouse, C57B1 (F) 5 mo Näslund *et al.* (1987)	Cotton swab soaked in acetone (controls) or 1% solution of benzo[*a*]pyrene in acetone, twice/wk 10 or 76/group	0/10, 17/76 (22%; invasive cervical C)	+	NR (acetone)
Intrafetal injection				
Mouse, Swiss (M, F) 12 wk Rossi *et al.* (1983)	0, 0.4, 4.0, 9.9, 19.8 nmol [0, 0.1, 1, 2.6, 5.2 µg]/animal, 1 × 43–56/group	Lung A (M + F combined): 0/37, 1/39 (3%), 10/42 (25%), 10/38 (26%), 12/31 (39%)	+	> 99% (trioctanoin-acetone mixture (1:1))

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; bw, body weight; C, carcinoma; d, day or days; DMSO, dimethyl sulfoxide; F, female; H, hepatoma; K, keratocanthoma; M, male; min, minute or minutes; mo, month or months; NH_4OH, ammonium hydroxide; NR, not reported; P, papilloma; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; SGA, sebaceous gland adenoma; T, tumour; *vs*, versus; wk, week or weeks; yr, year or years

Table 3.2 Summary of reports of malignant tumours clearly induced in experimental animals by benzo[*a*]pyrene

Organ site/ species	Lung	Trachea	Larynx	Forestomach	Liver	Lymphoid tissue (lymphoma)	Sarcoma (injection site)	Skin	Mammary gland
Mouse	x			x	x	x	x	x	
Rat	x						x		x
Hamster	x	x	x	x			x		

3.4 Intraperitoneal injection

In a series of studies in newborn and adult mice, intraperitoneal injection of benzo[*a*]pyrene increased the incidence of liver (adenomas and carcinomas) and lung (adenomas and adenocarcinomas) tumours and, occasionally, forestomach (squamous cell papillomas and carcinomas) and lymphoreticular tumours (Vesselinovitch *et al.*, 1975a, b; Wislocki *et al.*, 1986; Lavoie *et al.*, 1987; Busby *et al.*, 1989; Rippe & Pott, 1989; Mass *et al.*, 1993; Nesnow *et al.*, 1995; Ross *et al.*, 1995; Weyand *et al.*, 1995; Rodriguez *et al.*, 1997; Von Tungeln *et al.*, 1999).

In one study in rats with a single intraperitoneal injection of benzo[*a*]pyrene, a high incidence of abdominal mesotheliomas and sarcomas was observed (Roller *et al.*, 1992).

3.5 Inhalation

In a lifetime inhalation study (Thyssen *et al.*, 1981) in male hamsters, benzo[*a*]pyrene induced dose-related increases in the incidence of papillomas and squamous-cell carcinomas in both the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, oesophagus and forestomach).

3.6 Intrapulmonary injection

Dose-related increases in the incidence of malignant lung tumours (mainly epidermoid and squamous-cell carcinomas and a few pleomorphic sarcomas) were found after injection of benzo[*a*]pyrene into the lung of rats (Deutsch-Wenzel *et al.*, 1983; Iwagawa *et al.*, 1989; Wenzel-Hartung *et al.*, 1990; Horikawa *et al.*, 1991).

3.7 Intratracheal administration

Intratracheal administration of benzo[*a*]pyrene alone or mixed with particulates and suspended in saline with or without suspendents resulted in benign and malignant respiratory tumours in mice (Heinrich *et al.*, 1986a), rats (Pott *et al.*, 1987; Steinhoff *et al.*, 1991) and in numerous studies in hamsters (IARC, 2010). This treatment also induced forestomach tumours in hamsters (Saffiotti *et al.*, 1972; Sellakumar *et al.*, 1973; Smith *et al.*, 1975a, b, Stenbäck & Rowland, 1979). Larger benzo[*a*]pyrene particles were generally more effective than smaller ones.

Mice that lack the nucleotide excision-repair gene *XPA* (*XPA*$^{-/-}$ mice) showed a stronger lung-tumour response after intratracheal instillation of benzo[*a*]pyrene than did their similarly treated *XPA*$^{+/+}$ and *XPA*$^{+/-}$ counterparts (Ide *et al.*, 2000).

3.8 Buccal pouch application

Repeated application of benzo[*a*]pyrene to the buccal pouch mucosa of male hamsters resulted in a high incidence of forestomach papillomas (Solt *et al.*, 1987).

3.9 Subcutaneous tracheal grafts transplantation

In one study conducted in rats transplanted with subcutaneous rat tracheal grafts exposed to beeswax pellets containing various amounts of benzo[*a*]pyrene, a high incidence of squamous-cell carcinomas was reported (Nettesheim *et al.*, 1977).

3.10 Intramammilary administration

In three studies in rats, benign and malignant mammary gland tumours developed after intrammilary injection of benzo[*a*]pyrene (Cavalieri *et al.*, 1988a, b, 1991).

3.11 Intracolonic instillation

In three experiments in mice, intracolonic instillation of benzo[*a*]pyrene induced lymphomas and a variety of benign and malignant tumours in various organs including the forestomach (Toth, 1980; Anderson *et al.*, 1983).

3.12 Intravaginal application

Intravaginal application of benzo[*a*]pyrene in mice produced invasive cervical carcinoma; no such tumours were seen in controls (Näslund *et al.*, 1987).

3.13 Intrafetal injection

In one study in male and female Swiss mice, intrafetal injection of benzo[*a*]pyrene produced lung adenomas (Rossi *et al.*, 1983).

4. Other Relevant Data

Benzo[*a*]pyrene is a carcinogen that induces tumours in many animal species. Some of the examples relevant for this review are: lung tumours in mice, rats, and hamsters; skin tumours in mice; liver tumours in mice; forestomach tumours in mice and hamsters; and mammary gland tumours in rats (Osborne & Crosby, 1987; IARC, 2010). In humans, occupational exposures to benzo[*a*]pyrene-containing mixtures have been associated with a series of cancers: coke production: lung; coal gasification: lung, bladder; paving and roofing: lung; coal tar distillation: skin; soots: lung, oesophagus, haematolymphatic system, skin; aluminum smelting: lung, bladder; tobacco smoking: lung, lip, oral cavity, pharynx, oesophagus, larynx, bladder (IARC, 1984, 1985, 1986, 2010).

Studies on the mechanisms of action of benzo[*a*]pyrene have been reviewed (Xue & Warshawsky, 2005; IARC, 2010).

4.1 Metabolism

Benzo[*a*]pyrene is metabolized by both phase-I and phase-II enzymes to form a series of arene oxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulfate, and glucuronide (Osborne & Crosby, 1987). Benzo[*a*]pyrene-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on benzo[*a*]pyrene-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of benzo[*a*]pyrene-7,8-diol-9,10-epoxides, which form one class of ultimate carcinogenic metabolites of benzo[*a*]pyrene.

Both CYPs and peroxidases (e.g. prostaglandin-H synthase) can oxidize benzo[*a*]pyrene. The major cytochrome P450s involved in the formation of diols and diolepoxides are CYP1A1, CYP1A2 and CYP1B1 (Eling *et al.*, 1986; Shimada, 2006). Cytochrome P450s are inducible by benzo[*a*]pyrene and other PAHs through binding to the aryl hydrocarbon-receptor (AhR) nuclear complex, leading to changes in gene transcription of CYPs and phase-II enzymes. Mice lacking the AhR receptor are refractory to benzo[*a*]pyrene-induced tumorigenesis (Shimizu *et al.*, 2000). Both CYPs and peroxidases can form radical cations by one-electron oxidation. These cations comprise another class of ultimate carcinogenic metabolites (Cavalieri & Rogan, 1995). Some polymorphisms in human CYPs and phase-II enzymes (glutathione *S*-transferases, uridine 5′-diphosphate glucuronosyltransferases and sulfotransferases modulate susceptibility to cancer (Shimada, 2006). In another metabolic pathway, benzo[*a*]pyrene-7,8-dihydrodiol is oxidized to benzo[*a*]pyrene-7,8-quinone by enzymes of the aldo-keto reductase (AKR1) family. Among these, gene polymorphisms that influence susceptibility have been identified. NAD(P)H: quinone oxidoreductase-1 (NQO1) catalyses the reduction of benzo[*a*]pyrene quinones to hydroquinones, which may be re-oxidized and generate reactive oxygen species. Polymorphisms in this gene have also been described (Penning & Drury, 2007; IARC, 2010).

The current understanding of mechanisms underlying benzo[*a*]pyrene-induced carcinogenesis in experimental animals is almost solely based on two complementary pathways: those of the diolepoxides and the radical cations. Each provides a different explanation for the effects observed in experimental animals in specific tissues.

4.2 Diolepoxide mechanism

The diolepoxide mechanism for benzo[*a*]pyrene features a sequence of metabolic transformations: benzo[*a*]pyrene → benzo[*a*]pyrene-7,8-oxide (by CYP1A1 and CYP1B1) → benzo[*a*]pyrene-7,8-diol (by epoxide hydrolase) → benzo[*a*]pyrene-7,8-diol-9,10-epoxides (by CYP1A1 and CYP1B1) (Xue & Warshawsky, 2005). Each class of metabolic intermediate has been shown to be genotoxic and carcinogenic (Osborne & Crosby, 1987). The stereochemistry of the metabolic transformation of benzo[*a*]pyrene to diols and diolepoxides is an important component of this mechanism of action. Due to the creation of chiral carbons during the metabolic conversions, many of the metabolic intermediates of benzo[*a*]pyrene have multiple streochemical forms (enantiomeric and diastereomeric). As the metabolism proceeds the complexity of the stereo-chemical forms increases, eventually leading to four benzo[*a*]pyrene-7,8-diol-9,10-epoxides [(+)- and (-)-*anti*, (+)- and (-)-*syn*]. Diolepoxides react with DNA, mainly with the purines, deoxyguanosine and deoxyadenosine, and each diolepoxide can form both *cis* and *trans* adducts thus giving a total of 16 possible benzo[*a*]pyrene-7,8-diol-9,10-epoxide DNA adducts. However, in most cases far fewer DNA adducts are actually observed. The most ubiquitous benzo[*a*]pyrene adduct detected in isolated mammalian DNA after metabolic conversion in metabolically competent mammalian cells in culture, or in mammals, is the N^2-deoxyguanosine adduct, (+)-N^2-10*S*-(7*R*,8*S*,9*R*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene)-yl)-2'-deoxyguanosine (BPDE-deoxyguanosine), derived from 7*R*,8*S*-dihydroxy-9*R*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide, or BPDE). This adduct was first fully identified after isolation from benzo[*a*]pyrene-treated human and bovine bronchial explants (Jeffrey *et al.*, 1977). This diolepoxide is considered to be an ultimate, DNA-reactive,

metabolite of benzo[*a*]pyrene (Osborne & Crosby, 1987). The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide can form both stable and unstable (so-called 'depurinating') adducts with DNA, mediated by electrophilic carbonium ions (Chakravarti *et al.*, 2008). *In vivo*, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide produces stable adducts that were formed primarily with guanines in many species and organs (IARC, 2010).

Mice treated with benzo[*a*]pyrene had *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-N^2-deoxyguanosine adducts in their lung tissue, while the lung tumours induced by benzo[*a*]pyrene had G→T and G→A mutations in the K_i-*Ras* gene at codon 12 (Mass *et al.*, 1993). In mice treated with benzo[*a*]pyrene the major stable DNA adduct in the epidermis was the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-deoxyguanosine adduct (Melendez-Colon *et al.*, 1999). Skin tumours from benzo[*a*]pyrene-treated mice or in preneoplastic skin from benzo[*a*]pyrene-treated mice had G→T mutations in codon 13 and A→T mutations in codon 61 of the Ha-*Ras* gene (Chakravarti *et al.*, 2008).

Benzo[*a*]pyrene-induced skin tumours harboured G→T transversion mutations in the *Tp53* tumour-suppressor gene (Ruggeri *et al.*, 1993). The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts occurred at guanine positions in codons 157, 248, and 273 of the *TP53* gene in *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-treated human HeLa cells. The same positions are the major mutational hotspots found in human lung cancers (Denissenko *et al.*, 1996).

4.3 Radical-cation mechanism

The radical-cation mechanism for benzo[*a*]pyrene has been studied exclusively in connection with mouse-skin tumorigenesis (Cavalieri & Rogan, 1995). One-electron oxidation of benzo[*a*]pyrene by CYPs or peroxidases creates a radical cation localized on carbon 6, as a consequence of the ionization potential and geometric configuration. In mouse skin, this radical cation gives rise to the formation of covalent adducts with guanine (at the C8 carbon and N7 nitrogen) and adenine (at the N7 nitrogen). These adducts are unstable and are thought to generate apurinic sites in mouse skin. However, only low levels of apurinic sites were measured in the epidermis of mice treated with benzo[*a*]pyrene (Melendez-Colon *et al.*, 1999) and no studies to date have shown an increase in the number of apurinic sites in lung tissues treated with benzo[*a*]pyrene. In two *in vivo* studies, rats treated intraperitoneally with benzo[*a*]pyrene were shown to excrete 7-(benzo[*a*]pyrene-6-yl)-*N7*-guanine in faeces and urine, while the same adduct was detected in lung tissue of mice treated intraperitoneally with benzo[*a*]pyrene (Rogan *et al.*, 1990; Banasiewicz *et al.*, 2004). Skin papillomas obtained from mice treated topically with benzo[*a*]pyrene showed mutations (at guanine and/or adenine) at codons 12, 13 and 61 in the Ha-*Ras* oncogene (Wei *et al.*, 1999). Similar studies in preneoplastic skin from benzo[*a*]pyrene-treated mice showed Ha-*Ras* mutations at codons 13 and 61 (Chakravarti *et al.*, 2008). The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide can also form depurinating DNA adducts at guanine and adenine (at the N7 nitrogen). The distribution and chemical nature of the depurinating adducts (from both radical-cation and diolepoxide intermediates) in mouse skin and the distribution and chemical nature of the specific benzo[*a*]pyrene-induced mutations in mouse-skin papillomas have been reported (Chakravarti *et al.*, 2008).

4.4 Other activation mechanisms of benzo[*a*]pyrene

4.4.1 Meso-region mechanism

The mechanism of meso-region biomethylation and benzylic oxidation features biomethylation of benzo[*a*]pyrene to 6-methylbenzo[*a*]

pyrene, with *S*-adenosylmethione as the carbon donor (Flesher *et al.*, 1982). This process has been shown to occur *in vitro*, and *in vivo* in rat liver (Stansbury *et al.*, 1994). 6-Methylbenzo[*a*]pyrene is further metabolized by CYPs to 6-hydroxymethylbenzo[*a*]pyrene (Flesher *et al.*, 1997) and then conjugated to sulfate by 3′-phosphoadenosine-5′-phosphosulfate sulfotransferase to 6-[(sulfooxy)methyl]-benzo[*a*]pyrene. This reactive sulfate ester forms DNA adducts *in vivo* (Stansbury *et al.*, 1994). These benzo[*a*]pyrene-DNA adducts have only been measured in rat liver (Surh *et al.*, 1989), which is not a target for benzo[*a*]pyrene-induced carcinogenesis. There is no evidence to date that this mechanism operates in lung.

4.4.2 Mechanism via formation of ortho-quinone/ reactive oxygen species

This mechanism features enzymatic oxidation of benzo[*a*]pyrene-7,8-diol to the *ortho*-quinone, benzo[*a*]pyrene-7,8-quinone, by aldo-keto reductases (Mangal *et al.*, 2009). Benzo[*a*]pyrene-7,8-quinone can react with DNA to yield both stable and depurinating DNA adducts *in vitro* (McCoull *et al.*, 1999; Balu *et al.*, 2006) and can also undergo repetitive redox cycling which generates reactive oxygen species that damage DNA (Penning *et al.*, 1999). In human A549 lung-tumour cells benzo[*a*]pyrene-7,8-quinone increased the formation of 8-oxo-deoxyguanosine and DNA strand-breaks (Park *et al.*, 2008; Mangal *et al.*, 2009). In a yeast reporter-assay, benzo[*a*]pyrene-7,8-quinone (in the presence of redox cycling) induced 8-oxo-deoxyguanosine formation and G→T transversions in the *Tp53* tumour-suppressor gene. The mutational spectra induced in the yeast reporter-assay closely matched those seen in DNA from human lung tumours (Shen *et al.*, 2006). Benzo[*a*]pyrene-7,8-quinone inhibited the activity of protein kinase C in MCF-7 cell lysates suggesting an ability to alter cell signalling (Yu *et al.*, 2002). Rats treated with benzo[*a*]pyrene showed increased urinary concentrations of 8-oxo-deoxyguanosine, but lower levels in liver and lung tissues. This suggested that reactive oxygen species are generated during the CYP-dependent metabolism of benzo[*a*]pyrene, but induction of DNA-repair mechanisms may reduce these levels in target tissues (Briedé *et al.*, 2004). To date this mechanism has been studied only in in-vitro systems.

It is noted that formation of reactive oxygen species is not limited to the redox cycling of the *ortho*-quinone of benzo[*a*]pyrene (benzo[*a*]pyrene-7,8-quinone). There are several other sources of benzo[*a*]pyrene-induced reactive oxygen species. *In vivo*, both mice and rats metabolize benzo[*a*]pyrene to benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone and benzo[*a*]pyrene-6,12-quinone and these quinones enter into redox cycling and induce mutations (Osborne & Crosby, 1987; Joseph & Jaiswal, 1998). Many of the reactive intermediates of benzo[*a*]pyrene (oxides, diol-epoxides, radical cations) and quinone-generated reactive oxygen species can disrupt the balance of cellular oxidants and anti-oxidants by reducing the anti-oxidant levels thus leading to an imbalance and an excess of reactive oxygen species.

4.4.3 Aryl hydrocarbon-receptor mechanism

The AhR regulates the transcription of a series of genes including *Cyp1A1*, *Cyp1A2*, *Nqo1*, *Aldh3a1* (encoding aldehyde dehydrogenase 3A1), *UGT1a6* (uridine 5′-diphosphate-glucuronosyl transferase), and *Gsta1* (glutathione *S*-transferase A1). All these genes are activated by AhR-ligands, including benzo[*a*]pyrene, via the AhR-mediated aromatic hydrocarbon response element. The AhR plays a role in the response to oxidative stress in cell-cycle regulation and apoptosis. In addition, the CYP1A1/1A2-mediated metabolism generates oxidative stress (Nebert *et al.*, 2000). Mitochondrial hydrogen-peroxide production was induced by an AhR-ligand in

wild-type mice but not in *AhR*$^{-/-}$ knockout mice (Senft *et al.*, 2002). These mice were shown to be refractory to benzo[*a*]pyrene-induced carcinogenicity (Shimizu *et al.*, 2000). Benzo[*a*]pyrene induced oxidative stress in mouse lung (Rajendran *et al.*, 2008).

4.4.4 Immunosuppression mechanism

Benzo[*a*]pyrene induces immunosupression in adult mice by altering the cell-mediated responses (Wojdani & Alfred, 1984). Immune development in offspring is also altered following *in utero* exposure to benzo[*a*]pyrene (Urso & Gengozian, 1984). It is postulated that PAHs, including benzo[*a*]pyrene, act principally through their AhR-mediated CYP-derived metabolites (diolepoxides, quinones) to activate oxidative and electrophilic signalling pathways in lymphoid and nonlymphoid cells, including myeloid cells, epithelial cells, and other cell types. Furthermore, there is evidence that PAHs suppress immunity by p53-dependent pathways, by modulating signalling pathways in lymphocytes via non-genotoxic mechanisms, and by oxidative stress (Burchiel & Luster, 2001).

4.4.5 Epigenetic mechanisms

Benzo[*a*]pyrene and/or its metabolites have been shown to increase cell proliferation in several human cell lines, including terminally differentiated human bronchial squamous epithelial cells and in lung-cancer cells where increased expression of the *Cdc25B* gene (cell-division cycle 25B) was observed, along with reduced phosphorylation of Cdk1 (cyclin-dependent kinase 1) (Oguri *et al.*, 2003). Treatment with benzo[*a*]pyrene increased the number of human embryo lung-fibroblasts in the G1–S transition via the activation of c-Jun, through the p53-dependent PI-3K/Akt/ERK (phosphatidylinositol-3-kinase/protein kinase β/extracellular signal-regulated kinase) pathway (Jiao *et al.*, 2008).

Benzo[*a*]pyrene and/or its metabolites also affect apoptosis. Benzo[*a*]pyrene induced apoptosis in human MRC-5 lung fibroblasts via the JNK1/FasL (c-Jun N-terminal kinase 1/Fas Ligand) and JNK1/p53 signalling pathways (Chen *et al.*, 2005). Apoptosis induced by *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide in H460 human lung-cancer cells was associated with induction of Bak (BCL2-antagonist/killer) and with activation of caspase, but it was independent of Bcl-2 (Xiao *et al.*, 2007).

Altered DNA methylation has been reported to be associated with exposure to benzo[*a*]pyrene and/or its metabolites. After treatment of immortalized bronchial epithelial cells with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide, the concentration of cytosine-DNA methyltransferase-1 was increased and was associated with hypermethylation of the promoters of 5–10 genes, including members of the cadherin gene-family (Damiani *et al.*, 2008).

4.5 Human exposure to PAH-rich mixtures

4.5.1 Biomarkers of exposure and effect

Molecular-epidemiological studies of cancer associated with occupational and environmental exposures to PAH have provided biomarkers that may be used to estimate internal exposure as well as to inform about molecular mechanisms that may be relevant to cancer causation, particularly in defining the early events in the carcinogenesis process. Biomarkers can be detected in the target organ, in surrogate tissues, or in tumours. These can be categorized into *biomarkers of exposure*, which are generally specific to the PAH of concern (e.g. DNA or protein adducts), *biomarkers of effect* (e.g. genotoxic and cytogenetic effects, 8-oxo-deoxyguanosine, sister chromatid exchange (SCE), micronuclei, chromosomal aberrations, mutations in oncogenes, tumour-suppressor genes, or indicator genes),

and *biomarkers of susceptibility* (DNA-repair enzymes, e.g. XPA, XPC – *xeroderma pigmentosum* complementation groups A and C), bioactivation enzymes (e.g. CYPs), detoxification enzymes (e.g. GSTs), and mutagenic metabolites in urine (Kalina *et al.*, 1998; Pilger *et al.*, 2000; Simioli *et al.*, 2004; Raimondi *et al.*, 2005; Vineis & Husgafvel-Pursiainen, 2005; Matullo *et al.*, 2006; Farmer & Singh, 2008; Gyorffy *et al.*, 2008). Although biomarkers of effect and susceptibility are generally not unique to any specific PAH exposure, several these biomarkers may provide insight into the mechanism of carcinogenesis induced in humans by PAHs or PAH-rich exposures.

4.5.2 Exposure to benzo[a]pyrene and relationship with specific biomarkers

Biomarkers of exposure to complex mixtures that contain benzo[*a*]pyrene have been studied in populations exposed in industrial settings: coke production, coal-tar distillation, the aluminium industry, roofing and paving with coal-tar pitch, coal gasification, chimney sweeping, and iron and steel founding. Most if not all of these biomarkers are genotoxic markers. Populations of patients who undergo coal-tar therapy and groups exposed to combustion emissions, and tobacco smokers have also been evaluated. Studies on biomarkers of exposure are dominated by those focusing on the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct, the most commonly studied PAH-DNA adduct because of the availability of specific analytical methods and standards (Gyorffy *et al.*, 2008). In one study the depurinating adducts resulting from radical-cation formation, *viz.* 7-(benzo[*a*]pyrene-6-yl) guanine and 7-(benzo[*a*]pyrene-6-yl)adenine were found in the urine of women exposed to coal smoke (Casale *et al.*, 2001). Concomitantly, several biomarkers of effect have also been evaluated in these studies: chromosomal aberrations, sister chromatid exchange (Kalina *et al.*, 1998), DNA damage (measured by the comet assay) and 8-oxo-deoxyguanosine formation (Marczynski *et al.*, 2002). It is important to note that these genotoxic effects observed in humans in relation to exposure to benzo[*a*]pyrene-containing mixtures have also been observed in experimental studies where benzo[*a*]pyrene or *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide has been shown to induce sister chromatid exchange (Pal *et al.*, 1980; Brauze *et al.*, 1997), chromosomal aberrations, micronuclei (Kliesch *et al.*, 1982), DNA damage (Nesnow *et al.*, 2002), and 8-oxo-deoxyguanosine (Thaiparambil *et al.*, 2007). Tobacco smoke, dietary habits and indoor ambient air are also important sources of exposure to benzo[*a*]pyrene, which has been implicated as one of the components of tobacco smoke related to the induction of lung cancer in smokers (Watanabe *et al.*, 2009). In a large study of 585 smokers and nonsmokers, smoking and diet were highly correlated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct levels (Pavanello *et al.*, 2006). Several studies have demonstrated moderately increased levels of 8-oxo-deoxyguanosine from lungs, sperm, and leukocytes of smokers. Increased urinary excretion of 8-oxo-deoxyguanosine has also been reported (Hecht, 1999). In rats exposed to benzo[*a*]pyrene via oral, intratracheal and dermal routes, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts were formed in white blood cells independently of the exposure route and their numbers correlated with those found in lung DNA, suggesting that *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct levels in white blood cells may be used as a surrogate for pulmonary *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts (Godschalk *et al.*, 2000).

4.5.3 Relationship of biomarkers to human cancer

Mutations in *TP53* are common in lung cancers from smokers and less common in nonsmokers. These mutations are G→T transversions with hotspots in codons 157, 248 and 273 (Hainaut & Pfeifer, 2001; Pfeifer *et al.*, 2002) and they are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts. The active metabolite *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide causes a unique spectrum of *TP53* mutations distinct from those found in cancers that are not associated with smoking (Campling & el-Deiry, 2003). Similar G→T mutations have been reported in lung tumours from nonsmoking Chinese women whose tumours were associated with exposure to PAHs from smoke generated by burning smoky coal in unventilated homes. The mutations were clustered at the CpG rich codons 153–158 of the *TP53* gene, and at codons 249 and 273. The mutation spectrum was fully consistent with exposure to PAHs (DeMarini *et al.*, 2001).

4.6 Synthesis

Benzo[*a*]pyrene is metabolically activated to a series of reactive intermediates by CYP450 and related enzymes under control of the aryl-hydrocarbon receptor. There is strong evidence that the benzo[*a*]pyrene diolepoxide mechanism operates in mouse-lung tumorigenesis, while there is also strong evidence that both the radical-cation and the diolepoxide mechanisms are involved in mouse-skin carcinogenesis. The meso-region mechanism has been studied only in rat liver, while the mechanism that involves the formation of *ortho*-quinone/reactive oxygen species has only been studied *in vitro*, although reactive oxygen species can be formed *in vivo* by other benzo[*a*]pyrene-mediated mechanisms. All these pathways reflect genotoxic mechanisms, as they involve alterations to DNA. Benzo[*a*]pyrene is pleotropic and has the ability to affect many cell- and organ-based systems. Therefore, there are probably many modes of carcinogenic action operating to different extents *in vivo*. These include mechanisms that involve AhR, oxidative stress, immunotoxicity and epigenetic events.

Based on the best available, consistent and strong experimental and human mechanistic evidence it is concluded that benzo[*a*]pyrene contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAH mixtures that contain benzo[*a*]pyrene. The most commonly encountered – and most widely studied – mechanistically relevant DNA lesion is the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct. The formation of this adduct is consistent with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-associated genotoxic effects in surrogate tissues and with the mutation pattern in the *TP53* gene in lung tumours from humans exposed to PAH mixtures that contain benzo[*a*]pyrene. The fact that those PAH mixtures and benzo[*a*]pyrene itself induce genotoxic effects like sister chromatid exchange, chromosomal aberrations, micronuclei, DNA damage (comet assay) and 8-oxo-deoxyguanosine, supports the notion that benzo[*a*]pyrene contributes to human cancer.

5. Evaluation

There is *sufficient evidence* for the carcinogenicity of benzo[*a*]pyrene in experimental animals.

[No epidemiological data on benzo[*a*]pyrene alone were available to the Working Group.]

The genotoxic mechanism of action of benzo[*a*]pyrene involves metabolism to highly reactive species that form covalent adducts to DNA. These anti-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts induce mutations in the K-*RAS* oncogene and the *TP53* tumour-suppressor gene in human lung tumours, and

in corresponding genes in mouse-lung tumours. Exposure to benzo[*a*]pyrene and benzo[*a*]pyrene-containing complex mixtures also induce other genotoxic effects, including sister chromatid exchange, micronuclei, DNA damage and 8-oxo-deoxyguanosine, all of which can contribute to the carcinogenic effects of benzo[*a*]pyrene and benzo[*a*]pyrene-containing complex mixtures in exposed humans.

Benzo[*a*]pyrene is *carcinogenic to humans (Group 1)*.

In making the overall evaluation, the Working Group took the following into consideration:

The strong and extensive experimental evidence for the carcinogenicity of benzo[*a*]pyrene in many animal species, supported by the consistent and coherent mechanistic evidence from experimental and human studies provide biological plausibility to support the overall classification of benzo[*a*]pyrene as a human carcinogen (Group 1).

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COAL GASIFICATION

Coal gasification was considered by previous IARC Working Groups in 1983, 1987, and 2005 (IARC, 1984, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

During coal gasification, coal is reacted with oxygen, steam and carbon dioxide to form a gas containing hydrogen and carbon monoxide. During this process, which is essentially incomplete combustion, the heat evolved is consumed and the sulfur and nitrogen in the coal are converted to hydrogen sulfide (rather than sulfur dioxide) and ammonia (rather than nitrogen oxides), respectively. These reduced forms of sulfur and nitrogen are easily isolated, captured and used, making gasification a clean-coal technology with a better environmental performance than coal combustion (Shadle *et al.*, 2002).

Depending on the type of gasifier (e.g. air-blown, enriched oxygen-blown) and the operating conditions, gasification can be used to produce a fuel gas that is suitable for several applications (e.g. low heating-value fuel gas for use as industrial fuel and for power production; medium heating-value fuel gas for use as a synthesis gas in the production of chemicals such as ammonia and methanol, and for transportation fuel; or high heating-value gas) (Shadle *et al.*, 2002).

Gasification takes place in fixed-bed, fluidized-bed, moving-bed, and entrained-flow gasifiers. The earliest gasification processes were developed by use of a counter-current, fixed-bed gasifier, in which coal was fed onto the top of the bed and travelled downwards against the flow of gases. Atmospheric fixed-bed gasifiers of various designs are still occasionally found in small-scale industries. On a large scale, several Lurgi fixed-bed pressurized gasification plants are currently operating commercially, e.g. in the Republic of South Africa and in the USA (Shadle *et al.*, 2002; Crelling *et al.*, 2005). Fluidized-bed gasification, invented in 1922 by Winkler at BASF in Germany, has the advantage of a fairly simple reactor design. In this process, the air and steam flow required for gasification is sufficient to fluidize the bed of coal, char and ash. Fluidization occurs when the gas-flow velocity lifts the particles and causes the gas–solid mixture to flow like a fluid (Shadle *et al.*, 2002; Crelling *et al.*, 2005). Entrained-flow gasification takes place in a flame-like reaction zone, usually at a very high temperature, to produce a liquid slag. For economical operations, a high-standard heat-recovery system is mandatory, but the gas product typically has a very low methane content and is free of tars, oils and phenols, which thereby considerably simplifies gas and water treatment. Entrained-flow gasifiers of the Koppers-Totzek design are operated at atmospheric pressure. They are used industrially in many countries to produce hydrogen or

synthesis gas (Shadle *et al.*, 2002; Crelling *et al.*, 2005).

The moving-bed gasifiers produce tars, oils, phenols and heavy hydrocarbons, and the concentrations in the gas product are controlled by quenching and water scrubbing. Fluidized-bed gasifiers produce significantly smaller amounts of these compounds because of higher operating temperatures. Entrained-flow gasifiers that operate at even higher temperatures (in excess of 1650 °C) can achieve carbon conversions of more than 99.5%, while generating essentially no organic compounds heavier than methane (Shadle *et al.*, 2002).

In addition to PAHs, workers in coal gasification may be exposed to many compounds, including asbestos, silica, amines, arsenic, cadmium, lead, nickel, vanadium, hydrocarbons, sulfur dioxide, sulfuric acid and aldehydes (IARC, 1984).

2. Cancer in Humans

2.1 Cohort studies of coal-gasification workers

Occupational exposure during coal gasification was evaluated in *IARC Monograph* Volume 92 (IARC, 2010). There was *sufficient evidence* in epidemiological studies for the carcinogenicity of occupational exposure during coal gasification. The main body of evidence came from two cohort studies of coal-gasification workers in the United Kingdom (Doll *et al.*, 1972) and Germany (Berger & Manz, 1992), and a case–control study nested within a cohort of French gas- and electricity-production workers (Martin *et al.*, 2000; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-10-Table2.1.pdf). In all studies an excess of lung cancer in association with coal gasification was found, which was not likely to be explained by confounding from tobacco smoking. There was evidence supporting a lung-cancer excess in a historical record-linkage study from the United Kingdom (Kennaway & Kennaway, 1947), in two smaller cohorts (Kawai *et al.*, 1967; Hansen *et al.*, 1986), and a large but inadequately reported Chinese study (Wu, 1988).

In addition to lung cancer, the study from the United Kingdom (Doll *et al.*, 1972) showed an excess of bladder cancer, and the German study (Berger & Manz, 1992) showed an excess of cancers of the stomach and colon-rectum.

No epidemiological studies of coal-gasification workers have been published since the previous evaluation (IARC, 2010).

2.2 Synthesis

In three large studies, a consistent excess of lung cancer was found in association with occupational exposure during coal gasification. This excess was not likely to be explained by tobacco smoking.

3. Cancer in Experimental Animals

Coal-tars from gas works were previously evaluated in *IARC Monograph* Volume 34 (IARC, 1984). As early as 1923 and in subsequent decades, crude coal-tars from gas-works were tested for carcinogenicity by skin application in six studies in mice and two studies in rabbits. These tars induced a high number of skin papillomas and carcinomas in all studies in mice (Deelman, 1923; Kennaway, 1925; Hieger, 1929; Woglom & Herly, 1929; Berenblum & Schoental, 1947; Grigorev, 1960) and in both studies in rabbits (Berenblum & Schoental, 1947; Grigorev, 1960). No new studies have been published since the previous evaluation.

Manufactured gas plant residues (MGP) were previously evaluated in *IARC Monograph*

Table 3.1 Carcinogenicity studies in mice exposed to manufactured gas plant residues

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 185 d Weyand *et al.* (1994)	Groups of 10 male and 10 female mice were fed a gel diet containing 0 (control) or 0.50% MGP.	Fore-stomach carcinomas (M): 0/10, 1/10 Alveolar epithelium hyperplasia (M): 0/10, 1/10	NS	
Mouse, A/J (F) 260 d Weyand *et al.* (1995)	Groups of 30 mice were fed a gel diet containing 0 (control), 0.1, or 0.25% MGP.	Lung adenoma: 4/19, 19/27*, 29/29* Lung adenoma multiplicity: 0.59, 1.19**, 12.17** tumours/mouse	*$P < 0.05$ **$P < 0.001$	Authors could not explain the decrease in body weight gain that led to increased mortality in basal gel diet controls. No fore-stomach tumours were observed.
Mouse, B6C3F$_1$ (F) 104 wk Culp *et al.* (1998)	Groups of 48 mice were fed a diet containing 0 (control), 0.01, 0.03, 0.1, 0.3, 0.6 or 1.0% of CT-1. Additional groups of 48 mice were fed a diet containing 0.03, 0.1 or 0.3% of CT-2.	Hepatocellular adenomas or carcinomas (mainly adenomas)**: 0/47, 4/48, 2/46, 3/48, 14/45*, 1/42, 5/43, 7/47, 4/47, 10/45* Alveolar/bronchiolar adenomas or carcinomas (mainly adenomas)**: 2/47, 3/48, 4/48, 4/48, 27/47*, 25/47*, 21/45*, 4/48, 10/48*, 23/47* Fore-stomach papillomas or carcinomas**: 0/47, 2/47, 6/45, 3/47, 14/46*, 15/45*, 6/41, 3/47, 2/47, 13/44* Fore-stomach carcinomas**: 0/47, 0/47, 0/45, 2/47, 7/46*, 10/45*, 4/41, 0/47, 1/47, 6/44* Small intestine adenocarcinomas***: 0/47, 0/46, 0/45, 0/47, 0/42, 22/36*, 36/41*, 0/47, 0/47, 1/37 Haemangiosarcomas**: 1/48, 0/48, 1/48, 1/48, 11/48*, 17/48*, 1/45, 1/48, 4/48, 17/48* Histiocytic sarcomas**: 1/48, 0/48, 0/48, 1/48, 7/48, 5/48, 0/45, 3/48, 2/48, 11/48* Sarcomas**: 1/48, 4/48, 3/48, 2/48, 7/48, 1/48, 2/45, 0/48, 4/48, 5/48	*$P < 0.05$ **P–value for dose-related trend significant (0.003-< 0.00001) for CT-1 and CT-2 ***P–value for dose related trend < 0.00001 for CT-1	CT-1 was a composite from seven MGP waste sites. CT-2 was a composite from two of the seven waste sites plus a third site that had a very high benzo[*a*]pyrene content. Haemangiosarcomas included those of the skin, mesentery, mesenteric lymph nodes, heart, spleen, urinary bladder, liver, uterus, thoracic cavity, ovary and skeletal muscle. Sarcomas included those of the mesentery, fore-stomach, skin and kidney.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 52 wk Rodriguez *et al.* (1997)	Groups of more than 30 mice were administered a single intraperitoneal injection of 1.995, 3.99, 7.98 mg MGP-7 or 7.98 mg MGP-4 in corn oil. A group of approximately 60 mice served as corn-oil controls.	Liver tumours (mainly adenomas): 4/34, 8/32*, 17/29*, 12/28*, 3/63	*[$P < 0.01$]	MGP-4 from a single MGP site. MGP-7 was a composite of seven MGP sites including site MGP-4. No fore-stomach tumours and few pulmonary adenomas were observed.

CT-1, coal tar mixture 1; d, day or days; F, female; M, male; MGP, manufactured gas plant residues; NS, not significant; wk, week or weeks

Volume 92 (IARC, 2010). MGP were tested in one feeding study in female B6C3F$_1$ mice, one feeding study in female A/J mice, one feeding study in B6C3F$_1$ mice of both sexes, and in one study in male B6C3F$_1$ mice that received the agent by intraperitoneal injection (Table 3.1). In the first feeding study, an increased incidence of hepatocellular adenomas and carcinomas combined, alveolar/bronchiolar adenomas and carcinomas combined, forestomach papillomas and carcinomas, small intestine adenocarcinomas, as well as haemangiosarcomas, histiocytic sarcomas, and sarcomas were observed in female B6C3F$_1$ mice (Culp *et al.*, 1998). In the second study, female A/J mice developed lung adenomas at an increased incidence and multiplicity (Weyand *et al.*, 1995). The third study, in male and female B6C3F$_1$ mice, did not show an increase in tumour incidence (Weyand *et al.*, 1994). In male mice, intraperitoneal injection of MGP produced a significant increase in liver tumours, mostly adenomas (Rodriguez *et al.*, 1997). No new studies have been published since the previous evaluation.

4. Other Relevant Data

4.1 Mechanistic evidence relevant to the carcinogenic hazards from occupational exposures during coal gasification

4.1.1 Experimental systems

As reported in *IARC Monograph* Volume 34 (IARC, 1984), coal-gasification samples from the process stream and waste by-products were found to be mutagenic in various strains of *Salmonella typhimurium* in the presence of an exogenous metabolic activation system. The mutagenicity was found primarily in the fractions containing polycyclic aromatic hydrocarbons and their alkylated derivatives. The basic and neutral fractions of tar condensed from a product-gas stream induced both 6-thioguanine-resistant and 8-azaadenine-resistant mutations in DNA repair-deficient Chinese hamster ovary cells in the presence of exogenous metabolic activation. However, neither the micronucleus frequency nor the number of chromosomal aberrations were significantly increased by this treatment.

Male B6C3F1 mice were fed a diet containing coal tar from a gas plant residue. A complex pattern of aromatic adducts was observed in liver, lung, and fore-stomach DNA of these animals, which increased with dose and duration of treatment. In lung DNA one adduct was tentatively identified as *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-deoxyguanosine. This adduct was also identified in fore-stomach DNA from female B6C3F1 mice fed coal tar-containing diets (Culp & Beland, 1994). The identity of this adduct was confirmed upon analysis of lung DNA of female B6C3F1 mice fed a diet containing coal tar from manufactured gas plant residue. However, based on the levels of this specific adduct it was suggested that benzo[*a*]pyrene (B[*a*]P) contributes only a small fraction to the DNA adducts formed in lung tissue of mice that were given coal tar (Beland *et al.*, 2005). Male B6C3F1 mice were fed diets containing 0.1–1% (w/w) coal tar for 15 days. Adduct formation in the lung, but not in the fore-stomach, was dose-related. The B[*a*]P content in the coal tar could not account by itself for the aromatic DNA-adduct levels measured (Weyand *et al.*, 1991). Strain A/J mice formed aromatic DNA adducts in the lungs after ingestion of coal tar from manufactured gas plant residue via the diet. Three major DNA adducts were identified as being derived from benzo[*b*]fluoranthene, benzo[*a*]pyrene, and benzo[*c*]fluorene (Koganti *et al.*, 2000; Koganti *et al.*, 2001). In another study, female ICR mice received topical application of manufactured gas plant residue. In the complex pattern of lung DNA adducts, one was identified as being derived from 7H-benzo[*c*]fluorene.

However, detailed quantitative results after chromatographic separation of the residue into seven fractions suggested that components other than 7H-benzo[*c*]fluorene played an important role in adduct formation in lung DNA (Cizmas *et al.*, 2004). A retrospective comparison of tumour induction and DNA-adduct formation by B[*a*]P and coal tars in several experimental protocols indicated that tumour outcomes were not predicted by the formation of total DNA adducts or by the DNA adducts formed by B[*a*]P. These data suggest that B[*a*]P content by itself is not predictive of tumour outcome (Goldstein *et al.*, 1998).

In *IARC Monograph* Volumes 32 and 92 (IARC, 1983, 2010), benzo[*b*]fluoranthene was evaluated and found to be both genotoxic and carcinogenic in experimental studies. 7H-benzo[*c*]fluorene was carcinogenic to mice (IARC, 2010), but gave inconclusive results as a bacterial mutagen in *Salmonella typhimurium* strains TA98 and TA100 in the presence of an Aroclor-1254-induced rat-liver S9 (IARC, 1983). While a 7H-benzo[*c*]fluorene-DNA adduct was observed in mice that received topical applications of manufactured gas plant residue (Cizmas *et al.*, 2004), the structure of this adduct is unknown, although a diol epoxide structure has been proposed (Wang *et al.*, 2002).

Polycyclic aromatic hydrocarbons in the ambient air in gas works have been analysed (IARC, 1984) and several of these have been shown to be mutagenic (i.e. benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*ghi*]perylene) and carcinogenic (i.e. benz[*a*]anthracene, benzo[*a*]pyrene) in experimental studies (IARC, 1983, 2010; Platt & Grupe, 2005; Platt *et al.*, 2008a, b). These polycyclic aromatic hydrocarbons may contribute to the genotoxic and tumorigenic activities of tars from coal gasification.

Naphthalene has been reported to be a constituent of tar from coal gasification (IARC, 1984). Naphthalene is genotoxic and induces tumours in experimental animals (IARC, 1982; Brusick *et al.*, 2008).

4.1.2 Humans

There are no studies that describe specific effects in workers exposed to emissions associated with coal gasification.

4.2 Synthesis

There is strong evidence from experimental studies for a genotoxic mode of action for coal-gasification samples. Although there are no human studies, it is highly likely that genotoxicity is the mechanism relevant to the carcinogenic hazards from exposures to emissions of coal gasification.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of coal gasification. Coal gasification causes cancer of the lung.

There is *sufficient evidence* in experimental animals for the carcinogenicity of coal-tars from gas-works and manufactured gas plant residues.

There is strong evidence for a genotoxic mechanism for coal gasification samples based on experimental studies. Although there are no human studies, it is highly likely that genotoxicity is the mechanism for the carcinogenic effects of coal-gasification emissions, predominantly due to the presence of mutagenic PAHs.

Coal gasification is *carcinogenic to humans (Group 1).*

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OCCUPATIONAL EXPOSURES DURING COAL-TAR DISTILLATION

Occupational exposures during coal-tar distillation were considered by a previous Working Group in 2005 (IARC, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Manufacturing process

Coal tar is obtained by cooling the gas that is formed during the destructive distillation of coal to approximately ambient temperature. It is a black, viscous liquid composed primarily of a complex mixture of condensed-ring aromatic hydrocarbons. It may contain phenolic compounds, aromatic nitrogen bases and their alkyl derivatives, and paraffinic and olefinic hydrocarbons. Coal-tar pitch is the residue from the distillation of coal tar (Betts, 1997).

The largest source of tar and pitch is the pyrolysis or carbonization of coal. Until the end of the Second World War, coal tar was the main source of benzene, toluene, xylenes, phenol, cresols and cresylic acids, pyridine and methylpyridines, naphthalene, anthracene, creosote, tar paints, road tars and pitch binders (Betts, 1997). However, by the 1990s, over 90% of the world production of these aromatic chemicals was derived from the petrochemical industry, and coal tar became chiefly a source of anti-corrosion coatings, wood preservatives, feed-stocks for the manufacture of carbon black and binders for electrodes (Betts, 1997).

Coal-tar distillation products comprise essentially the distillate (primarily a complex mixture of mono- and polycyclic aromatic hydrocarbons) and the residue from the distillation (pitch) (Betts, 1997).

The part of coke-oven tar that is normally distillable at atmospheric pressure boils at up to ~400 °C and contains principally aromatic hydrocarbons. These include (in order of the distillation fraction): benzene, toluene and the xylene isomers, tri- and tetra-methylbenzenes, indene, hydrindene (indane), and coumarone; polar compounds, including tar acids (phenol and cresols) and tar bases (pyridine, picolines (methylpyridines) and lutidines (dimethylpyridines)); naphthalene, contaminated with small but significant amounts of thionaphthene, indene and other compounds; methylnaphthalene isomers; biphenyl, acenaphthene and fluorene; anthracene and phenanthrene; pyrene and fluoranthene (Betts, 1997).

Much less is known about the composition of pitch, the residue from coal-tar distillation. Studies of coke-oven pitch indicate that it contains: four-membered aromatic hydrocarbon

Table 1.1 Concentrations of PAHs in the air and urine of workers in coal-tar distillation

Reference Country Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Air (µg/m³)		Urinary 1-hydroxypyrene (µmol/mol creatinine)	
						Mean	Range	Mean	Range
van de Ven & Nossent (1984) the Netherlands NR	Coal-tar distillation Operators, cleaners, maintenance	NR	49	NR	Sum of 11 PAHs	31	< 1–277		
Jongeneelen *et al.* (1986) the Netherlands NR	Coal-tar distillation		*Air; urine*		Sum of 11 PAHs; pyrene 1-Hydroxypyrene				
	Operator pitch unit	1	8; 2	0		26; 8.5	< 2–280;	3.7	NR
	Operator batch distillery	1	6; 2	1		14; 5.1	< 2–96	11.8	NR
	Operator pump station	1	5; 4	0		4.7; 1.4		4.0	NR
	Cleaner	1	4; 4	1		16; 5.2		4.6	NR
Price *et al.* (2000) United Kingdom 1998	Tar distillation								
	Low-temperature	8	8	1	Total 19 PAHs	12.17	3.99–38.59		
					HSE 11[a]	0.008	< 0.004–0.008		
					Benzo[*a*]pyrene	ND	ND		
					Pyrene	0.037	0.013–0.068		
					1-Hydroxypyrene			0.36	0.21–1.05
	High-temperature	12	12	4	Total 19 PAHs	279.04	51.9–1130.5		
					HSE 11[a]	0.95	0.15–4.87		
					Benzo[*a*]pyrene	0.283	0.019–0.642		
					Pyrene	1.24	0.14–6.73		
					1-Hydroxypyrene			2.60	0.78–5.69
Preuss *et al.* (2003) Germany 1999–2001	Tar distillation	18	NR	NR		*Median*	*90%*	*Median*	*90%*
					Sum of 16 PAHs	63.62	455.17	0.78	2.28
					1-Hydroxypyrene			3.65	12.36
					Sum of hydroxy-phenanthrene				

[a] Benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene, anthanthrene, cyclopenta[*cd*]pyrene

ND, not detected; NR, not reported; PAHs, polycyclic aromatic hydrocarbons

Conversions used for 1-hydroxypyrene: 1 µmol/mol creatinine = 1.93 µg/g creatinine = 0.013 µmol/L = 2.84 µg/L = 2.84 ng/mL

ring systems (e.g. chrysene, fluoranthene, pyrene, triphenylene, naphthacene and benzanthracene); five-membered ring systems (picene, benzo[*a*]pyrene and benzo[*e*]pyrene, benzofluoranthenes and perylene); six-membered ring systems (dibenzopyrenes, dibenzofluoranthenes and benzoperylenes); and seven-ring systems (coronene). Other aromatic chemicals present in pitch include methyl- and polymethyl-derivatives, mono- and polyhydroxy-derivatives, and heterocyclic compounds (Betts, 1997).

For a more detailed description of the coal-tar distillation process, see the previous *IARC Monograph* (IARC, 2010).

1.2 Occupational exposure

Concentrations of PAHs in the ambient air and in urine of workers in coal-tar distillation have been measured in several studies. The results are summarized in Table 1.1. The levels of PAH exposures overall were similar in installations that used the high-temperature process and much lower in the low-temperature distillation facility. A study from Germany (Preuss *et al.*, 2003) showed high air concentrations of total PAHs as well as urinary 1-hydroxypyrene levels.

2. Cancer in Humans

A previous Working Group (IARC, 2010) concluded that there was *sufficient evidence* in humans for the carcinogenicity of occupational exposures during coal-tar distillation. Two large surveillance programmes provided evidence of an increased risk for skin cancer among coal-tar distillers. Notifications of skin cancer in England during 1911–38 were analysed in relation to occupation and more than 700 skin cancers attributed to exposure to coal tar among coal-tar distillers had been recorded; crude mortality rates of scrotal cancer were very high among coal-tar distillers (Henry, 1946). Occupational health surveillance in a German coal-tar distillation plant identified 606 individuals with skin lesions during 1946–96, a third of whom had also malignant skin tumours (squamous-cell and basal-cell carcinoma); 20 cases of scrotal cancer (squamous-cell) were observed (Letzel & Drexler, 1998). No indication of an increased risk for skin cancer was found in more recent cohort-mortality studies, but mortality studies are not sufficiently sensitive to identify potential risks for skin cancer. The findings for other cancer sites were inconsistent. A modest, non-significant increase in mortality was reported for lung cancer in one British and one Dutch study (Maclaren & Hurley, 1987; Swaen & Slangen, 1997), and a significant excess in the incidence of buccal cavity and pharyngeal cancers was reported in a French study (Moulin *et al.*, 1988).

Since the previous evaluation (IARC, 2010) there have been no further relevant studies of cancer risk associated with occupational exposures during coal-tar distillation.

3. Cancer in Experimental Animals

Three high-temperature tars – one undiluted and two as benzene extracts – produced skin tumours, including carcinomas, when applied to the skin of mice. Each one of five blast-furnace tars and two extracts of blast-furnace tars produced skin tumours, including carcinomas, after topical application to mice (IARC, 1985). Likewise, each one of five pharmaceutical coal-tar preparations caused skin tumours, including carcinomas, when applied to the skin of mice (IARC, 1985).

Two unspecified coal tars caused skin tumours, including carcinomas, after application to the skin of mice. Lung tumours but not skin tumours were produced in rats after application of coal tar to the skin. In one study, an

unspecified coal tar produced tumours when applied to the ears of rabbits (IARC, 1985).

No data were available to the Working Group on the carcinogenicity of distillation fractions of low-temperature tars or of products derived from these tars (IARC, 1985).

In addition, several individual polynuclear aromatic compounds for which there is *sufficient evidence* of carcinogenicity in experimental animals have been measured at high concentrations in air samples taken from certain areas in coal-tar distillation plants (IARC, 2010).

4. Other Relevant Data

4.1 Mechanistic considerations relevant to the carcinogenic hazards of exposures during coal-tar distillation

4.1.1 Experimental systems

In previous *IARC Monographs* (IARC, 1985, 1987, 2010) coal-tar pitch and roofing-tar emissions were found to be mutagenic in *Salmonella typhimurium* (in the presence of an exogenous metabolic activation system) and in two mammalian cell systems (in the presence and absence of an exogenous metabolic activation system). Coal-tar pitch and roofing-tar emissions induced sister chromatid exchange in Chinese hamster ovary cells and enhanced viral transformation in Syrian hamster embryo cells (both in the absence and presence of an exogenous metabolic activation system). Samples of therapeutic coal-tars, extracts of coal-tar shampoos, an industrial coal-tar-pitch and vapours escaping from a coal-tar sample at 37 °C were all mutagenic in *Salmonella typhimurium* in the presence of an exogenous metabolic activation system.

Coal tar applied topically to the skin of male Parkes mice produced a complex pattern of DNA adducts in skin and lung tissues when analysed by means of ^{32}P-postlabelling (Schoket *et al.*, 1988), with one adduct spot tentatively identified as benzo[*ghi*]perylene (Hughes *et al.*, 1993).

Benzo[*ghi*]perylene was shown to be photomutagenic in *Salmonella typhimurium* strains. It induced DNA strand-breaks (comet assay) in V79 Chinese hamster lung fibroblasts with photo-activation (Yan *et al.*, 2004; Platt & Grupe, 2005; Platt *et al.*, 2008a; IARC, 2010). The major metabolic intermediate of benzo[*ghi*]perylene is the K-region oxide, 3,4-epoxy-3,4-dihydrobenzo[*ghi*]perylene (Platt *et al.*, 2008b).

Male B6C3F1 mice fed a diet containing coal tar from manufactured gas plant residue produced a complex pattern of aromatic adducts in DNA of the liver, lung, and fore-stomach, which increased with dose and time of treatment; one of the adducts was identified as the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-deoxyguanosine-adduct (Culp & Beland, 1994; Culp *et al.*, 2000). In a similarly designed feeding study, a complex pattern of aromatic DNA adducts was produced in the lung, fore-stomach, and spleen tissues of mice; it was demonstrated that the benzo[*a*]pyrene content alone in the coal tar could not account for the aromatic DNA-adduct levels (Weyand *et al.*, 1991). In the lung of female A/J mice fed a diet containing coal tar from manufactured gas plant residue, three major aromatic DNA adducts were identified as being derived from benzo[*b*]fluoranthene, benzo[*a*]pyrene, and *7H*-benzo[*c*]fluorene (Weyand & Wu, 1995; Koganti *et al.*, 2000). The *7H*-benzo[*c*]fluorene-DNA adduct was also observed in mice who received topical application of manufactured gas plant residue (Cizmas *et al.*, 2004); the structure of this adduct is unknown, although a diol epoxide structure has been proposed (Wang *et al.*, 2002).

In the previous *IARC Monograph* (IARC, 2010) benzo[*b*]fluoranthene was found to be both genotoxic and carcinogenic in experimental studies. *7H*-benzo[*c*]fluorene gave inconclusive results when tested for mutagenicity in *Salmonella*

typhimurium strains TA98 and TA100 in the presence of rat liver S9 (IARC, 1983), but it was found to be carcinogenic to mice (IARC, 2010).

Coal tar applied topically to *lambda-lacZ* transgenic mice (MutaMouse®) strongly increased the mutation frequency in epidermal cells. Isolated epidermal cells from C3H/Tif/hr hairless mice that had been given coal tar had higher levels of DNA strand-breaks (as analysed in the comet assay) (Thein *et al.*, 2000). In female B6C3F1 mice fed diets containing coal tar, cell proliferation was increased in the small intestine. *K-ras*, *H-ras*, and *p53* mutations were observed in the coal tar-induced tumours, the most abundant being *K-ras* mutations in fore-stomach and lung tumours (Culp *et al.*, 2000).

Chemical analyses of high-temperature coal tars have identified a series of polycyclic aromatic hydrocarbons that are both genotoxic and carcinogenic in experimental studies. Among these compounds are benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, chrysene, and indeno[1,2,3-*cd*]pyrene (IARC, 1983, 1985, 2010). These polycyclic aromatic hydrocarbons may contribute in part to the genotoxic and tumorigenic activities of coal tars.

Both naphthalene and benzene are constituents of coal tars produced by various processes (IARC, 1985). Both chemicals are genotoxic and induce tumours in experimental animals (IARC, 1982, 2002). Benzene is considered to be carcinogenic to humans (IARC, 2002).

4.1.2 Humans

In previous *Monographs* (IARC, 1985, 2010) short-term assays were reviewed to assess the genotoxic effects of coal-tar exposure in humans. Patients treated with coal-tar ointments produced urine that was mutagenic in *Salmonella typhimurium* strains TA98 and YG1024 in the presence of an Aroclor–1254-induced rat-liver metabolic activation system. In the *S. typhimurium* strain YG1024, *GSTM1*-null patients had higher levels of mutagens in their urine than *GSTM1*-positive patients.

DNA-adduct measurements that involved coal-tar workers or patients undergoing coal-tar therapy have focused solely on detection of benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts. In the skin and white blood cells (monocytes, lymphocytes, and granulocytes) of a group of eczema patients topically treated with coal-tar ointments the presence of aromatic DNA adducts was demonstrated by means of ^{32}P-postlabelling analysis. One of the adducts co-migrated with the benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct (Godschalk *et al.*, 1998). Analysis of the same adduct by use of an HPLC/ fluorescence method in a group of 26 psoriasis patients showed that the percentage of subjects with adducts did not exceed the 95 percentile control-subject value (Pavanello *et al.*, 1999). The white blood cells of 23 psoriasis patients undergoing clinical coal-tar therapy were examined for the presence of benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts with an enzyme-linked immunosorbent assay (ELISA). These adducts were detected and the adduct levels decreased with time after treatment, but no relationship could be ascertained between the level of exposure and the number of adducts and no difference in DNA-adduct formation was found between smoking and non-smoking patients (Paleologo *et al.*, 1992). PAH diolepoxide-DNA adducts and *GSTM1* genotype were determined in white blood cells of 57 psoriasis patients and 53 controls by use of ELISA and polymerase chain reaction (PCR), respectively. These DNA adducts were slightly elevated in patients compared with controls. There was no relationship between the presence of the *GSTM1* gene and the number of adducts (Santella *et al.*, 1995). Skin-biopsy samples from 12 psoriasis patients receiving coal-tar ointments contained aromatic DNA adducts, measured by ^{32}P-postlabelling analysis (Schoket *et al.*, 1990). In another ^{32}P-postlabelling study no significant

effects were reported of a similar treatment of psoriasis patients on the levels of benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts in peripheral blood lymphocytes (Pavanello & Levis, 1994). In a study of 111 Korean painters using coal-tar-based paint, the levels of aromatic DNA adducts measured by ^{32}P-postlabelling analysis were slightly higher compared with 17 on-site control workers (Lee *et al.*, 2003).

In lymphocytes of 49 coal-tar workers a significant increase of chromosomal aberrations, sister chromatid exchange, and satellite associations was observed, compared with values in non-exposed controls (Yadav & Seth, 1998).

Increased levels of p53 peotein were found in skin biopsies of atopic eczema patients treated topically with coal-tar; a correlation was observed between p53 and aromatic DNA-adduct levels measured in the same tissue by ^{32}P-postlabelling analysis (Godschalk *et al.*, 2001).

4.2 Synthesis

In experimental systems, coal tars were mutagenic in bacteria and mammalian cells, and induced sister chromatid exchange and morphological cell transformation in cultured mammalian cells. Coal tar was also mutagenic *in vivo*, in transgenic mice. Mouse-lung tumours induced by coal-tar treatment had mutations in the K-*ras* proto-oncogene.

Epidemiological studies in humans and studies in experimental animals were consistent with respect to coal-tar exposures being carcinogenic to the lung. Coal tars produced lung and skin tumours in mice and rats after exposure by inhalation, lung tumours in rats after dermal treatment, and skin tumours in mice after dermal treatment. Coal tar was a mouse skin-tumour initiator (see Section 3).

Indications on the role of PAH in the mechanism of action of exposure to coal tar are based on the detection of DNA adducts of several PAHs, e.g. benzo[*ghi*]perylene, benzo[*b*]fluoranthene, *7H*-benzo[*c*]fluorene and benzo[*a*]pyrene in *in vitro* studies, with DNA adducts of benzo[*b*]fluoranthene, benzo[*c*]fluorene and benzo[*a*]pyrene being detected in lung tissues of exposed animals. The benzo[*a*]pyrene DNA adduct was identified as an *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-deoxyguanosine-adduct.

In studies in humans, the urine from patients undergoing coal-tar treatments was mutagenic in bacteria. Peripheral blood lymphocytes of workers occupationally exposed to coal tars had increased chromosomal damage. Measurements of PAH-DNA adducts in human studies are based exclusively on detection of benzo[*a*]pyrene-DNA adducts, in particular *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-deoxyguanosine.

In conclusion, studies in experimental systems and in surrogate tissues of humans provide strong evidence for a genotoxic/mutagenic mechanism underlying the effects of occupational exposures during coal-tar distillation.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during coal-tar distillation. Occupational exposures during coal-tar distillation cause cancer of the skin (including, but not limited to, cancer of the scrotum).

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

Studies in experimental systems and in tissues of humans provide strong evidence for a genotoxic mechanism underlying the effects of occupational exposures during coal-tar distillation in humans. The detection of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-DNA adducts in the peripheral blood lymphocytes of exposed humans suggests the participation of benzo[*a*]pyrene in the genotoxic mechanism of this exposure in humans.

Occupational exposures during coal-tar distillation are *carcinogenic to humans (Group 1).*

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COAL-TAR PITCH

Coal-tar pitch and associated exposures were considered by previous IARC Working Groups in 1984, 1987, and 2005 (IARC, 1985, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Studies of coke-oven pitch indicate that coal-tar pitch contains: four-membered aromatic hydrocarbon ring systems (e.g. chrysene, fluoranthene, pyrene, triphenylene, naphthacene and benzanthracene); five-membered ring systems (picene, benzo[*a*]pyrene (B[*a*]P) and benzo[*e*] pyrene, benzofluoranthenes and perylene); six-membered ring systems (dibenzopyrenes, dibenzofluoranthenes and benzoperylenes); and seven-ring systems (coronene). Other aromatic chemicals in coal-tar pitch include methylated and polymethylated derivatives, mono- and polyhydroxylated derivatives, and heterocyclic compounds (Betts, 1997).

For a more detailed description of the coal-tar distillation process, including coal-tar pitch, the reader is referred to *IARC Monograph* Volume 92 (IARC, 2010), and to the *Monograph* on Occupational Exposures during Coal-tar Distillation in this volume.

1.2 Human exposure

1.2.1 Occupational exposure

Coal-tar pitch is used in electrode manufacture, roofing and paving.

Studies examining exposures during roofing and paving published since the previous *IARC Monograph* (IARC, 2010) are summarized below.

(a) Roofing

The exposures associated with roofing are the result of two operations. First, the old roof is removed by cutting, prying and scraping the existing material from the roof, and discarding it. A new roof is then installed by melting solid blocks of coal-tar pitch, pumping or carrying buckets of the molten material to the roof, where layers of roofing felt and liquid coal-tar pitch are spread upon the surface to produce a cover (NIOSH, 2000; IPCS, 2004). Roofers are primarily exposed to PAHs. Other exposures include silica, diesel exhaust, asbestos and organic solvents. Recent studies on airborne concentrations of PAHs measured in roofing are summarized in Table 1.1.

Studies in the USA showed that long after cessation of applying coal-tar pitch on roofs,

Table 1.1 Concentrations of PAHs in the ambient air of workers in roofing with coal-tar pitch

Reference Country Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Dermal Exposure (ng/cm²)		Urinary 1-OH-pyrene (µmol/mol creatinine)
						Mean	Range	
McClean *et al.* (2007) USA 1998	Tear-off of coal-tar roofs	26						
	Roof workers	21	71	NR	Polycyclic aromatic compound	898	48–30014	
			71		Pyrene	11.0	< 2.4–221	
			71		B[*a*]P	3.3	< 0.5–59	
	Tear-off	NR	41	NR	Polycyclic aromatic compound	886	49–33538	
			41		Pyrene	11.5	< 2.4–168	
			41		B[*a*]P	4.6	< 0.5–84	
	Put-down	NR	56	NR	PAC	344	48–21437	
			55		Pyrene	3.8	< 2.4–150	
			54		B[*a*]P	1.0	< 0.5–59	
	Kettlemen	5	19	NR	Polycyclic aromatic compound	299	40–4558	
			19		Pyrene	4.5	< 2.4–34	
			18		B[*a*]P	0.9	< 0.5–20	
Toraason *et al.* (2001) USA 1998	Tear-off of coal-tar roofs							Mean (SD)
	coal-tar tear-off exposure		16		start wk			0.74 (0.86)
			15		end wk			3.55 (2.17)
	asphalt-fume only exposure		6		start wk			0.26 (0.13)
			5		end wk			0.58 (0.29)
	controls		3		start wk			0.08 (0.12)
			3		end wk			0.12 (0.12)

B[*a*]P, benzo[*a*]pyrene; NR, not reported; SD, standard deviation; wk, week or weeks

workers can still be exposed to coal tar during tear-off of existing roofs (Toraason *et al.*, 2001; McClean *et al.*, 2007). Working with coal-tar pitch was associated with a sixfold increase in PAH exposure, an eightfold increase in pyrene exposure and a 35-fold increase in benzo[*a*] pyrene exposure. Urinary 1-OH-pyrene concentrations in workers and controls showed a fivefold difference at post-shift and a 7.2-fold difference several hours later, which shows the importance of dermal exposure resulting in a prolonged uptake after the end-of-shift.

(b) Paving

Roadway paving can be conducted by several methods, including hot-mix laying and chip sealing. In hot-mix laying, the mixture of a binder (coal tar, bitumen or a blended product containing both) and aggregate (stone chips) is spread on the roadway by a paving machine, followed by a roller. In the chip-sealing process (also known as surface dressing), the liquid binder (coal tar, bitumen, or a mixture of the two) is sprayed directly onto the road surface, then the aggregate stone is spread on top and rolled (Darby *et al.*, 1986).

Detailed information on cessation of coal-tar use in the European paving industry has been collected in the course of an IARC study on cancer mortality among asphalt workers. Table 1.2 presents the last reported year-of-use of coal tar in paving by any company that participated in the cohort study. The data originated from a company questionnaire and its ensuing evaluation by country-specific experts (Burstyn *et al.*, 2003). A gradient in cessation of use can be seen, with Scandinavian countries ending use earlier than central and southern European countries, such as the Netherlands, France and Germany. However, even within countries, large differences in the use of coal tar have occurred between companies, depending on the supplier of the asphalt mixes and the presence of coke ovens in the neighbourhood. Even after cessation of the use of coal tar, workers in road paving have continued to be exposed to this substance due to the use of recycled coal-tar asphalt in some countries.

Table 1.2 Cessation of use of coal tar in asphalt paving (surface dressing)

Country	Last year of use
Finland	1965
Denmark	1974
Sweden	1974
Norway	1984
the Netherlands	1990
France	1992
Germany	1995

From Burstyn *et al.* (2003)

2. Cancer in Humans

In *IARC Monograph* Volume 92 (IARC, 2010) it was concluded that there is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during paving and roofing with coal-tar pitch. This was based on studies of pavers and roofers who presumably had been exposed to coal-tar pitch (and often also to bitumen), which suggested increased cancer risks in these occupations. An excess risk for lung cancer was reported in studies of members of a roofer's union in the USA, in analyses of registry-based data on pavers in the United Kingdom and roofers in the USA, and in follow-up studies of cancer incidence among pavers in Finland and the Netherlands (Kennaway & Kennaway, 1947, 1951; Hammond *et al.*, 1976; Milham, 1982; Pukkala, 1995; Swaen & Slangen, 1997; Stern *et al.*, 2000). An increased mortality from urinary bladder, laryngeal or skin (non-melanoma) cancer was observed in one or more of these cohorts, but this finding was not widely supported by other studies. In three case–control studies conducted in the USA, a tobacco-smoking-adjusted increase in the risk for lung

cancer was reported among roofers (Schoenberg *et al.*, 1987; Zahm *et al.*, 1989; Morabia *et al.*, 1992) however, none of these increases was statistically significant. A meta-analysis of the case–control studies reported a statistically significant meta-relative risk (Partanen & Boffetta, 1994).

Since the previous evaluation (IARC, 2010) a few additional studies have been published with information on paving with coal-tar pitch and associated cancers.

2.1 Cohort Studies

Roofing and flooring, and paving of roads involve the use of bitumen and coal-tar pitch. Although coal-tar pitch has been phased out in most countries, some studies published since the previous *IARC Monograph* (IARC, 2010) have included workers in road-paving exposed to both petroleum-based bitumen and coal-tar pitch.

In studies in France, Norway, and Sweden a higher lung cancer mortality or incidence was found among workers first employed while the asphalt mixes still contained some coal tar (before 1970 in France and before 1965 in Norway and Sweden). None of the elevated rates were statistically significant (Bergdahl & Järvholm, 2003; Randem *et al.*, 2003; Stücker *et al.*, 2003). In the French study a higher but statistically non-significant mortality from stomach cancer was found in workers hired before 1970 (Stücker *et al.*, 2003; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-12-Table2.1.pdf).

In a study in Finland raod-paving workers were ranked by coal-tar exposure (see Table 2.1 on-line). Relative to those not exposed to coal tar, the risk for lung cancer was 1.49 for workers exposed to very low level of coal tar (assessed semiquantitatively on the basis of score–years) and 10.7 for those who exposed to intermediate levels of coal tar (p for trend = 0.05) (Kauppinen *et al.*, 2003).

Bladder-cancer incidence by estimated average and cumulative benzo[*a*]pyrene exposure levels was evaluated in paving cohorts from Denmark, Finland, Israel, and Norway (see Table 2.1 on-line). An internal comparison showed indications of a trend with average exposure to benzo[*a*]pyrene (*P* for trend not significant) (Burstyn *et al.*, 2007). The overall incidence of bladder cancer in European asphalt workers was similar to that expected (SMR 1.05; Boffetta *et al.*, 2003).

3. Cancer in Experimental Animals

Six coal-tar pitches and three extracts of coal-tar pitches all produced skin tumours, including carcinomas, when applied to the skin of mice. An extract of roofing-tar pitch had both initiating and promoting activity in separate experiments (IARC, 1985).

4. Other Relevant Data

4.1 Mechanistic considerations relevant to the cancer hazards from exposure during roofing and paving with coal-tar pitch

4.1.1 Experimental systems

In previous *IARC Monographs* (IARC, 1985, 1987) short-term tests to assess the genotoxicity of coal-tar pitch were reviewed. Coal-tar pitch and roofing-tar emissions were mutagenic in bacteria in the presence of an exogenous metabolic activation system, and in mammalian cells with and without metabolic activation. These agents also induced sister chromatid exchange in Chinese hamster ovary cells and enhanced viral transformation in Syrian hamster embryo cells,

both in the absence and presence of an exogenous metabolic activation system.

Chemical analyses of high-temperature coal-tar pitches identified several polycyclic aromatic hydrocarbons that are genotoxic and carcinogenic in experimental studies (IARC, 1985). These include benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, chrysene, and indeno[1,2,3-*cd*]pyrene (IARC, 1983, 2010). These polycyclic aromatic hydrocarbons may contribute to the genotoxic and tumorigenic activities of coal-tar pitches.

4.1.2 Humans

DNA strand-breaks (measured by single-cell gel electrophoresis; comet assay) and the 8-oxo-deoxyguanosine/deoxyguanosine (8-oxo-dG/dG) ratio (measured by means of HPLC with electrochemical detection) were determined in peripheral blood leukocytes of roofers exposed to dust from coal-tar pitch (coal tar) during removal of existing roofs before applying hot asphalt. When the workers were stratified by 1-hydroxypyrene excretion in the urine, the amount of DNA strand-breaks in their leukocytes increased, and the 8-oxo-dG/dG ratio decreased in a dose-dependent manner (Toraason *et al.*, 2001).

4.2 Synthesis

There is strong evidence from experimental data that coal-tar pitch has a genotoxic mechanism of action. There is moderate evidence in humans for a genotoxic mechanism underlying the effects of exposures during roofing and paving with coal-tar pitch, based on one study.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of coal-tar pitch as encountered in paving and roofing. Coal-tar pitch as encountered in paving and roofing causes cancer of the lung.

Also, a positive association has been observed between exposure to coal-tar pitch as encountered in paving and roofing, and cancer of the bladder.

There is *sufficient evidence* in experimental animals for the carcinogenicity of coal-tar pitch.

There is strong evidence from experimental data that coal-tar pitch has a genotoxic mechanism of action. There is moderate evidence in humans for a genotoxic mechanism underlying the effects of exposures during roofing and paving with coal-tar pitch, based on one study.

Coal-tar pitch is *carcinogenic to humans (Group 1).*

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COKE PRODUCTION

Coke production and associated exposures were considered by previous IARC Working Groups in 1983, 1987, and 2005 (IARC, 1984, 1987, 2010). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Production process

Coke was first produced commercially in England in the early eighteenth century. By the early to mid-1800s, coke was being widely produced in Europe and the United States of America as the major fuel for blast furnaces.

Coal carbonization is a process that yields metallurgical coke for use in iron-making blast furnaces and other metal-smelting processes. Carbonization entails heating the coal to temperatures as high as 1300 °C in the absence of oxygen to distill out tars and light oils. A gaseous by-product, referred to as coke-oven gas, together with ammonia, water and sulfur compounds are also removed thermally from the coal. The coke that remains after this distillation largely consists of carbon in various crystallographic forms, but also contains the thermally modified remains of various minerals that were in the original coal. These mineral residues, commonly referred to as coke ash are not combustible and are left after the coke is burned. Coke also contains part of the sulfur from the coal. Coke is principally used as a fuel, as a reducing agent and support for other raw materials in iron-making blast furnaces. A much smaller amount of coke is used similarly in cupola furnaces in the foundry industry. The carbonization by-products are usually refined within the coke plant to commodity chemicals such as elemental sulfur, ammonium sulfate, benzene, toluene, xylene and naphthalene. Subsequent processing of these chemicals produces a large number of other chemicals and materials. Coke-oven gas is a valuable heating fuel that is used mainly within steel plants, for example, to fire blast-furnace stoves, to soak furnaces for semi-finished steel, to anneal furnaces and lime kilns as well as to heat the coke ovens themselves (Kaegi *et al.*, 1993).

Coke is mostly produced from slot-type by-product coke ovens. Above the ovens is a roof system from which coal is discharged into each oven. Modern technology includes telescopic charging chutes to minimize dust emissions during charging. Many facilities also include automatic removal and replacement of the charging-hole lid. Volatile gases generated from the coal during carbonization flow to the top of the oven, into the free space and out through standpipes connected to large collecting mains that transport the gases to the by-product plant in which they are processed into various materials. Water is sprayed into the mains to cool the

Table 1.1 Concentrations of PAHs in the air and in urine of workers in coke ovens

Reference Country Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Air levels (μg/m³)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Yrjänheikki *et al.* (1995) Finland 1987–90	Coke oven 4 working areas 10 working areas	160	510 dust; 90 gas	NR	 Fluorene Phenanthrene Benzo[*a*]pyrene	 0.58–24.64 0.16–18.76 0.05–10.30			
Liu *et al.* (2006) China NR	Coke oven	47	47	12	**1-Hydroxypyrene** All Non-smokers Smokers			**Median** 5.7 3.0 6.8	 1.4–12 0.6–6.9 2.6–14.5 (s)
Yang et al. (2007) China NR	Coke oven	101	101	77	Total PAH			**Mean**	
	Top	4 (20)				22.83	SD, 0.86	8.49	3.90
	Side	4 (27)				8.70	SD, 2.22	5.2 [a]	NR
	Bottom	4 (25)				6.04	SD, 1.85	1.79	3.71
	Adjunct workplaces	8 (29)				5.60	SD, 0.87	1.51	3.60
Marczynski *et al.* (2009) Germany 1999–2003	Coke oven	37	37	27	Sum of 16 PAHs 1-Hydroxypyrene Sum of hydroxy-phenanthrene Benzo[*a*]pyrene	22.5 1.0	15.9–91.0 0.4–2.5	 2.0 3.5	 1.1–3.6 2.1–7.5
Rossella *et al.* (2009) Poland NR	Coke oven/ maintenance	50/5	54	54	1-Hydroxypyrene			15.4 [b]	1.1–147.1

NR, not reported; PAH, polycyclic aromatic hydrocarbons; SD, standard deviation

[a] Read from figure

[b] Median

Conversions used for 1-hydroxypyrene: 1 μmol/mol creatinine = 1.93 μg/g creatinine = 0.013 μmol/L = 2.84 μg/L = 2.84 ng/mL.

gases and to condense out some of the tar. At the end of the coking cycle – which ranges from about 15 to 30 hours –, the coke is pushed into a hot car (quench car). The hot car may or may not have a moveable roof or partial roof to minimize gaseous and particulate emissions. The hot coke is then quenched before being dropped onto a conveyor system for transportation to a blast furnace, storage pile or out of the plant (Kaegi *et al.*, 1993; Crelling *et al.*, 2005). For a more detailed description of the coke production process and coke ovens, the reader is referred to the previous *IARC Monograph* (IARC (2010).

In 1990, total worldwide coke production was about 378 million tonnes, a production volume that had remained essentially unchanged since 1970. The former USSR was the largest coke producer (80 million tonnes), followed by the People's Republic of China (73 million tonnes), Japan (53 million tonnes) and the United States of America (USA) (27 million tonnes). Since 1970, production in the former USSR has remained in the 75–85 million tonne range, but massive shifts in production have occurred in the USA, Japan and China. Between 1970 and 1990, production in the USA decreased by more than 50% while Japanese production increased by 50%. During the same period, China increased coke production more than threefold (Kaegi *et al.*, 1993). By 1999, worldwide coke production had declined to about 326 million tonnes, of which 121 million tonnes were produced in China (Terjung, 2000).

1.2 Human exposure

Coke-oven workers are primarily exposed to polycyclic aromatic hydrocarbons (PAHs). Concentrations of PAHs in the ambient air and in urine of workers in coke ovens are summarized in Table 1.1. In addition to PAHs, coke-oven workers may be exposed to a large number of compounds, including asbestos, silica, amines, arsenic, cadmium, lead, nickel, vanadium, hydrocarbons, sulfur dioxide, sulfuric acid and aldehydes (IARC, 1984).

More than 30 studies on occupational exposure of coke-oven workers have been reported since 1983, of which six included profiles of three PAHs or more; seven others reported levels of pyrene, benzo[*a*]pyrene or both; the other studies reported composite measures (benzene-soluble fraction, cyclohexane-soluble material) or provided data on biological measurements only. A variety of sites across the coke plants were sampled, and the overall pattern (regardless of the exposure measured) was that topside workers (including lidmen, tar chasers and Larry-car operators) had the highest exposures, followed by workers by the side of the ovens (such as coke-side machine operators, bench-men, door repairers, wharf-men, quenchers, pushers and temperature controllers). Workers in other areas of the plant such as maintenance, office and control workers had the lowest exposures (Table 1.1). Modernization of coke plants, including improved control measures, can substantially reduce exposures (Quinlan *et al.*, 1995c).

2. Cancer in Humans

Occupational exposures during coke production were evaluated most recently in *IARC Monograph* Volume 92 (IARC, 2010). There was *sufficient evidence* in epidemiological studies for the carcinogenicity of occupational exposures during coke production, although the evidence was not uniform. A marked excess of lung cancer and a clear trend of increasing risk for this cancer with increasing duration of exposure was found in a large cohort study from the USA and Canada (Costantino *et al.*, 1995; see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-13-Table2.1.pdf). In an even larger study from China there was also a marked lung-cancer excess (Wu, 1988). In cohort studies

from France (Chau *et al.*, 1993), Italy (Franco *et al.*, 1993), Japan (Sakabe *et al.*, 1975), and the Netherlands (Swaen *et al.*, 1991) increased risks for lung cancer were found consistently, although not all results were statistically significant. No excess of lung cancer was observed in three cohort studies of coke-plant workers in the United Kingdom (Buck & Reid, 1956; Davies, 1977; Hurley *et al.*, 1983), but an excess had been found in an earlier record-linkage study in the United Kingdom (Kennaway & Kennaway, 1947). A smoking-adjusted excess risk for lung cancer and a positive association with duration of exposure to coke-oven emissions was found in a population-based case–control study of Chinese women (Wu-Williams *et al.*, 1993; see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-13-Table2.2.pdf). No further epidemiological studies of coke-oven workers have been published since the previous *IARC Monograph* (IARC, 2010).

Overall, an increased risk for lung cancer was found in three large studies, two of which showed evidence of a dose–response; one study was adjusted for smoking. A lung-cancer excess is supported by several but not all other available studies. Thus, there is evidence from epidemiological studies that lung cancer is causally associated with occupational exposures during coke production. The evidence is inadequate to allow and evaluation for cancer of the bladder and skin.

3. Cancer in Experimental Animals

Samples of tar from coke ovens were previously evaluated in *IARC Monograph* Volume 34 (IARC, 1984). No new studies have been published since then.

3.1 Skin application

Two samples were collected from a modern coke oven: a topside air-particulate sample and a coke-oven main sample. The coke-oven topside sample was found to contain 478 mg/kg benzo[*a*]pyrene (Nesnow *et al.*, 1982, 1983).

The coke-oven main sample was applied topically to groups of 40 male and 40 female SENCAR mice at doses of 0 (control), 0.1, 0.5, 1.0, 2.0 or 4.0 mg (in acetone) per mouse on a weekly basis, except for the highest dose, which was applied twice weekly at 2.0 mg per application, for 50–52 weeks. The incidence of skin carcinomas ranged from 5–98% in groups of treated mice. No carcinomas were observed in the control groups (Nesnow *et al.*, 1983).

The tumour-promoting activity of the coke-oven main sample was tested by repeated weekly dermal applications of 0 (control), 0.1, 0.5, 1.0, 2.0 and 4.0 mg (in acetone) for 34 weeks to groups of 40 male and 40 female SENCAR mice pre-treated (one week earlier) with a dermal dose of 50.5 µg benzo[*a*]pyrene. Skin papilloma incidence was reported to increase from 3–100% over the dose range of the coke-oven main sample tested. No papillomas were observed in the control groups (Nesnow *et al.*, 1983).

The tumour-initiating activity of both samples mentioned above was studied with groups of 40 male and 40 female SENCAR mice that were skin-painted once with 0 (control), 0.1, 0.5, 1.0, 2.0 or 10 mg of each sample dissolved in acetone. The 10-mg dose was administered in split doses of 2.0 mg each for 5 days. Beginning one week later, 2 µg of 12-*O*-tetradecanoylphorbol-13-acetate were administered topically, twice weekly. The incidence of skin papillomas and carcinomas was reported after six months and one year, respectively. Both the topside and main samples produced dose-related increases in both papillomas and carcinomas. For the topside sample, the papilloma incidence ranged from 10–100% and the carcinoma incidence was 0–20%, while

for the coke-oven main sample, the incidence of papillomas ranged from 31–100% and of carcinomas from 10–65%. In the control groups, the incidences of papillomas and carcinomas ranged from 5–8% and 0–5%, respectively (Nesnow *et al.*, 1982, 1983).

3.2 Inhalation

3.2.1 Mouse

The carcinogenicity of coke-oven tar (from a USA by-product coke oven) was investigated in C3H mice. In the untreated control group, all 30 mice survived for the duration of the experiment (71 weeks) and did not develop any squamous-cell tumours of the lung. A second group of 33 mice was exposed to a coal-tar aerosol at 300 mg/m^3 for two hours daily, three times weekly, during weeks 35–71 of the experiment. Five squamous-cell tumours of the lung, one of which was malignant, occurred in this group (Horton *et al.*, 1963).

In another experiment, the tar (Tar 1) from the same source as that in the study by Horton *et al.* (1963) and another tar (Tar 2) were separated into phenolic (P-Tar) and non-phenolic (N-Tar) fractions. Both tars were derived from USA by-product coke ovens. Aerosols were produced from these fractions and animals were exposed to the aerosols, separate or in combination, at 120–200 mg/m^3, three times per week for 55 weeks. Six groups of 50 male C3H/HeJ mice were treated with air only (control) or with aerosols of Tar 1, N-Tar 1, N-Tar 1 + P-Tar 1, N-Tar 1 + P-Tar 2 or N-Tar 2 + P-Tar 1. The experiment was terminated at 55 weeks. Lung tumours were recorded in animals that survived beyond 46 weeks. Lung-adenoma incidence was 0/32 (control), 12/13, 16/20, 14/19, 14/25 and 14/23, respectively. Lung-adenocarcinoma incidence was 0/32 (control), 3/13, 0/20, 1/19, 1/25, 0/23, respectively (Tye & Stemmer, 1967).

Groups of 75 CAF_1-JAX and 75 ICR-CF1 female mice were exposed for 90 days to concentrations of coal-tar aerosol of 0.2, 2.0 and 10 mg/m^3 (99% of the droplets were ≤ 5 μm in size), and were observed for a further 21 weeks. The aerosol consisted of a composite mixture of benzene/toluene/xylene fractions of coal tars collected from the effluents of several different coke ovens in the USA. Unexposed controls of each strain of mice were included in the study. Lung-tumour data were not available. The incidence of skin tumours [not further specified] was 1/61, 14/75 and 44/55, respectively, in the exposed ICR-CF1 mice, and 3/225 in the controls. In the exposed groups of CAF_1-JAX mice, the skin-tumour incidences were 0/75, 3/65 and 18/43, respectively, with 0/225 in the controls (MacEwen *et al.*, 1976). [The Working Group noted the lack of solvent controls.]

A group of 75 female ICR-CF1 mice and a group of 50 female CAF_1-JAX mice were exposed to the coal-tar aerosol described above at a concentration of 10 mg/m^3 for six hours per day, five days per week, for 18 months; unexposed controls of both strains were available. Alveologenic carcinomas were observed in 26/61 exposed ICR-CF1 mice, 27/50 exposed CAF_1-JAX mice, 3/68 ICR-CF1 controls and 8/48 CAF_1-JAX controls. The exposed and control groups did not differ in the incidence of other types of tumours, including skin tumours (MacEwen *et al.*, 1976). [The Working Group noted the lack of solvent controls.]

3.2.2 Rat

A group of 40 male and a group of 40 female CFE strain Sprague-Dawley weanling rats were exposed to the aerosol described above at a concentration of 10 mg/m^3 for six hours per day, five days per week, for 18 months; 40 male and 40 female unexposed controls were available. Among treated rats, 38/38 males and 31/38 females developed squamous-cell carcinomas of the lung. In the control groups, no such tumours were observed in 36 males and 37 females (MacEwen *et al.*, 1976). [The Working Group noted the lack of solvent controls.]

4. Other Relevant Data

4.1 Mechanistic evidence relevant to the carcinogenic hazard from occupational exposures during coke production

4.1.1 Experimental systems

Experimental studies of the effects of exposure to coke-oven emissions have been evaluated in previous *IARC Monographs* (IARC, 1984, 1987, 2010). Emissions from coke ovens were mutagenic to *Salmonella typhimurium* TA98 and TA100, both with and without exogenous metabolic activation, and in several mammalian cell systems: L5178Y mouse lymphoma $Tk^{+/-}$ cells with and without metabolic activation, Chinese hamster ovary cells (6-thioguanine resistance), and BALB/c 3T3 cells (ouabain resistance). Coke-oven emissions induced DNA strand-breakage in Syrian hamster embryo cells, and sister chromatid exchange in Chinese hamster ovary cells with and without exogenous metabolic activation. These emissions also caused morphological transformation in BALB/c 3T3 cells without activation.

PAHs are a component of coke-oven mixtures and many carcinogenic PAHs are genotoxic in *in-vitro* and *in-vivo* bioassay systems. Examples are benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*c*]phenanthrene, benzo[*b*]fluoranthene, and benzo[*j*]fluoranthene (IARC, 1984). Many of the genotoxic effects induced by coke-oven emissions in experimental systems have also been found after exposure to individual carcinogenic PAHs (IARC, 1983; Osborne & Crosby, 1987; Harvey, 1991). The extent to which PAHs contribute to the genotoxic activity of coke-oven emissions is not known, but the available evidence strongly suggests that they do make a contribution. Some insight into the role of PAHs in genotoxicity and cancer associated with coke production can be obtained from DNA-adduct studies in experimental animals exposed to coke-oven emissions. In rats exposed to such emissions by inhalation one major adduct was detected by the [^{32}P]-postlabelling procedure. This adduct had the same chromatographic mobility as the major *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-(BPDE)-N^2-deoxyguanosine adduct resulting from incubation of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide with calf thymus DNA. This adduct was observed in DNA from lung, heart, and liver of the exposed rats. However, the autoradiograms also showed evidence of a complex mixture of unidentified aromatic adducts (Binková *et al.*, 1994). Mice topically exposed to coke-oven emissions showed some evidence of the formation of a BPDE-DNA adduct (Lewtas *et al.*, 1993). Although the studies in exposed rats and mice provided some evidence with respect to an *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide adduct, many other DNA adducts remained unidentified and their contribution to the total DNA-adduct profile cannot be ascertained. In addition, these DNA-adduct studies are dependent on the availability of fully characterized PAH-DNA adduct standards and in many [^{32}P]-postlabelling experiments only the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adduct was used as a reference. This adduct has been correlated with specific mutations in the oncogene, K_i-*Ras*, found in lung tumours induced by benzo[*a*]pyrene in mice (Ross & Nesnow, 1999). Moreover, K_i-*RAS* mutations are common in human lung tumours (Vineis & Caporaso, 1995).

4.1.2 Humans

As was previously reported (IARC, 1987), peripheral blood lymphocytes of coke-oven workers had increased frequencies of sister chromatid exchange (SCE) compared with age-matched controls. Also, in one study the urine of coke-oven workers was mutagenic in *Salmonella typhimurium* in the presence of an exogenous

metabolic system. Numerous subsequent studies largely confirmed these results.

In an Italian study, no differences in frequencies of chromosome aberrations and micronuclei were found in cultured lymphocytes of 92 coke-oven workers in a steel-production plant and a group of 19 controls from a non-oven plant in the same area (Forni *et al.*, 1996). In Germany, a group of 29 coke-oven workers and a control group were studied for frequencies of DNA single-strand breaks, DNA–protein crosslinks (measured with an alkaline filter-elution assay), SCE, and DNA adducts in lymphocytes. DNA strand-breaks in lymphocytes of coke-oven workers were significantly higher than in controls (Popp *et al.*, 1997). Eleven of 31 male non-smoking coke-oven workers in Poland produced urine that was mutagenic in *Salmonella typhimurium* strain YG1024 in the presence of an exogenous source of metabolic activation, compared with urine samples of 31 male non-smoking controls (Simioli *et al.*, 2004). 8-Oxodeoxyguanosine levels in white blood cells and the amount of DNA damage (measured with the comet assay) in lymphocytes were higher in 20 German coke-oven workers than in 55 controls (Marczynski *et al.*, 2002). Urinary 8-oxodeoxyguanosine concentrations were higher in 55 Taiwan, Chinaese topside coke-oven workers than in 162 side-oven workers (Wu *et al.*, 2003). In another study from Taiwan, China, elevated levels of 8-oxodeoxyguanosine in urine, micronuclei in lymphocytes, and glutathione *S*-transferase in serum were found in 47 workers exposed to coke-oven emissions, compared with the levels in 31 controls (Liu *et al.*, 2006). In a study of 49 Polish coke-oven workers the micronucleus frequency in peripheral blood lymphocytes was related to specific *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct formation in the same subject (Pavanello *et al.*, 2008a). Chinese coke-oven workers (n = 141) had higher frequencies of micronuclei, nucleoplasmic bridges, and nuclear buds compared with the values in 66 controls; the number of nucleoplasmic bridges and nuclear buds correlated with 1-hydroxypyrene concentrations in urine (Duan *et al.*, 2009).

A significant correlation was found between the concentrations of 1-hydroxypyrene and 8-oxodeoxyguanosine in urine from a group of 91 coke-oven workers in Taiwan, China, compared with 49 controls (Hu *et al.*, 2004), while a Japanese/Chinese study of 119 coke oven workers and 37 controls could not find a correlation between 8-oxodeoxyguanosine levels in leukocytes and 1-hydroxypyrene concentrations in urine (Zhang *et al.*, 2003). In a group of 37 German coke-oven workers the level of 8-oxodeoxyguanosine and the number of DNA strand-breaks (measured with the comet assay) were higher than in 48 controls (Marczynski *et al.*, 2009).

The mutant frequency at the *HPRT* locus (6-thioguanine resistance) in lymphocytes from a population of 43 Italian coke-oven workers was increased compared with the frequency in 26 non-exposed controls, but the difference was not significant. The percentages of the different types of gene alteration were also similar in exposed and non-exposed subjects, based on an analysis of mutations in 161 *HPRT* clones derived from the two groups. Only the frequency of splice mutations in mutant clones derived from coke-oven workers was significantly higher than in controls (Zanesi *et al.*, 1998). No difference in the plasma concentrations of either p53 (mutated or wild-type) or p21(WAF1) protein was found between 66 coke-oven workers and 44 controls in the Czech Republic, but significantly higher amounts of these proteins were found in the subgroup exposed to carcinogenic PAHs at concentrations of < 1 μg/m^3 compared with the group exposed to carcinogenic PAHs > 1 μg/m^3. Overall, a negative correlation between the concentration of p53 protein in plasma and personal exposure to carcinogenic PAHs was found (Rössner *et al.*, 2003). In another study from the Czech Republic, cytogenetic markers (chromosomal aberrations, SCE,

cells with a high frequency of SCE, the heterogeneity index SCE, and polymorphism of the genes *GSTM1* and *NAT2*) were evaluated in the peripheral lymphocytes of 64 coke-oven workers and 34 control subjects from the same plant. All the cytogenetic markers were significantly increased in the exposed workers compared with the control group, also when smoking status was taken into account. No effects of the *GSTM1* and *NAT2* genotypes on the cytogenetic markers were noted (Kalina *et al.*, 1998).

The effect of polymorphisms in genes involved in DNA repair was evaluated by means of the comet assay in isolated peripheral blood lymphocytes from 94 coke-oven workers in North-eastern China and 64 controls. One of the *XRCC1* genotypes, Arg399Gln, was associated with an increased frequency of micronuclei in the coke-oven workers (Cheng *et al.*, 2009).

Coke-oven workers have been extensively studied for the presence of biomarkers of exposure, in some cases with mixed results due to methodological issues, small sample size, inter-individual variability, and confounding factors, such as smoking and diet. However, there are several key studies that show clear relationships between exposures to coke-oven emissions and certain biomarkers. The literature is dominated by studies that examined *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts as a biomarker of exposure. The amount of this adduct has been found to be increased in peripheral blood lymphocytes of male coke-oven workers compared with controls in several studies of different worker populations (Pavanello *et al.*, 1999a, 2004; Chen *et al.*, 2003). In some of these studies, analysis of genetic polymorphisms showed that the *GSTM1-null* detoxifying genotype and some low-activity nucleotide excision-repair (NER) genotypes were associated with higher *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct formation (Pavanello *et al.*, 1999b, 2004, 2005).

In 35 Dutch coke-oven workers a significant induction of aromatic DNA adducts in lymphocytes and of micronuclei in exfoliated urothelial cells was found compared with the levels in 37 controls (van Delft *et al.*, 2001). When 89 French coke-oven workers were compared with 18 power-plant workers with respect to *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct levels and genetic polymorphisms in the *CYP1A1*, *GSTM1* and *GSTT1* genes, higher numbers of DNA adducts were detected in individuals with the combined *CYP1A1(1/*2* or **2A/*2A)-GSTM1-null* genotype (Rojas *et al.*, 2000). In Polish coke-oven workers the influence was studied of four polymorphisms of nucleotide excision-repair genes and of *GSTM1* on *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct levels in lymphocytes and monocytes. The increase in DNA-adduct levels was significantly related to lack of GSTM1 activity and to the low nucleotide excision-repair capacity of the *XPC-PAT*$^{+/+}$ genotype (Pavanello *et al.*, 2005). In other studies by the same group, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA-adduct levels were significantly increased in peripheral blood lymphocytes of coke-oven workers compared with controls, after adjustment for smoking status and diet (Pavanello *et al.*, 2004, 2008a). In one study from China, formation of the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-albumin adduct was associated with exposure to coke-oven emissions, after adjustment for smoking status (Wang *et al.*, 2007).

Based on the available evidence from studies in experimental systems, coke-oven emissions are mutagenic in bacteria and in mammalian cells, induce DNA damage, SCE, and morphological cell transformation. DNA-adduct data from experimental studies suggest that coke-oven emissions produce a complex mixture of aromatic adducts, one being identified as *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-N^2-deoxyguanosine. Genotoxic effects of exposures to coke-oven emissions have been studied in

surrogate tissues from populations in industrial settings. Measured end-points include 8-oxodeoxyguanosine levels, DNA strand-breaks, SCE, micronuclei, chromosomal aberrations, and urinary mutagenicity. The increased 8-oxodeoxyguanosine levels reflect increased oxidative stress. This could be a result of redox cycling of PAH quinones and the damaging effects of reactive intermediates on cellular antioxidant levels (Joseph & Jaiswal, 1998; Klaunig & Kamendulis, 2004; Park *et al.*, 2009).

Chronic exposure to PAH in Polish non-smoking coke-oven workers induced both gene-specific (e.g. in the *TP53* gene) and global methylation changes in peripheral blood lymphocytes. These changes were correlated with BPDE-DNA adduct levels and micronuclei in the same subjects (Pavanello *et al.*, 2009).

4.2 Synthesis

Overall, these data strongly indicate a mutagenic/genotoxic mode of action for occupational exposures during coke production, based on experimental and human studies. The data also identify lung as a target, given the major route of human exposure, based on both experimental and human studies. There is ample mechanistic support for the respiratory carcinogenic effects of occupational exposures during coke production in humans, in part through analysis of exposure to benzo[*a*]pyrene. This is based on direct measurement of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts in peripheral blood lymphocytes (surrogate tissue) and on the identification of genotoxic effects consistent with those induced by *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide or benzo[*a*]pyrene. It is also consistent with the known carcinogenic activity of this epoxide in lung tissues in experimental animals. Moreover, the influence of *GST* polymorphisms on levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts is suggestive of the presence of reactive electrophilic intermediates, such as *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide. Since coke-oven emissions are complex mixtures, these exposures could have more than one underlying mechanism of action. The fact that chronic exposure to PAH in Polish non-smoking coke-oven workers induced both gene-specific (e.g. in the *TP53* gene) and global methylation changes in peripheral blood lymphocytes, suggests an epigenetic mechanism.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of coke production. Coke production causes cancer of the lung.

There is *sufficient* evidence in experimental animals for the carcinogenicity of samples of tar taken from coke ovens.

There is strong evidence for a genotoxic mechanism underlying the effects of occupational exposures during coke production, based on both experimental and human studies.

The detection of anti-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-DNA adducts in the peripheral blood lymphocytes in exposed populations suggests the participation of benzo[*a*]pyrene in the genotoxic mechanism for this exposure in humans.

Coke production is *carcinogenic to humans (Group 1).*

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MINERAL OILS, UNTREATED OR MILDLY TREATED

Mineral oils were considered by previous IARC Working Groups in 1983 and 1987 (IARC, 1984, 1987). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Mineral oils (also known as base oils, mineral base oils or lubricant base oils) are chemical substances prepared from naturally occurring crude petroleum oil. Crude oil is distilled first at atmospheric pressure and then under high vacuum to yield vacuum distillates and residual fractions that can be further refined to mineral oils. Mineral oils refined from petroleum crude oils are complex and variable mixtures of straight and branched-chain paraffinic, naphthenic (cycloparaffinic), and aromatic hydrocarbons having carbon numbers of 15 or more and boiling points in the range of 300–600°C (IARC, 1984).

Mineral oils are described by several dozen generic "petroleum stream" Chemical Abstracts Service (CAS) numbers. Many mineral oils may have more than one CAS number because different refiners submitted slightly different descriptions for similar refining streams when CAS numbers were being assigned (both in the United States of America and Europe) (CONCAWE, 1997).

The hydrocarbon composition and physical characteristics of a mineral oil depend on both the composition of the original crude oil and the processes used during refining (e.g. solvent extraction, hydro-treatment) (CONCAWE, 1997). Production processes of mineral oils have changed substantially over time (IARC, 1987; Tolbert, 1997). In the past, many mineral oils were only mildly refined and contained significant levels of polycyclic aromatic hydrocarbons (PAHs). Acid treatment was initially used to remove PAHs and other impurities and to improve the technical properties of the finished oils. In recent decades, acid treatment has largely been replaced by extensive refining with solvent extraction and/or hydro-treatment, which has further reduced the level of PAHs and other contaminants. Mineral oils have been produced by means of the severe hydro-treatment procedure since the 1960s (Kane *et al.*, 1984; Mackerer *et al.*, 2003). Regulatory pressures in the USA further encouraged the move to highly refined mineral oils in the mid-1980s (Woskie *et al.*, 2003).

There are several assays that can be used to determine if a mineral oil is highly or severely refined (Mackerer *et al.*, 2003). Two useful

short-term assays that are widely used by mineral-oil manufacturers are the modified Ames test (ASTM, 1996) and the IP346 assay (Institute for Petroleum, 1985, 1993; CONCAWE, 1994). The modified Ames test measures the amount of extractable mutagenic activity in a mineral-oil sample; mineral oils with a mutagenicity index ≤ 1.0 in this assay are considered highly or severely refined. The IP346 assay measures the amount of material extractable in dimethyl sulfoxide (DMSO): mineral oils with a DMSO-extractable content < 3% in the IP346 assay are considered highly or severely refined. Naphthenic mineral oils tend to have higher non-mutagenic DMSO extractables and some naphthenic oils can give a false-positive result in the IP346 assay. The modified Ames test can give conclusive results if the outcome of the IP346 assay is high.

1.2 Uses

A wide variety of mineral oil-containing products including lubricants as well as products intended for non-lubricant purposes are manufactured for different applications. Lubricant products include engine oils, transmission fluids, gear oils, hydraulic fluids, as well as metalworking fluids (also known as metal-removal fluids). Metalworking fluids may be different from other mineral oil-containing products due to the types of additives used, the additive treatment rates, and contaminants – including those of microbial origin – that are associated with use. "Non-lubricant" products include agricultural spray oils, printing inks, tyre oils, etc. Oil mists or aerosols can arise from the use of mineral oil both as lubricant and as non-lubricant. In practice, oil aerosols may be generated by several mechanisms such as aeration, contact with a fast-moving surface, or by heating. Important applications associated with potential generation of oil aerosols are metal-working, textile machinery, rock drills, aerosol lubrication, agriculture sprays, concrete mould release agents, corrosion preventatives, printing inks, rubber extenders, lubricant-blending in open processes, and applications in food and pharmaceutical preparations (CONCAWE, 1986; Urbanus *et al.*, 2003; ACGIH, 2007). The particle size of the mists, aerosols or fogs is likely to differ for each of these processes (IARC, 1984).

1.3 Human exposure

1.3.1 Occupational exposure

There are several occupational environments in which an oil mist can be generated. In these situations the opportunities for dermal exposure or inhalation exposure, with concomitant ingestion, are substantial. Such occupations include metalworking, printing-press operating, and cotton- and jute-spinning (Tolbert, 1997). According to the US National Occupational Exposure Survey (1981–83), approximately 1 million workers (including approximately 390,000 women) in the USA were potentially exposed to mineral oil (NIOSH, 1990).

A small number of studies have evaluated respiratory morbidity from exposure to mineral-oil mist among newspaper pressmen, marine engineers, cable oilers, and tunnel blasters. Mineral-oil aerosol concentrations in these studies ranged from approximately 0.3 mg/m^3 (Bakke *et al.*, 2001) to about 3 mg/m^3 (Skyberg *et al.*, 1992; Svendsen & Hilt, 1997, 1999; Bukowski, 2003). Values of up to >20 mg/m^3 were recorded in earlier studies (Goldstein *et al.*, 1970).

Ambient mineral-oil mist concentrations were measured in the engine rooms of ships: the typical lubricating oil (b.p. 300–700°C) is a solvent-refined mineral oil containing paraffins, cycloparaffins, aromatic hydrocarbons, and additives. The air concentrations of oil mist in the engine rooms of different ships varied from not detectable to 0.53 mg/m^3 (mean 0.24 mg/m^3). The levels of hydrocarbons varied from 0.2 to

14.5 mg/m^3 (Svendsen & Børresen, 1999). [The level of refinement of these oils was not reported.]

1.3.2 Non-occupational exposure

The non-occupationally involved general population may be exposed to mineral oils through ingestion of contaminated foodstuffs. In a study conducted in Switzerland, Grob *et al.* (2001) analysed mineral oil in the fat or in a raw extract from animal feed or foodstuffs. The average concentration in the feed was 100 mg/kg, with a maximum of 1000 mg/kg, 25 mg/kg in animal body fat (maximum, 150 mg/kg) and 30 mg/kg in the fat phase of eggs (maximum, 80 mg/kg). Paraffin oil is used for feed production, which may account for part of the contamination problem (e.g. in eggs). [The level of refinement of these mineral oils was not reported.]

2. Cancer in Humans

2.1 Introduction

Mineral oils comprise a diverse set of agents used for a wide range of industrial operations. There is evidence that mineral oils vary in their potential to cause cancer with the degree of treatment or processing. Hydro-treatment and solvent extraction reduce the PAH content, and thus the carcinogenicity of the oils. Untreated and mildly treated oils have been classified as Group-1 carcinogens, with *sufficient evidence* from studies in humans that mineral oils (containing various additives and impurities) that have been used in occupations such as mule-spinning, metal machining and jute-processing are carcinogenic to humans.

A major challenge in making an overall assessment of the carcinogenicity of mineral oils is this diversity in processing, with incomplete information on the extent of processing in specific industrial applications. Mineral oils are typically used as part of a complex mixture for such applications as metalworking, lubrication, and cooling. The additional processing and combining with other agents makes attribution of risk specifically to mineral oils difficult (Woskie *et al.*, 2003).

2.2 Cancer of the skin/scrotum

The evidence from a series of case reports and case series for skin cancer, particularly of the scrotum, over the period from the early 1900s through the 1960s, was reviewed in *IARC Monograph* Volume 34 (IARC, 1984). Five large case series of mule-spinners had been reported, each with over 100 scrotal cancers (Green, 1910; Southam & Wilson, 1922; Henry & Irvine, 1936; Henry, 1947), with sizable numbers of cases in other exposed populations. Despite the inherent limitations in case series as a source of information, the numbers of cases observed, the rarity of scrotal cancer, and the intensity of the direct exposure of the skin in these jobs during that time period render these case series highly informative. Scrotal cancer is virtually pathognomonic for occupational exposure, in part as a result of these historical case series.

Over the intervening period since the 1960s nothing has challenged the assessment of *sufficient evidence* of human carcinogenicity based on the historical evidence pertaining to skin cancer. In a cohort of 792 Swedish metalworkers exposed to oil mist there were four cases of scrotal cancer, all among 242 men employed as turners, versus 0 expected overall (Järvholm *et al.*, 1981). The same group (Järvholm *et al.*, 1985) reported on a cohort of 682 bearing-ring industry workers and found working as a lathe operator to be associated with scrotal cancer (four observed, 0.3 expected, $P < 0.001$). Zhao *et al.* (2005) studied a cohort of 5049 male aerospace workers in the USA and found a significantly increased risk for skin melanoma (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/

vol100F/100F-14-Table2.1.pdf). Case series indicative of an increased risk for scrotal cancer continue to be published based on historical exposures. Pressmen working in a wax-manufacturing department in an oil refinery in the USA had a marked excess of scrotal cancer based on 11 cases in men working >10 years during the period 1937–56, which corresponds to a crude rate of 806 per 100,000 relative to a general population rate estimated at 0.15 per 100,000 (Hendricks *et al.*, 1959). Tool setters and tool fitters in the West Midlands area of England showed notably elevated risk for scrotal cancer over the period 1936–1976 (Waldron *et al.*, 1984).

Several epidemiological studies were able to detect the expected increased risk for skin cancer in general, or scrotal cancer in particular, but since these cancers are rarely fatal, studies based on cancer mortality were of limited use to address the question. Roush *et al.* (1982) studied squamous-cell carcinoma of the scrotum in a case–control study in Connecticut, USA, among men diagnosed between 1935 and 1973 (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.2.pdf). Occupations associated with exposure to cutting oils, including tool or machine setters, screw-machine operators, machinists, and machine operators, were examined and showed an odds ratio of 10.5 (95%CI: 4.0–36.9).

2.3 Other cancers

The more rigorous epidemiological studies pertain to the occupations in which mineral oils are used in various formulations and in different degrees, including metal workers, machinists, jute workers, and others. Given the time period and setting, the mineral oils studied were likely to be highly treated. At the time of the previous *IARC Monograph* there were several studies of workers in these industries, mostly based solely on job title and industry of employment and limited in detail regarding exposure (IARC, 1984). Exposure to mineral oil was inferred based solely on job title or self-reported exposure. Whereas dermal exposure is the primary route of exposure for skin/scrotal cancer, for other sites and under improved hygienic conditions, aerosols are of equal or greater concern.

Focusing on studies that made attempts to address exposure to mineral oil directly, there has been sporadic and inconsistent support for an association with bladder cancer (Ugnat *et al.*, 2004; Friesen *et al.*, 2009; see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.3.pdf and Table 2.4 at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.4.pdf), stomach cancer (Zhao *et al.*, 2005; see Table 2.5 at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.5.pdf and Table 2.6 at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.6.pdf), rectal cancer (Gerhardsson de Verdier *et al.*, 1992; Eisen *et al.*, 2001; see Table 2.7 at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.7.pdf and Table 2.8 at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.8.pdf), pancreatic cancer (Yassi *et al.*, 2003), sinonasal cancers (Roush *et al.*, 1980), laryngeal cancer (Ahrens *et al.*, 1991; Eisen *et al.*, 1992), and lung cancer (Rønneberg *et al.*, 1988; Acquavella *et al.*, 1993; see Table 2.9, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.9.pdf and Table 2.10 at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-14-Table2.10.pdf). Individual studies have suggested that mineral oil may be related to a range of other cancers, including those of the larynx and pancreas, based on studies of metal-workers and related manufacturing occupations. For each of these, however, there are studies of equal or higher quality that do not show associations, and in many cases there are inconsistent results within the same study across exposure indices. There have been varyious interpretations of the strength of the

evidence linking exposure to metalworking fluids to a range of cancer sites (Tolbert, 1997; Calvert *et al.*, 1998; Mirer, 2003; Savitz, 2003). The diversity in exposures to other agents, including synthetic oils and contaminants, and the presence of accompanying unrelated occupational exposures make the relevance of many studies tenuous in the assessment of the carcinogenic hazards of exposures to mineral oils.

Lung cancer is the most extensively investigated cancer in these occupations. While there are several studies supporting an association with exposed workers in certain occupations (Coggon *et al.*, 1984; Rønneberg *et al.*, 1988; Acquavella *et al.*, 1993; Droste *et al.*, 1999; Zhao *et al.*, 2005), the exposures are not generally to mineral oils alone, given the use of other compounds in the metal-working trade (see Table 2.9, online), the most detailed study did not show an association (Schroeder *et al.*, 1997; Eisen *et al.*, 2001), and smoking was not controlled for in some of the studies that did show an association (Coggon *et al.*, 1984; Zhao *et al.*, 2005; see Table 2.10 online).

The case–control studies have typically been better able to address confounding, whereas the cohort studies have tended to examine exposure in more detail. However, even in the most sophisticated studies, it was not possible to isolate highly-treated from untreated or mildly-treated oils, nor are mineral oils used in isolation from other agents. As noted in several reviews (Tolbert, 1997; Woskie *et al.*, 2003), in metal-working there is concomitant exposure to a range of chemicals including biocides, metal dusts, and by-products of oil heating. Even in the study of the Michigan automobile-manufacturing workers – the most detailed study of metal-workers – the authors identified categories of exposure to straight-chain and soluble lubricating fluids, but were not able to separate or characterize mineral-oil exposures in particular (Eisen *et al.*, 1992).

2.4 Synthesis

There is consistent evidence that untreated or mildly-treated mineral oils cause cancer of the skin, specifically of the scrotum, in humans. The association is highly unlikely to be due to chance, bias, or confounding, given the large case series, supportive epidemiological studies, the rarity of scrotal cancer, and the intensity of exposure during the period of interest.

There were insufficient data regarding cancer at other sites to draw conclusions on the carcinogenicity in humans for untreated, mildly-treated, or highly-treated mineral oils. Recent studies did address highly-treated mineral oils, but were limited in their assessment of this agent, instead addressing all aspects of the work environment. Exposure to mineral oils in metal-working and other industries has not been easy to assess, and other agents of concern are known to be present in such work environments. Given these limitations in assessing exposure to mineral oils, and the lack of consistency in study findings by cancer site, the evidence for carcinogenicity of highly-treated mineral oils remains insufficient to draw conclusions.

3. Cancer in Experimental Animals

Petroleum-derived base oils and formulated products have been tested for their potential carcinogenicity in mice and other experimental animals, by skin application, in feeding studies, by inhalation exposure, and by subcutaneous and intra-peritoneal injection. In *IARC Monograph* Volume 33 (IARC, 1984), the Working Group divided petroleum materials into eight classes plus two subclasses (6.2, 7.2) based on the extent of refinement. Class 8 covers petroleum-derived materials not otherwise classified, and this category is not considered in this *Monograph*. This categorization scheme is still useful and applied here despite the fact that the terminology used

to describe the material tested in recent studies may not be easily conducive to allocate it to a given class. Table 3.1 presents the most representative animal cancer bioassays with mineral oils evaluated in *IARC Monograph* Volume 33 (IARC, 1984), as well as studies published since that time.

3.1 Earlier studies

Vacuum-distillate fractions [class 1], either naphthenic or paraffinic in nature, produced a significant skin-tumour response. De-waxing of these distillates did not appreciably alter their activity (Halder *et al.*, 1984; IARC, 1984; Kane *et al.*, 1984). Early studies demonstrated that both light and heavy fractions of paraffinic oils induced benign and malignant skin tumours (Twort & Ing, 1928). De-waxed paraffinic distillates induced both benign and malignant skin tumours in mouse skin (Gradiski *et al.*, 1983). Jute-batching oil induced benign and malignant skin tumours and promoted tumours in mice pre-treated with 7,12-dimethylbenz[*a*]anthacene (Roe *et al.*, 1967).

Acid-treated oils [class 2] of either naphthenic or paraffinic origin, induced benign and malignant skin-tumour responses (Twort & Lyth, 1939; Bingham *et al.*, 1965; Bingham & Horton, 1966), unless severe acid treatment had been applied (Twort & Lyth, 1939).

Solvent-refined oils (raffinates) [class 3], either naphthenic or paraffinic in nature, generally did not produce skin tumours (Gradiski *et al.*, 1983). However, in one study skin application of solvent-extracted paraffinic oil induced one malignant tumour, which suggests possible skin tumour-inducing activity (Doak *et al.*, 1983).

Hydro-treated oils [class 4], principally paraffinic in nature, induced a moderate incidence of skin tumours when treatment of the distillates was mild (Halder *et al.*, 1984; Kane *et al.*, 1984), while no tumour was induced by severely hydro-treated oils. The combination of mild hydro-treatment and solvent extraction appears to reduce or eliminate skin tumorigenicity.

White oils and petrolatums [class 5], which are produced from oils that have undergone the most severe acid and/or hydro-treatment, showed no activity in the skin-tumour bioassay (Doak *et al.*, 1983). Single subcutaneous injection of three different grades of medicinal petrolatum into mice induced no treatment-related tumours during the following 18 months (Oser *et al.*, 1965). Similarly, a lifetime study in rats involving subcutaneous injection of liquid paraffin and yellow petrolatum did not show local tumours, except a single osteosarcoma near the site of yellow petrolatum injection (Schmähl & Reiter, 1953). Intra-peritoneal injection of two highly refined food-grade mineral oils into certain strains of mice induced plasma-cell neoplasms and reticulum-cell sarcomas (Potter & Boyce, 1962; Bober *et al.*, 1976). Mice receiving repeated intra-peritoneal injections of liquid paraffin developed peritoneal reticulum-cell sarcomas, plasma-cell leukaemia, myeloid leukaemia and lymphocytic leukaemia (Rask-Nielsen & Ebbesen, 1965). In two feeding studies in which three different samples of medicinal-grade petrolatum and liquid paraffin were fed to rats for two years at either 2% or 5% of the diet, no significant increase in tumour incidence was observed (Schmähl & Reiter, 1953; Oser *et al.*, 1965). Although the experimental design was considered inadequate and the exposure period was short, inhalation of light white naphthenic aerosol (100 mg/m^3) by mice, rats, hamsters, and rabbits from 6–13 months did not produce a significant increase in tumours in any of the species tested (Wagner *et al.*, 1964).

Solvent extracts [class 6.1], which are by-products of solvent refining and sometimes called aromatic oils, induced a significant increase in incidence of skin tumours (Gradiski *et al.*, 1983). The same response was produced with highly concentrated aromatic extracts of medicinal-grade petrolatums (Kane *et al.*, 1984).

Table 3.1 Carcinogenicity studies of mineral oils in experimental animals

Species, strain (sex) Duration Reference	Route Oil Type Dosing regimen, Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rats, Fisher (M, F) 104 wk Shoda *et al.* (1997)	Oral administration of 0, 2.5 or 5% liquid paraffin (class 1) in the diet. Controls received basal diet 50 animals/sex/group	All neoplastic lesions were similar to those that occur spontaneously; no significant difference was detected.	NS	Granulomatous inflammation was observed on the mesenteric lymph nodes, but this was not associated with any neoplastic lesion.
Chester-Beatty stock mice (M) 84 wk Roe *et al.* (1967)	Skin application 14 applications of 0.25 ml of JBO (class 1) during 9½ wk Untreated, JBO, DMBA, JBO + DMBA 24 animals/group	Mice with malignant tumours Untreated 0/24, JBO 6/24*, DMBA 2/24, JBO + DMBA 11/24**	* $P = 0.01$ ** $P = 0.004$	DMBA Benzo[*a*]pyrene content of JOB < 1 mg/kg.
Mice, C3H, C57BL, and Rockland Farm (M, F) 310 d Gilman & Vesselinovitch (1955)[1]	Skin application of soluble cutting oil (class 7.1) 3×/wk Different mouse strains were untreated or treated with 1 of 2 undiluted or diluted oils containing additives. 20–40 animals/group	Oil, strain:% mice with skin tumours,% carcinomas Controls: 0,0 1 Undiluted, C3H: 61,22 1 Diluted, C3H: 19,3 2 Undiluted, C3H: 58,19 2 Undiluted, C57BL: 27,3 2 Undiluted, RF: 33,7	NR	Sulfurized mineral-oil base from straight-run distillate in 40% water emulsion. Usually further diluted with eight parts of water before use.
Mice, CFLP (F) 104 wk Grimmer *et al.* (1982b) [1]	Skin application of used gasoline-engine oil (class 7.2), twice/wk of 0.1 ml of 3:1 mixture of acetone/ cyclohexane containing doses of 0, 0.625, 1.875, 5.625 mg of engine oil artificially aged 65 animals/group	Papillomas/carcinomas,% tumour-bearing mice 0/1, 1.5 3/0, 4.6 8/9, 26.6 14/29, 69.4	Dose-related increase $P < 0.01$	No comparison with unused oil, but the strong dose-response and the large number of animals per group are noted
Mice, Swiss Albino (F) 1 d initiation, 30 wk promotion study Agarwal *et al.* (1985)	Topical application of JBO (class 1) and six of its reconstituted fractions. Mice were initiated with single application of 100 µl of vehicle, JBO, or one of six reconstituted fractions. This was followed by 5 µg TPA 3×/wk for 30 wk 10 animals/group	Treatment: papilloma + keratoacanthoma/mice Untreated: 0/10 Vehicle control: 0/9 JBO: *4/7 (57%) 6 Fractions: 4/9 (44%), 0/3, 0/5, 1/9 (11%), 2/6 (33%), 3/8 (37%) DMBA (positive control): 7/7 (100%)	*[$P = 0.01$]	Tumours were considered benign. JBO obtained from Indian refinery. Chemical analysis not given, but physicchemical characteristics were provided. Fourteen original fractions combined to six, which were tested in a tumour initiation–promotion assay. Cause of death of missing animals not indicated.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Oil Type Dosing regimen, Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, Swiss Albino (F) 40 wk, plus 14 wk tumour promotion. Agarwal *et al.* (1988)	Topical application of 50 µl JBO-P (Class 1), one of four JBO-P fractions or vehicle 3×/wk for 40 wk. JBO-P fractions were designed to limit PAH type content. Controls and three groups treated with fractions were further treated 3×/wk for 14 wk with 5 µg TPA; 20 animals/group	Oil: tumour incidence (+TPA) Untreated: 0/20 (0/12) Vehicle: 0/20 (0/14) JBO-P: 12/20 JBO-P reconstituted: 10/20 JBO-P PAH free: 2/20 (0/7) JBO-P 2–3-ring PAH: 0/20 (0/9) JBO-*P* > 3-ring PAH: 0/20 (7/7)	[NS]	All tumours were considered benign and identifiable as squamous-cell papillomas and keratoacanthomas. Source of JBO same as in Agarwal *et al.* (1985).
Mice, Swiss EOPS (F) 18 mo Gradiski *et al.* (1983) [1]	Topical application of 0.05 ml, 3×/wk for 1 mo and 0.05 ml, twice/wk for 11 mo of paraffinic distillates (class 1), Mineral ("White oil" (F) and 5 (A-E) solvent extractions (class 6) of Middle East crude oil containing different levels of PAHs and benzo[*a*]pyrene Oil,% PAH/B[*a*]P ppb A, 43.5 /1100; B, 9.15/270 C, 3.08/33; D, 2.06/1; E, 0.80/0.2; F, 0.26/NP Groups A-F, 30 animals/group; 60 controls	Tumours classified as benign, malignant, or benign & malignant for oils A-F Controls: 0, 0, 0; A: 10, *5, 10; B: 8, 2, 3; C: 1, 0, 0; D: 0, 0, 0 E: 0, 0, 0; F: 0, 0, 0 Aromatic extract (class 6.1) produced 15 malignant tumours in 30 mice	*[$P = 0.0032$]	Malignant tumours were squamous cell carcinomas, sarcomas, and mixed tumours. Oil refinement considered same as commercially used in mineral-oil production. Dermal application of oils had marked irritating effect. Benign tumours were acanthosic, papillomatous, hyperkeratotic.
Mice, SPF, CFLP (F) 104 wk Grimmer *et al.* (1982a)[1]	Topical application of 0.1 ml, twice/wk of used engine oil (class 7.2) 1) Untreated 2) Solvent, 3) Used oil, 0.6, 1.8, 5.6 mg 4) PAH free, 0.5, 1.7, 5.1 mg 5) PAH (2, 3) 0.04, 0.1, 0.4 mg 6) PAH (> 3 ring) 6, 20, 60 ug 7) Recons oil, 0.6, 1.8, 5.6 mg 8) B[*a*]P 3, 7, 15 ug Mice/group at start (NR), total 1300 animals	Papilloma/Carcinoma 1) 0/0 2) 0/1 3) 3/0, 8/9, 14/29 4) 0/1, 0/2, 1/0 5) 2/2, 1/1, 4/2 6) 0/0, 7/2, 20/13 7) 1/2, 4/2, 16/19 8) 6/20, 6/44, 6/54	Tumour induction time using log-rank test and Wilcoxon test show significant effect of used oil, >3-ring PAH, reconstituted oil, and B[*a*]P. *P*-value not provided.	Oil aged in gasoline-driven car. Oil dissolved in cyclohexane and PAH extracted with nitro-methane. Tumours occurred only at site of treatment and were described as papillomas and carcinomas. Authors concluded that >3-ring PAH content accounted for 70% carcinogenicity and B[*a*]P content accounted for 18%. Tumour incidence may be misleading as the number of mice/group was unknown.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Oil Type Dosing regimen, Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, Swiss (F, M) Lifespan Lijinsky *et al.* (1966)[1]	Skin application of 60 µl petrolatum or 20 µl fractionated solutions in iso-octane Amber Petrolatum (National Formulary grade) (class 5) twice/wk fractionated into aliphatic and aromatic fractions. Controls received 20 µl iso-octane 30 treated animals/group; 50 controls	Material: Total tumours/carcinomas (F, M) Isooctane: 0/0, 2/1 Petrolatum: 2/0, 3/0 Filtrate: /0, 0/0 Adsorbate: 26/9[a], 3/0 Nitromethane: 10/1, 31/9[b] Cyclohexane: 8/5[c], 4/1	[a][P < 0.0001; carcinomas (F)] [b][P < 0.0001; carcinomas (M)] [c][P < 0.0059; carcinomas (M)]	Skin tumours were papillomas, kerato-acanthomas and malignant squamous-cell carcinomas. Internal tumours within limits of controls.
Mice, C3H (M) 24 mo. McKee *et al.* (1989)	Skin application of 37.5 µl of naphthenic crude oil (class1), light and heavy vacuum distillates of the crude, and hydro-treated (mild or severe) (class 4) products, twice/wk for 24 mo. White mineral oil (- control) B[*a*]P (+ control) 1. unrefined light 2. hydro-treated light mild 3. hydro-treated light severe 4. unrefined heavy 5. hydro-treated heavy mild 6. hydro-treated heavy severe 7. unrefined heavy (viscous) 8. hydro-treated heavy mild 9. hydro-treated heavy severe 40 animals/group	Number of mice with carcinoma/papilloma White mineral oil: 0/0 B[*a*]P: 21/1* 1. 20/0* 2. 0/0 3. 0/0 4. 21/3* 5. 0/0 6. 0/0 7. 1/1 8. 0/0 9. 0/0	*[$P < 0.0001$]	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Oil Type Dosing regimen, Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, Swiss (F) 15 wk Mehrotra *et al.* (1987)	Topical application of JBO-C (class 1) 3×/wk for 15 wk Tumour promotion assay groups: 1. Untreated 2. Acetone, 0.1 ml 3. JBO-C, 50 µl 4. TPA, 15 nmole in 0.1 ml acetone 5. Urethane, 1mg/g bw in 50 µl saline 6. Urethane+JBO-C 7. Urethane+TPA 8. 3MC, 4 µg/g 9. 3MC once+JBO-C 10. 3MC once+TPA 15 animals/group	Mice in groups 1, 2, 3, 4, 5, and 7 did not develop tumours. Group 6 had 3 squamous cell papillomas and 1 keratoacanthoma. Group 7 had 3 squamous-cell papillomas and 2 keratoacanthomas. Group 9 had 2 squamous-cell papillomas, 1 keratoacanthoma, and 1 malignant fibrosarcoma. Group 10 had 1 fibroma and 2 keratoacanthomas.	Probability of tumour development was determined as $P = r/n$ (r = mice with tumours, n = total of mice). Statistical methods described, but no P-value provided. Results of statistical analysis not presented.	TPA or 3MC were administered once subcutaneously as initiators. Groups 2, 3, 4 promotion; 5, 6 initiation; 4, 5, 9, 10 initiation+promotion. Although not stated that results were statistically significant, authors concluded that JBO-C acted as tumour promoter following initiation by 3MC or urethane.
Mice, Swiss (F) Study 1, 20 wk; study 2, 14 wk Mehrotra *et al.* (1988)	Topical application JBO-P (class 1) 3×/wk for 20 wk (Exp. 1) or 14 wk (Exp. 2) Experiment 1 a. 50 µl acetone b. 5 µl B[*a*]P in 50 µl acetone c. 30 µl neat JBO-P Experiment 2 a. 1mg/g urethane in 50 µl saline, single s.c. b. ureth.+30 µl paraffin c. ureth.+30 µl crot oil d. ureth.+30 µl JBO-P e. 30 µl neat JBO 10 animals/group	Experiment 1 a. 0/10 b. 3 /9 (33%) c. 8/9 (89%) Experiment 2 a. 0/10 b. 0/10 c. 6/10 (60%) d. 5/9 (56%) e. 2 /10 (20%)	NR	Most tumours are benign squamous-cell papillomas and keratoacanthomas. One JBO-P and 2 B[*a*]P exposed mice developed malignant squamous-cell carcinomas. Results indicate that JBO-P is tumorigenic and acts as a tumour promoter. The short duration of the exposure was noted.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Oil Type Dosing regimen, Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, C3H, (M) 2 yr Nessel *et al.* (1998)	Topical application of mineral oil (MO), heavy (class 5) clarified oil (HCO), straight-run kerosene (SRK), straight-run gas oil (SRGO), light cycle oil (LCO) 50 µl of MO or HCO at 100% 2x/wk 50 µl of SRK, SRGO, LCO at 100% 2x/wk, at 50% 4x/wk, or at 28.5% 7x/wk 50 animals/group	MO and SRK 0 HCO, 42 carcinomas, 3 keratoacanthomas, 37 papillomas SRG, 8 carcinomas, 4 fibrocarcinomas SRGO, 2 carcinomas, 3 papillomas LCO, 12 carcinomas, 5 fribrosarcomas, 14 papillomas	sHCO, SRK, and LCO, $P < 0.01$ SRGO, $P < 0.05$	Large, well-designed study addressing the role of dermal irritation in carcinogenesis. Diluted oils produced reduced irritation and fewer tumours with significant tumour formation in 100% or 50% solutions only. Results support conclusion that non-irritating oils with low PACs are not tumorigenic
Mice, CD-1 (M) 54 wk Nessel *et al.* (1999)	Topical application of C10-C14 normal paraffins (NP) (class 1); steam-cracked gas oil (SCGO); light refined paraffinic (LRPO), or jet fuel (JF) Tumour promotion initiated with 25 µg DMBA and promoted with 25 µl TPA (+ control) or 75 µl of NP at 100% twice/wk, at 50% 4×/wk, or at 28.5% 7×/wk, or 75 µl SCGO, LRPO, or JF at 100% twice/wk or at 28.5% 7×/wk 30 animals/group	Control 0/30 DMBA + TPA 29/30 (97%) ** DMBA 1/30 NP 15/30 (50%) **, 1/30 (3%), 3/30 (10%) SCGO 17/30 (56%) **, 9/30 (30%) ** LRPO 7/30 (23%) *, 0/30 JF 11/30 (37%) **, 0/30	*$P < 0.05$ vs control ** $P < 0.01$ vs control	Large, well-designed tumour-promotion study. Undiluted (100%) solutions more irritating and tumorigenic than diluted solutions (28.5%). Most tumours were papillomas and to a lesser extent squamous-cell carcinomas
Mice, DBA/2, CBA (F) 24 mo Rask-Nielsen & Ebbesen (1965)[1]	Intra-peritoneal injections of high-visocosity oil (Primol D & Bayol F) (class 5) Three injections of 0.5 ml Primol D to mice at 10, 15, and 21 wk 36 DBA/2 and 12 CBA	DBA/2 mice: 15 (42%) had peritoneal-cell sarcomas, 3 had plasma-cell leukaemia, 3 myeloid leukaemia, 2 lymphocytic leukaemia. CBA mice: 1 had reticulum-cell sarcoma, and 1 had lymphocytic leukaemia	NR	No mention of controls IC and C3H mice injected with Bayol F developed oil-granulomas but not plasma-cell tumours (Hermann, 1966)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Oil Type Dosing regimen, Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, BALB/c (F) 14 mo Potter & Boyce (1962)[1]	Intraperitoneal injections of two refined mineral oils US Pharmacopeia (Bayol F and Primol D) (class 5) Mice received Bavol F as single injection of 0.4 or 0.5 ml, or three injections of 0.5 ml at intervals of two months. 40 or 32 animals/group One group of >56 mice received injections of Primol D	Bavol F: 8, 2, 22 plasma-cell neoplasms in each of the three groups receiving injections. Primol D: plasma-cell neoplasms in 13/56 (23%) mice Corn-oil control: no neoplasms	NR	Tumour morphology same as those induced by mixture of Freund's adjuvant and *Staphylococcus* (Potter & Robertson, 1960) Tumours appeared to arise from mesenteric oil-granulomas (Potter & MacCardle, 1964)
Mice, BALB/c (F) 7 or 12 mo Bober *et al.* (1976)[1]	Intra-peritoneal injections of mineral oil Primol D (class 5) Mice received three 0.5 ml injections of oil or saline. Two wk after oil injection mice were immunized with plaque-forming units of bacteriophage T2	Plasma-cell tumours from Primol D alone: Experiment 1, 0/21 Experiment 2, 7/27 (26%)		Injections of bacterial endotoxins enhanced the incidence of tumours in BALB/c mice injected with Primol D. Effect of oil alone was inadequately described. Experiments 1 and 2 differed on source of mice and study duration; 7 and 12 mo, respectively.

bw, body weight; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; JBO, jute-batching oil (mineral oil obtained from the distillation of crude petroleum); M, male; MC, methylcholanthrene; mo, month or months; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; vs, versus; wk, week or weeks

High-boiling fractions from catalytically cracked oils [class 6.2] – also classified as aromatic oils – induced increasing numbers of skin tumours in mice with increasing boiling-ranges above 370°C. Further fractionation established that the activity is maximal for oils boiling at 500–520°C and is concentrated in the aromatic fraction of the oils. High-boiling, catalytically cracked oils also produced skin tumours in rabbits and monkeys (Smith *et al.*, 1951). Additional studies confirmed the increased tumorigenic activity of oils with boiling ranges 370–500°C. Promoting activity was also detected for some oil fractions in mice and rabbits (Shubik & Saffiotti, 1955; Saffiotti & Shubik, 1963).

Unused gasoline-engine oil [class 7.1] applied in several studies to mouse skin did not give a tumorigenic response with the exception of one single tumour (Saffiotti & Shubik, 1963; Kane *et al.*, 1984). Mice exposed via inhalation to unused diesel-engine oil for 11 months exhibited no increase in tumour incidence (Lushbaugh *et al.*, 1950). In contrast, unused cutting oils (also class 7.1), which are often formulated products consisting of blends of base oils and chemical additives, produced skin tumours (Gilman & Vesselinovitch, 1955; Jepsen *et al.*, 1977).

Used gasoline-engine oil [class 7.2] had stronger tumorigenic activity than unused oil; solvent extraction of polyaromatic hydrocarbons almost eliminated the tumorigenic activity (Grimmer *et al.*, 1982a). Similarly, used cutting oil [also class 7.2] tended to be more active than a comparable, unused oil (Gilman & Vesselinovitch, 1955; Jepsen *et al.*, 1977).

3.2 Studies published since the previous evaluation

Studies conducted since *IARC Monograph* Volume 33 (IARC, 1984) show a marked improvement in experimental design and reporting, and confirmed previous findings. Exposure to batching oil [class 1] induced benign and malignant skin tumours and promoted tumour formation in mice pre-treated with urethane (Mehrotra *et al.*, 1987; 1988). Subsequent studies with the same oil reported only benign tumours in mice treated with jute-batching oil, which occurred with and without pre-treatment with 7,12-dimethylbenz[*a*]anthacene (Agarwal *et al.*, 1985). Additionally, in mice treated dermally with fractions of the same jute-batching oil for 40 weeks, the PAH-containing fractions induced tumours only when treatment was followed by 12-*O*-tretradecanoylphorbol-13-acetate (Agarwal *et al.*, 1988). Unrefined naphthenic crude oil applied to the skin of mice induced papillomas and carcinomas (McKee *et al.*, 1989). Dietary exposure to liquid paraffin did not induce an increased tumour incidence (Shoda *et al.*, 1997).

Both mild and severe hydro-treatments of naphthenic oils [class 4] eliminate the tumorigenicity, which is associated with a decrease in PAH content (McKee *et al.*, 1989). Despite the association, PAH content alone was not considered predictive of carcinogenicity.

Most tumours observed after exposure to white oils and petrolatums [class 5] produced from oils that have undergone the most severe acid and/or hydrogen treatment, were papillomas and to a lesser extent squamous cell carcinomas (Nessel *et al.*, 1998, 1999).

4. Other relevant data

4.1 Humans

A group of 31 male glassmakers (smokers and non-smokers) and a group of suitably matched controls exposed to aerosols of mineral oils were examined for chromosomal abnormalities in peripheral blood lymphocytes. Chromosomal damage, including chromatid breaks, chromosome breaks, and chromosome exchanges

(di-centrics and reciprocal translocations) were increased in the exposed workers compared with controls (Srám *et al.*, 1985).

Workers from a cold-rolling steel plant (smokers and non-smokers) exposed to mineral oils were examined for mutagenic activity in the urine by means of *Salmonella typhimurium* strain TA98 in the presence of an exogenous source of metabolic activation. There was a significant difference in urinary mutagenicity between the exposed workers and the controls. While among non-smokers the mutagenic activity was not increased in exposed compared with unexposed workers, the overall results suggested a synergistic effect of smoking and exposure to mineral oils (Pasquini *et al.*, 1985).

Overall, there is weak evidence on the mechanism underlying the effects of exposures to mineral oils in humans. This evidence is based on genotoxic (mutagenic) activity of mineral oils in bacteria and a single cytogenetic study of glassworkers exposed to aerosols of mineral oils.

4.2 Experimental systems

Samples of vacuum distillates (class 1), solvent-refined oils (class 3), hydro-treated oils (class 4), used hardening oil (class 7.2) and used crankcase oils (class 7.2) were mutagenic in *Salmonella typhimurium* in the presence and absence (class 7.2 only) of an exogenous source of metabolic activation. Samples of a white oil (class 5), a refined steel-hardening oil (class 7.1) and of unused crankcase oils (class 7.1) were not mutagenic in *S. typhimurium* strain TA98 in the presence or absence of exogenous metabolic activation (IARC, 1984, 1987).

Naphthenic distillates (raw or acid-treated) were tested for mutagenicity in the *S. typhimurium* assay in the presence and absence of an exogenous source of metabolic activation. With metabolic activation, both untreated and acid-treated naphthenic distillates were mutagenic. The naphthenic distillates contained approximately 12% (w/w) polycyclic aromatic hydrocarbons (Granella & Clonfero, 1991). A series of 15 high-viscosity mineral oils obtained from naphthenic distillates, including used, recycled and pooled oils, were examined for mutagenic activity in the *S. typhimurium* assay with strains TA98 and TA100 in the presence and absence of an exogenous source of metabolic activation. Four of the samples (three acid-treated naphthenic oils and one recycled fraction of a used oil) showed significant mutagenic activity in the presence metabolic activation (Granella *et al.*, 1995).

An extensive evaluation of the mutagenic activities of 13 mineral oils obtained from various processes was conducted with a modified *S. typhimurium* assay. The modification consisted of extracting the mineral-oil samples with dimethyl sulfoxide and increasing the concentrations of both $NADP^+$ and the liver post-mitochondrial fraction (S9). The mutagenic activities of the mineral-oil samples were significantly correlated with the amount of 3–7-ring polycyclic aromatic compounds for a subgroup of oil samples (Blackburn *et al.*, 1984; Roy *et al.*, 1988).

Six mineral-oil samples were evaluated in a *S. typhimurium* assay activated with washed liver microsomes from Aroclor-1254-induced rats. Five of the six samples were mutagenic. The results showed an empirical correlation between increasing mutagenicity index, carcinogenicity and the polycyclic aromatic hydrocarbon content of the oils (Brooks *et al.*, 1995).

Three mineral-oil samples (N11, N1, and R1) and several fractions of the N1 oil – obtained from silica-gel column chromatography and high-performance liquid chromatography – were evaluated by means of ^{32}P-postlabelling for their ability to form DNA adducts after a single dermal application on the skin of TO mice. Both the R1 and N1 oils had formed unidentified DNA adducts at 24 hours. The 2–3-ring fraction produced more adducts than the 4–6-ring fraction of N1. The adduct levels first increased

and then decreased with time after treatment (Ingram *et al.*, 2000).

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of untreated or mildly treated mineral oils. Untreated or mildly treated mineral oils cause cancer of the skin (observed in the scrotum).

There is *sufficient evidence* in experimental animals for the carcinogenicity of untreated vacuum distillates, acid-treated oils, and aromatic oils, including extracts from solvent treatment of distillates and the high-boiling fraction of catalytically cracked oils [classes 1, 2 and 6].

There is *sufficient evidence* in experimental animals for the carcinogenicity of mildly hydrotreated oils [class 4].

There is *sufficient evidence* in experimental animals for the carcinogenicity of used gasoline-engine oil [class 7.2].

There is weak evidence on the mechanism underlying the effects in humans of exposures to mineral oils. This evidence is based on genotoxic (mutagenic) activity of mineral oils in bacteria and a single cytogenetic study of glassworkers exposed to aerosols of mineral oils.

Untreated and mildly treated mineral oils are *carcinogenic to humans (Group 1).*

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SHALE OILS

Shale oils were considered by previous IARC Working Groups in 1984 and 1987 (IARC, 1985, 1987). Since that time, new data have become available, which have been incorporated into this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 68308-34-9
Chem. Abstr. Serv. Name: Shale oils

Crude shale oil is the product of thermal processing of raw oil shale. Oil shale is sedimentary rock containing mainly mineral components and organic matter called kerogen, which has a low solubility in organic solvents. Oil shale has a low boiling-point and it produces liquid organic products (oils) on thermal decomposition. Crude shale oils differ principally from crude petroleum in that they contain higher concentrations of organic nitrogen compounds and arsenic. Materials encountered in oil-shale processing include raw oil shale, crude shale oil, spent shale, oil-shale ash, synthetic crude oil (or 'syncrude') and refined products. Operations include retorting, upgrading and refining (IARC, 1985).

Crude shale oils are viscous, waxy liquids made up of hydrocarbons (alkanes, alkenes and aromatic compounds) and polar components (organic nitrogen, oxygen and sulfur compounds). Crude shale oils are very complex mixtures, and only few of the compounds have been identified.

To recover the oil from the shale, its organic portion (kerogen) must be decomposed thermally. This thermal decomposition, known as retorting, converts the solid organic material of the shale into liquid and gaseous fractions and a solid carbonaceous residue. The liquid fraction, the so-called shale oil, consists of condensable hydrocarbons (C_{5+}) and small quantities of decomposition water. The gaseous product is a mixture of carbon monoxide, carbon dioxide, hydrogen, nitrogen, hydrogen sulfide, methane, and other hydrocarbons (C_{4-}). The carbonaceous residue, a coke-like material, is obtained in a mixture with the inorganic minerals of the original oil shale (Weiss, 2005).

1.2 Uses

Oil shales occur in many parts of the world. The areas where they are found range in size from small occurrences of little or no economic value to thousands of square miles. Oil shales also differ in geologic age, from Cambrian (570–500 million years ago) to Tertiary (65–2 million years ago). Total world resources of extractable oil shale are conservatively estimated at 2.6 trillion barrels (AAPG, 2009).

Early applications of shale oils included use as a source of paraffin waxes and burning oils for lamps, as well as for medicinal purposes. Later on, shale oils were used to prepare gasoline, diesel oils and lubricants from its light, intermediate and heavy distillates, respectively. The fuel oils, representing the major part of liquid products from the internal combustion (gas-generator type) retorts, have been used in the manufacture of gas-turbine fuel oil, automobile gasoline, and additives for high-sulfur petroleum fuel oils. (Aarna, 1978; Öpik & Kaganovich, 1981). In the People's Republic of China, shale oil has been used to generate electric power and as a refinery feedstock (Dickson, 1981). Products included gasoline, kerosene, diesel fuel and coke (Qian, 1982; IARC, 1985).

Oil-shale industries are operating in Europe (Estonia), South America (Brazil) and Asia (China). The largest operations are in Estonia, where approximately 12 million tonnes of oil shale is mined annually (underground and open-pit mining). About 85% of this material is burned as fuel in electric power-plants in north-eastern Estonia; the remainder is retorted for shale oil and used to manufacture fuels and petrochemicals. In Brazil, oil shale is mined in open pits and is retorted for shale oil, liquefied petroleum gas, sulfur and fuel gas (AAPG, 2006).

1.3 Human exposure

According to the US National Occupational Exposure Survey (1981–83), approximately 350 workers (including approximately 150 women) were potentially exposed to shale oil (NIOSH, 1990).

Very few studies were identified that assessed occupational exposures to shale oil. Most studies of this industry assessed exposures to other contaminants: free crystalline silica in the form of quartz; asbestos (Kangur, 2007), diesel exhaust (Scheepers *et al.*, 2002; Muzyka *et al.*, 2003, 2004; Knudsen *et al.*, 2005), trace elements (arsenic, cadmium, lead, mercury, nickel), hydrogen sulphide, uranium and radon, carbon monoxide, phenol, polynuclear aromatic compounds, such as benzo(*a*)pyrene and 1-nitropyrene (Kuljukka *et al.*, 1996, 1998; Anderson *et al.*, 1997; Boffetta *et al.*, 1997; Kivistö *et al.*, 1997; Scheepers *et al.*, 2002, 2003), aromatic compounds, such as benzene and toluene (Anderson *et al.*, 1997; Kivistö *et al.*, 1997; Marcon *et al.*, 1999; Scheepers *et al.*, 2002; Sørensen *et al.*, 2004), sulfur dioxide, and dust in shale mines, retorts, tips and brick-works (Louw *et al.*, 1986).

2. Cancer in Humans

Most of the literature addressing the carcinogenicity of shale oils dates back to the early part of the 20th century, with compelling evidence from case series of skin cancer, particularly of the scrotum, in the United Kingdom. These case series are substantial in scale, including 65 cases of scrotal cancer that occurred in the period 1900–21 in the Scottish shale-oil industry (Scott, 1922a, b). Exposure of cotton-textile workers (mule spinners) exposed under non-hygienic conditions to lubricating oil that included shale oil during varying time periods, was strongly associated with cancer of the scrotum (Southam, 1928; Brockbank, 1941). Even in the absence of methodologically rigorous epidemiologic studies, this association has become accepted as causal.

As the oil-shale industry has risen and fallen over time in various parts of the world, a modest epidemiological literature has been developed. The size and prospects for the industry have fluctuated dramatically over time in relation to the price of oil, resulting in a haphazard approach to epidemiology that addresses the health of workers in the industry. Furthermore, varying technologies for releasing the oil from the shale have different implications for exposures of

the workforce. A morbidity survey of several hundred workers in the USA employed at an oil-shale demonstration facility suggested an increased risk for abnormal sputum cytology and no excess of skin cancer (Rom *et al.*, 1985).

The most extensive epidemiological study of cancer among oil-shale workers addressed causes of death among 6359 Scottish workers employed in the period 1950–62 and followed for vital status through 1982 (Miller *et al.*, 1986). The focus was on the subset of 3161 men who worked in mining, retorting, or refining, with maximum potential exposure to shale oil. A total of 1868 of these men died, 802 before the start of mortality follow-up and 1066 during the follow-up, i.e. between January 1968 to December 1982. Mortality of the workers from lung, stomach, colon, rectum, bladder, and kidney cancers was similar to or lower than that of an external population. Only skin cancer, with six observed deaths, was in excess, with an estimated relative risk of 4.9 (95%CI: 2.2–10.9). This elevation in skin-cancer mortality is consistent with the case series noted previously. A more detailed evaluation of the exposure conditions for 212 lung-cancer cases compared with 221 men with other diseases, mostly cardiovascular problems, revealed no associations with occupational exposures. In contrast to the skin-cancer mortality excess, a survey of 1664 living workers from the same cohort revealed no increase in self-reported skin tumours (Seaton *et al.*, 1986). No lung-cancer excess was reported among workers in the shale-oil industry employed at a facility in central Sweden between 1942 and 1966. Comparison of 51 lung-cancer deaths with 206 referent deaths did not indicate any association of shale-oil exposure with lung cancer (Seldén, 1987).

Overall, based on the case series, with some corroborating epidemiological studies, there is evidence that exposure to shale oil is causally associated with skin cancer, particularly of the scrotum. Data pertaining to other cancer sites, including lung, remain inadequate to draw a conclusion on any association due to the limited research done thus far, the limited quality of the exposure data, and the poor precision of the estimates in the completed studies.

3. Cancer in Experimental Animals

A large number and a wide variety of animal studies have been conducted to analyse shale-derived oil and its precursors, or the by-products of shale-oil processing. The resulting picture is quite complex, as experimental results are affected by several factors, including: (1) the nature of the material under study (raw shale, spent shale, crude, hydro-treated or refined shale oil, retort process-water, or oil-shale ash); (2) the mineral composition of the original shale and the type of retort process; and (3) the fractionation procedure used to separate and/or characterize the active constituents of the complex mixture.

Table 3.1 includes some of the studies considered as the most representative of the carcinogenicity of shale oils as well as studies published since the previous evaluation (IARC, 1985).

3.1 Raw and spent oil-shale

3.1.1 Skin application

Solvent extracts from both raw and spent oil-shale containing benzo[*a*]pyrene were applied to mouse skin and induced skin papillomas and carcinomas (Berenblum & Schoental, 1944; Hueper, 1953; Rowland *et al.*, 1980; IARC, 1985).

3.1.2 Intratracheal administration

Crude shale oil and its aromatic fractions were enclosed in bee's wax pellets – which allow slow release of the content – and implanted in the lungs of rats. The substances induced a

Table 3.1 Carcinogenicity studies of shale-oils in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen, Shale oil Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Raw and spent oil shale				
Mouse, Swiss, (F) Lifetime Rowland *et al.* (1980)[a]	Dermal application of raw shale oil and TOSCO II retort 2.5 mg in 1/60 ml, twice/wk Raw shale oil contains 0.66 µg/ml B[*a*]P Spent shale oil contains 1.4 µg/ml B[*a*]P 50 or 100 animals/group	Control: 0/100; sham control: 0/50 Spent shale: 3/50 (6%) papillomas, *3/50 (6%) carcinomas; Raw shale oil: 0/50 Number of survivors not indicated	*[$P = 0.0356$ vs control]	Raw oil shale from Colony Oil shale development, Parachute Creek, Colorado
Rats, Wistar (F) 24 mo Dagle *et al.* (1990)	Intra-tracheal administration of 0.2 ml of beeswax pellet containing 0, 0.6, 6, 60 mg crude shale oil, three of its chemically derived fractions, or crude petroleum. Equal volumes of beeswax and tricaprylin were used in the vehicle-control pellets. 30 animals/group	Rats with lung epidermoid carcinomas at three doses Control, 0; Crude, 1, 3, 9; Neutral, 0, 1, 6; Basic, 2, 2, 8; PNA, 1, 9, 10 Number of survivors not indicated	$P < 0.05$	Crude shale oil from Anvil Points Mine, Colorado. Chemically derived fractions were: neutral, PNA, and basic. Number of effective rats not noted.
Rat, Fisher 344, sex (NR) 24 mo Holland *et al.* (1983)[a]	Inhalation of raw oil shale and spent oil shale, 0 or 90 mg/m^3 respirable fraction 5 h/d, 4 d/wk for 24 mo. Positive control, 10 mg/m^3 quartz 62 animals/group	Lung adenomas, SCC, or adenocarcinomas Control: 1/17 adenoma; sham control: 0/15; positive control: 4/57 (7%) adenomas, 13/57 (23%) carcinomas Raw: 1/50 (2%) adenoma; 11/50 (22%) carcinomas; spent: 3/57 (5%) adenomas, 10/57 (17%) carcinomas	[NS]	Shale from Anvil Points, Colorado No information on survival or latency period was provided.
Hamster, Syrian golden, sex (NR) 16 mo Holland *et al.* (1983)[a]	Inhalation of raw oil shale and spent oil shale 0 or 50 mg/m^3, 4 hr/d, 4 d/wk	No lung tumours in any of the groups. Number of survivors not indicated	–	Results are preliminary
Crude shale-oils from low-temperature retorting				
Mouse, strain A, C57BL, hairless, sex (NR) 20 mo Hueper (1953)[a]	Dermal application of two Green River crude shale oils, once/wk for one yr Unspecified amount in xylene (6 mo) then in ethyl ether (6 mo) 42 untreated strain A mice 1) NTU crude-shale oil 100 strain A, 25 C57BL 2) Fisher-assay crude-shale oil 50 strain A, 30 hairless	Skin tumours Untreated A, 0/42 1) A, 1/38 (3%) papilloma; C57BL, 4/19 (21%) squamous-cell carcinomas 2) A, 2/45 (4%) squamous-cell carcinomas; hairless, 1/10 (10%) squamous cell carcinoma	–	1) Nevada-Texas-Utah (NTU) retort processed at 538–816 °C 2) Fischer-assay retort processed at 371–538 °C No controls for C57BL and hairless mice

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Shale oil Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, C3H/He (F, M) 700 d Wilson & Holland (1988)	Dermal application of 2.5–5 mg shale derived crude oils in 50 µl of 60% acetone and 40% cyclohexane (v/v), 3 × /wk for 700 d. In addition to solvent-exposed controls there were untreated controls and B[*a*]P positive controls. 20 animals/sex/group	% M, F with skin papillomas and/or carcinomas at 2.5, or 5 mg 1) 95/85, 95/95 2) 70/75, 90/85 3) 60/35, 60/75 No tumours in the solvent- or untreated-control groups	$P < 0.05$, Significant differences between male and female occurred only in time to tumour (not shown)	Oak Ridge oil-shale repository: 1) PCSO, Anvil Points,Colorado 2) OCSO, Piceance Creek Basin, Colorado 3) PCSO-UP, hydrated PCSO
Mouse, A/Jax (M) 23–25 wk Smith & Witschi (1983)[a]	Intratracheal administration of paraho crude shale-oil 0, 500, 1250, 2500 mg/kg in corn oil, 3 × / wk for 8 wk 30 animals/group	Lung tumours 0, 7/16 (44%)*, 6/12 (50%)*, 3/5 (60%)*	*$P < 0.05$, all three exposed compared with control	
Shale-oil distillates, blends and other commercial products				
Mouse, C3H/HeN (F, M) 105 wk Clark *et al.* (1988)	Dermal application of seven shale-oil derived distillates, 25 mg, 3 × /wk for 21 wk Mineral oil (USP) was used on controls. Benzo[*a*]pyrene was used as a positive control. 25 animals/sex/group	Tumours at injection site Oil,% surviving mice with carcinoma/fibrosarcoma Control 0/2; benzo[*a*]pyrene 96/4; Crude oil, 50/4*; Hydro-treated oil, 0/0; Naptha, 12/4*; JP-4, 24/21*; Jet-A, 4/8*; Diesel, 1/1; Residum, 6/0*	*$P < 0.05$ carcinoma and/or fibrosarcoma	Hydrotreated Syncrude was prepared from crude shale oil retorted from shale oil in a procedure designed to simulate commercial production
Mouse, CC57Bl (M, F) Lifetime Bogovski *et al.* (1990)	Dermal application of crude shale oil or industrial residue produced from a blend of shale oils In benzene, 0 or 18 mg, twice/wk for 6 mo (50 times); one group received benzene and another was untreated 29 M and 40 F/group	Skin carcinomas: 0/61 (benzene control); 2/60 (3%) (crude shale oil); 3/56 (5%) (industrial residue)	NS	

[a] Summarized in IARC (1985)

B[*a*]P, benzo[*a*]pyrene; d, day or days; F, female; h, hour or hours; M, male; mo, month or months; NR, not reported; NS, not significant; SCC, squamous-cell carcinoma; vs, versus; wk, week or weeks; yr, year or years

dose-dependent increase in lung cancer (epidermoid carcinomas) (Dagle *et al.*, 1990).

3.1.3 Inhalation

An aerosol generated from a Write Dust Feed packed with a raw-shale sample from Anvil Points, Colorado, and one spent-shale sample from a direct-heated retort induced lung adenomas and carcinomas in rats during 24 months of exposure, but not in hamsters during 16 months of exposure (Holland *et al.*, 1983).

3.2 Crude shale oils from low-temperature retorting

3.2.1 Skin application

Crude shale oils from a variety of locations around the world and processed by heat transfer or retort combustion at temperatures below 1000 °C consistently induced squamous cell papillomas and carcinomas when repeatedly applied to the skin of mice (Hueper, 1953; IARC, 1985). Shale-derived crude oils and a hydrotreated product induced papillomas and carcinomas in mouse skin during nearly two years of treatment (Wilson & Holland, 1988).

The inner surface of rabbit ears painted with the heavy fraction of the generator (semi-coking) oil obtained from the Estonian oil-shale in gas generators at Kohtla-Järve induced squamous cell carcinomas in 8% of the rabbits. In one surviving rabbit, metastases of the carcinomas were found in the regional lymph nodes, the liver, and the lungs (Vahter, 1959; IARC, 1985)

3.2.2 Intratracheal administration

Intratracheal administration of a crude shale oil at three dose levels significantly increased the incidence of lung tumours in mice across all dose groups (Smith & Witschi, 1983; IARC, 1985).

3.3 Crude shale oils from high-temperature retorting

3.3.1 Skin application

Crude shale oils processed in chamber ovens above 900 °C induced squamous cell papillomas and carcinomas when applied to the skin of mice (Larionov, 1947; Bogovski, 1958, 1961; Turu, 1961; Bogovski & Vinkmann, 1979; IARC, 1985). Chamber-oven oil applied to the inner surface of rabbit ears resulted in multiple squamous-cell papillomas and keratoacanthomas and cornifying and non-cornifying squamous-cell carcinomas in 22% of the rabbits. In one rabbit, metastases in the lung and liver were found (Vahter, 1959; IARC, 1985).

3.4 Shale-oil fractions

Assessment of fractionations of shale oil were undertaken to determine the extent to which exposure to fractions containing known carcinogens such as benzo[*a*]pyrene correlates with carcinogenic activity.

3.4.1 Skin application

Chromatographic fractions of shale oil prepared by adsorption on aluminium oxide and elution with various solvents induced benign and malignant skin tumours in mice (Berenblum & Schoental, 1943; IARC, 1985). Chromatographic fractions of high-temperature (800–1000 °C, chamber-oven) shale oil were collected on silica-gel column and further fractionated into five fractions on aluminium oxide eluted with various solvents. Some of the fractions induced carcinomas and sarcomas with metastases in mice, and papillomas but not carcinomas in rabbits (Bogovski, 1961, 1962; IARC, 1985).

3.4.2 Subcutaneous and/or intramuscular administration

Intramuscular injection of various thermo-distillation products and multiple chromatography fractions of crude shale oil into the thigh of mice induced sarcomas at the site of injection (Hueper & Cahnmann, 1958; IARC, 1985). Chromatographic fractions of chamber-oven tar injected intramuscularly into the thigh of mice induced sarcomas at the injection site and lung tumours in some of the mice, which were also reported in historical controls (Bogovski, 1961, 1962; IARC, 1985).

3.5 Shale-oil distillates, blends and other commercial products

3.5.1 Skin application

Application to the skin of mice of individual distillates and blends of distillates from shale oil – including products such as 'green' oil, 'blue' oil, unfinished gas oil, machine lubricating oil, fuel oil, wood-impregnating oil, tar, bitumen, coke, and lacquer – induced papillomas, spindle-cell sarcoma, and squamous-cell carcinomas (Twort & Ing, 1928; Hueper, 1953; IARC, 1985). Heavy fractions of shale oils appeared to be more carcinogenic than light fractions. The latter induced only benign tumours while heavy fractions induced benign and malignant tumours with a shorter latency period (IARC, 1985).

Crude oil, naptha, and jet fuels derived form shale induced squamous-cell carcinomas and fibrosarcomas when applied to the skin of mice, whereas hydro-treated and diesel-distilled shale oil did not produce tumours (Clark *et al.*, 1988). Crude shale oil and industrial residue derived from a blend of shale oils induced two and three skin carcinomas (in 60 and 56 animals), respectively. No tumours were observed in controls (Bogovski *et al.*, 1990).

Tolichthtol, a product obtained from the acid residue of rectification of shale-oil aromatic fractions – containing up to 22% (w/w) sulfur compounds – did not induce tumours during 24 months after application to the skin (Vinkmann & Mirme, 1975)

3.5.2 Intratracheal administration

Shale-oil coke (a raw-shale distillation residue) did not produce tumours in Syrian golden hamsters after intra-tracheal instillation (Rowland *et al.*, 1980).

3.6 Synthesis

Inhalation of either raw oil shale or spent oil shale produced lung tumours in rats. Application of an extract of spent oil shale produced skin tumours in mice. Skin application of crude oils from both low- and high-temperature retorting induced skin tumours in mice and rabbits; the oils obtained from high-temperature retorting had higher carcinogenic activity. A low-temperature crude oil produced lung tumours in mice after intra-tracheal instillation. Various fractions of shale oils were carcinogenic when applied to the skin of mice and rabbits. Shale-oil distillates, residues, blends and commercial products of the oil-shale industry were tested in mice by dermal application, and produced skin tumours. Distillation fractions from less highly refined shale oils were more carcinogenic than the more highly refined products.

4. Other Relevant Data

4.1 Humans

Shale oil-plant workers in Estonia were examined for chromosomal damage and aneuploidy in peripheral blood cells by means of tandem-labelling fluorescence in situ hybridization. One

group of 12 workers was engaged in benzene production from shale oils and another group, of five workers, engaged in coke operations. The control group of eight was from a nearby village. No significant difference in the extent of DNA breakage was detected in nucleated cells in blood smears of exposed *vs* control subjects. In contrast, modest but significantly increased frequencies of breakage affecting both chromosomes 1 and 9 were observed in the cultured lymphocytes of the benzene-exposed workers compared with the unexposed controls (Marcon *et al.*, 1999). [The Working Group noted that workers were likely to be also exposed to other carcinogens such as benzene and coke-oven emissions.]

Peripheral blood lymphocytes from 49 smoking and non-smoking coke-oven workers from a shale-oil plant in Estonia and 10 controls from a nearby village were examined for the presence of aromatic DNA adducts by use of the [^{32}P]-postlabelling techniques. Mean DNA-adduct levels in the exposed group did not differ from those in the controls; however, smokers had significantly higher levels of DNA adducts compared with non-smokers (Kuljukka *et al.*, 1998).

4.2 Experimental systems

Low-temperature shale-derived crude oils and oil-shale retort waters showed mutagenic activity in bacteria, fungi, and mammalian cells in culture. These two agents also induced chromosomal effects in mammalian cells *in vitro* and *in vivo* (IARC, 1985, 1987). Data on the genotoxic activities of raw and spent shale and oil-shale ash, and oil-shale retort-process waters were inconclusive at the time.

Dichloromethane extracts of oil-shale ash were evaluated in a matagenicity assay with an arabinose-resistant *Salmonella typhimurium* strain. These extracts were highly mutagenic in the absence of an exogenous metabolic activation system. Similar results were obtained with oil-shale ash extracts prepared with ethyl acetate/methanol as the extraction solvent (Whong *et al.*, 1983).

Shale oil and acid-base-neutral solvent fractions were evaluated with the morphological cell-transformation assay in Syrian hamster embryo cells in the presence of an exogenous source of metabolic activation. The unfractionated crude oil, the basic fraction, and the PAH fraction produced a positive response in the assay (Frazier & Andrews, 1983).

Genotoxicity assays were conducted on industrial Kitiver shale oil and its two fractions, the low-temperature fraction (230–350 °C) and the rectification residue obtained in the laboratory, as well as the industrial rectification residue. Human lymphocytes exposed to the shale oil and each of the rectification samples had significantly increased frequencies of sister chromatid exchange compared with the controls, irrespective of the presence or absence of exogenous metabolic activation. Shale oil and the industrial rectification samples also significantly increased the frequency of chromosomal abnormalities compared with the controls, with and without metabolic activation (Bogovski *et al.*, 1990).

4.3 Synthesis

Shale oils are genotoxic in experimental systems. There are only few data to determine an underlying mechanism for the carcinogenicity of shale oils.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of shale oils. Shale oils cause cancer of the skin (observed in the scrotum).

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

Shale oils are genotoxic in experimental systems. There is weak evidence to determine a mechanism of action underlying the carcinogenic effects of shale oils, based on two studies with lymphocytes in exposed workers.

Shale oils are *carcinogenic to humans (Group 1).*

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SOOT, AS FOUND IN OCCUPATIONAL EXPOSURE OF CHIMNEY SWEEPS

Soot was considered by previous IARC Working Groups in 1972, 1984, and 1987 (IARC, 1973, 1985, 1987). Chimney sweeping and other exposures to soot were evaluated in 2005 (IARC, 2010). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

For hundreds of years, chimneys have been swept with long steel brushes inserted manually into the chimney from the top and from the bottom. Chimney sweeps are exposed to soot, with concurrent exposure to sulfur dioxide and arsenic (Bagchi & Zimmerman, 1980).

Soot is black particulate matter that is formed as a by-product of combustion or pyrolysis of organic (carbon-containing) materials, such as coal, wood, fuel oil, waste oil, paper, plastics and household refuse. The chemical composition and properties of soots are highly variable and depend on the type of starting material and the conditions of combustion. Soots vary considerably with respect to their relative content of carbon, their particle type, size and shape, and the types of organic and inorganic compounds adsorbed onto the particles. In general, soots have a total carbon content of up to 60%, a high content of inorganic material, and a soluble organic fraction. The latter is extractable with organic solvents and consists largely of PAHs and their derivatives. Inorganic constituents may include oxides, salts, metals, sulfur and nitrogen compounds, water, and other adsorbed liquids and gases (IARC, 1985; Watson & Valberg, 2001).

Table 1.1 summarizes several recent studies that investigated exposure of chimney sweeps to PAHs. Knecht *et al.* (1989) assessed exposures in the breathing zone of chimney sweeps during so-called 'dirty' or 'black work' on 11 working days. Samples were taken per 'job category', based on the type of fuel fired: oil fuel, oil/solid or solid fuels. Twenty PAHs were quantified in a total of 115 samples. Higher concentrations were seen in soots that originated from burning of solid fuels. A bio-monitoring study carried out in Germany (n = 93) and Poland (n = 7) in 1995 reported 1-hydroxypyrene concentrations in the urine ranging from below the detection limit (0.1 µg/L) up to 12.8 µg/L (Letzel *et al.*, 1999). Urinary concentrations in the samples from Poland were on average five times higher, most probably due to the fact that coal and wood are used more often as fuels in Poland. The concentrations in urine samples from workers in Germany were relatively low. The use of personal protective devices among this group of 100 chimney sweeps was not mentioned. In an Italian study, Pavanello *et al.* (2000) analysed the urine of 27 chimney

Table 1.1 Concentrations of PAHs in the ambient air and in urine of chimney sweeps

Reference Country Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Air levels (μg/m³)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Median	Range
Knecht *et al.* (1989) Germany NR	Chimney sweeps	NR							
	Oil fuel		37		Benzo[*a*]pyrene	0.36			
	Oil/solid fuel		34			0.83			
	Solid fuel		44			0.82			
Göen *et al.* (1995) Germany	Chimney sweeps	27						0.36	0.05–1.40
Letzel *et al.* (1999) Germany, Poland 1995	Chimney sweeps	100	100	42	1-Hydroxypyrene			[0.25]*	[0.04–4.5]*
	Apprentices, journeymen	79	79	NR				[0.28]*	
	District master	21	21	NR				[0.11]*	
	From Germany	93	93	38				[0.14–0.32]*	
	From Poland	7	7	4				[1.23]*	
	Smokers	42	42	42				[0.39]*	
	Non-smokers, ex-smokers	58	58	0				[0.19]*	
Pavanello *et al.* (2000) Italy NR	Chimney sweeps	27	27	0				Mean	
					1-Hydroxypyrene			0.56	0.04–2.34

* Originally reported in μg/L. Conversion factor: 1 μmol/mol creat. = 1.93 μg/g creat. = 0.013 μmol/L = 2.84 μg/L

NR, not reported

sweeps: the 1-hydroxypyrene concentrations were in the same wide range as those reported for the chimney sweeps in Germany and Poland (Letzel *et al.*, 1999).

Increased concentrations of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans were found in blood lipids of 227 chimney sweeps from Bavaria (Wrbitzky *et al.*, 2001).

2. Cancer in Humans

In *IARC Monograph* Volume 92 (IARC, 2010), epidemiological studies of cancer in humans were considered to provide *sufficient evidence* for the carcinogenicity of occupational exposure as a chimney sweep. The evidence partly came from a large series of reports on cases of scrotal skin cancer in this occupational group. Soot was first noted as a cause of scrotal cancer in humans by Pott (1775). Many case reports of scrotal and other skin cancers among chimney sweeps appeared subsequently in several different countries (e.g. Earle, 1808; Butlin, 1892; Henry & Irvine, 1936; Henry, 1937, 1946, 1947). A total of 1487 cases of scrotal cancer were reported to the Registrar General for England and Wales from 1911–1935 (Henry, 1937). Of these, 6.9% had occurred in chimney sweeps; the estimated proportion of chimney sweeps in England and Wales in 1921 and 1931 was about 0.06% of all adult males, indicating a large excess of scrotal cancer among workers in this profession.

Evanoff *et al.* (1993) conducted large cohort study of Swedish chimney sweeps and found an excess of cancer of the lung, bladder, oesophagus and haematolymphatic organs; a study from Finland corroborated these findings (Pukkala, 1995). These studies did not include individual adjustments for tobacco smoking, but in the Swedish study an adjustment was made for smoking at the group level, whereas in the Finnish study adjustment was for social class. Both analyses indicated that confounding from tobacco smoking did not explain the findings regarding lung cancer. In two Danish cohort studies an excess of total cancer was found, but the studies were too small to evaluate individual cancer sites (Hansen *et al.*, 1982; Hansen, 1983; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-16-Table2.1.pdf).

Pukkala *et al.* (2009) reported on a record-linkage study from the Nordic countries encompassing 15 million people aged 30–64 identified from the censuses in 1960, 1970, 1980/81, and 1990, and followed for cancer in the national cancer registries until 2005. A total of 5498 male chimney sweeps from Denmark, Finland, Norway and Sweden were identified in the cohort. Statistically significant excesses of cancers of the lung, oesophagus, pharynx, bladder, and colon were found. There was no excess of non-melanoma skin cancer. There was not a large heterogeneity in risk between countries, and no adjustment for smoking was made.

The above-mentioned study by Pukkala *et al.* (2009) – which included information from the earlier study (Pukkala, 1995) – adds to the previous evidence of an excess of cancer of the lung, bladder and oesophagus among chimney sweeps. Despite the classical risk for scrotal cancer in chimney sweeps, studies of this occupational group under modern working conditions show no such excesses.

Overall, considering a consistently observed increased lung-cancer risk in several studies, and on the basis of a large cohort study that demonstrated an internal dose–response after group-level adjustment for smoking, there is evidence from human epidemiological studies that lung cancer is causally associated with occupational exposure during work as a chimney sweep. No internal dose–response was observed for bladder cancer in the large Swedish study, and the evidence for an excess bladder cancer among chimney sweeps must be considered as

limited. The incidence of oesophageal cancer is highly correlated with smoking and alcohol consumption, and in the absence of control for these two factors, the evidence of an association with occupational exposure as a chimney sweep is inadequate. From historical case reports there is sufficient evidence of an increased risk for (scrotal) skin cancer among chimney sweeps.

3. Cancer in Experimental Animals

Coal soot was tested in two experiments in mice by whole-body exposure, but these studies were inadequate for evaluation. Coal-soot extracts applied to the skin of mice produced skin tumours in two studies (IARC, 1985).

In limited studies, subcutaneous implants of wood soot in female rats produced a few local sarcomas. Similar implants in the scrotal sac of rats did not produce tumours. One study of wood-soot extract applied to the skin of mice was uninformative (IARC, 1985).

One study of an extract of fuel-oil soot applied to the skin of mice was uninformative. Extracts of soot from the combustion of oil shale produced skin tumours in mice after dermal application and lung tumours in rats after intratracheal instillation (IARC, 1985).

Extracts of soot from the combustion of heating oil produced from shale oil produced skin tumours in mice in two skin-application experiments (IARC, 1985).

4. Other Relevant Data

4.1 Mechanistic evidence relevant to the carcinogenic hazards from occupational exposure as a chimney sweep

4.1.1 Experimental systems

Experimental studies on soots have been evaluated in *IARC Monograph* Volume 35 and in Supplement 7 (IARC, 1985, 1987). In one study, extracts of soot samples from domestic sources were mutagenic in *Salmonella typhimurium*, both in the presence and absence of an exogenous metabolic system. Extracts of an experimentally-derived soot were mutagenic in forward-mutation assays in *S. typhimurium* and in cultured human lymphoblasts in the presence of metabolic activation (IARC, 1985). Extracts of particulate emissions from wood-combustion induced sister chromatid exchange in Chinese hamster ovary cells, transformation of Syrian hamster embryo cells, and mutation in *S. typhimurium*. An experimentally prepared, intact particulate soot and an extract of this material were both mutagenic in a human lymphoblastoid cell line (IARC, 1987).

Chemical analyses of chimney-soot extracts have identified several polycyclic aromatic hydrocarbons that are genotoxic and carcinogenic in experimental studies. These include benz[*a*]anthracene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, chrysene, and indeno[1,2,3-*cd*]pyrene (IARC, 1983, 1985, 2010). These polycyclic aromatic hydrocarbons may contribute to the genotoxic and tumorigenic activities of soots.

4.1.2 Humans

The frequency of micronuclei in peripheral B- or T-lymphocytes was studied in 71 Swedish chimney sweeps. Genetic polymorphisms in enzymes involved in metabolic activation were

investigated to explain some of the variation in micronucleus formation. The sweeps did not have higher frequencies of micronuclei in either cell type when the results were adjusted for age and smoking, and there was no association between years of work and micronucleus formation (Carstensen *et al.*, 1993).

The same group of workers was studied for the presence of aromatic DNA adducts and micronuclei, and also genotyped for *CYP1A1* and *GST1*. While no specific DNA adducts were identified, the sweeps had higher total DNA-adduct levels in white blood cells, but the increase was not statistically significant. There were no systematic differences in DNA-adduct patterns between the sweeps and the controls. DNA adducts in sweeps were moderately but statistically significantly correlated with micronuclei in both T- and B-lymphocytes. The correlation between adduct-levels and micronuclei was most marked in T-lymphocytes of individuals lacking the *GST1* gene (Ichiba *et al.*, 1994).

Groups of 45 Swedish chimney sweeps and 49 controls were investigated for micronucleus formation in blood lymphocytes stimulated by phytohaemagglutinin or pokeweed mitogen, and by analysis of lymphocyte subgroups and neutrophilic leukocytes. There were higher frequencies of micronuclei among sweeps than in controls, with both methods of stimulation. The effect on micronucleus formation in lymphocytes was more significant in cells stimulated with pokeweed mitogen, suggesting that the T4 lymphocytes were preferentially damaged by the occupational exposure (Holmén *et al.*, 1994).

Analysis of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide-DNA adducts in a group of 19 chimney sweeps showed that four of them (21%) had adduct levels exceeding the 95 percentile control-subject value (Pavanello *et al.*, 1999a). These higher levels were associated with the lack of GSTM1 activity: three of the chimney sweeps had the *GSTM1* *0/*0 genotype (Pavanello *et al.*, 1999b).

4.2 Synthesis

Extracts of soots contain carcinogenic polycyclic aromatic hydrocarbons and are genotoxic. Based on a small number of genotoxicity studies in exposed humans, there is moderate evidence of a genotoxic mode of action for the carcinogenic hazards associated with occupational exposures as a chimney sweep. The detection of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-DNA adducts in the peripheral blood lymphocytes of chimney sweeps suggests involvement of benzo[*a*]pyrene in the genotoxic effect of this exposure in humans.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of soot as found in occupational exposure of chimney sweeps. Soot, as found in occupational exposure of chimney sweeps, causes cancer of the skin (observed in the scrotum), and of the lung.

Also, a positive association has been observed between exposure to soot as found in occupational exposure of chimney sweeps and cancer of the bladder.

There is *inadequate* evidence in experimental animals for the carcinogenicity of soot.

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

Extracts of soots contain carcinogenic polycyclic aromatic hydrocarbons and are genotoxic. Based on a small number of genotoxicity studies in humans there is moderate evidence for a genotoxic mechanism for occupational exposures as a chimney sweep.

Soot as found in occupational exposure of chimney sweeps is *carcinogenic to humans (Group 1)*.

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OCCUPATIONAL EXPOSURES DURING ALUMINIUM PRODUCTION

Aluminium production was considered by previous IARC Working Groups in 1983, 1987, and 2005 (IARC, 1984, 1987, 2010). Since 2005 new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

The aluminium-production industry as referred to in this *Monograph* involves processes such as the electrolytic reduction of alumina to aluminium, and the casting of aluminium into ingots. The mining of bauxite, production of alumina from bauxite, alloying and fabrication of sheet metal, wire, foil and other such products are not considered.

1.1 Natural occurrence

Aluminium, the third most abundant element in the earth's crust, occurs in nature in combination with silicon and oxygen (i.e. as aluminium silicate). When subject to tropical weathering, aluminium silicate may react to form aluminium hydroxide. Rock containing high concentrations of aluminium hydroxide is called bauxite. This rock is the usual starting material for the production of aluminium. Metallurgical-grade alumina (Al_2O_3) extracted from bauxite by the Bayer process is generally referred to as the ore (Sanders, 2002).

1.2 Manufacturing processes

The electrolytic process by which aluminium is produced was described in *IARC Monograph* Volume 34 (IARC, 1984). The process is briefly summarized here. Since 1886, nearly all aluminium has been produced by electrolysis of alumina dissolved in a molten cryolite (Na_3AlF_6)-based bath (also known as the Hall–Héroult process). Molten aluminium is deposited on the carbon cathode, which also serves as the melt container, and oxygen is simultaneously deposited on and consumes the carbon–carbon anode(s) of the electrolytic cell (Sanders, 2002).

A modern alumina-smelting cell consists of a rectangular steel shell lined with refractory insulation surrounding an inner lining of baked carbon. Electric current enters the cell through the anode (either pre-baked or continuously self-baking Søderberg anode) and leaves through steel (collector) bars connected to the carbon cathode at the bottom. Pre-baked anodes are produced by moulding petroleum coke and coal-tar pitch binder into blocks which are baked at 1000–1200 °C. Søderberg anodes are formed continuously from a paste of petroleum coke and coal-tar pitch. The paste is typically added

Table 1.1 Concentrations of PAHs in the ambient air and in the urine of workers in the aluminium industry[a]

Reference Country	Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Air levels (µg/m³)		Urine levels (µmol/mol creatinine)	
							Mean	Range	Mean	Range or SD
Göen *et al.* (1995) Germany	since 1990	Aluminium smelter workers	25	25	NR	1-Hydroxypyrene			[4.2]	[0.05–65]
Schoket *et al.* (1999) Hungary	before 1991	Two aluminium plants	70	NR		1-Hydroxypyrene				
		Plant I	24						4.1	3.6 (SD)
		Plant II	45						22.2	14.2 (SD)
Carstensen *et al.* (1999a), Alexandrie *et al.* (2000) Sweden	1995	Pot-room workers				*Particulate phase*	mg/m³	mg/m³		
			97	97	31%	Total 22	13.2[c]	0.01–270		
			93	93		Benzo[*a*]pyrene	0.97[c]	0.02–23.5		
			94	94		Pyrene	1.11[c]	0.07–34.4		
						Gaseous phase	µg/m³	µg/m³		
			96	96		Total 7	16.3[c]	0.01–132		
			95	95		Pyrene	1.56[c]	0.01–9.5		
						1-Hydroxypyrene				
			94	94		pre-shift			3.4[c]	0.1–26.6
			96	96		post-shift			4.3[c]	0.1–17.7
Friesen *et al.* (2006) Canada	1975–2001	All jobs		CTPV, 2624; B[*a*]P, 1275			CPTV model, 1977–2000	B[*a*]P model, 1977–2000		
		Jobs in pot-room (anode operator/ assistant, controlman, studblast operator, equipment operator, pot operator, foreman)					50–2000	0.2–11		
		Other jobs in pot-rooms					50–700	0.2–11		
		Other jobs, not in pot-rooms					50–150	0.2–0.7		

[a] All studies are for the Søderberg process.

[b] Conversions used: 1-OHP: 1 µmol/mol creatinine = 1.93 µg/g creatinine = 0.013 µmol/l = 2.84 µg/l = 2.84 ng/mL

[c] Median

B[*a*]P, benzo[*a*]pyrene; NR, not reported; SD, standard deviation

to the top of the rectangular steel shell and bakes to form carbon as it passes through the casing, replacing the anode that is being consumed. Molten aluminium is generally removed from the cells daily by siphoning into a crucible (Sanders, 2002).

1.3 Human exposure

Workers in aluminium production are primarily exposed to polycyclic aromatic hydrocarbons (PAHs). Occupational exposures in this industry and the related carbon electrode-manufacturing industry have been monitored most intensively with respect to PAHs. Biomonitoring studies have focused primarily on exposures in the aluminium industry itself (IARC, 1984, 2010; Table 1.1) and in anode-manufacturing for the aluminium industry (IARC, 1984, 2010; Table 1.2). Other potential exposures in these occupational settings include: sulfur dioxide and fluorides; aluminium fluoride; fibrous sodium aluminium tetrafluoride particles; fluorspar; alumina; carbon monoxide; carbon dioxide; various trace metals, such as vanadium, chromium and nickel; asbestos; extreme heat; and high static magnetic fields (Benke *et al.*, 1998; Dufresne *et al.*, 1996).

Exposures to PAHs, sulfur dioxide and fluorides have decreased over time (Benke *et al.*, 1998). At two plants that operated the vertical stud Søderberg pot-rooms in Norway, exposures have decreased fourfold on average between the late 1950s and the late 1980s (Romundstad *et al.*, 1999). The decrease in exposure can be attributed to the implementation of improved control technology, increased use of effective devices for personal protection, and the increasing predominance of pre-bake pot-rooms (Benke *et al.*, 1998), although this may only apply to the anode pre-baking plants. Concentrations of 1-hydroxypyrene in urine of workers in anode-manufacturing for the aluminium industry did not decrease considerably between the mid-1980s and mid-1990s (Table 1.2). In a review of 15 studies, it was concluded that the use of biological monitoring has not led to a reduction in exposure (Hopf *et al.*, 2009). The exposure models by Friesen *et al.* (2006) cover 25 years of extensive monitoring in a Canadian Söderborg smelter, and show a rapid decline in inhalation exposures before the early/mid-1980s, but a considerable levelling off more recently.

Dermal exposure to PAHs and the ensuing uptake through the skin may contribute to the internal exposure of workers to PAHs. Vanrooij *et al.* (1992) showed that dermal exposure does not necessarily correlate with exposure by inhalation of workers in the pot-rooms and the anode pre-bake plants. Levels of benzo[*a*]pyrene on the wrists of workers in the bake-oven area were twice as high as those of workers from the paste plant. The exposure of bake-oven workers to benzo[*a*]pyrene by inhalation, however, appeared to be four times lower than that of workers in the paste plant. Exposure to pyrene by both inhalation and dermal contact was higher in the paste plant. No information was available for temporal trends in dermal exposure in these workplaces.

2. Cancer in Humans

The cancer hazards associated with exposures in aluminium production were evaluated in *IARC Monograph* Volume 92 (IARC, 2010). There was *sufficient evidence* from epidemiological studies of a carcinogenic effect of occupational exposure in aluminium production, based on a relatively large number of studies that showed a consistent excess of cancer of the bladder and a somewhat less consistent excess of lung cancer. The following review is based on studies of aluminium-smelter workers included in *IARC Monograph* Volume 92 (IARC, 2010) and those published later.

Table 1.2 Concentrations of PAHs in the ambient air and in the urine of workers in anode-manufacturing for the aluminium industry

Reference Country Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Air levels (µg/m³)		Urinary levels (µmol/mol creatinine)[a]	
						Mean	Range or SD	Mean	Range
Göen *et al.* (1995) Germany	Carbon-electrode production	23	23	NR	1-Hydroxypyrene			[5.8–12.7]	[1.1–65]
Bentsen-Farmen *et al.* (1999) Norway	Electrode paste-plant workers	17	17	NR	Sum of 17 PAHs 1-Hydroxypyrene pre-shift post-shift	38.0	41.6 (SD)	 3.93 10.20	 3.20 (SD) 6.58 (SD)
Friesen *et al.* (2006) Canada 1975–2001						CPTV model, 1977–2000	B[a]P model, 1977–2000		
	carbon plant jobs					50–300	0.2–5		

[a] Conversions used: 1-OHP: 1 µmol/mol creatinine = 1.93 µg/g creatinine = 0.013 µmol/l = 2.84 µg/l = 2.84 ng/mL

B[*a*]P, benzo[*a*]pyrene; CPTV, coal-tar pitch volatiles; SD, standard deviation

2.1 Cancer of the urinary bladder

A large cohort study from Québec, Canada (Gibbs *et al.*, 2007) showed an excess of bladder-cancer mortality with a statistically significant linear trend with cumulative exposure to benzo[*a*]pyrene B[*a*]P (see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-17-Table2.1.pdf). The excess risk was evident only in workers who had been first employed before 1950, and smaller risks were noted in those first employed later (Gibbs & Sevigny, 2007a, b). An increased risk for bladder cancer and a significant exposure-response trend was found in a cohort study from British Columbia, Canada (Spinelli *et al.*, 2006; Friesen *et al.*, 2007). Both Canadian studies derived smoking-adjusted risk estimates. A significant excess for bladder cancer with a positive exposure-response trend was also found in a study of a Norwegian aluminium smelter (Romundstad *et al.*, 2000). Supporting evidence of a bladder-cancer excess comes from cohort studies from France (Mur *et al.*, 1987; Moulin *et al.*, 2000) and the United States of America (USA) (Rockette & Arena, 1983). A recently reported cohort study from Australia showed no excess of bladder cancer, although the follow-up was relatively short (Friesen *et al.*, 2009; Sim *et al.*, 2009). No bladder-cancer excess was found in a Swedish study (Björ *et al.*, 2008).

2.2 Cancer of the lung

An excess of lung cancer in aluminium-production workers has been reported although the data were less consistent than for bladder cancer. The large Quebec cohort showed a smoking-adjusted excess of lung cancer with an exposure-response trend (Gibbs *et al.*, 2007; Gibbs & Sevigny, 2007b; Armstrong & Gibbs, 2009). An excess of lung cancer, but no clear trend, was noted in the Swedish cohort (Björ *et al.*, 2008). The cohort from British Columbia Canada showed no overall excess of lung cancer, but a trend with increasing cumulative exposure to B[*a*]P (Spinelli *et al.*, 2006; Friesen *et al.*, 2007). The Australian cohort showed no excess but a positive trend with exposure to dust, but not to B[*a*]P (Friesen *et al.*, 2009; Sim *et al.*, 2009). No excess of lung cancer was evident in the studies from France (Mur *et al.*, 1987; Moulin *et al.*, 2000), Norway (Romundstad *et al.*, 2000) or the USA (Rockette & Arena, 1983).

2.3 Synthesis

Overall, the cohort studies strongly support an association between work in aluminium smelters and bladder-cancer risk. Confounding or chance is not likely to explain the findings. There is an increased risk for cancer of the bladder from occupational exposure in aluminium smelters.

An increased risk for lung cancer has been found in several but not all epidemiological studies in the aluminium-production industry. Some studies also show a dose–response trend in terms of B[*a*]P–years. Confounding from smoking or chance is not likely to explain the findings. Based on these observations, there is evidence that risk for cancer of the lung is causally associated with work in aluminium smelters.

The exposure circumstances, especially levels of PAH in aluminium smelters, vary between industrial departments and also depend on the process used. However, data are not sufficient to disentangle the cancer risks associated with these different exposure situations.

3. Cancer in Experimental Animals

Two samples of airborne particulate polynuclear organic matter were collected from two sites in an aluminium-production plant. Each sample was tested by topical application of 50 mg in toluene (1:1) twice weekly to the skin of twenty C3H mice. Samples containing 0.11% and

0.62% B[*a*]P induced 15/18 and 15/17 malignant skin tumours, respectively. The average time of appearance of the first tumours was 24 and 18 weeks, respectively. No tumours were observed in 37 toluene-treated controls (Bingham *et al.*, 1979; IARC, 1984) [Duration of the study and sex of the animals unspecified; the control animals were from a different study of the same laboratory, with the same protocol.]

In addition, several individual polynuclear aromatic compounds for which there is *sufficient evidence* of carcinogenicity in experimental animals have been measured at high levels in air samples taken from certain areas in aluminium-production plants (IARC, 1984, 2010).

4. Other Relevant Data

4.1 Mechanistic evidence relevant to the carcinogenic hazard from occupational exposures during aluminium production

4.1.1 Experimental systems

Air-emission samples from an aluminium-smelting facility were mutagenic in *Salmonella typhimurium* strains TA98 and TA100. All samples were mutagenic in both strains in the presence of an exogenous metabolic activation system and some samples were mutagenic in strain TA98 in the absence of metabolic activation (Alfheim & Wikstrom, 1984). Air-particle samples collected on filters in the anode-paste plant and pot-room in a Søderberg aluminium-production facility were also mutagenic in strains TA100 and TA98, mainly after metabolic activation; some positive results were also obtained in TA98 without S9-mix (Krøkje *et al.*, 1985).

PAHs have been detected and their concentrations measured in the atmosphere of different locations in an aluminium-production plant (IARC, 1984). These PAHs may contribute, in part, to the genotoxic and tumorigenic activities of particulates collected from such plants.

Naphthalene, which is genotoxic and carcinogenic in experimental studies, has also been detected in the indoor atmosphere of an aluminium-production plant (IARC, 2002; Brusick *et al.*, 2008).

4.1.2 Humans

No increase in the frequency of sister chromatid exchange or chromosomal aberrations in peripheral blood lymphocytes was observed in workers in the aluminium industry. No effects on sperm morphology, sperm counts, or double Y-bodies were noted in aluminium-production workers compared with matched controls. There were mixed reports on the mutagenic activity in the urine of workers in the aluminium industry (IARC, 1987).

Human studies on the genotoxic effects of exposures during aluminium production have been reviewed in *IARC Monograph* Volume 92 (IARC, 2010). In several studies, aromatic DNA adducts were analysed in peripheral blood lymphocytes of aluminium-production workers, with mixed results. In 172 Hungarian aluminium-plant workers, higher aromatic DNA-adduct levels were measured compared with those in controls (Schoket *et al.*, 1999). Earlier studies from Hungary had observed aromatic DNA adducts in the lymphocytes of aluminium-plant workers at different locations, at different times of the year, and in different job categories (Schoket *et al.*, 1993a, b, 1995). A significant linear correlation was observed between the total amount of aromatic DNA adducts in lymphocytes and the concentration of 1-hydroxypyrene in urine of Hungarian pot-room workers with the *GSTM1*-null genotype (Schoket *et al.*, 2001). Other populations of aluminium-plant workers have also been studied, with generally positive results with respect to detection of aromatic DNA adducts (Kriek *et al.*, 1993; Ovrebø *et al.*,

1995; van Schooten *et al.*, 1995). Ninety-eight Swedish pot-room workers were examined for the presence of aromatic DNA adducts and gene polymorphisms. No significant differences were observed in the levels of total or individual DNA adducts between pot-room workers and controls (Tuominen *et al.*, 2002). Only one sample from the lymphocytes of 30 aluminium-plant workers was found to contain B[*a*]P-7,8-diol-9,10-oxide-DNA adducts (Vahakangas *et al.*, 1985). In a group of 36 aluminium anode-plant workers, the percentage of subjects with DNA-adduct levels exceeding the 95 percentile control-subject value was small and not significant (Pavanello *et al.*, 1999). Antibodies against B[*a*]P-7,8-diol-9,10-oxide-DNA were detected in the serum of 13.3% of 105 aluminium-plant workers (Galati *et al.*, 2001).

The lymphocytes of 42 Italian aluminium-plant workers were examined for micronucleus formation and DNA-damage induction (single-cell gel electrophoresis assay). While none of the workers showed significant changes in the frequency of micronuclei, significant increases in DNA damage were noted, but only when the lymphocytes were cultured in the presence of cytosine arabinoside, a nucleoside analogue that blocks DNA synthesis (Crebelli *et al.*, 2002).

Ninety-eight Swedish pot-room workers and 55 controls were examined for the presence of polymorphisms in genes encoding biotransformation enzymes, of gene mutations, DNA strand-breaks, and micronuclei in mononuclear blood cells, and of 8-oxodeoxyguanosine in urine. No correlations were found between any of the genotoxicity biomarkers and any of the exposure measures, e.g. length of employment in the pot-room, 1-hydroxypyrene in urine, or PAH-DNA adducts in peripheral lymphocytes, even when different genotypes for biotransformation enzymes were considered (Carstensen *et al.*, 1999b).

4.2 Synthesis

Air-emission samples from aluminium smelters were mutagenic in bacteria. There were mixed reports on the mutagenicity of urine from exposed workers. DNA-adduct studies of blood samples from aluminium-smelter workers also gave mixed results.

Based on both experimental and human studies, there is weak-to-moderate evidence for a genotoxic mechanism underlying the effects of occupational exposures during aluminium production.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during aluminium production. Occupational exposures during aluminium production cause cancer of bladder, and of the lung.

There is *sufficient evidence* in experimental animals for the carcinogenicity of airborne particulate polynuclear organic matter from aluminium-production plants.

Air-emission samples from aluminium smelters were mutagenic in bacteria. There were mixed reports on the mutagenicity of urine from exposed workers. DNA-adduct studies of blood samples from aluminium-smelter workers also gave mixed results.

Based on both experimental and human studies, there is weak-to-moderate evidence for a genotoxic mechanism underlying the effects of occupational exposures during aluminium production.

Occupational exposures during aluminium production are *carcinogenic to humans (Group 1)*.

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Vahakangas K, Haugen A, Harris CC (1985). An applied synchronous fluorescence spectrophotometric assay to study benzo[a]pyrene-diolepoxide-DNA adducts. *Carcinogenesis*, 6: 1109–1115. doi:10.1093/carcin/6.8.1109 PMID:3926334

van Schooten FJ, Jongeneelen FJ, Hillebrand MJX *et al.* (1995). Polycyclic aromatic hydrocarbon-DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: relation with job category and synergistic effect of smoking. *Cancer Epidemiol Biomarkers Prev*, 4: 69–77. PMID:7894326

Vanrooij JGM, Bodelier-Bade MM, De Looff AJA *et al.* (1992). Dermal exposure to polycyclic aromatic hydrocarbons among primary aluminium workers. *Med Lav*, 83: 519–529. PMID:1297067

AFLATOXINS

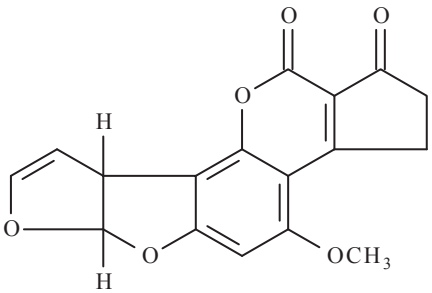
Aflatoxins were considered by previous IARC Working Groups in 1971, 1975, 1987, 1992 and 2002 (IARC, 1972, 1976, 1987, 1993 and 2002). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

1.1.1 Aflatoxin B1

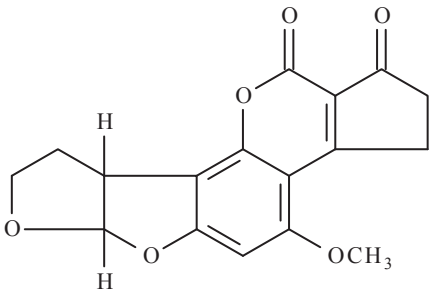
Chem. Abstr. Serv. Reg. No.: 1162-65-8
Chem. Abstr. Serv. Name:
(6a*R*,9a*S*)-2,3,6a,9a-Tetrahydro-4-methoxycyclopenta[*c*]furo-(3′,2′:4,5)furo[2,3-*h*][*l*]benzopyran-1,11-dione



$C_{17}H_{12}O_6$
Relative molecular mass: 312.3

1.1.2 Aflatoxin B2

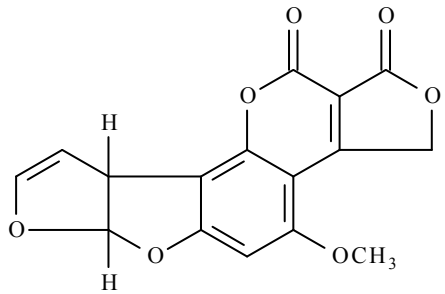
Chem. Abstr. Serv. Reg. No.: 7220-81-7
Chem. Abstr. Serv. Name:
(6a*R*,9a*S*)-2,3,6a,8,9,9a-Hexahydro-4-methoxycyclopenta[*c*]-furo[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione



$C_{17}H_{14}O_6$
Relative molecular mass: 314.3

1.1.3 Aflatoxin G1

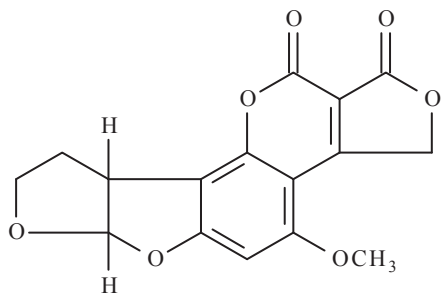
Chem. Abstr. Serv. Reg. No.: 1165-39-5
Chem. Abstr. Serv. Name: (7a*R*,10a*S*)-3,4,7a,10a-Tetrahydro-5-methoxy-1*H*,12*H*-furo-[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][*l*]benzopyran-1,12-dione



$C_{17}H_{12}O_7$
Relative molecular mass: 328.3

1.1.4 Aflatoxin G2

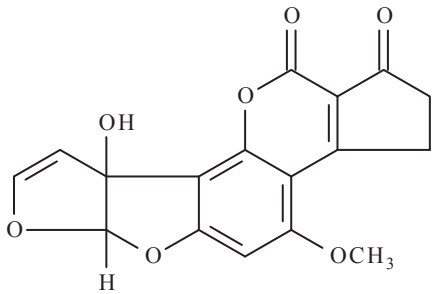
Chem. Abstr. Serv. Reg. No.: 7241-98-7
Chem. Abstr. Serv. Name: (7a*R*,10a*S*)-3,4,7a,9,10,10a-Hexahydro-5-methoxy-1*H*,12*H*-furo[3′,2′:4,5]furo[2,3-*h*]pyrano[3,4-*c*][*l*]benzopyran-1,12-dione



$C_{17}H_{14}O_7$
Relative molecular mass: 330.3

1.1.5 Aflatoxin M1

Chem. Abstr. Serv. Reg. No.: 6795-23-9
Chem. Abstr. Serv. Name: (6a*R*,9a*R*)-2,3,6a,9a-Tetrahydro-9a-hydroxy-4-methoxycyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*][*l*]benzopyran-1,11-dione



$C_{17}H_{12}O_7$
Relative molecular mass: 328.3

Description: Aflatoxins form colourless to pale-yellow crystals. Intensely fluorescent in ultraviolet light, emitting blue (aflatoxins B1 and B2) or green (aflatoxin G1) and green-blue (aflatoxin G2) fluorescence, from which the designations B and G were derived, or blue-violet fluorescence (aflatoxin M1).

Solubility: Very slightly soluble in water (10–30 µg/mL); insoluble in non-polar solvents; freely soluble in moderately polar organic solvents (e.g. chloroform and methanol) and especially in dimethyl sulfoxide (IARC, 2002).

1.2 Sources and uses

Aflatoxins are produced primarily by the common fungus *Aspergillus flavus* and the closely related species *A. parasiticus*. These are well defined species: *A. flavus* produces only B aflatoxins and sometimes the mycotoxin cyclopiazonic acid (CPA), while *A. parasiticus* produces both B and G aflatoxins, but not CPA. Aflatoxin M1 is a metabolite of aflatoxin B1 that can occur in milk and milk products from animals

consuming feed contaminated with B aflatoxins (IARC, 2002).

Aspergillus species capable of producing aflatoxins include *A. flavus*, *A. parasiticus*, *A. nomius*, *A. pseudotamarii*, *A. bombycis*, *A. ochraceoroseus*, and *A. australis* (IARC, 2002). *A. flavus* and *A. parasiticus* are responsible for the largest proportion of aflatoxins found in foodstuffs throughout the world. Of the other species, only *A. australis*, which appears to be widespread in the southern hemisphere and is common in Australian peanut soils, may also be an important source of aflatoxins in some countries (IARC, 2002).

Because of the importance of aflatoxins, *A. flavus* has become the most widely reported foodborne fungus – even with the proviso that *A. parasiticus* is sometimes not differentiated from *A. flavus* in general mycological studies. *A. flavus* is especially abundant in the tropics. Levels of *A. flavus* in warm temperate climates such as in the USA and Australia are generally much lower, while the occurrence of *A. flavus* is uncommon in cool temperate climates, except in foods and feeds imported from tropical countries (IARC, 2002).

The major hosts of *A. flavus* among food and feed commodities are maize, peanuts, and cottonseed [Note: the terms maize and peanuts will be used throughout this Volume for corn and groundnuts, respectively]. In addition, various spices sometimes contain aflatoxins, while tree nuts are contaminated less frequently. Small amounts of aflatoxins may be found in a wide range of other foods (IARC, 2002).

It seems probable that although *A. parasiticus* occurs in the same geographical range as *A. flavus*, it is less widely distributed. In particular, it has been found only rarely in south-eastern Asia. The food-related hosts of *A. parasiticus* are similar to those of *A. flavus*, except that *A. parasiticus* is very uncommon in maize (IARC, 2002).

With maize, peanuts, and cottonseed, invasion of plants and developing seed or nut by *Aspergillus spp.* may occur before harvest, resulting in potentially high levels of aflatoxins in these commodities and the continuing difficulty to eliminate aflatoxins from these products. With other crops, prevention of the formation of aflatoxins relies mainly on avoidance of contamination after harvest by use of rapid drying and good storage practice (IARC, 2002).

Apart from natural formation, aflatoxins are produced only in small quantities for research purposes, by fermentation of *A. flavus* or *A. parasiticus* on solid substrates or media in the laboratory. Aflatoxins are extracted by solvents and purified by chromatography (IARC, 1993).

1.3 Human exposure

1.3.1 Exposure of the general population

Dietary intake is the primary non-occupational source of human exposure to aflatoxins. Intakes in the range of nanograms to micrograms per day occur mainly through consumption of maize and peanuts, which are dietary staples in some tropical countries (IARC, 2002).

Aflatoxins have been found in a variety of agricultural commodities, but the most pronounced contamination has been encountered in maize, peanuts, cottonseed, and tree nuts. An extensive review of the amounts of aflatoxins in commodities in North America, South America, Europe, Asia and Africa was included in *IARC Monograph* Volume 56 (IARC, 1993). More recent data were compiled in *IARC Monograph* Volume 82 (IARC, 2002).

Surveys of selected foods for the presence of aflatoxins in many countries have continued to detect some level of contamination; the amounts are highly variable, ranging from < 0.1 µg/kg to hundreds of µg/kg depending on source, food type, climate, storage conditions, and other factors (IARC, 2002). The fraction of samples with detectable levels of aflatoxin B1 or total aflatoxins (B1, B2, G1 and G2) can range from a few percent (e.g. 6.9% of imported peanuts

Table 1.1 Estimated numbers of workers exposed to aflatoxins in the European Union

Industry, occupational activity	
Education services	740
Research and scientific institutes	460
Food manufacturing	320
Water transport	200
Medical, dental, other health, veterinary services	100
Land transport	20
TOTAL	1840

From: CAREX (1999)

in Japan, 1999–2000; Okano *et al.*, 2003) to as much as 30% or more (e.g. maize in some parts of Latin America and Asia (IARC, 2002). Data on the occurrence of aflatoxin M1 in milk were summarized in the previous *IARC Monograph* (IARC, 1993).

From the point of view of dietary intake, aflatoxins in staple foods such as maize are almost all pervading. This contamination poses a far greater problem in the tropics than in temperate zones of the world. However, because of the movement of agricultural commodities around the globe, no region of the world is free from aflatoxins (IARC, 2002).

International exposure estimates on the intake of aflatoxins were summarized in *IARC Monograph* Volume 82 (IARC, 2002). These estimates include data from the 1995 compendium, Worldwide Regulations for Mycotoxins and the 1998 and 2001 reports of the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1998, 2001). The occurrence and assessment of aflatoxins in human biological fluids and tissues (e.g. cord blood, cord serum, and breast milk) were summarized in the previous *IARC Monograph* (IARC, 2002).

Several recent studies have addressed the early detection, prevention and control of aflatoxins in the food and feed chain around the world (Williams *et al.*, 2004; Kabak *et al.*, 2006; Magan, 2006; Strosnider *et al.*, 2006; Bryden, 2007; Kendra & Dyer, 2007; Magan & Aldred, 2007; Wagacha & Muthomi, 2008). These publications described pre- and post- harvest strategies (such as field management, use of biological and chemical agents, improved drying and storage conditions, irradiation, moisture control, biocompetitiveness and biotechnology (e.g. transgenic expression of maize-specific genes)) and early detection methods (such as molecular imprinted polymers, lateral-flow devices, and molecular-based technology).

1.3.2 Occupational exposure

Occupational exposure to aflatoxins can occur during processing and handling of contaminated grains, particularly animal feed. Airborne concentrations at the workplace are typically in the ng/m^3-range, but higher concentrations (up to µg/m^3) have been reported.

Estimates of the number of workers potentially exposed to aflatoxins in Europe have been developed by CAREX, an international information system on occupational exposures to known and suspected carcinogens collected in the period 1990–1993. This CAREX (CARcinogen EXposure) database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for aflatoxins in the European Union (CAREX, 1999).

Few studies have evaluated occupational exposures to aflatoxins (IARC, 2002).

Selim *et al.* (1998) collected dust samples from 28 farms in the United States during harvest and unloading, animal feeding, and bin cleaning. Aflatoxin concentrations ranged from 0.00004 to 4.8 μg/m^3. The lowest concentrations were detected during harvest and unloading, the highest during bin cleaning.

Brera *et al.* (2002) collected and analysed a total of 44 full-shift samples (26 personal samples, 18 ambient-air samples) to determine airborne concentrations of aflatoxins B1, B2, G1, and G2 in dust collected at three food-processing plants (cocoa, coffee, and spices) in Tuscany, Italy. Concentrations ranged from below the detection limit (< 0.002 ng/m^3), to 0.130 ng/m^3.

2. Cancer in Humans

2.1 Hepatocellular carcinoma

2.1.1 Previous evaluation

Aflatoxins were last evaluated in *IARC Monograph* Volume 82 (2002) and confirmed as a Group-1 agent. The weight of evidence for the classification of the aflatoxins as Group-1 carcinogens was driven by statistically significantly increased risks for hepatocellular carcinoma (HCC) in individuals exposed to aflatoxins, as measured by aflatoxin-specific biomarkers in cohort studies in Shanghai and Taiwan, China (Ross *et al.*, 1992; Qian *et al.*, 1994; Wang *et al.*, 1996). This effect was independent of exposure to hepatitis B virus (HBV); however, when HBV status was included in the analysis, a greater than multiplicative interaction between aflatoxin exposure and HBV infection was found.

2.1.2 Cohort studies

See Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-18-Table2.1.pdf

There has been no recent update of the cohort studied by Ross *et al.* (1992) and Qian *et al.* (1994). However, the cohort of Wang *et al.* (1996) has been extensively updated in three subsequent reports (Wu *et al.* 2007a, b, 2009). In these studies, the risk for HCC was significantly elevated for subjects with high concentrations of aflatoxin metabolites in the urine. Subjects who were seropositive for the hepatitis-B surface antigen (HBsAg) and had high aflatoxin exposure were at higher risk than those with high aflatoxin exposure only, or HBsAg-seropositivity only. There seemed to be no correlation with polycyclic aromatic hydrocarbon(PAH)-albumin-adduct formation (Wu *et al.* 2007a). The risk was elevated in those with urinary concentrations of the biomarker 8-oxodeoxyguanosine (8-oxodG) above the median, who were also HBsAg-positive (Wu *et al.*, 2007b). In one small cohort the risk for HCC from aflatoxin exposure was also elevated (Ming *et al.* 2002).

2.1.3 Case-series and case–control studies

(a) Aflatoxin-specific TP53 mutations

In recent years, epidemiological and experimental studies have linked exposures to aflatoxin with the formation of a specific mutation in codon 249 in the *TP53* tumour-suppressor gene, which has provided an important biological target for risk assessment. The identification of a strong mechanistic link between exposure to aflatoxin and mutation in *TP53* has triggered analyses of this codon-249 mutation in tumour tissues and blood samples in populations at high risk for HCC. In case-series of HCC patients in China, the prevalence of this mutation ranged from 36–54% (Jackson *et al.*, 2001, 2003; Stern *et al.*, 2001; Ming *et al.*, 2002). In the one case–control study in China, Huang *et al.* (2003) found an adjusted odds ratio of 22.1 (95%CI: 3.2–91.7) for the presence of a codon-249 *TP53* mutation among HCC cases compared with controls. In contrast, case-series in Africa found a much

lower prevalence of this type of mutation in some populations, ranging from 1% in one study in Egypt to 35% in The Gambia, West Africa.

(b) Metabolic polymorphisms and HCC risk from aflatoxin

See Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-18-Table2.2.pdf.

The availability of aflatoxin-specific biomarkers has enhanced the possibility to monitor individual exposure to this agent. In three case–control studies (two nested within cohorts) an analysis of a variety of genetic polymorphisms as probable modifiers of risk from aflatoxin, has been undertaken in regions of high HCC incidence (Sun *et al.*, 2001; McGlynn *et al.*, 2003; Kirk *et al.*, 2005). These polymorphisms are predicated on the hypothesis that enhanced detoxication or activation pathways of aflatoxin exposure will be a surrogate biomarker of exposure. All studies were limited because of small numbers of subjects in high-risk strata, but two studies were consistent in finding an increased risk for HCC among those with the *GSTM1*-null genotype, and in one of these studies the risk was elevated among those with the highest consumption of peanuts (an index of consumption of aflatoxin-contaminated food).

(c) Aflatoxin biomarkers of exposure

See Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-18-Table2.3.pdf.

Biomarkers of exposure to aflatoxin have been evaluated for association with risk for HCC in two case–control studies. The risk was significantly higher in those who were HBAsG-positive (Omer *et al.*, 2001, 2004; Liu *et al.*, 2008), in those who carried the *GSTM1*-null genotype (Omer *et al.*, 2001), and in those with oxidative stress (Liu *et al.*, 2008). In one study, it was determined that the attributable risk for the effects of exposure to aflatoxin and HBsAg-positivity was of the order of 80% (Omer *et al.*, 2004).

2.2 Synthesis

Geographically distinct cohort studies in Shanghai and Taiwan, China have independently found statistically significant effects of exposure to aflatoxin on the development of HCC. These results, buttressed by the information from several case-series and case–control studies also confirm that in the presence of HBV exposure, as judged by HBsAg status, there is a greater than multiplicative interaction between aflatoxin and HBV, increasing the risk for HCC. Further evidence of the role of aflatoxins in the development of HCC was gained from studies that demonstrated the ability of aflatoxins to induce a specific mutation in codon 249 of the *TP53* tumour-suppressor gene.

3. Cancer in Experimental Animals

3.1 Previous evaluations

Carcinogenicity studies in experimental animals, with administration of aflatoxin mixtures and aflatoxin B1, B2, M1, G1, or G2 to rats, mice, hamsters, salmon, trout, ducks, tree shrews, woodchucks and monkeys by several routes of exposure have been previously reviewed (IARC, 1993, 2002).

See Table 3.1.

The two previous IARC evaluations concluded that there was *sufficient evidence* for the carcinogenicity in experimental animals of naturally occurring mixtures of aflatoxins and of the individual aflatoxins B1, G1, and M1; there was *limited evidence* for aflatoxin B2, and *inadequate* evidence for aflatoxin G2. This *Monograph* reviews relevant carcinogenicity studies published since 2002.

Table 3.1 Carcinogenicity studies in experimental animals exposed to aflatoxins

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 59 wk Wogan *et al.* (1971)	0, 3, 750 µg (total dose) aflatoxin B2 5 d/wk, ip for 8 wk	Hepatocellular carcinomas: 0/10 and 3/9 at 57–59 wk	[NS]	Group size NR
Rat, F344 (M) 68 wk Wogan *et al.* (1971)	0, 700, 1400, 2000 µg (total dose) aflatoxin G1 by oral gavage 4 d/wk for 2.5 or 8 wk	Hepatocellular carcinomas: 0/10, 0/3, 3/5, 18/18 Kidney: adenocarcinomas: 4/26 dosed animals	[$P < 0.05$], two higher doses –	Group size NR
Rat, F344 (M) 100 wk Wogan & Paglialunga *et al.* (1974)	0 (control) or 25 µg aflatoxin M1 5 d/wk for 8 wk, oral gavage 12–29/group	Liver tumours: 0/12, 1/29	[NS]	Purity > 99%
Rat, F344 (M, F) 18 mo Frayssinet & Lafarge-Frayssinet (1990)	30% peanut-oil cake (control) or diet with 1000 ppb aflatoxin B1 and 170 ppb aflatoxin G1 19–20/group (M), 10–11/group (F)	Liver carcinomas (M): 0/20, 18/19 Liver carcinomas (F): 0/10, 5/11	[$P < 0.0001$] [$P < 0.05$]	
Rat, Wistar WAG (MF) 18 mo Frayssinet & Lafarge-Frayssinet (1990)	30% peanut oil cake (control) or diet with 1000 ppb aflatoxin B1 and 170 ppb aflatoxin G1 17–20/group (M), 10–11/group (F)	Liver carcinomas (M): 0/20, 17/17 Liver carcinomas (F): 0/10, 9/11	[$P < 0.0001$] [$P < 0.005$]	
Rat, F344 (M) 21 mo Hsieh *et al.* (1984)	0 (control), 5 or 50 µg/kg of diet aflatoxin M1 18–25/group	Benign and malignant liver tumours: 0/21, 0/25, 6/18*	*[$P < 0.01$]	* includes 2 hepatocellular carcinomas
Rat, MRC (M, F) 100–105 wk Butler *et al.* (1969)	0 (control), 20 or 60 µg aflatoxin G1/animal in the drinking-water, 5 d/wk/20 wk 11–15/group/sex	Benign and malignant liver tumours 0/15, 2/15, 9/11 (M) 0/15, 1/15, 12/15 (F)	[significant], high dose (M, F)	Liver tumours were mainly hepatocellular carcinomas
Rat, Wistar 64 wk Hao *et al.* (2009)	0 (control) or 100–200 µg aflatoxin B1/kg bw 1–3×/wk, ip 11–25/group	Hepatocellular carcinomas: control, 0/11; treated, 19/25	[$P < 0.0001$]	Sex NR
Trout (*S. gairdneri*) up to 16 mo Ayres *et al.* (1971)	0 (control), 4 ppb aflatoxin B1, 8 ppb aflatoxin B1, and 20 ppb aflatoxin G1 in diet 20–57/group	Liver hepatomas (12 mo): 0/20; 10/40; 40/57; 1/20 Liver hepatomas (16 mo): 0/40; 14/40; 32/40; 7/40	[$P < 0.05$], [$P < 0.0001$], [NS] [$P < 0.0001$],[$P < 0.0001$], [$P < 0.05$]	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Trout (fry) up to 12 mo Bailey *et al.* (1994a)	Positive controls received 4 µg/kg aflatoxin B1 in diet for 12 mo. Positive controls received 20 µg/kg aflatoxin B1 in diet for 2 wk. Positive controls received aflatoxin M1 (80 or 800 µg/kg) for 2 wk. Positive controls received 64 µg/kg aflatoxin B1 for 2 wk Control and treated groups received a maximum of 8 µg/kg aflatoxin M1 for 2 wk (n = 110, total)	Liver: 34% (39/116) tumours at 12 mo Liver: 37% (68/186) tumours at 9 mo Liver: 5.7% (11/193) and 50% tumours, respectively at 9 mo Liver: 29% (80/278) tumours at 12 mo Liver: no tumours (0/110) at 12 mo in both groups	[significant] [significant] [significant] [significant] –	Study was designed to look at treatment of food source to reduce effect of aflatoxin contamination of feed. Liver-tumour data shown here are only for the 'positive controls' given aflatoxins in the diet. Liver tumours were predominantly hepatocellular carcinomas and mixed carcinomas (> 70%).
Trout (*O. mykiss*, Shasta strain) (fry) 9 mo Bailey *et al.* (1994b)	0, 4, 8, 16, 32, 64 ng aflatoxin B1 or aflatoxicol in diet for two wk 200 controls/group; 400 treated/group	Liver tumours: 0/192, 25/382, 98/387, 194/389, 287/389, 302/383 for aflatoxin B1. Aflatoxicol also caused liver tumours.	[significant]	Liver tumours were predominantly hepatocellular carcinomas and mixed carcinomas (> 80%).
Trout (*O. mykiss*, Shasta strain) (fry) 13 mo Bailey *et al.* (1994b)	0, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5 µg/ml aflatoxin B1 or aflatoxicol solution exposure of embryo for 1 h and diet exposure at swimup for 13 mo 400 treated/group	Liver tumours: 1/349, 15/346, 59/348, 131/343, 191/343, 254/347, 252/313 for aflatoxin B1 Aflatoxicol also caused liver tumours	[significant]	Diet exposure unclear. Liver tumours were predominantly hepatocellular carcinomas and mixed carcinomas (> 70%).
Trout (fry) up to 12 mo Bailey *et al.* (1998)	4–64 µg/kg of aflatoxin B1, aflatoxicol, aflatoxin M1, aflatoxicol M1 in diet for 2 wk 120 treated/group	Liver tumour response: aflatoxin B1 (1.000); aflatoxicol (0.936); aflatoxin M1 (0.086); aflatoxicol M1 (0.041)		Tumour response is relative to aflatoxin B1, 1.000. Liver tumours were predominantly malignant (> 80%).
Trout (fry) 13 mo Tilton *et al.* (2005)	0 (control) or 0.5 µg/mL aflatoxin B1 in 0.01% ethanol for 30 min (exposure in tank), ≈400/group	Liver tumours: control 0/~400; treated, 20/~400 (30% hepatocellular carcinomas, 70% mixed carcinomas)	[significant]	Limited reporting of study

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Transgenic mouse TGF-β1 and wild type (C57Bl/6J x CBA) 12 mo Schnur *et al.* (1999)	Aflatoxin B1 (6 µg/kg bw) given as a single ip injection to wild type and transgenic mice 11 wild type, 12 transgenic (exposed) 9 wild type, 19 transgenic (controls)	Liver neoplasms: 0/9 wild type and 0/19 transgenic controls; 3/11 wild type and 3/12* exposed transgenic animals	*[$P < 0.05$]	Limited reporting of study. Transgenic mice overexpress TGF-β1. Liver tumours were mainly adenomas. Sex unspecified.
Transgenic mouse (+/–) FVB/N (wild type), p53 (+/–), HBVTg, and HBVTg-p53 (+/–) (M, F) 12–13 mo Cullen *et al.* (2009)	FVB/N; FVB/N + 1 mg/kg bw aflatoxin B1, single injection, ip; HBVTg, HBVTg + aflatoxin B1; p53 (+/–); p53 (+/–) + aflatoxin B1; HBVTg-p53 (+/–); HBVTg-p53 (+/–) + aflatoxin B1 15–30/group	Liver neoplasms (M): 0/19, 2/21, 0/32, 3/20, 1/30, 1/15, 0/29, 5/24* Liver neoplasms (F): 0/21, 0/20, 0/23, 0/19, 0/19, 0/17, 0/22, 2/29	*$P < 0.01$	Liver neoplasm only in groups exposed to aflatoxin B1. Aflatoxin B1 increased the incidence in HBVTg and p53 (+/–) mice.
Transgenic mouse with C3H/HeN background 11 mo Takahashi *et al.* (2002)	XPA+/–, +/–, –/– with 0.6 or 1.5 mg/kg bw aflatoxin B1 as single injection, ip 11–30/group	Liver carcinomas 0.6 mg/kg: 0%, 13%, 50%* 1.5 mg/kg: 6%, 6%, 38%*	*$P < 0.05$	Also significant for benign liver tumours and tumour multiplicity at 0.6 mg/kg. Also significant for liver tumour multiplicity at 1.5 mg/kg.
Transgenic mouse Hupki (human *TP53* knock-in) 18 mo Tong *et al.* (2006)	Wild type (129/Sv background); Hupki; Wild type + 6 µg aflatoxin B1, as single ip injection; Hupki + 6 µg aflatoxin B1, as single ip injection 21–46/group	Hepatocellular adenomas: 0/30, 0/46, 9/21, 6/34 Hepatocellular carcinomas: 0/30, 0/46, 4/21, 15/34	$P = 0.041$ in Hupki with aflatoxin B1 compared with wild type with aflatoxin B1 $P = 0.057$ in Hupki with aflatoxin B1 compared with wild type with aflatoxin B1	Sex NR
Mouse NIH 58–74 wk Huang *et al.* (2004)	Aflatoxin G1: 0 (control), 3 µg/kg bw or 30 µg/kg bw by gavage 3x/wk for 24 wk 10–14/group	Lung adenocarcinomas: 0% (0/11), 30% (3/10), and 43% (6/14)	High dose, $P = 0.02$	Sex NR
Tree shrew 160 wk Su, *et al.* (2004)	Dietary (milk) dose of 0 (control) or 200–400 µg aflatoxin B1/kg bw/d 20–29/group	Hepatocellular carcinomas: control, 0/20; aflatoxin B1-treated, 6/29	[$P < 0.05$]	Sex NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Tree shrew (M, F) 160 wk Li *et al.* (1999)	Control, HBV+, aflatoxin B1-treated, HBV+/aflatoxin B1-treated Aflatoxin B1 (150 µg/kg bw/d) in feed for 105 wk 4–11/sex/group	Hepatocellular carcinomas in 67% (14/21) of males and females (combined) that were injected with HBV and fed aflatoxin B1. Aflatoxin alone resulted in 30% (3/10) hepatocellular carcinomas (male and female combined). No tumours in the two other groups	$P < 0.01$ (HBV and aflatoxin B1 group compared with aflatoxin B1 group).	Age NR
Tree shrew 150 wk Duan *et al.* (2005)	Dietary (milk) dose of 0 (control) or 150 µg/kg bw/d, 5 x/wk for 105 wk 13–48/group	Hepatocellular carcinomas: control, 0/13; aflatoxin B1, 35/48	[$P < 0.0001$]	Age and sex NR
Tree shrew (M, F) 90 wk Li *et al.* (2008)	Dietary (milk) dose of 0 (control) or 400 µg/kg bw/d 12–15/group	Hepatocellular carcinomas: control, 0/12; aflatoxin B1, 11/15	[$P < 0.0001$]	Age NR
Woodchuck (M, F) 25 wk Bannasch *et al.* (1995)	Control, WHV+, aflatoxin B1 (20–40 µg/kg bw in diet) and WHV+/aflatoxin B1 treated 6/group	Liver tumours: 0/9, 5/9, 0/5, 2/5		Animals were 10 mo of age

bw, body weight; d, day or days; HCC, hepatocellular carcinoma; ip, intraperitoneal; min, minute or minutes; mo, month or months; NR, not reported; NS, not significant; WHV, woodchuck hepatitis virus; wk, week or weeks; XPA, *Xeroderma pigmentosum* (a protein, involved in nucleotide excision-repair)

3.2 Aflatoxin B1

3.2.1 Transgenic mouse

An 11-month study was conducted with transgenic mice deficient in the XPA (*Xeroderma pigmentosum* A) protein. This protein recognizes various types of DNA damage, binds to the damaged DNA region and functions in the first step of the nucleotide excision-repair process. Treatment of these *XPA*$^{-/-}$ mice with a single dose of aflatoxin B1 given by intraperitoneal injection resulted in an increased incidence of liver carcinomas compared with the incidence in wild-type mice (Takahashi *et al.*, 2002). An 18-month study in Hupki (human *TP53* knock-in) transgenic mice that received a single dose of aflatoxin B1 by intraperitoneal injection, showed increased incidences of hepatocellular adenomas (P = 0.041) and carcinomas (P = 0.057) (Tong *et al.*, 2006). A 12–13 month study in FVB/N and *p53*$^{+/-}$ mice (with or without transgenic hepatitis-B virus expression) exposed to a single dose of aflatoxin B1 by intraperitoneal injection, showed liver tumours (hepatocellular adenomas and carcinomas combined) in *p53*$^{+/-}$ HBV-transgenic male mice (Cullen *et al.*, 2009).

These three studies in mice confirm earlier findings of Schnur *et al.* (1999) of an increased incidence of liver tumours (mainly adenomas) in TGF-β1-transgenic mice given aflatoxin B1.

3.2.2 Rat

An intraperitoneal study in Wistar rats confirmed that aflatoxin B1 is a liver carcinogen in this species (Hao *et al.*, 2009).

3.2.3 Tree shrew

A carcinogenicity study to detect alterations in the *p53* and *p21* genes in hepatocellular carcinomas in tree shrews infected with HBV showed an increased incidence of hepatocellular carcinomas in animals that had received aflatoxin by the oral route (Su *et al.*, 2004). This finding was confirmed by Duan *et al.* (2005) and Li *et al.* (2008) in similar studies.

3.2.4 Trout

A study by Tilton *et al.* (2005) confirmed that aflatoxin B1 is a liver carcinogen in trout. In this study, trout embryos were exposed for 30 minutes to water containing 50 ppb aflatoxin F1, and kept for a further 13 months.

3.3 Aflatoxin G1

3.3.1 Mouse

A 58–74-week study in NIH mice given aflatoxin G1 by gavage resulted in an increased incidence in lung adenocarcinomas (Huang *et al.*, 2004).

3.4 Synthesis

Table 3.1 lists the more recent studies described above and also summarizes several of the previously evaluated studies.

Results of additional carcinogenicity studies in animals reported since the previous IARC evaluations are consistent with the conclusions of previous Working Groups. Studies performed with trouts (whole-body exposure), in transgenic mouse models (by intraperitoneal injection), in mice (by gavage), and in tree shrews (via the diet) strengthen the original conclusions of *sufficient evidence* for carcinogenicity in experimental animals of aflatoxin B1 and G1. Aflatoxin B1 increases the incidence of liver cancer in rats, tree shrews, trouts, and transgenic mice. Aflatoxin G1 increases the incidence of liver cancer in rats.

4. Other Relevant Data

Experimental studies on aflatoxins have been reviewed in previous *IARC Monographs* (IARC, 1993, 2002). There is an extensive body of information related to the mechanism of aflatoxin-induced carcinogenicity, encompassing data on toxicokinetics, metabolism, genotoxicity, molecular biology, interactive effects with HBV, and human susceptibility factors. Aflatoxins are naturally occurring mycotoxins that are well documented hepatocarcinogens in humans (IARC, 1993, 2002; Gomaa *et al.*, 2008). At least 13 different types of aflatoxin are found naturally. Aflatoxin B1 is considered the most potent of the aflatoxins and is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin B1 is genotoxic in prokaryotic and eukaryotic systems *in vitro*, including cultured human cells, and *in vivo* in humans and in a variety of animal species. Exposure to aflatoxin B1 induces adducts to DNA and albumin, gene mutations and chromosomal alterations including micronuclei and sister chromatid exchange, and mitotic recombination. Exposure to aflatoxin B1 is mechanistically associated with a specific AGG→AGT transversion mutation in codon 249 of the *TP53* gene in human hepatocellular carcinoma, providing mechanistic support for a causal link between exposure and disease (Gomaa *et al.*, 2008).

The key steps in the mechanism of carcinogenicity of aflatoxins involve metabolism to the reactive *exo*-epoxide, binding of the *exo*-epoxide to DNA resulting in formation of DNA adducts, and miscoding in replicating DNA, which leads to development of mutations with eventual progression to tumours. Biological interactions with HBV also play a role in the hepatic carcinogenicity of aflatoxins in humans (IARC, 2002).

4.1 Toxicokinetics

Rigorous quantitative comparisons of dietary intakes and the amount of aflatoxin metabolites in body fluids following absorption and distribution are lacking. As noted in previous *Monographs* (IARC, 1993, 2002), aflatoxin M1 concentrations in human urine and human breast milk have been correlated with dietary aflatoxin intake (Gan *et al.*, 1988; JECFA, 2001). Using aflatoxin-specific monoclonal antibody-based immunoaffinity chromatography, Wild *et al.* (1992) measured aflatoxin concentrations in cooked foods in a village in The Gambia. Estimated intakes of aflatoxins were less than those derived from the levels of aflatoxin–serum adducts and the concentrations in urine of the same individuals (Wild *et al.*, 1992). In humans, as in other species, the DNA binding and carcinogenicity of aflatoxin B1 result from its conversion to the 8,9-epoxide by cytochrome P450 (CYP) enzymes (Essigmann *et al.*, 1982; Guengerich, *et al.*, 1998). There is interindividual variation in the rate of activation of aflatoxins, including differences between children and adults. These differences may be relevant to the pharmacokinetics of aflatoxins, which in humans have still not been fully elucidated (Ramsdell & Eaton, 1990; Wild *et al.*, 1990).

Factors that explain differences in the response to aflatoxin between human individuals and animal species and strains include the proportion of aflatoxin metabolized to the 8,9-*exo*-epoxide (mainly by CYP enzymes) relative to other, much less toxic metabolites, and the prevalence of pathways that lead to the formation of non-toxic conjugates with reduced mutagenicity and cytotoxicity (Guengerich *et al.*, 1998).

After dermal application, aflatoxin B1 is absorbed via the skin in rats (Wei *et al.*, 1970). Aflatoxins are absorbed from the gut of sheep (Wilson *et al.*, 1985) and rats (Kumagai, 1989) and distributed via the blood, not by the lymphatic

system. In rats, absorption after intratracheal instillation is more rapid than after an oral dose, but the body distribution and excretion patterns are not different for these two routes of administration (Coulombe & Sharma, 1985). When a tracheally administered dose was first adsorbed onto dust, the binding of aflatoxin B1 to lung and tracheal DNA was increased and retention in the trachea was prolonged, compared with administration of microcrystalline aflatoxin B1 alone (Coulombe *et al.*, 1991). Aflatoxin is also rapidly absorbed after inhalation by the rat, resulting in the formation of hepatic DNA adducts (Zarba *et al.*, 1992). Aflatoxin B1 as weIl as aflatoxin M_1 are concentrated in the liver of rats 30 minutes after an intraperitoneal or oral dose of 7 mg/kg bw ^{14}C-aflatoxin B1; at 24 hours, both aflatoxins were detected only as traces (Wogan, 1969). In-vitro studies with bovine melanin have shown that unmetabolized aflatoxin B1 binds reversibly to this pigment (Larsson *et al.*, 1988).

More aflatoxin-B1 metabolites are usually excreted in rat faeces than in urine after intraperitoneal injection of [^{14}C]-ring-labelled aflatoxin B1 (Wogan, 1969). Intraperitoneal co-injection of [^{3}H]-glutathione and aflatoxin B1 (AFB1) in rats showed that the excretion of [^{3}H]-GSH–AFB1conjugates proceeds almost exclusively through the bile: 14% of the radioactivity was excreted as the conjugate by this route, and only traces were found in urine (Emerole, 1981). Degradation of aflatoxin B1–glutathione conjugate by enzymes of the mercapturic-acid pathway has been described in rat-kidney preparations *in vitro* (Moss *et al.*, 1985). The extent of urinary excretion of aflatoxin B1–mercapturate, together with the sulfate and glucuronide conjugates, correlates with species-sensitivity to aflatoxin B1 (Raj & Lotlikar, 1984).

In a more recent study, aflatoxin B1 (AFB1) was administered to rats by gavage for nine consecutive days at eight dose levels ranging from 50 pg/kg bw to 55 µg/kg bw (Scholl *et al.*, 2006). The dose–response relationship was linear-quadratic, with an upward curvature at higher doses. The adduct yield [(pg Lys-AFB1/mg albumin)/(µg AFB1/kg body wt)] increased sixfold, nonlinearly with the dose between the 0.05- and 55-µg AFB_1/kg bw groups, and showed the onset of saturation in the highest dose group, where the adduct yield was approximately 2%.

A recent study by Jubert *et al.* (2009) investigated aflatoxin-B1 pharmacokinetics in human volunteers by use of microdosing techniques and Accelerator Mass Spectrometry (AMS). The kinetics of low-dose aflatoxin B1 were investigated in three volunteers who received an oral dose of 30 ng [^{14}C]-labelled aflatoxin. AMS was used to measure the levels of aflatoxin equivalents in plasma and urine. Pharmacokinetic modelling of absorption and disposition showed that excretion was rapid, with 95% of the total urinary aflatoxin-B1 equivalents produced within the first 24 hours. Absorption of aflatoxin-B1 equivalents into the systemic circulation was also rapid, with peak concentrations being reached within approximately 1 hour. Changes in plasma concentrations of aflatoxin-B1 equivalents following intervention in each subject mirrored those seen in urine. The authors did not discriminate between free aflatoxin B1 and its various metabolites or conjugates. Based on total [^{14}C] equivalents, aflatoxin B1 was rapidly absorbed into plasma in all volunteers, with first-order kinetics.

4.2 Metabolism

The metabolism of aflatoxin B1 in humans and laboratory animals has been well characterized (Essigmann *et al.*, 1982; Eaton & Gallagher, 1994; McLean & Dutton, 1995; Gallagher *et al.*, 1996; Code *et al.*, 1997; Guengerich *et al.*, 1998; Ueng *et al.*, 1998; IARC, 2002). CYP1A2, 2B6, 3A4, 3A5, 3A7 and GSTM1 are enzymes that mediate aflatoxin metabolism in humans. The overall contribution of these enzymes to aflatoxin-B1 metabolism *in vivo* will depend not

only on their affinity but also on their expression level in human liver, where CYP3A4 is predominant. This enzyme mediates the formation of the *exo*-epoxide and aflatoxin Q_1, while CYP1A2 can generate some *exo*-epoxide but also a high proportion of *endo*-epoxide and aflatoxin M_1. *In vitro* evidence that both these enzymes are responsible for aflatoxin metabolism in humans has been substantiated by biomarker studies. Aflatoxins M_1 and Q_1, produced by CYP1A2 and 3A4, respectively, are present in the urine of individuals exposed to aflatoxin (Ross *et al.*, 1992; Qian *et al.*, 1994). In humans, as in other species, the DNA-binding and carcinogenicity of aflatoxin B1 result from its conversion to the aflatoxin B1 8,9-*exo*-epoxide by CYP3A4 (Essigmann *et al.*, 1982). This epoxide is highly reactive and is the main mediator of cellular injury (McLean & Dutton, 1995).

CYP3A5, in contrast to CYP3A4, metabolizes aflatoxin B1 mainly to the *exo*-8,9-epoxide but is about 100-fold less efficient in catalysing 3-hydroxylation of aflatoxin B1 to yield the aflatoxin Q_1 metabolite (Wang *et al.*, 1998). Hepatic CYP3A5 expression differs markedly between individuals. Factors that explain the variation in response to aflatoxin among human individuals, animal species and strains include the proportion of aflatoxin metabolized to the 8,9-*exo* and *endo*-epoxide relative to other, much less toxic metabolites and the prevalence of pathways forming non-toxic conjugates with reduced mutagenicity and cytotoxicity (Eaton & Gallagher, 1994; McLean & Dutton, 1995; Guengerich *et al.*, 1998).

The expression of enzymes involved in aflatoxin metabolism can be modulated with chemopreventive agents, resulting in inhibition of DNA-adduct formation and hepatocarcinogenesis, as has been demonstrated in rats. Oltipraz is a chemopreventive agent that increases glutathione conjugation and inhibits the activity of some cytochrome P450 enzymes (e.g. CYP1A2). Results from clinical trials with oltipraz in the People's Republic of China are consistent with experimental data in showing that, following dietary exposure to aflatoxins, modulation of the metabolism of aflatoxins can lead to reduced levels of DNA adducts (IARC, 2002; Kensler *et al.*, 2005).

There are marked interspecies differences in sensitivity to aflatoxin-induced carcinogenesis (Gorelick, 1990; Eaton & Gallagher, 1994; Eaton & Groopman, 1994). For example, the adult mouse is almost completely refractory to tumour formation except under conditions of partial hepatectomy, or as a result of liver injury through expression of transgenically induced hepatitis-B virus antigens. In contrast, the rat is extremely sensitive. A considerable part of this interspecies variation is understood in terms of differences in activation and detoxification activities of aflatoxin-metabolizing enzymes in the pathways described above (IARC, 2002). Microsomal preparations from mice show a higher specific activity for aflatoxin-B1 8,9-epoxide production than those from the rat (Ramsdell & Eaton, 1990). However, in the mouse, the resistance to aflatoxin carcinogenesis is largely, if not exclusively, explained by the constitutive hepatic expression of an α-class GST, mGSTA3–3, a detoxifying enzyme with a high affinity for aflatoxin B1 8,9-epoxide (Buetler & Eaton, 1992; Hayes *et al.*, 1992). In contrast, rats do not constitutively express a GST isoform with high epoxide-conjugating activity, but they do express an inducible α-class GST (rGSTA5–5) with high activity. The induction of this enzyme plays a major role in the resistance of rats to aflatoxin-B_1-induced hepatocarcinogenicity following treatment with enzyme inducers including oltipraz, ethoxyquin and butylated hydroxyanisole (Kensler *et al.*, 1986, 1987; Hayes *et al.*, 1991, 1994; Pulford & Hayes, 1996).

Current knowledge of the molecular mechanisms of aflatoxin-induced carcinogenesis contributes to the understanding of the nature of the biological interaction between hepatitis B virus (HBV) and aflatoxins in determining the

risk for hepatocellular carcinoma (IARC, 2002). In Asia and Africa, where the majority of cases are found, aflatoxins and hepatitis viruses (HBV and HCV) are important factors giving rise to extraordinarily high incidence rates (24.2–35.5/100000) of hepatocellular carcinoma. In these areas, HBV-induced chronic active hepatitis and cirrhosis constitute major risk factors for liver cancer.

Infection with HBV may increase aflatoxin metabolism. In HBV-infected children in The Gambia there was a higher level of aflatoxin–albumin adducts than in non-infected children, an observation consistent with altered aflatoxin metabolism (Allen *et al.*, 1992; Turner *et al.*, 2000). However, similar studies in adults did not show such differences (Groopman *et al.*, 1992; Wild *et al.*, 2000). Glutathione *S*-transferase activity is reduced in human liver in the presence of HBV infection (Zhou *et al.*, 1997). In HBV-transgenic mice, liver injury is associated with increased expression of cytochrome P450 enzymes (Kirby *et al.*, 1994).

4.3 Aflatoxin–albumin adducts

4.3.1 Aflatoxin–albumin adducts as biomarkers of exposure in children

Gong *et al.* (2003) conducted a cross-sectional study in Benin and Togo to investigate aflatoxin exposure in children around the time of weaning and correlated these data with food consumption, socioeconomic status, agro-ecological zone of residence, and anthropometric measures. Blood samples from 479 children (age, 9 months to 5 years) from 16 villages in four agro-ecological zones were assayed for aflatoxin–albumin adducts as a measure of recent (2–3 months) past exposure. Aflatoxin–albumin adducts were detected in 475/479 (99%) children (geometric mean 32.8 pg/mg, 95%CI: 25.3–42.5). Adduct levels varied markedly across agro-ecological zones, with mean values being approximately four times higher in the central than in the northern region. The aflatoxin–albumin adduct level increased with age up to three years, and was significantly (P = 0.0001) related to weaning status of the 1–3-year age group: weaned children had approximately twofold higher mean aflatoxin–albumin adduct levels (38 pg aflatoxin–lysine equivalents per mg of albumin [pg/mg]) than those receiving a mixture of breast milk and solid foods, after adjustment for age, sex, agro-ecological zone, and socioeconomic status. A higher intake of maize, but not peanuts, in the preceding week was correlated with higher aflatoxin–albumin adduct levels in the children. The prevalence of stunted growth (height for age Z-score, HAZ) and being underweight (weight for age Z-score, WAZ) were 33% and 29%, respectively, by World Health Organization criteria. Children in these two categories had 30–40% higher mean aflatoxin–albumin levels than the remainder of the children, and strong dose–response relationships were observed between aflatoxin–albumin levels and the extent of stunting and being underweight. Polychronaki *et al.* (2008) investigated aflatoxin exposure in Egyptian children (n = 50; age, 1–2.5 years) by assessing urinary aflatoxin metabolites (AFM1, AFB1, AFB2, AFG1, AFG2). Samples from Guinean children (n = 50; age, 2–4 years) were analysed in parallel, providing a comparison with a region of established, frequent exposure to aflatoxin. Overall, aflatoxins were less frequently present in Egyptian (38%) than in Guinean urine samples (86%) (P < 0.001). For AFM1, the geometric mean level in Guinea (16.3 pg/ml; 95%CI: 10.1–26.6 pg/ml) was six times higher (P < 0.001) than in Egypt (2.7 pg/ml; 95%CI: 2.5–2.8 pg/ml).

4.3.2 Aflatoxin–albumin adducts as biomarkers of exposure in intervention trials

The aflatoxin-biomarker studies in populations at high risk for HCC have stimulated the development of interventions to reduce exposure to aflatoxins. In the study by Turner *et al.* (2005), aflatoxin biomarkers were used to assess whether post-harvest measures to restrict aflatoxin contamination of peanut crops could reduce exposure in the lower Kindia region of Guinea. Farms from 20 villages were included, ten of which implemented a package of post-harvest measures to restrict aflatoxin contamination of the peanut crops; ten controls followed usual post-harvest practices. The concentrations of aflatoxin–albumin adducts from 600 people were measured immediately after harvest, and three and five months later, to monitor the effectiveness of the intervention. In control villages the mean aflatoxin–albumin concentration increased from 5.5 pg/mg (95%CI: 4.7–6.1) immediately after harvest to 18.7 pg/mg (17.0–20.6) five months later. By contrast, the mean aflatoxin–albumin concentration in intervention villages after five months of peanuts storage was similar to that immediately post-harvest (7.2 pg/mg [6.2–8.4] *vs* 8.0 pg/mg [7.0–9.2]). At five months, the mean adduct concentration in intervention villages was less than 50% of the values in control villages (8.0 *vs* 18.7 pg/mg; $P < 0.0001$). About a third of the people had non-detectable aflatoxin–albumin concentrations at harvest. At five months, five persons (2%) in the control villages had non-detectable adduct concentrations, compared with 47 (20%) of the subjects in the intervention group ($P < 0.0001$).

4.4 Aflatoxin–DNA adducts

Formation of DNA adducts through reaction with metabolically activated aflatoxin is well characterized. The primary site of adduct formation in DNA is the *N7* position of the guanine base (Guengerich *et al.*, 1998). Aflatoxin B1 is activated to its 8,9-*exo*-epoxide, which reacts with DNA to form the 8,9-dihydro-8-(*N7*-guanosinyl)-9-hydroxy aflatoxin B1 (AFB1–*N7*-Guo) adduct. This adduct represents more than 98% of the total adducts formed by the 8,9-*exo*-epoxide (Guengerich *et al.*, 1998).

The positively charged imidazole ring of the guanosine adduct promotes depurination and consequently, apurinic site formation. As a result, the purine-adduct aflatoxin-*N7*-guanine can be measured in the urine (see below). Under slightly alkaline conditions, the imidazole ring of AFB1–*N7*-Guo is opened and forms the more stable – not depurinating – ring-open aflatoxin B1–formamidopyrimidine adduct (Groopman *et al.*, 1981).

DNA and protein adducts of aflatoxin have been detected in many studies in human liver and in body fluids. Some studies related the level of adducts to polymorphisms in metabolizing enzymes, to investigate interindividual susceptibility to aflatoxin (IARC, 1993, 2002).

4.4.1 Aflatoxin–DNA adducts as biomarkers in intervention trials

Egner *et al.* (2001) reported on a clinical trial with chlorophyllin in Qidong County, People's Republic China. Chlorophyllin is a mixture of semisynthetic, water-soluble derivatives of chlorophyll that has been shown in animal models to be an effective inhibitor of aflatoxin-induced hepatocarcinogenesis by blocking the bioavailability of the carcinogen. A total of 180 adults from Qidong were randomly assigned to ingest 100 mg of chlorophyllin or a placebo three times a day for four months. The primary endpoint was modulation of levels of aflatoxin-N7-guanine adducts in urine samples collected three months into the intervention. Chlorophyllin consumption at each meal led to an overall 55% reduction ($P = 0.036$) in median urinary levels of this

aflatoxin biomarker compared with concentrations in the urine of those taking the placebo.

Kensler *et al.* (2005) described a randomized, placebo-controlled chemoprevention trial aimed at testing whether drinking hot-water infusions of three-day-old broccoli sprouts, containing defined concentrations of glucosinolates, could alter the disposition of aflatoxin. Two hundred healthy adults drank infusions containing either 400 µmol or < 3 µmol glucoraphanin (control value) nightly for two weeks. An inverse association was observed for excretion of dithiocarbamates and aflatoxin-DNA adducts ($P = 0.002$; $R = 0.31$) in individuals who consumed broccoli-sprout glucosinolates.

4.5 Mutagenicity

Aflatoxin B1 induces mutations in *Salmonella typhimurium* strains TA98 and TA100, and causes unscheduled DNA synthesis, chromosomal aberrations, sister chromatid exchange, micronucleus formation and cell transformation in various *in vivo* and *in vitro* mammalian systems. For its mutagenicity, aflatoxin B1 is strongly dependent on metabolic activation with a rat-liver S9 fraction: the mutagenicity in *Salmonella* tester strains TA98 and TA100 without S9 was approximately 1000 times lower than in the presence of S9 (IARC, 1993, 2002).

Aflatoxin B1 can induce mitotic recombination in addition to point mutations (IARC, 2002). This has been demonstrated in both yeast and mammalian cells. In human lymphoblastoid cells, aflatoxin B1 treatment resulted in mitotic recombination and loss of heterozygosity. A reversion assay demonstrated aflatoxin B1-induced intrachromosomal recombination in a mutant cell-line derived from V79 cells that harbour an inactivating tandem-duplication in the *Hprt* gene. Aflatoxin B1 also induced recombination in minisatellite sequences in yeast expressing recombinant human CYP1A2. Liver tumours in HBV-transgenic mice – accumulating hepatitis-B surface antigen in the endoplasmic reticulum of the hepatocytes – treated with aflatoxin B1 transplacentally contained rearrangements in minisatellite sequences after transplacental exposure to aflatoxin B1; no such alterations were seen in non-treated animals (Kaplanski *et al.*, 1997). These findings suggest that aflatoxin can induce genetic instability in addition to point mutations. Mitotic recombination and genetic instability may therefore be two mechanisms by which aflatoxin may contribute to genetic alterations, such as loss of heterozygosity, in hepatocollular carcinoma.

Efforts to correlate biomarkers of aflatoxin exposure (i.e. adduct levels) with mutation indiction have given mixed results. In human subjects from Qidong County, People's Republic of China, aflatoxin exposure was determined as high or low by measuring aflatoxin–albumin adduct levels in serum in comparison with the *HPRT* mutant frequency in lymphocytes. A higher *HPRT* mutant frequency was observed in subjects with high compared with low aflatoxin exposure (Wang *et al.*, 1999). In a study in The Gambia, chromosomal aberrations, micronuclei and sister chromatid exchange were studied in 35 adults, 32 of whom had measurable concentrations of aflatoxin–albumin adducts. There was no correlation within this group between the cytogenetic alterations and aflatoxin–albumin adducts in peripheral blood at the individual level. In a further study, blood samples of 29 individuals of the same group were tested for DNA damage in the single-cell gel electrophoresis (comet) assay, but no correlation was observed with aflatoxin–albumin adducts or *GSTM1* genotype (Anderson *et al.*, 1999).

4.6 Molecular lesions

It has been suggested that exposure to aflatoxin B1 can lead to hepatocellular carcinoma through induction of a specific mutation in codon 249 of the *TP53* tumour-suppressor gene (Gomaa

et al., 2008). Indeed, molecular analyses of human hepatocellular carcinomas have revealed a high prevalence of an AGG→AGT (Arg → Ser) transversion at codon 249 of the *TP53* tumour-suppressor gene (249ser mutation) in tumours from areas of the world where aflatoxin exposure was reported to be high (Montesano *et al.*, 1997). A large number of studies have been published on aflatoxin exposure and *TP53* mutations; two meta-analyses examined the relationship between aflatoxin exposure, HBV infection and *TP53* mutations in 20 (Lasky & Magder, 1997) and in 48 published studies (Stern *et al.*, 2001).

In geographical correlation studies, exposure to aflatoxin was associated with a specific G→T transversion in codon 249 of the *TP53* gene in human hepatocellular carcinoma. This alteration is consistent with the formation of the major aflatoxin B1–*N7*-guanine adduct and the observation that G→T mutations are predominant in cell culture and animal model systems. The high prevalence of the codon-249 mutation in human hepatocellular carcinoma, however, is only partly explained in experimental studies by sequence-specific binding and mutation induced by aflatoxin B1, or by an altered function of the p53 protein in studies of hepatocyte growth and transformation.

Preneoplastic lesions have been examined to define the time point in the natural history of hepatocellular carcinoma when the *TP53* mutation occurs. Hulla *et al.* (1993) examined six hyperplastic nodules from rat liver that had developed three weeks after intraperitoneal injection with aflatoxin B1 followed by partial hepatectomy. No mutations at the codon-249 equivalent were found. In other studies mice received intraperitoneal injections of aflatoxin B1 and were examined for tumours six to 14 months later (Tam *et al.*, 1999). Of the 71 lung tumours examined, 79% showed positive nuclear p53-staining. Analysis of microdissected tumour samples revealed mutations in different codons in exons 5, 6 and 7. Direct sequencing showed 26 mutations, which included nine G:C to A:T transitions, 11 A:T to G:C transitions and five transversions (two G:C to T:A, two T:A to A:T and one A:T to C:G). The high mutation frequency and heterogeneous staining pattern suggested that *TP53* mutations occur relatively late in aflatoxin-B1-induced mouse lung tumorigenesis.

Investigations have been conducted to establish which DNA adduct is the most likely precursor of the mutations induced by aflatoxin B1. In several experimental systems these mutations are certainly consistent with the main carcinogen-binding occurring at guanine in DNA, leading to G→T transversions (IARC, 1993, 2002). When a pS189 shuttle vector was modified by aflatoxin B1 and then replicated in human Ad293 cells, predominantly G→T transversions were detected (Trottier *et al.*, 1992). However, other types of mutation have also been observed with aflatoxin B1. For example, Levy *et al.* (1992) transfected an aflatoxin-B_1-modified shuttle vector into DNA repair-deficient (XP) or -proficient human (GM0637) fibroblasts, and examined mutations in the *SUP-F* marker gene. Higher mutation frequencies were observed in the DNA repair-deficient cells and the location of mutations was significantly affected by repair proficiency. The majority of mutations were at GC base pairs: 50–70% were G →T transversions, but G→C transversions and G→A transitions were also frequent. A polymerase stop-assay was used to examine the of aflatoxin-B_1-binding site within the shuttle vector: no strong correlation was found between initial binding sites and subsequent hotspots for mutation. This suggests that processing of adducts, e.g. during DNA replication and repair, can influence not only the overall mutation frequency but also the distribution of mutations within a gene.

A host-mediated assay was used to determine the pattern of mutagenesis induced by aflatoxin B1 in the *lacI* gene of *E. coli* bacteria recovered from rat liver. Most of the 281 forward mutations analysed were base substitutions at GC base

pairs; over half were GC→TA transversions, with other mutations evenly divided between GC→AT transitions and GC→CG transversions (Prieto-Alamo *et al.*, 1996).

In a human lymphoblastoid cell line (h1A2v2) expressing the human recombinant CYP1A1 enzyme, aflatoxin B1 (4 ng/mL; 25 hours) produced a hotspot GC→TA transversion mutation at base pair 209 in exon 3 of the *HPRT* gene in 10–17% of all mutants. This hotspot occurred at a GGGGGG sequence (target base underlined) (Cariello *et al.*, 1994).

Bailey *et al.* (1996) studied the induction of mutations resulting from two of the principal forms of DNA damage induced by aflatoxin B1, namely the AFB1–*N7*-Guo adduct and the ensuing apurinic sites, by site-directed mutagenesis. Single-stranded M13 bacteriophage DNA containing a unique AFB1–*N7*-Guo adduct or an apurinic site was used to transform *E. coli*. The predominant mutations with AFB1–*N7*-Guo were G→T transversions targeted to the site of the original adduct (approximately 74%), with lower frequencies of G→A transitions (13–18%) and G→C transversions (1–3%). Using *E. coli* strains differing in biochemical activity of the UmuDC- and MucAB proteins – involved in processing of apurinic sites by insertion of dAMP – the authors showed that the mutations observed with AFB1–*N7*-Guo were not predominantly a simple result of depurination of the initial adduct. A significant number of base substitutions were located at the base 5′ to the site of the original adduct, representing around 13% of the total mutations. This induction of mutation at the base adjacent to the original site of damage was not observed with apurinic sites as the mutagenic lesion. It was suggested that this reflects interference with DNA replication following the intercalation of aflatoxin-B1–8,9-epoxide (Gopalakrishnan *et al.*, 1990).

4.7 Synthesis

Several key steps in the development of hepatocellular carcinoma induced by exposure to aflatoxin are well accepted (Wild & Montesano, 2009), and provide strong evidence that the mechanism of action of this agent involves metabolic activation to a genotoxic metabolite, formation of DNA adducts, and modification of the *TP53* gene. The concurrent presence of hepatitis B virus increases the incidence of hepatic tumours in humans. Aflatoxin B1 is the most common and potent of the aflatoxins. It is metabolized predominantly in the liver to an AFB1–8,9-*exo*-epoxide, which forms a promutagenic AFB1-*N7*-guanine DNA adduct that results in G→T transversion mutations. In human hepatocellular cancers in areas where aflatoxin exposure is high, up to 50% of tumours have been shown to harbour a specific AGG→AGT point mutation in codon 249 of the *TP53* tumour-suppressor gene (codon 249Ser mutation) (Hussain *et al.*, 2007; Wild & Montesano, 2009).

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of aflatoxins. Aflatoxins cause cancer of the liver (hepatocellular carcinoma).

There is *sufficient evidence* in experimental animals for the carcinogenicity of naturally occurring mixtures of aflatoxins, and of aflatoxin B1, G1 and M1.

There is *limited evidence* in experimental animals for the carcinogenicity of aflatoxin B2.

There is *inadequate evidence* in experimental animals for the carcinogenicity of aflatoxin G2.

There is strong evidence that the carcinogenicity of aflatoxins operates by a genotoxic mechanism of action that involves metabolic activation to a genotoxic epoxide metabolite, formation of DNA adducts, and modification of the TP53 gene. In human hepatocellular

carcinoma from areas where exposure to aflatoxins is high, up to 50% of tumours have been shown to harbour a specific point mutation in the *TP53* tumour-suppressor gene.

Aflatoxins are *carcinogenic to humans (Group 1)*.

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BENZENE

Benzene was considered by previous IARC Working Groups in 1981 and 1987 (IARC, 1982, 1987). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 71–43–2
Chem. Abstr. Serv. Name: Benzene
IUPAC Systematic Name: Benzene

C6H6
Relative molecular mass: 78.1
From O'Neil (2006) and Lide (2008), unless otherwise stated
Description: Clear, colourless, volatile, highly flammable liquid
Solubility: Slightly soluble in water; miscible with acetone, chloroform, diethyl ether and ethanol; soluble in carbon tetrachloride
Octanol/water partition coefficient: log K_{ow}, 2.13 (Hansch *et al.*, 1995)
Conversion factor: ppm = 0.313 × mg/m^3

1.2 Uses

Historically, benzene has been used as a component of inks in the printing industry, as a solvent for organic materials, as starting material and intermediate in the chemical and drug industries (e.g. to manufacture rubbers, lubricants, dyes, detergents, pesticides), and as an additive to unleaded gasoline (NTP, 2005; ATSDR, 2007; Williams *et al.*, 2008).

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 (Burridge, 2007). In the United States of America, 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 United States of America (accounting for 22% of demand) is in the manufacture of cumene (isopropylbenzene), nearly

Table 1.1 Estimated numbers of workers exposed to benzene in the European Union (top 10 industries)

Industry, occupational activity	
Personal and household services	942500
Wholesale and retail trade and restaurants and hotels	248300
Land transport	42800
Manufacture of plastic products	17000
Iron and steel basic industries	14900
Manufacture of other chemical products	12700
Manufacture of industrial chemicals	12500
Manufacture of machinery, except electrical	9600
Construction	8300
Education services	7400
TOTAL	1367800

all of which is consumed in phenol production. Benzene is also used to make chemical intermediates: 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). 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).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to benzene occurs via inhalation or dermal absorption of solvents in the rubber, paint (including paint applications) and parts-manufacturing industries. It also occurs during crude-oil refining and chemical manufacturing, a large component of which entails exposure to gasoline. Workers involved in the transport of crude oil and gasoline and in the dispensing of gasoline at service stations, as well as street workers, taxi drivers and others employed at workplaces with exposure to exhaust gases from motor vehicles also experience exposure to benzene (Nordlinder & Ramnäs, 1987).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for benzene in the EU by industry for the top-10 industries (CAREX, 1999). Exposure to benzene is defined as inhalation or dermal exposure at work to benzene likely to exceed significantly non-occupational exposure due to inhaling urban air or filling in gasoline stations (long-term exposure usually below 0.01 ppm)].

From the US National Occupational Exposure Survey (1981–1983), it was estimated that approximately 272300 workers (including 143000 women) were potentially exposed to benzene in the United States of America. Industries where potential exposure occurred included agricultural services, oil and gas extraction, construction (includes general building and special trades

contractors), food products, tobacco manufacturing, textile mills, lumber and wood, printing and publishing, chemical and allied products, petroleum and coal products, rubber manufacturing, leather manufacturing, transportation, and health services (NIOSH, 1990).

van Wijngaarden & Stewart (2003) conducted a critical review of the literature on occupational exposures to benzene in the 1980s in the USA and Canada. The data indicated that workers in most industries experienced exposure levels below the regulatory limit (1 ppm) of the US Occupational Safety and Health Administration (OSHA), with a weighted arithmetic mean of 0.33 ppm across all industries. It was noted that little information was available on exposure levels and their determinants for many industries with potential exposure.

Williams *et al.* (2008) summarized the values of the benzene content of selected petroleum-derived products based on published literature between 1956 and 2003. A total of 22 studies were identified, which contained 46 individual data sets and evaluated potential occupational exposure to benzene in the USA during the handling or use of these petroleum-derived products. All mean (or median) airborne concentrations were less than 1 ppm, and most were < 0.1 ppm. Table 1.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table1.2.pdf) summarizes airborne benzene concentrations from studies and governmental reports published between 1981 and 2006.

Capleton & Levy (2005) tabulated typical benzene-exposure levels in different occupational groups in various areas in Europe and North America (Table 1.3). The values are similar to those reported by van Wijngaarden & Stewart (2003) and Williams *et al.* (2008) for exposures of 1 hour or more.

Williams *et al.* (2005) reviewed available industrial-hygiene data describing exposure during the marine transport of benzene-containing products. Although there were differences in sampling strategies and in benzene content of the liquids being transported, typical benzene concentrations in air (personal time-weighted average) were in the range of 0.2–2.0 ppm during closed loading and 2–10 ppm during open loading-operations.

Liang *et al.* (2005) reviewed and tabulated benzene exposures by industry in the People's Republic of China, using data published between 1960 and 2003. The five industries with the highest reported exposures were those producing leather products, electronic devices, machinery, shoes, and office supplies and sports equipment. Median ambient concentrations in these industries were, respectively: 124.8 mg/m^3, 98.7 mg/m^3, 75.4 mg/m^3, 50.4 mg/m^3, and 50.3 mg/m^3. [The Working Group noted that all data were collected with sampling methods of very short duration (1–20-minute time-weighted averages). In addition, a considerable part of the surveys were follow-up studies of benzene poisonings. Therefore, these data cannot be considered as representative and cannot be compared with the information reported from the USA.] Levels of short-term exposure to benzene varied considerably between industries (Table 1.4) and showed generally a downward trend over time (Fig. 1.1).

Urinary *trans,trans*-muconic acid (*t,t*-MA) and *S*-phenylmercapturic acid (*S*-PMA) are sensitive markers for recent exposure to benzene at low levels (Qu *et al.*, 2005).

1.3.2 Non-occupational exposure

The major sources of benzene in the atmosphere are anthropogenic and include fixed industrial sources, fuel evaporation from gasoline filling-stations and automobile exhaust. Benzene has been measured in outdoor air at various locations in the USA at concentrations ranging from 0.02 ppb (0.06 µg/m^3) in a rural area, to 112 ppb (356 µg/m^3) in an urban area. Exposure to benzene is highest in areas of heavy motor-vehicle traffic and around gasoline filling-stations. Based on

Table 1.3 Typical benzene exposure levels in different occupational groups/areas in Europe and North America[a]

Occupational group/area	Year	Long-term exposure levels (mg/m^3)					Short-term exposure levels (mg/m^3)					Reference
		N	AM	GM	Min	Max	N	AM	GM	Min	Max	
Upstream petrochemical industry												Verma *et al.* (2000)
Conventional oil/gas	1985–96	198	0.206	0.036	0.003	7.78	23	0.662	0.021	< 0.004	7.954	
Conventional gas	1985–96	608[b]	0.089	0.024	0.006	6.868	40	2.328	0.144	< 0.02	35.2	
Heavy oil processing	1985–96	236	0.112	0.051	< 0.003	1.60	24	0.056	0.027	< 0.017	0.731	
Pipeline	1985–96	8	0.392	0.350	0.160	1.540	-	-	-	-	-	
Downstream petrochemical industry												CONCAWE (2000, 2002), Merlo *et al.* (2001)
Refinery												
On-site operators	1993–98	97	0.22	-	0.008	7.88	-	-	-	-	-	
	1999–2001	-	-	-	-	-	6	1.0	0.9	0.8[c]	1.4[c]	
Off-site operators	1993–98	321	0.32	-	0.008	23.3	49	2.19	-	0.08	11.8	
	1999–2001	-	-	-	-	-	7	0.7	0.7	0.6[c]	0.8[c]	
Maintenance workers	1993–98	373	0.41	-	0.008	18.1	7	2.62	-	0.23	8.6	
Laboratory technicians	1993–98	628	0.30	-	0.0015	5.0	5	1.93	-	0.28	4.6	
Marine and rail car loading												
Deck crew, open loading	1993–98	41	0.56	-	0.08	5.4	4	0.23	-	0.23	0.3	
Deck crew, closed loading	1993–98	2	0.56	-	0.51	0.6	-	-	-	-	-	
Marine loading	1993–98	32	0.51	-	0.023	3.7	2	0.7	-	0.23	1.2	
Jetty staff	1993–98	46	0.37	-	0.023	1.7	24	0.79	-	0.23	5.8	
Rail car terminal operators (toploading with VR)	1999–2001	21	0.5	0.4	0.2[c]	0.7[c]	3	0.5	-	0.5	0.5	
Road tanker distribution												
Terminal supervisors	1993–98	151	0.36	-	0.001	3.1	8	2.20	-	0.23	11.2	
Drivers, bottom loading & VR	1999–2001	33	0.6	0.4	0.2[c]	1.2[c]	15	1.8	1.4	0.5[c]	3.8[c]	
Drivers, delivery	1999–2001	-	-	-	-	-	7	0.7	0.4	0.2[c]	1.6[c]	

Table 1.3 (continued)

Occupational group/area	Year	Long-term exposure levels (mg/m³)					Short-term exposure levels (mg/m³)					Reference
		N	AM	GM	Min	Max	N	AM	GM	Min	Max	
Service station												CONCAWE (2000, 2002), Merlo *et al.* (2001) Contd.
Attendants	1999–2000	78	0.102	-	0.0115	0.478	-	-	-	-	-	
Cashiers	1993–98	268	0.05	-	0.001	1.92	-	-	-	-	-	
Miscellaneous workers	1999–2001	6	0.2	0.1	0.1[c]	0.2[c]	-	-	-	-	-	
Gasoline pump maintenance	1993–98	2	0.55	-	0.16	0.93	6	3.8	-	0.19	11.8[d]	
Coke oven industry												Hotz *et al.* (1997)
Coke plant	1994–95	19	0.13[e]	-	ND[f]	1.76[f]	-	-	-	-	-	
Coke plant	1994–95	17	1.79[e]	-	0.52[f]	23.82[f]	-	-	-	-	-	
By-product plant	1994–95	21	1.17[e]	-	0.20[f]	5.30[f]	-	-	-	-	-	
Motor mechanics	1994–98	243	0.362	-	< 0.005	13.0	8	10.15[g]	-	1.2	46	CONCAWE (1986), Nordlinder & Ramnäs (1987), Popp *et al.* (1994), Hotz *et al.* (1997), Javelaud *et al.* (1998)
	1981	-	-	-	-	-	-	0.52	-	0.33	1.50	
Aviation												CONCAWE (2000), Egeghy *et al.* (2003)
Civilian airport operators	1993–98	10	0.10	-	0.008	0.60	-	-	-	-	-	
Military fuel maintenance workers	2003	114	0.252[e]	-	0.006	6.63	-	-	-	-	-	
Military fuel handling, distribution, recovery & testing workers	2003	38	0.007[e]	-	0.001	1.85	-	-	-	-	-	
Firefighters	1991–2002	43	-	-	< 0.37[h]	6.14[h]	22[i]	-	-	< LOD	68.25	Jankovic *et al.* (1991), Bolstad-Johnson *et al.* (2000), Caux *et al.* (2002)
							96[j]	1.24	-	0.228	6.468	

Table 1.3 (continued)

Occupational group/area	Year	Long-term exposure levels (mg/m³)					Short-term exposure levels (mg/m³)					Reference
		N	AM	GM	Min	Max	N	AM	GM	Min	Max	
Urban workers												Fustinoni *et al.* (1995), Carrer *et al.* (2000), Crebelli *et al.* (2001), Merlo *et al.* (2001)
Traffic police/wardens	1994–2000	236	0.020	-	0.009	0.316	-	-	-	-	-	
Bus drivers	1998–2000	152	0.0238	-	0.003	0.092	-	-	-	-	-	
Office workers	1994–2000	289	0.016	-	0.002	0.115	-	-	-	-	-	

[a] When selecting typical benzene exposure values, preference has been given to studies published within the previous 10 years and for which greater than 10 subjects were sampled. Where appropriate, data sets have been combined to give an overall mean exposure.
[b] Data for which an arithmetic mean was available
[c] 10th and 90th percentile values.
[d] The mean was strongly influenced by one high exposure level of 46 mg/m3, if this is excluded the mean exposure is 5.03 mg/m3 (range:1.2–14.0 mg/m3).
[e] Median value.
[f] 5th and 95th percentile values.
[g] Small spillage associated with the highest result.
[h] Exposure estimated from biological monitoring.
[I] Exposure during the knockdown phase of fire fighting.
[j] Exposure during the overhaul phase of fire fighting.
AM, arithmetic mean; GM, geometric mean; Max, maximum; Min, minimum; N, number of samples; VR, vapour recovery
From Capleton & Levy (2005)

Table 1.4 Comparison of the average benzene concentrations (mg/m³) by industry

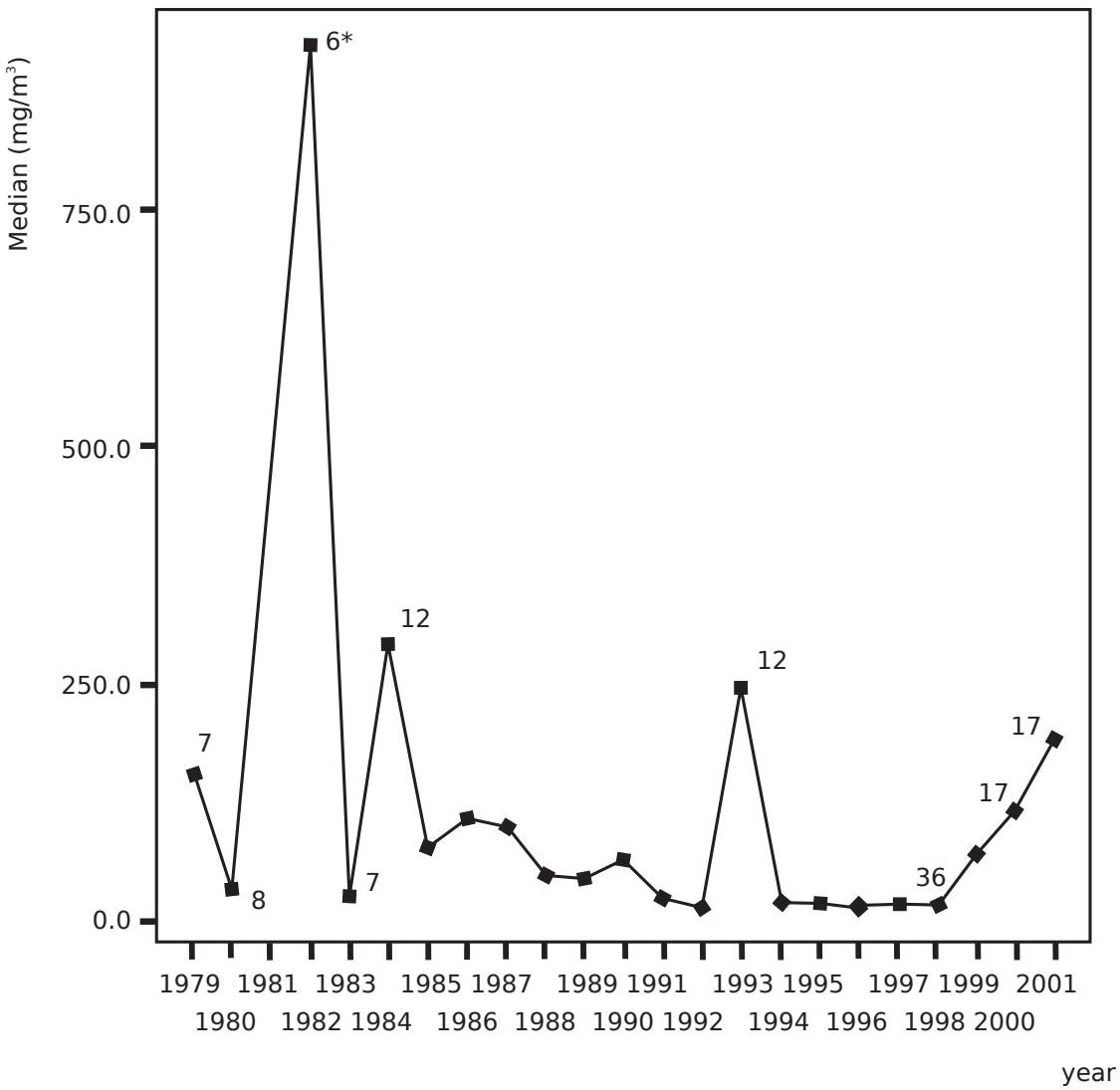
Type of industry	No. of sets	No of samples	Median	Average (range)
Leather products[a]	18	1487	124.8	124.1 (3.7–267.8)
Electronic devices manufacturing[a]	6	1930	98.7	120.2 (4.5–254.9)
Machinery manufacturing[a]	6	6815	75.4	75.6 (4.2–152.7)
Shoes manufacturing, leather[a]	70	12 197	50.4	149.9 (1.3–1488.6)
Office supplies and sports equipment[a]	6	106	50.3	79.4 (10.7–256.0)
Spray painting	29	1186	39.8	53.4 (0–226.8)
Furniture manufacturing	8	618	39.3	36.6 (2.0–72.0)
Misc. electronic parts manufacturing	7	197	33.6	50.5 (3.0–105.6)
Automobile manufacturing	6	3478	32.8	56.8 (0–196.1)
Organic chemical industry	19	650	23.8	39.3 (12.8–130.5)
Rubber products manufacturing	15	182	22.9	114.6 (0.1–633.6)
Other industries	10	6799	18.5	23.8 (2.2–85.5)
Paint manufacturing	37	525	13.2	23.9 (1.0–127.5)
Chemical industry	18	859	7.6	19.3 (0–123.9)
Printing industry	8	6416	6.5	7.2 (0–23.6)
Metal-based products processing	10	77	1.4	7.5 (0–38.0)
Toy manufacturing	2	2531	132.9	132.9 (1.5–264.3)
Coal products manufacturing	3	23	96.0	79.8 (12.8–130.5)
Crude oil processing	3	992	62.6	54.4 (7.4–93.2)
Petroleum & geological prospecting	3	22	57.2	41.9 (5.8–62.6)
Other textile industries/printing & dyeing	1	178	26.2	26.2
Civil engineering & construction	3	137	20.3	122.2 (1.2–345.2)
Pottery & porcelain products manufacturing	3	26	20.2	22.4 (7.1–40.0)
Electronic circuit manufacturing	3	26	20.2	22.4 (7.1–40.0)
Plastic products manufacturing	2	1216	15.2	15.2 (2.3–28.2)
Other precision instruments manufacturing	2	44	14.3	14.3 (8.7–19.9)
Household metal hardware manufacturing	1	1139	2.3	2.3

[a] The top five industries with more than six measurement sets in an individual industry. Industries following the blank space (after Metal-based processing) are those for which fewer than six data sets were available.
From Liang *et al.* (2005)

an average benzene concentration of 12.5 ppb (40 μg/m³) in the air and an exposure of 1 hour per day, the daily intake of benzene from driving or riding in a motor vehicle is estimated to be 40 μg. Exposure is higher for people who spend significant time in motor vehicles in areas of congested traffic (NTP, 2005; ATSDR, 2007).

The primary sources of exposure to benzene for the general population are ambient air containing tobacco smoke, air contaminated with benzene (for example, in areas with heavy traffic, around gasoline filling-stations), drinking contaminated water, or eating contaminated food. The median level of benzene was 2.2 ppb (7 μg/m³) in 185 homes without smokers and 3.3 ppb (10.5 μg/m³) in 343 homes with one or more smokers. Amounts of benzene measured per cigarette ranged from 5.9 to 75 μg in mainstream smoke and from 345 to 653 μg in sidestream smoke. Benzene intake from ingestion of water and foods is very low, compared with intake from ambient air (ATSDR, 1997; NTP, 2005). Residential exposure to benzene can also occur from leaking underground gasoline-storage tanks. Benzene concentrations in homes from such exposures have been estimated to

Fig. 1.1 Overall trend in median benzene exposure in Chinese industry, 1979–2001. The star indicates the number of measurement sets in the database



From Liang *et al.* (2005)

Table 1.5 Benzene in breath, blood and urine samples in the general population without occupational or known exposure to benzene[a]

Country	Analyte	Median/Mean	Reference
People's Republic of China	Urine	120 ng/L	Kim *et al.* (2006a)
People's Republic of China	Urine	69 ng/L	Waidyanatha *et al.* (2001)
People's Republic of China and Malaysia	Urine	1.49 ng/L	Ong *et al.* (1995)
Estonia	Blood Breath Urine	12 nmol/L 7 nmol/L 0.1 nmol/L	Kivistö *et al.* (1997)
Italy	Blood	110 ng/L (NS) 219 ng/L (S)	Brugnone *et al.* (1998)
Italy	Urine	1155 ng/L	Gobba *et al.* (1997)
Mexico	Blood	0.63 µg/L (service attendants) 0.30 µg/L (street vendors) 0.17 µg/L (office workers)	Romieu *et al.* (1999)
Singapore	Blood Urine	1.27 nmol/L 1.29 nmol/L	Ong *et al.* (1996)
Thailand	Blood	65.6 ppt	Navasumrit *et al.* (2005)

[a] Including control workers
NS, non-smoker; S, smoker
From Johnson *et al.* (2007)

range from 0–42 ppm (1–136 mg/m^3) (Patel *et al.*, 2004).

Duarte-Davidson *et al.* (2001) assessed human exposure to benzene in the general population of the United Kingdom. It was estimated that infants (< 1 year old), the average child (11 years old), and non-occupationally exposed adults receive average daily doses of benzene in the range of 15–26 µg, 29–50 µg, and 75–522 µg, respectively. These values correspond to average airborne benzene concentrations in the range of 3.40–5.76 µg/m^3, 3.37–5.67 µg/m^3, and 3.7–41 µg/m^3 for these three groups, respectively.

Benzene concentrations in breath, blood and urine samples collected among the general populations (without occupational or known exposure to benzene) in Asia, Europe and North America are presented in Table 1.5 (Johnson *et al.*, 2007).

2. Cancer in Humans

In *IARC Monographs* Volume 29 (IARC, 1982) the Working Group concluded there was *sufficient evidence* in humans for the carcinogenicity of benzene, noting that a series of cohort and case–control studies showed statistically significant associations between occupational exposure to benzene and benzene-containing solvents and leukaemia (predominantly myelogenous leukaemia). In *IARC Monographs* Supplement 7 (IARC, 1987) benzene was classified as a Group-1 carcinogen, citing additional evidence of an increased incidence of acute nonlymphocytic leukaemia (ANLL) in workers exposed to benzene in three cohort studies, including an update of a cohort cited in Volume 29 (IARC, 1982). Since 1987, there have been numerous reports from cohort studies in populations exposed to benzene, including updates of earlier reports, and new case–control studies of leukaemia or its subtypes, non-Hodgkin lymphoma (NHL), multiple myeloma, and to a

lesser extent other tumours in adults. There have also been several case–control studies of childhood leukaemia with data on benzene, solvents, gasoline, and other related exposures. In addition, several meta-analyses have been published of one or more tumour sites.

[The Working Group decided to restrict its review to those case–control studies of paediatric cancers that included estimates of environmental benzene exposure, rather than surrogate exposures such as proximity to petrol stations or traffic. Also, the Working Group weighed more heavily the findings from studies with estimates of occupational exposure to benzene rather than broader measures (e.g. to solvents) in case–control studies. It was also decided not to rely in general on case–control studies where exposure assessment was limited to asking study subjects directly if they had been exposed to particular chemicals. Furthermore, the Working Group did not consider cohort studies of workers in synthetic rubber-manufacturing due to the difficulty of separating out effects from benzene *vs* those of other chemicals that may cause haematological malignancies. The Working Group decided not to take into consideration a series of meta-analyses of studies of petroleum workers (Wong & Raabe, 1995, 1997, 2000a, b). There were methodological concerns about the expansion from paper to paper of additional studies, cohorts, and countries, and the overall approach may dilute out the risks associated with relatively highly exposed subgroups of these populations that in general were not identified. In addition, an increased risk of ANLL – or the alternative classification, Acute Myelogenous Leukaemia (AML), which is more restrictive but still constitutes most of ANLL – was not detected in the initial meta-analysis by Wong & Raabe (1995), this body of work was not considered relevant for assessing what additional cancers may be associated with exposure to benzene beyond ANLL/AML. Abd finally, the Working Group noted that some meta-analyses of the same tumour came to opposite conclusions, which could be due to different inclusion/exclusion criteria, focusing on different subgroups of the study populations, or to different approaches to selecting risk estimates for inclusion (e.g. Lamm *et al.*, 2005; Steinmaus *et al.*, 2008), thus complicating the overall assessment of the literature. The Working Group therefore decided not to rely in general on results of meta-analyses in its evaluations.]

2.1 Leukemias and lymphomas

2.1.1 Acute non-lymphocytic leukaemia/ acute myelogenous leukaemia

Since 1987, additional analyses of previously published 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; see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.1.pdf). It was also noted that the NCI-CAPM cohort study found evidence of an increased risk for the combined category of ANLL and myelodysplastic syndromes (Hayes *et al.*, 1997). Case–control studies do not add substantively to these conclusions (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.2.pdf). 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 online). 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).

2.1.2 Acute lymphocytic leukaemia

Acute Lymphocytic Leukaemia (ALL) is now considered one subtype of NHL in the WHO-classification of lymphomas. 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; Divine *et al.*, 1999b; Lewis *et al.*, 2003; Kirkeleit *et al.*, 2008; Yin *et al.*, 1996; Guénel *et al.*, 2002; Gun *et al.*, 2006; see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.3.pdf). [The Working Group noted that the magnitude of the risk-estimate in the NCI-CAPM cohort (Yin *et al.*, 1996) was similar to the risk observed for ANLL in the same study, which was statistically significant. This approach has been suggested when attempting to interpret the association between occupational exposure to benzene and hematological subtypes that are less common than AML (Savitz & Andrews, 1997).]

In one case–control study in adults in Shanghai, a significant increased risk for ALL was found for the group with 15 or more years of self-reported occupational exposure to benzene (Adegoke *et al.*, 2003); another study in the USA had only three exposed cases (Blair *et al.*, 2001; Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.4.pdf). In a case–control study of childhood ALL no association was found with maternal self-reported occupational exposure to benzene, but a borderline significant association was noted with exposure to gasoline (Shu *et al.*, 1988; see Table 2.4 online). No association with self-reported maternal exposure to benzene was found in a large study of childhood ALL in the USA (Shu *et al.*, 1999; see Table 2.4 online). A case-control study of childhood cancer in Denmark did not find an association of estimated environmental exposure to benzene from air pollution with ALL (Raaschou-Nielsen *et al.*, 2001).

2.1.3 Chronic myelogenous leukaemia

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; see Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.5.pdf). 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://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.6.pdf).

2.1.4 Chronic lymphocytic leukaemia

Chronic Lymphocytic Leukaemia (CLL) – also referred to as small lymphocytic lymphoma (SLL) – is now considered as a subtype of NHL in the WHO-classification of lymphomas. CLL can be an indolent disease of the elderly, which raises questions about cohorts that are not followed up until the study population is relatively old and about studies that use mortality instead of incident data. In addition, the diagnosis of CLL was less frequently made in the past, until complete blood counts were routinely obtained in recent decades.

Several cohort studies in the petroleum industry showed mixed results, with some non-significantly increased risks reported and other studies showing no association (see Table 2.7 available at http://monographs.iarc.fr/ENG/

Monographs/vol100F/100F-19-Table2.7.pdf). 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). Updates of two cohort studies in the Southern US found an increased risk for CLL, which was significant in one cohort for workers hired before 1950, but not in the other (Huebner *et al.*, 2004).

A case–control study in Italy showed evidence of a dose–response relationship between the extent of benzene exposure with the number of years worked with benzene (Costantini *et al.*, 2008) and in a large multicentre international study in Europe a significant excess in risk for CLL was found with increasing exposure to benzene, but the dose–response was not significant (Cocco *et al.*, 2010; see Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.8.pdf). Blair *et al.* (2001) conducted a study in the Midwestern USA and found no association with benzene exposure although there were only three cases in the high-exposure category. In a study of women in Connecticut, a non-significantly increased risk for CLL was found with increasing exposure to benzene (Wang *et al.*, 2009; see Table 2.8 online).

2.1.5 Non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of histological subtypes, and the definition of both NHL and its subtypes has evolved over the last several decades with the application and discontinuation of several classification schemes, which complicates the assessment of exposure to benzene and risk for NHL. For example, CLL – now classified by the WHO as a subtype of NHL – has generally not been combined with other types of NHL in reports from cohort studies of benzene-exposed workers or in earlier case–control studies of NHL. Further, given the indolent nature of some NHL subtypes, cohorts with only mortality data may underestimate associations with NHL. In most cohort studies an increased risk for NHL was not detected, one particular exception being the NCI-CAPM cohort study in China (Hayes *et al.*, 1997; Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.9.pdf). An excess of NHL was not detected in the Pilofilm cohort (Rinsky *et al.*, 2002) or in the Australian Health Watch study in an analysis of NHL combined with multiple myeloma (two-thirds of which were NHL cases) (Glass *et al.*, 2003).

Of 14 independent case–control studies that were considered informative, five showed evidence of increased risk with benzene exposure, two (Fabbro-Peray *et al.*, 2001; Dryver *et al.*, 2004) for NHL as a whole (Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.10.pdf). Data on histological subtypes of NHL have generally not been reported in publications of occupational cohort studies of benzene-exposed workers, but they have been mentioned in some case–control studies. For various benzene-exposure metrics, slightly increased, but non-significant risks for NHL were found in a case–control study among women in Connecticut, as well as higher risks – also non-significant – for follicular lymphoma

and diffuse large B-cell lymphoma (DLBCL), two common NHL subtypes (Wang *et al.*, 2009). Cocco *et al.* (2010) conducted an analysis of a large multicentre case–control study of NHL in Europe and found no significant increase in risk for B-cell NHL or DLBCL, but an elevated risk, albeit not statistically significant, for follicular lymphoma associated with exposure to benzene (see Table 2.10 online), and a significant association between combined exposure to benzene/toluene/xylene and follicular lymphoma. Other case–control studies showed increased, non-significant risks for one or both of these histological subtypes, and in one study in Italy a significant association was found between medium/high exposure to benzene and the risk for diffuse lymphoma (Miligi *et al.*, 2006; OR = 2.4, 95%CI: 1.3–1.5).

2.1.6 Multiple myeloma

Most cohort studies showed no association with multiple myeloma (MM) (Table 2.11 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.11.pdf). However, there was a statistically significant excess of MM reported for the Pliofilm cohort (SMR 4.1; 95%CI: 1.1–10.5, based upon four deaths) (Rinsky *et al.*, 1987), which did not persist in the most recent update (Rinsky *et al.*, 2002; see Table 2.11 online). In a cohort study among chemical workers at the Monsanto chemical company suggestive evidence was found of a dose–response relationship (Collins *et al.*, 2003), while in a cohort study of Norwegian workers in the upstream petroleum industry (i.e. the phases of oil extraction and initial transportion, which entail extensive exposure to crude oil) a significant increased risk for MM was found (Kirkeleit *et al.*, 2008).

Case–control studies of MM with estimates of exposure to benzene largely show no association (Table 2.12 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.12.pdf). An exception was an early study in which a significant association was found between risk for MM and the proportion of cases and controls with "solvent/benzene" exposure (La Vecchia *et al.*, 1989). In another study, borderline significant effects were detected (Costantini *et al.*, 2008). In a large multicentre case–control study of NHL in Europe there was no association of benzene exposure with MM (Cocco *et al.*, 2010).

A meta-analysis by Infante (2006) analysed data from seven well defined "benzene cohorts" outside of petroleum refining and found a statistically significant increase in risk for MM (RR 2.1; 95%CI: 1.3–3.5).

2.1.7 Hodgkin disease

There are sparse data on Hodgkin disease in studies of benzene-exposed cohorts, with most studies having very small numbers of cases and showing no association (see Table 2.13 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.13.pdf). Overall, there is no evidence of an increased risk. The relatively few case–control studies in adults also show no association (see Table 2.14 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.14.pdf). In a case–control study of childhood cancer in Denmark, an increased risk for Hodgkin disease was detected in association with estimated environmental exposures to benzene (Raaschou-Nielsen *et al.* (2001) (see Table 2.14 online).

2.2 Cancer of the lung

Cohort studies with information on potential or estimated benzene exposure and lung cancer are shown in Table 2.15 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.15.pdf). Although most studies show no association, in two cohorts with quantitative exposure-assessment evidence of a dose–response relationship was found (Hayes *et al.*, 1996; Collins

et al., 2003) and in two others statistically significant increases in risk were observed (Lynge *et al.*, 1997; Sorahan *et al.*, 2005). A case–control study from Canada showed no association of exposure to benzene with lung cancer overall or with the major histological subtypes (Gérin *et al.*, 1998; see Table 2.16 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.16.pdf).

2.3 Cancer of the kidney

Cohort studies with results on kidney cancer are shown in Table 2.17 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.17.pdf). Results generally do not show any association. In a case–control study among males in Germany an association was found between exposure to benzene and an increased risk for kidney cancer (Pesch *et al.*, 2000), but in a study in Montreal, Canada, there was little evidence of an association (Gérin *et al.*, 1998) (see Table 2.18 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-19-Table2.18.pdf).

2.4 Other cancers

In the evaluation of the cohort studies that provided data on the cancer sites considered above, it was apparent that associations have occasionally been found with other cancer sites including malignant melanoma (Schnatter *et al.*, 1996; Consonni *et al.*, 1999; Lewis *et al.*, 2003), nose and stomach cancer (Fu *et al.*, 1996) and prostate cancer (Collingwood *et al.*, 1996), but overall there was no consistency across the cohorts.

3. Cancer in Experimental Animals

Studies on the carcinogenesis of benzene in rats and mice after exposure by inhalation, intragastric gavage, skin application, and by intraperitoneal or subcutaneous injection have been reviewed in *IARC Monographs* Volume 29 and in Supplement 7 (IARC, 1982, 1987). In Supplement 7 it was concluded that there is *sufficient evidence* in experimental animals for the carcinogenicity of benzene. Results of adequately conducted carcinogenicity studies reported before and after 1987 are summarized in Tables 3.1, 3.2, 3.3, 3.4.

Exposure to benzene by inhalation increased the incidence of Zymbal gland carcinomas, liver adenomas, and forestomach and oral cavity carcinomas in female rats (Maltoni *et al.*, 1982a, c, 1983, 1985, 1989). It also increased the incidence of lymphohaematopoietic (lymphoma, myelogenous) neoplasms in male and female mice (Snyder *et al.*, 1980; Cronkite *et al.*, 1984, 1989; Farris *et al.*, 1993), and Zymbal gland carcinomas, squamous cell carcinomas of the preputial gland, and lung adenomas in male mice (Snyder *et al.*, 1988; Farris *et al.*, 1993).

Oral administration of benzene increased the incidence of Zymbal gland carcinomas and oral-cavity papillomas and carcinomas in rats of both sexes, of carcinomas of the tongue, papillomas and carcinomas of the skin and of the lip and papillomas of the palate in male rats, of forestomach acanthomas in both sexes of the rat, and of forestomach carcinomas in female rats (Maltoni & Scarnato, 1979; Maltoni *et al.*,1982b, 1983, 1988, 1989; NTP, 1986; Maronpot, 1987; Huff *et al.*, 1989; Mehlman, 2002). Given by the oral route, benzene also increased the incidence of Zymbal gland carcinomas, forestomach papillomas, lymphomas, and lung adenomas and carcinomas in mice of both sexes, of liver carcinomas, adrenal gland pheochromocytomas, harderian gland adenomas and preputial gland squamous cell carcinomas in male mice,

Table 3.1 Carcinogenicity studies in experimental animals exposed to benzene by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley, (M, F) 150 wk Maltoni *et al.* (1982a, c, 1983, 1985, 1989)	Three different treatment groups (n = 54–75) and 2 controls (breeder controls, n = 60; embryo controls, n = 149–158). Pregnant breeders (Group 1) and embryos exposed transplacentally (Group 2) were exposed 4 h/d, 5 d/wk for 7 wk at 200 ppm; then postpartum breeders and offspring were exposed 7 h/d, 5 d/wk for 12 wk during weaning at 200 ppm; after weaning, breeders and offspring were exposed 7 h/d, 5 d/wk for 85 wk at 300 ppm. Group 3 were embryos exposed 4 h/d, 5 d/wk for 7 wk at 200 ppm transplacentally then 7 h/d, 5 d/wk for 8 wk at 200 ppm. Therefore, the embryos were exposed transplacentally during pregnancy and the offspring were exposed by inhalation and possibly by ingestion via milk.	Zymbal's gland carcinomas Group 1 (breeders 104 wk): F–1/60 (controls), 3/54 Group 2 (embryos 104 wk): M–2/158 (controls), 6/75 F–0/149 (controls), 8/65 Group 3 (embryos 15 wk); M–2/158 (controls), 4/70 F–0/149 (controls), 1/59 Liver adenomas Group 1: F–0/60, 1/54 Group 2: M–1/158, 2/75 F–0/149, 5/65 Group 3: M–1/158, 2/70 F–0/149, 5/59 Oral cavity carcinomas Group 1: F–0/60, 2/54 Group 2: M–0/158, 1/75 F–0/149, 10/65 Group 3: M–0/158, 2/70 F–0/149, 6/59	[NS] [NS] [significant] [NS] [NS] [NS] [NS] [significant] [NS] [significant] [NS] [NS] [significant] [NS] [significant]	Breeders were 13 wk old at the start of exposure; embryos were 12 days old at the start of the exposures

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley, (M, F) 150 wk Maltoni *et al.* (1982a, c, 1983, 1985, 1989) Contd.		Forestomach carcinomas (in situ) Group 1: F–0/60, 0/54 Group 2: M–0/158, 0/75 F–0/149, 3/65 Group 3: M–0/158, 0/70 F–0/149, 0/59	 [NS] [NS] [significant] [NS] [NS]	
Mouse, C57BL/6J (M) Lifetime Snyder *et al.* (1980)	0 (filtered air) or 300 ppm benzene, 6 h/d, 5 d/wk 40/group	Total lymphohaematopoietic: 2/40, 8/40 - Lymphocytic lymphoma: 2/40, 6/40 - Plasmocytoma 0/40, 1/40 - Leukaemia 0/40, 1/40	$P < 0.005$, X^2-test $P < 0.001$, X^2-test NS NS	Purity unspecified Exposed mice had body weight gain depression relative to the controls throughout the study. Exposed mice had a median survival of 41 wk vs 75 wk for the controls.
Mouse, CD-1 Lifetime Goldstein *et al.* (1982)	0 or 300 ppm benzene 6 h/d, 5 d/wk 40/group	Myelogenous leukaemia: 0/40, 3/40	NS	Purity unspecified. Sex unspecified Although the incidence of 3/40 is not significantly higher than the 0% incidence observed in control animals ($P = 0.147$), these preliminary findings do give credence to the myeloleukaemogenic effect of benzene due to the lack of observation of spontaneous myeloproliferative disorders in the animal strain under study.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, C57Bl/6 BNL (F) Lifetime Cronkite *et al.* (1984)	0 or 300 ppm for 16 wk, 6 h/d, 5 d/wk 88–90/group	Total lymphohaematopoietic malignancies: 0/88, 8/90	[$P < 0.01$]	Purity unspecified
		- Thymic lymphoma: 0/88, 6/90	[$P < 0.05$]	
		- Lymphoma (unspecified): 0/88, 2/90	[NS]	
Mouse, CD-1 (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 1200 ppm benzene, 6 h/d, 5 d/wk for 10 wk 50 exposures total 80/group	Lung adenomas: 17/71, 33/71	$P < 0.001$	Purity unspecified
		Zymbal's gland carcinomas: 0/71, 4/71	$P < 0.05$	
Mouse, CD-1 (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 300 ppm benzene, 6 h/d, 5 d/wk for 1 wk followed by 2 wk of non exposure for life 60/group	Lung adenomas: 3/46, 14/54	$P < 0.005$	Purity unspecified
		Leukaemia/lymphomas: 1/46, 7/54	NS	
		Zymbal's gland carcinomas: 0/46, 2/54	NS	
Mouse, C57Bl (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 1200 ppm benzene, 6 h/d, 5 d/wk for 10 wk 80/group	Zymbal's gland carcinomas: 0/67, 4/68	NS	Purity unspecified
Mouse, C57Bl (M) Lifetime Snyder *et al.* (1988)	0 (filtered air) or 300 ppm benzene, 6 h/d, 5 d/wk for 1 wk followed by 2 wk of non exposure for life 60/group	Zymbal's gland carcinomas: 0/46, 19/54	$P < 0.001$	Purity unspecified

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, CBA/Ca BNL (M, F) Lifetime Cronkite *et al.* (1989)	0, 100 (M), 300 (M, F) ppm benzene for 16 wk 6 h/d, 5 d/wk. 60 – 85/group	100 ppm Myelogenous neoplasms: 0/70, 2/85 Other neoplasms, other than hepatoma and haematopoietic: 14/70, 38/85 300 ppm Myelogenous neoplasms: M–0/60, 11/57 F–1/60, 6/54 Other neoplasms, other than hepatoma and haematopoietic: M–13/60, 30/57 F–21/60, 43/54	 NS $P < 0.001$ $P < 0.001$ $P = 0.040$ $P < 0.001$ $P < 0.001$	Purity unspecified Medium lifespan in male (510 days) and female (580 days) mice exposed to 300 ppm was significantly reduced versus sham –exposed males (1 030 days) and females (1 100 days). Myelogenous neoplasms included acute myeloblastic and chronic granulocytic leukaemia. Other neoplasms included Zymbal's and Harderian gland tumours, squamous cell carcinoma, mammary gland adenocarcinoma, and papillary adenocarcinoma of the lung.
Mouse, CBA/Ca (M) 22 months Farris *et al.* (1993)	0, 300 ppm benzene 6 h/d, 5 d/wk for 16 wk 125/group	Malignant lymphomas: 2/119, 14/118 Preputial gland (squamous cell carcinomas): 0/118, 71/118 Lung (alveolar/bronchiolar adenomas): 17/119, 42/119 Zymbal's gland (carcinomas): 1/125, 14/125 Forestomach (squamous cell carcinomas): 0/125, 9/125 Harderian gland: 6/125, 7/125	$P < 0.002$ $P < 0.01$ $P < 0.01$ See comments See comments See comments	100% pure Exposure to benzene caused a significant decrease ($P < 0.01$) in survival and was a significant cause ($P < 0.01$) of early mortality during the first 9 mo post exposure. Most (12) of the lymphomas were of the lymphoblastic or lymphocytic type; two (2) were of the mixed type. Zymbal's gland, forestomach and Harderian gland were examined microscopically only when gross lesions were evident.

d, day or days; h, hour or hours; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; ppm, parts per million; wk, week or weeks; yr, year or years

Table 3.2 Carcinogenicity studies in experimental animals exposed to benzene by gavage

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989)	0, 50, 100, 200 mg/kg bw benzene in corn oil (M); 0, 25, 50, or 100 mg/kg bw in corn oil (F) 5 d/wk 60/group	***Zymbal's Gland:***		> 99.7% pure Groups of 10 rats/sex/group were removed at 51 wks for blood sampling and killed at 52 wks. Survival decreased with increasing dose in both sexes; survival of the high-dose females was significantly less than that of the controls; control females had a greater than average survival normally observed for female F344 rats. Final mean body weight of the high dose males was significantly less than that of the vehicle controls. Most of the dosed rats that died before 103 wks had neoplasms.
		Carcinoma: M–2/32, 6/46, 10/42, 17/42 F–0/45, 5/40, 5/44, 14/46	$P < 0.001$, $P = 0.193$, $P = 0.017$, $P < 0.001$ (M); $P < 0.001$, $P < 0.022$, $P < 0.018$, $P < 0.001$ (F)	
		Adenoma/Carcinoma: M–2/32, 7/46, 10/42, 18/42 F–0/45, 5/40, 6/44, 15/46	$P < 0.001$, $P = 0.131$, $P = 0.017$, $P < 0.001$ (M); $P < 0.001$, $P = 0.022$, $P = 0.010$, $P < 0.001$ (F)	
		Palate:		
		Papilloma: M–0/50, 4/50, 4/50, 9/50 F–1/50, 3/50, 5/50, 3/50	$P < 0.001$, $P = 0.064$, $P = 0.057$, $P < 0.001$ (M); $P = 0.103$, $P = 0.240$, $P = 0.053$, $P = 0.183$ (F)	
		Lip:		
		Papilloma: M–0/50, 2/50, 5/50, 5/50 F–0/50, 0/50, 2/50, 2/50	$P < 0.001$, $P = 0.216$, $P = 0.015$, $P = 0.008$ (M)	
		Carcinoma: M–0/50, 0/50, 0/50, 3/50 F–0/50, 0/50, 0/50, 0/50	$P = 0.002$, -,-, $P = 0.035$ (M)	
		Papilloma/Carcinoma: M–0/50, 2/50, 5/50, 8/50 F–0/50, 0/50, 2/50, 2/50	$P < 0.001$, $P = 0.216$, $P = 0.015$, $P = 0.008$ (M)	
		Tongue:		
		Papilloma: M–1/50, 0/50, 2/50, 2/50 F–0/50, 1/50, 1/50, 0/50		
		Carcinoma: M–0/50, 3/50, 4/50, 4/50 F–0/50, 0/50, 4/50, 4/50	$P = 0.039$, $P = 0.133$, $P = 0.051$, $P = 0.028$ (M)	
		Papilloma/Carcinoma: M–1/50, 3/50, 6/50, 6/50 F–0/50, 1/50, 5/50, 4/50	$P = 0.013$, $P = 0.328$, $P = 0.044$, $P = 0.028$ (M)	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989) Contd.		***Oral Cavity*** (overall rates): Papilloma: M–1/50, 6/50, 11/50, 13/50 F–1/50, 4/50, 8/50, 5/50	$P < 0.001$, $P = 0.058$, $P = 0.001$, $P < 0.001$ (M); $P = 0.017$, $P = 0.127$, $P = 0.006$, $P = 0.032$ (F)	
		Carcinoma: M–0/50, 3/50, 5/50, 7/50 F–0/50, 1/50, 4/50, 5/50	$P = 0.001$, $P = 0.133$, $P = 0.030$, $P = 0.001$ (M); $P = 0.003$, $P = 0.468$, $P = 0.047$, $P = 0.010$ (F)	
		Papilloma/Carcinoma: M–1/50, 9/50, 16/50, 19/50 F–1/50, 5/50, 12/50, 9/50	$P < 0.001$, $P = 0.012$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.068$, $P < 0.001$, $P = 0.001$ (F)	
		Skin: Papilloma: M–0/50, 2/50, 1/50, 5/50	$P < 0.001$, $P = 0.216$, $P = 0.451$, $P < 0.005$ (M)	
		Carcinoma: M–0/50, 5/50, 3/50, 8/50 =	$P < 0.001$, $P = 0.032$, $P = 0.098$, $P < 0.001$ (M)	
		Papilloma/Carcinoma: M–1/50, 7/50, 5/50, 12/50	$P < 0.001$, $P = 0.031$, $P = 0.076$, $P < 0.001$ (M)	
		Uterus: Endometrial stromal polyp: F–7/50, 7/50, 7/49, 14/50	$P = 0.001$, $P = 0.468$, $P = 0.420$, $P = 0.003$ (F)	
Rat, Wistar (M, F) Lifetime Maltoni *et al.* (1983, 1988, 1989), Mehlman (2002)	0, (control), 500 mg/kg bw benzene in olive oil once/d, 5 d/wk, 104 wk 40/group/sex	**Zymbal's gland:** Carcinoma: M–0/40, 7/40 F–0/40, 6/40	[$P < 0.01$]; [$P < 0.05$]	99.93% pure Mortality was higher in benzene-treated male and female rats. Benzene treated rats had lower body weights.
		Oral cavity: Carcinoma: M–1/40, 2/40 F–0/40, 4/40	[NS]	
		Nasal cavity: Carcinoma: M–0/40, 2/40 F–1/40, 1/40	[NS]	
		Malignant tumours: M–8/40, 19/40 F–10/40, 21/40	-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) Lifetime Maltoni *et al.* (1983, 1989), Maltoni & Scarnato (1979), Mehlman (2002)	Benzene in olive oil 0 (control), 50 or 250 mg/kg bw once/d, 4–5 d/wk for 52 wk 30 or 35/group	**Leukaemia:** M–0/28, 0/28, 4/33 F–1/30, 2/30, 1/32	[NS]	99.93% pure
		Zymbal's gland (carcinomas): M–0/28, 0/28, 0/33 F–0/30, 2/30, 8/32*	*[$P < 0.005$] (F)	
Rat, Sprague-Dawley (M, F) Lifetime Maltoni *et al.* (1989) Maltoni *et al.* (1983), Mehlman (2002), Maltoni *et al.* (1982b)	Benzene in olive oil 0 (control), 500 mg/kg bw once/d, 4–5 d/wk for 104 wk Controls, 50/group Treated, 40/group	**Leukaemia:** M–3/50, 1/40 F–1/50, 3/40	[NS]	99.93% pure
		Zymbal's gland: Carcinoma: M–1/50, 18/40 F–0/50, 16/40	[$P < 0.0001$] (M, F)	
		Nasal cavity: Carcinoma: M–0/50, 3/40 F–0/50, 1/40	[NS]	
		Oral cavity: Carcinoma: M–0/50, 21/40 F–0/50, 20/40	[$P < 0.0001$] (M, F)	
		Skin: Carcinoma: M–0/50, 9/40 F–1/50, 0/40	[$P < 0.001$] (M)	
		Liver: Hepatomas: M–3/50, 3/40 F–0/50, 1/40	[NS]	
		Angiosarcoma: M–0/50, 2/40 F–0/50, 3/40		
		Forestomach: Acanthoma/dysplasia: M–0/50, 10/40 F–0/50, 7/40	[$P < 0.005$] (M, F)	
		Carcinoma M–0/50, 1/40 F–0/50, 6/40	[$P < 0.01$] (F)	
		Total Malignant tumours: M–12/50, 68/40 F–11/50, 59/40	-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989)	0, 25, 50, or 100 mg/kg bw benzene in corn oil (M, F) 5 d/wk 60/group	***Zymbal's Gland:*** Carcinoma: M–0/43, 1/34, 4/40, 21/39 F–0/43, 0/32, 1/37, 3/31	$P < 0.001$, $P = 0.489$, $P = 0.012$, $P < 0.001$ (M); $P = 0.007$,–, $P = 0.450$, $P = 0.045$ (F)	> 99.7% pure Groups of 10 mice/sex/group were removed at 51 wks for blood sampling and killed at 52 wks. Survival decreased with increasing dose in both sexes; survival of the high dose males and high dose females was significantly less than those of the controls ($P < 0.001$ and $P = 0.004$, respectively). Final mean body weights of the high dose males and females were less than that of the vehicle controls. Increased incidence of neoplasms were observed at multiple sites for male and female mice.
		Preputial Gland: Squamous cell carcinoma: 0/21, 3/28, 18/29, 28/35	$P < 0.001$, $P = 0.225$, $P < 0.001$, $P < 0.001$ (M)	
		Carcinoma NOS: 0/21, 2/28, 1/29, 3/35	$P < 0.019$, $P = 0.359$, $P = 0.445$, $P = 0.043$ (M)	
		Carcinoma (all types): 0/21, 5/28, 19/29, 31/35	$P < 0.001$, $P = 0.091$, $P < 0.001$, $P < 0.001$ (M)	
		Ovary Tubular adenoma: 0/47, 0/44, 3/49, 3/48	$P = 0.008$, -, $P = 0.090$, $P = 0.047$	
		Granulosa cell tumour or carcinoma: 1/47, 1/44, 6/49, 8/48	$P < 0.001$, $P = 0.730$, $P < 0.040$, $P < 0.004$ (F)	
		Benign mixed cell tumour: 0/47, 1/44, 12/49, 7/48	$P < 0.001$, $P = 0.471$, $P < 0.001$, $P < 0.001$ (F)	
		Mammary gland: Carcinoma: F–0/49, 2/45, 5/50, 10/49	$P < 0.001$, $P = 0.202$, $P < 0.026$, $P < 0.001$ (F)	
		Carcinosarcoma: F–0/49, 0/45, 1/50, 4/49	$P < 0.001$, –, $P < 0.495$, $P < 0.017$ (F)	
		Harderian gland: Adenoma: M–0/49, 9/46, 13/49, 11/48 F–5/48, 6/44, 10/50, 6/47	$P < 0.001$, $P = 0.001$, $P < 0.001$, $P < 0.001$ (M); $P = 0.133$, $P = 0.369$, $P = 0.090$, $P = 0.204$ (F)	
		Carcinoma: M–1/49, 2/46, 0/49, 3/48 F–0/48, 0/44, 0/50, 4/47	$P < 0.001$, –, –, $P = 0.020$ (F	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989) Contd.		Adenoma/Carcinoma: M–1/49, 10/46, 13/49, 14/48 F–5/48, 6/44, 10/50, 10/47	$P < 0.001$, $P = 0.002$, $P < 0.001$, $P < 0.001$ (M); $P = 0.009$, $P = 0.369$, $P = 0.090$, $P = 0.017$ (F)	
		Lung (alveolar/bronchiolar): Adenoma: M–6/49, 6/48, 8/50, 12/49 F–4/49, 2/42, 5/50, 9/49	$P < 0.001$, $P = 0.499$, $P = 0.188$, $P = 0.005$ (M); $P = 0.003$, $P = 0.437$N, $P = 0.398$, $P = 0.011$ (F)	
		Carcinoma: M–5/49, 11/48, 12/50, 14/49 F–0/49, 3/42, 6/50, 6/49	$P < 0.001$, $P = 0.052$, $P = 0.017$, $P < 0.001$ (M); $P = 0.002$, $P = 0.084$, $P = 0.010$, $P = 0.004$ (F)	
		Adenoma/Carcinoma: M–10/49, 16/48, 19/50, 21/49 F–4/49, 5/42, 10/50, 13/49	$P < 0.001$, $P = 0.069$, $P = 0.007$, $P < 0.001$ (M); $P = < 0.001$, $P = 0.0.366$, $P = 0.039$, $P < 0.001$ (F)	
		Lymphohaematopoietic: Lymphoma: M–4/49, 9/48, 9/50, 15/49 F–15/49, 24/45, 24/50, 20/49	$P < 0.001$, $P = 0.075$, $P < 0.030$, $P < 0.001$ (M); $P = 0.031$, $P = 0.021$, $P = 0.025$, $P = 0.037$ (F)	
		Leukaemia: M–0/49, 1/48, 1/50, 0/49 F–0/49, 1/45, 2/50, 2/49	NR	
		Lymphoma/leukaemia: M–4/49, 10/48, 10/50, 15/49 F–15/49, 25/45, 26/50, 22/49	$P < 0.001$, $P = 0.048$, $P < 0.018$, $P < 0.001$ (M); $P = 0.014$, $P = 0.014$, $P = 0.012$, $P = 0.017$ (F)	
		Forestomach: Papilloma: M–2/45, 1/42, 2/44, 5/38 F–1/42, 3/40, 6/45, 5/42	$P = 0.003$, $P = 0.567$N, $P < 0.556$, $P < 0.014$ (M); $P = 0.022$, $P = 0.288$, $P = 0.038$, $P = 0.040$ (F)	
		Carcinoma: M–2/45, 1/42, 2/44, 5/38	NR	
		Papilloma/carcinoma: M–2/45, 2/42, 3/44, 5/38	$P = 0.004$, $P = 0.623$, $P = 0.335$, $P = 0.014$ (M)	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 103 wks NTP (1986), Maronpot (1987), Huff *et al.* (1989) Contd.		***Liver:*** Adenoma: M–7/49, 11/48, 6/50, 3/47 F–1/49, 8/44, 5/50, 4/49	$P = 0.156$, $P = 0.008$, $P = 0.079$, $P = 0.077$ (F)	
		Carcinoma: M–9/49, 8/48, 17/50, 8/47 F–3/49, 4/44, 8/50, 4/49	$P = 0.072$, $P = 0.589$, $P = 0.028$, $P = 0.293$ (M)	
		Adenoma/Carcinoma: M–15/49, 17/48, 22/50, 11/47 F–4/49, 12/44, 13/50, 7/49	$P = 0.076$, $P = 0.256$, $P = 0.029$, $P = 0.225$ (M); $P = 0.103$, $P = 0.014$, $P = 0.008$, $P = 0.086$ (F)	
		Adrenal Gland: Pheochromocytoma: M–1/47, 1/48, 7/49, 1/46 F–6/49, 1/44, 1/50, 1/48	$P = 0.096$, $P = 0.725$, $P = 0.010$, $P = 0.632$ (M)	
Mouse, A/J (M, F) 24 wk Stoner *et al.* (1986)	0 (control), 24 g/kg bw in tricaprylin vehicle 3x/wk for 8 wk 16/group	**Lung** (adenomas): M–3/15, 8/16 F–2/14, 5/15	NR	Purity NR
		tumours/ mouse: M–0.27 ± 0.59, 0.63 ± 0.72 F–0.14 ± 0.36, 0.53 ± 0.92	$P < 0.05$ NS	
Mouse, Swiss (M, F) Lifetime Maltoni *et al.* (1988)	0 (control), 500 mg/kg bw benzene in olive oil once/d, 5d/wk 40/group	**Mammary gland** (carcinomas): M–1/40, 0/40 F–2/40, 19/40	[$P < 0.0001$] (F)	99.93% pure
		Lung (adenomas): M–3/40, 16/40 F–4/40, 15/40	[$P < 0.01$] (M, F)	
		Zymbal's gland (carcinomas): M–0/40, 4/40 F–0/40, 1/40	[NS]	
		Malignant tumours: M–9/40, 14/40 F–11/40, 28/40	-	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, RF/J (M, F) Lifetime Maltoni *et al.* (1989), Mehlman (2002)	0 (control), 500 mg/kg bw benzene in olive oil once/d, 4–5 d/wk for 52 wk Male, 45/group Female, 40/group	**Mammary Gland** (carcinomas): M–0/45, 0/45 F–1/40, 9/40	[$P < 0.05$] (F)	99.93% pure
		Lung: All tumours: M–5/45, 23/45 F–3/40, 15/40	[$P < 0.005$] (M, F)	
		Adenocarcinomas: M–0/45, 0/45 F–0/40, 1/40	[NS]	
		Leukaemia: M–17/45, 26/45 F–14/40, 24/40	[NS]	
Mouse, C57Bl/6-Trp53 (F) 26 wk French & Saulnier (2000)	0 (control), 200 mg/kg bw benzene 5d/wk Controls – 20/group Dosed – 40/group	**Subcutis** (sarcomas): 0/20, 16/39	[$P < 0.001$]	> 99.9% pure vehicle unspecified
		Thymus (lymphomas): 0/20, 3/39	[NS]	
Mouse, haploinsufficient $p16^{Ink4a}/p19^{Arf}$ (M, F) 27 wk NTP (2007)	0 (control), 25, 50, 100, 200 mg/kg bw benzene in corn oil 5 d/wk 15/group	**Malignant lymphomas:** M–0/15, 0/15, 0/15, 0/15, 5/15	$P = 0.021$ (high dose) $P < 0.001$ (trend)	

d, day or days; F, female; i.p., intraperitoneal; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years; bw, body weight

Table 3.3 Carcinogenicity studies in experimental animals exposed to benzene by intraperitoneal injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, A/J (M, F) 24 wk Stoner *et al.* (1986)	0 (control), 480, 1 200, 2 400 mg/kg bw in tricaprylin vehicle 3x/wk for 8 wk 16/group	**Lung adenomas:** M–3/16, 5/15, 8/16, 10/16 F–4/16, 4/15, 4/16, 6/15	NR	Purity NR
		Tumours/ mouse: M–0.25 ± 0.58, 0.53 ± 0.92, 0.63 ± 0.72, 0.69 ± 0.60 F–0.31 ± 0.60, 0.44 ± 0.89, 0.25 ± 0.45, 0.47 ± 0.64	$P < 0.05$ (1 200 and 2 400 mg/kg) (M)	

F, female; M, male; mo, month or months; NR, not reported; wk, week or weeks; bw, body weight

Table 3.4 Carcinogenicity studies in experimental animals exposed to benzene via skin application

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, hemizygous and homozygous Tg.AC (v-Ha-*ras*) (M, F) 20 wk Blanchard *et al.* (1998)	200 µl of acetone: vehicle control 200 µl benzene, neat 2–7x/wk 10 mice/treated group	Skin papillomas: Hemizygous Tg.AC M–6/65, 3/10 F–2/65, 4/10 Homozygous Tg.AC M–NR, 10/10 F–NR, 9/10	 [NS] [$P < 0.01$] - -	Purity NR
Mouse, hemizygous Tg.AC (v-Ha-*ras*) (M, F) 26 wk Holden *et al.* (1998)	G1: Untreated (shaved) G2: acetone 200 µl, 7d/wk, 20 wk G3: 100 µl benzene, 3x/wk, 20 wk G4: 150 µl benzene, 3x/wk, 20 wk 10 mice/group	Skin (papillomas): M–0/10, 0/10, 0/10, 3/10 F–0/10, 0/10, 1/10, 1/10	$P \leq 0.05$, G4 vs negative controls	Purity NR
Mouse, homozygous, FVB/N-Tg.AC (v-Ha-*ras*) (F) 32 wk French & Saulnier (2000)	0 µl/wk: 200 µl acetone 1/d 3x/wk for 20 wk (control) 450 µl/wk: 150 µl in 50 µl acetone 1/d, 3x/wk for 20 wk 800 µl/wk: 200µl neat, 2/d, 2/wk for 20 wk 20 mice/group	Granulocytic leukaemia: 0/19, 4/14*, 11/15*	*$P \leq 0.05$	> 99.9% pure

d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years

and of benign and malignant ovarian tumours, mammary gland carcinomas and carcinosarcomas, and Harderian gland carcinomas in female mice (NTP, 1986; Stoner *et al.*, 1986; Maronpot, 1987; Maltoni *et al.*, 1988, 1989; Huff *et al.*, 1989; Mehlman, 2002).

Increased multiplicity of lung adenomas was observed in male mice after intraperitoneal injection of benzene (Stoner *et al.*, 1986).

Exposure of genetically altered, tumour-prone mice to benzene by oral administration, skin application, or inhalation resulted in increased incidences of skin tumours (Blanchard *et al.* 1998; Holden *et al.*, 1998; French & Saulnier, 2000) and lymphohaematopoietic malignancies (French & Saulnier, 2000; NTP, 2007; Kawasaki *et al.*, 2009).

4. Other Relevant Data

4.1 Genetic and related effects

Benzene induced chromosomal aberrations, micronuclei and sister chromatid exchange in bone-marrow cells of mice, chromosomal aberrations in bone-marrow cells of rats and Chinese hamsters and sperm-head anomalies in mice treated *in vivo*. It induced chromosomal aberrations and mutation in human cells *in vitro* but did not induce sister chromatid exchange in cultured human lymphocytes, except in one study in which high concentrations of an exogenous metabolic system were used. In some test systems, benzene induced cell transformation. It did not induce sister chromatid exchange in rodent cells *in vitro*, but it did induce aneuploidy and, in some studies, chromosomal aberrations in cultured Chinese hamster ovary 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). Chromosomal aberrations in human peripheral lymphocytes have been associated with occupational exposure to benzene for decades (Forni, 1979; IARC, 1982; Eastmond, 1993; Zhang *et al.*, 2002; Holecková *et al.*, 2004).

4.2 Leukaemogenic potential of benzene

Benzene is carcinogenic to the bone marrow causing leukaemia and myelodysplastic syndromes (MDS) and probably also to the lymphatic system causing non-Hodgkin lymphoma. Its carcinogenic mechanism of action is likely to be different for these two target tissues and probably multifactorial in nature. The metabolism of benzene will be summarized below and a review is presented of the current state of knowledge on the mechanisms of leukaemia and lymphoma induction by benzene. With regard to leukaemia, probable mechanisms of leukaemogenesis in the myeloid series, mainly acute myeloid leukaemia (AML) and MDS are discussed. Then, potential mechanisms by which benzene could cause acute lymphocytic leukaemia (ALL) in both adults and children are reviewed. Finally, mechanisms for the benzene-induced development of non-Hodgkin lymphoma are summarized, including that of chronic lymphocytic leukaemia (CLL), as it is now classified as a form of lymphoma.

4.2.1 Metabolism of benzene and its relevance to carcinogenicity

Benzene must be metabolized to become carcinogenic (Ross, 2000; Snyder, 2004). Its metabolism is summarized in Fig. 4.1. The initial metabolic step involves cytochrome P450 (CYP)-dependent oxidation to benzene oxide,

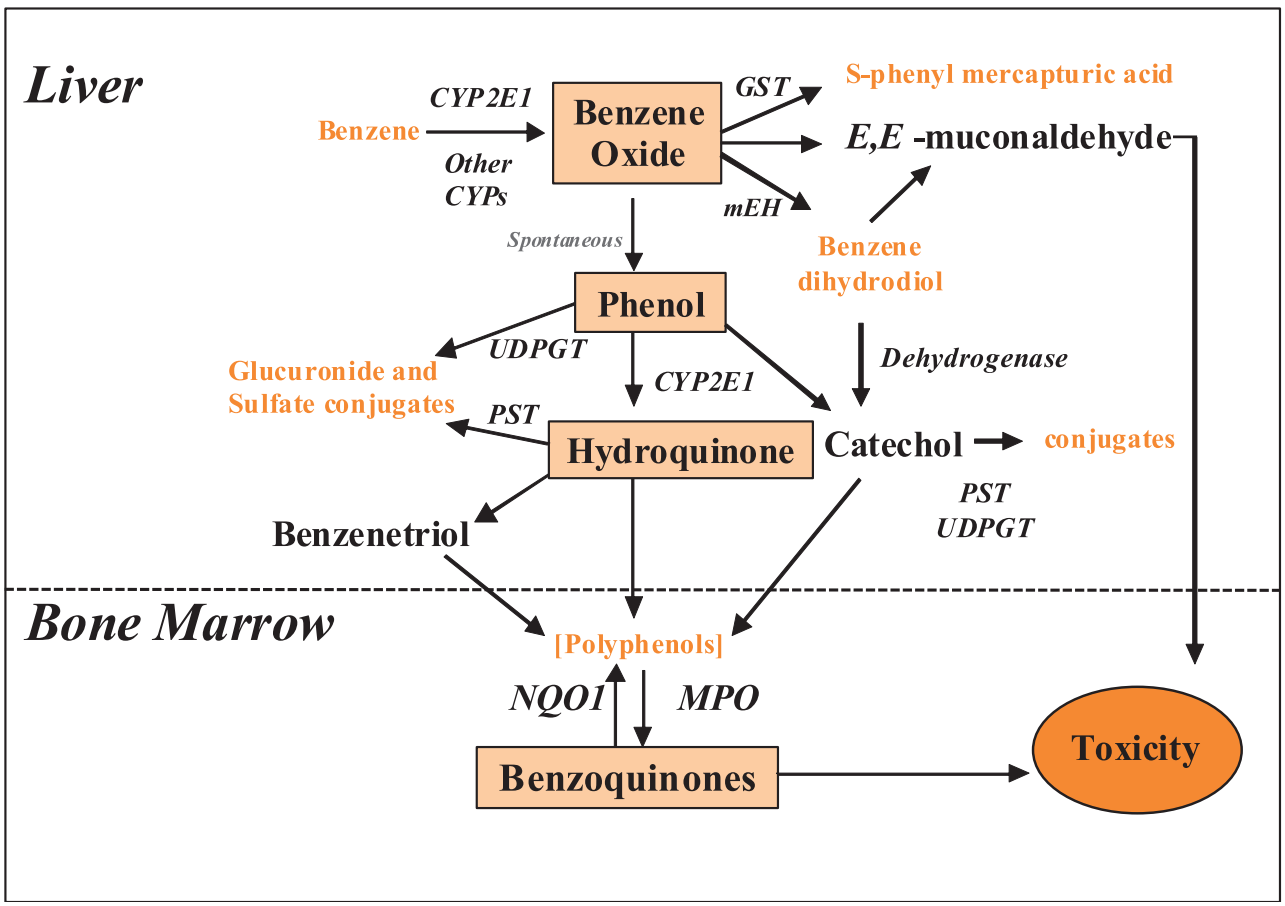
which exists in equilibrium with its tautomer oxepin. Most benzene oxide spontaneously rearranges to phenol, which is either excreted or further metabolized to hydroquinone and 1,4-benzoquinone. The remaining benzene oxide is either hydrolysed to produce benzene 1,2-dihydrodiol (catechol), which is further oxidized to 1,2-benzoquinone, or it reacts with glutathione to produce *S*-phenylmercapturic acid. Metabolism of oxepin is thought to open the aromatic ring, to yield the reactive muconaldehydes and *E,E*-muconic acid. Human exposure to benzene at concentrations in air between 0.1 and 10 ppm, results in urinary metabolite profiles with 70–85% phenol, 5–10% each of hydroquinone, *E,E*-muconic acid and catechol, and less than 1% of *S*-phenylmercapturic acid (Kim *et al.*, 2006b). Benzene oxide, the benzoquinones, muconaldehydes, and benzene dihydrodiol epoxides (formed from CYP-mediated oxidation of benzene dihydrodiol) are electrophiles that readily react with peptides, proteins and DNA (Bechtold *et al.*, 1992; McDonald *et al.*, 1993; Bodell *et al.*, 1996; Gaskell *et al.*, 2005; Henderson *et al.*, 2005; Waidyanatha & Rappaport, 2005) and can thereby interfere with cellular function (Smith, 1996). It remains unclear what role these different metabolites play in the carcinogenicity of benzene, but benzoquinone formation from hydroquinone via myeloperoxidase in the bone marrow has been suggested as being a key step (Smith, 1996). There is considerable evidence for an important role of this metabolic pathway that leads to benzoquinone formation, as the benzoquinone-detoxifying enzyme NAD(P)H:quinone oxidoreductase1 (NQO1) protects mice against benzene-induced myelodysplasia (Long *et al.*, 2002; Iskander & Jaiswal, 2005) and humans against the hematotoxicity of benzene (Rothman *et al.*, 1997). However, this does not rule out adverse effects from other metabolites.

Increased susceptibility to the toxic effects of benzene has been linked to genetic polymorphisms that increase the rate of metabolism of benzene to active intermediates, or decrease the rate of detoxification of these active intermediates (Rothman *et al.*, 1997; Xu *et al.*, 1998; Kim *et al.*, 2004).

Recently it has been shown that benzene is most likely metabolized initially to phenol and *E,E*-muconic acid via two enzymes rather than just one CYP enzyme, and that the putative, high-affinity enzyme is active primarily at benzene concentrations below 1 ppm (Rappaport *et al.*, 2009). CYP2E1 is the primary enzyme responsible for mammalian metabolism of benzene at higher levels of exposure (Valentine *et al.*, 1996; Nedelcheva *et al.*, 1999). CYP2F1 and CYP2A13 are reasonable candidate enzymes that are active at environmental levels of exposure below 1 ppm (Powley & Carlson, 2000; Sheets *et al.*, 2004; Rappaport *et al.*, 2009). These CYPs are highly expressed in the human lung. Despite much research, more work is needed to elucidate the different roles of multiple metabolites in the toxicity of benzene and the pathways that lead to their formation.

A role for the aryl-hydrocarbon receptor (AhR) is also emerging in the haematotoxicity of benzene. AhR is known mainly as the mediator for the toxicity of certain xenobiotics (Hirabayashi & Inoue, 2009). However, this transcription factor has many important biological functions and evidence is emerging that it has a significant role in the regulation of haematopoietic stem cells (Hirabayashi & Inoue, 2009; Singh *et al.*, 2009). It has been hypothesized that AhR expression is necessary for the proper maintenance of quiescence in these cells, and that AhR downregulation is essential for their "escape" from quiescence and subsequent proliferation (Singh *et al.*, 2009). It has been demonstrated that AhR-knockout (KO) ($AhR^{-/-}$) mice do not show any haematotoxicity after exposure to benzene (Yoon *et al.*, 2002). Follow-up studies have shown that mice that had been lethally irradiated and repopulated with marrow cells from AhR-KO mice did not display any sign of benzene-induced

Fig. 4.1 Simplified metabolic scheme for benzene showing major pathways and metabolizing enzymes leading to toxicity. CYP2E1, cytochrome P450 2E1; GST, glutathione-*S*-transferase; NQO1, NAD(P)H:quinone oxidoreductase 1; MPO, myeloperoxidase; UDPGT, Uridine diphosphate glucoronosyl transferase; PST, phenol sulphotransferase; mEH, microsomal epoxide hydrolase



haematotoxicity (Hirabayashi *et al.*, 2008). The most likely explanation for these findings is that the absence of AhR removes haematopoietic stem cells from their quiescent state and makes them susceptible to DNA damage from benzene exposure and subsequent cell death through apoptosis. Further research is needed to examine the effects of benzene and its metabolites on cycling and quiescent haematopoietic stem cells.

4.2.2 Mechanisms of myeloid leukaemia development

(a) General

AML and MDS are closely-related diseases of the bone marrow that arise de novo (without an obvious cause) in the general population or following therapy with alkylating agents, topoisomerase II inhibitors, or ionizing radiation (therapy-related AML and MDS, i.e. t-AML and t-MDS) (Pedersen-Bjergaard *et al.*, 2006, 2008). Occupational exposure to benzene is widely thought to cause leukaemias that are similar to various forms of t-AML and t-MDS (Irons

& Stillman, 1996; Larson & Le Beau, 2005; Zhang *et al.*, 2007). AML and MDS both arise from genetically altered CD34+ stem cells or progenitor cells in the bone marrow (Morgan & Alvares, 2005; Passegué & Weisman, 2005) and are characterized by many different types of recurrent chromosome aberrations (Pedersen-Bjergaard *et al.*, 2006; Mrózek & Bloomfield, 2008). These aberrations have been shown to often develop into the genetic mutations that produce leukaemia. Cytogenetic analysis of chromosome number and structure has therefore become important in diagnosis and treatment of MDS and AML (Pedersen-Bjergaard *et al.*, 2006; Mrózek & Bloomfield, 2008). Recent research has shown that the chromosome aberrations and gene mutations detected in therapy-related and de novo MDS and AML are identical, although the frequencies with which they are observed in different subtypes may differ (Pedersen-Bjergaard *et al.*, 2008). Hence, therapy-related and de novo MDS and AML are considered identical diseases (Pedersen-Bjergaard *et al.*, 2008).

At least three cytogenetic categories of AML and MDS are commonly observed: those with unbalanced aberrations, with balanced rearrangements, and with normal karyotype:

Unbalanced chromosome aberrations comprise primarily the loss of various parts of the long arm or loss of the whole chromosome 5 or 7 (5q–/–5 or 7q–/–7) and gain of a whole chromosome 8 (+8) (Pedersen-Bjergaard *et al.*, 2006, 2007, 2008). These cases often have a complex karyotype and carry point mutations of *TP53* or *AML1*. Unbalanced chromosome aberrations are common after therapy with alkylating agents.

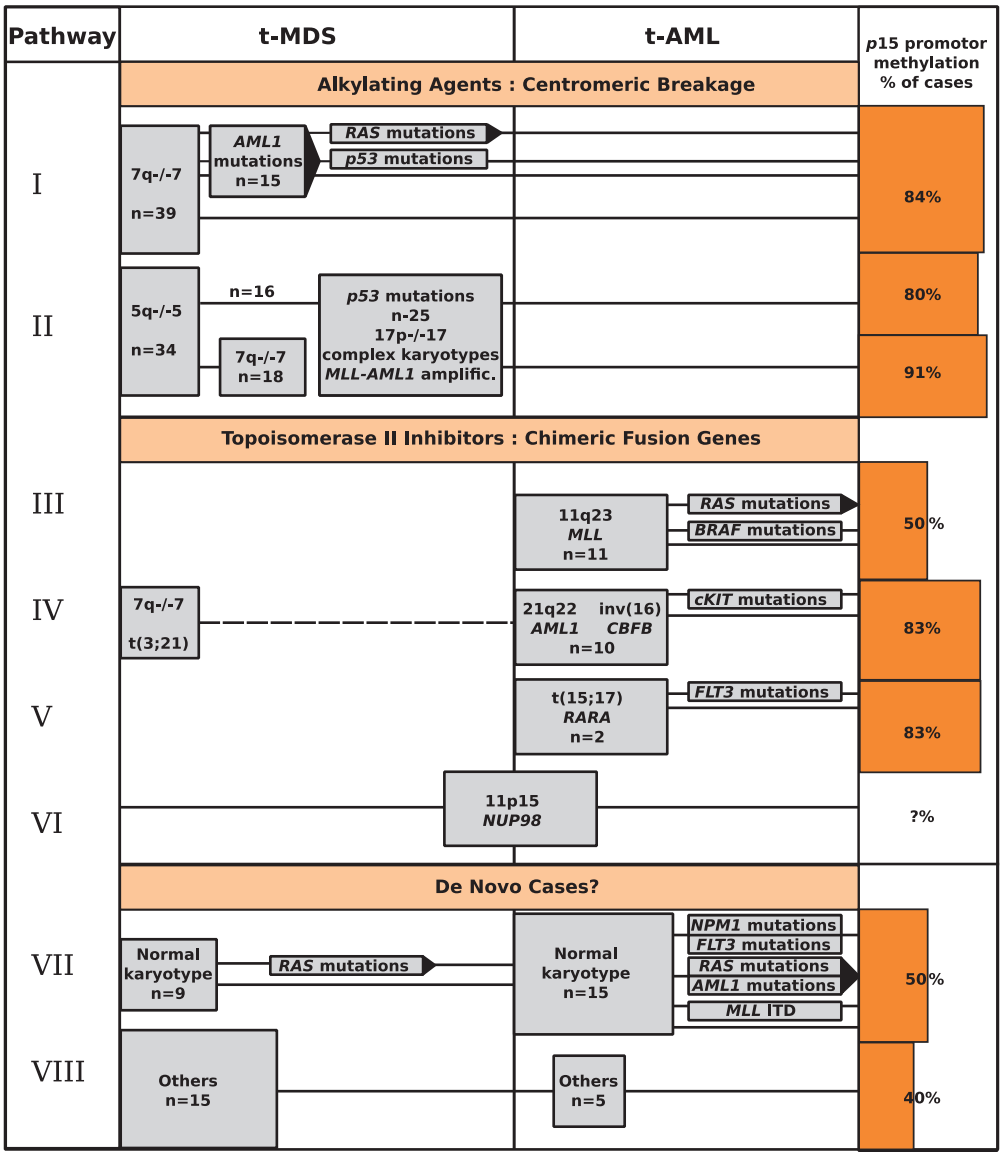
Balanced rearrangements are recurrent balanced translocations [e.g. t(11q23), t(8;21) and t(15;17)] or inversions [e.g. inv(16)], which arise, at least in the therapy-related subset of cases, as illegitimate gene recombinations related to functional inhibition of topoisomerase II (Pedersen-Bjergaard *et al.*, 2006, 2008). Among the most important rearranged transcription-factor genes are the mixed-lineage leukaemia (*MLL*) at 11q23, the *AML1* at 21q22, the retinoic-acid receptor-α *RARA* at 17q21 and the core-binding factor subunit-β (*CBFB*) at 16q22 (Pedersen-Bjergaard *et al.*, 2007).

Cases with a normal karyotype often harbour mutations of the *NPM1* gene (which encodes nucleophosmin), internal tandem duplications of the *FLT3* gene (which encodes fms-related tyrosine kinase), and/or point mutations or an altered methylation status of the *C/EBPα* gene (which encodes CCAAT/enhancer binding protein α) (Cuneo *et al.*, 2002; Pedersen-Bjergaard *et al.*, 2006, 2007, 2008; Hackanson *et al.*, 2008).

Within these three cytogenetic categories there are at least eight different genetic pathways that lead to MDS and AML, as defined by the specific chromosome aberrations present in each (Pathways I –VIII in Fig. 4.2). As more becomes clear about the molecular cytogenetics of leukaemia, it seems likely that many other pathways to AML and MDS will be discovered. For example, recent unbiased high-resolution genomic screens have identified many genes not previously implicated in AML that may be relevant for pathogenesis, along with many known oncogenes and tumour-suppressor genes (Ley *et al.*, 2008; Mardis *et al.*, 2009; Walter *et al.*, 2009).

Another classical pathway to AML is through the transformation of a myeloproliferative disorder (MPD) (Abdulkarim *et al.*, 2009), although there is less evidence for this pathway as a relevant mechanism to benzene-induced AML. MPDs include Philadelphia chromosome (Ph)-positive chronic myelogenous leukaemia (CML) and the Ph-negative conditions *polycythemia vera*, essential trombocythemia and idiopathic myelofibrosis. It is well established that AML may occur as a late complication in all these disorders. Over the first ten years after diagnosis, the incidence of leukaemic transformation is reported to be higher in patients with idiopathic myelofibrosis (8–23%) compared with

Fig. 4.2 Genetic Pathways to Myelodysplastic Syndromes (MDS) and Acute Myeloid Leukaemia



From Pedersen-Bjergaard *et al.* (2006)

patients with essential trombocythemia (0.5–1%) and *polycythemia vera* (1–4%) (Abdulkarim *et al.*, 2009). Thus, benzene may first produce an MPD, which later transforms into AML.

An important role for epigenetic changes is also emerging in association with the development of leukaemia. Functional loss of the CCAAT/enhancer binding protein α (*C/EBPα*) (also known as CEBPA), a central regulatory transcription factor in the haematopoietic system, can result in a differentiation block in granulopoiesis and thus contribute to leukaemic transformation (Fröhling & Döhner, 2004). Recent work has shown that epigenetic alterations of *C/EBPα* occur frequently in AML and that *C/EBPα* mRNA is a target for miRNA-124a (Hackanson *et al.*, 2008). This miRNA is frequently silenced by epigenetic mechanisms in leukaemia cell lines. *C/EBPα* is also capable of controlling *miRNA-223* expression, which is vital in granulocytic differentiation (Fazi *et al.*, 2005). Altered expression of several miRNAs is also observed in some forms of AML (Dixon-McIver *et al.*, 2008; Marcucci *et al.*, 2008).

(b) Mechanisms of benzene-induced myeloid leukaemia development

There is strong evidence that benzene can induce AML via pathways I, II and IV, considerable supporting evidence for pathway V, some evidence for pathway III, but little information regarding pathways VI–VIII (see Fig. 4.2). Exposure to benzene has been associated with higher levels of the chromosomal changes commonly observed in AML, including 5q–/–5 or 7q–/–7, +8, and t(8;21) in the blood cells of highly exposed workers (Smith *et al.*, 1998; Zhang *et al.*, 1999, 2002). The benzene metabolite hydroquinone produces these same changes in cultured human cells, including cultures of CD34+ progenitor cells (Smith *et al.*, 2000; Stillman *et al.*, 2000). This provides strong evidence for the induction by benzene of AML via pathways I, II and IV (see Fig. 4.2).

Pathways III, IV and V are related to the inhibition of the DNA-related enzyme topoisomerase II, which is essential for the maintenance of proper chromosome structure and segregation; it removes knots and tangles from the genetic material by passing an intact double helix through a transient double-stranded break that it creates in a separate segment of DNA (McClendon & Osheroff, 2007; Bandele & Osheroff, 2009). To maintain genomic integrity during its catalytic cycle, topoisomerase II forms covalent bonds between active-site tyrosyl residues and the 5′-DNA termini created by cleavage of the double helix (Bandele & Osheroff, 2009). Normally, these covalent topoisomerase II-cleaved DNA complexes (known as cleavable complexes) are fleeting intermediates and are tolerated by the cell. However, when the concentration or longevity of cleavage complexes increases significantly, DNA double-strand breaks occur (Lindsey *et al.*, 2004). If topoisomerase II–induced double-strand breaks are incorrectly repaired, two unrelated (nonhomologous) chromosomes are fused together to produce translocations or inversions (Deweese & Osheroff, 2009).

There are different types of topoisomerase-II inhibitors. Epidophyllotoxins, such as etoposide, cause chromosome damage and kill cells by increasing physiological levels of topoisomerase II-DNA cleavage complexes (Baker *et al.*, 2001; Felix, 2001; Deweese & Osheroff, 2009). These drugs are referred to as topoisomerase-II poisons to distinguish them from catalytic inhibitors of the enzyme because they convert this essential enzyme to a potent cellular toxin. Other drugs, such as merbarone, act as inhibitors of topo-II activity but, in contrast to etoposide they do not stabilize topoisomerase II-DNA cleavable complexes. Nevertheless, they are potent clastogens both in vitro and in vivo (Wang *et al.*, 2007).

Several studies have shown that benzene in vivo, and its reactive metabolites hydroquinone

and 1,4-benzoquinone in vitro, inhibit the functionality of topoisomerase II and enhance DNA cleavage (Chen & Eastmond, 1995; Frantz *et al.*, 1996; Hutt & Kalf, 1996; Eastmond *et al.*, 2001; Fung *et al.*, 2004; Lindsey *et al.*, 2004, 2005; Whysner *et al.*, 2004). Bioactivation of hydroquinone by myeloperoxydase to 1,4-benzoquinone enhances topoisomerase-II inhibition (Eastmond *et al.*, 2005). Indeed, 1,4-benzoquinone was shown to be a more potent topoisomerase-II inhibitor than hydroquinone in a cell-free assay system (Hutt & Kalf, 1996; Baker *et al.*, 2001). These findings demonstrate that benzene through its reactive quinone metabolites can inhibit topoisomerase II and probably cause leukaemias with chromosome translocations and inversions known to be generated by topoisomerase-II inhibitors, including AMLs harbouring t(21q22), t(15;17) and inv(16) in a manner consistent with pathways IV and V (Andersen *et al.*, 2002; Voltz *et al.* 2004; Mistry *et al.*, 2005; Pedersen-Bjergaard *et al.*, 2007, 2008). The evidence for rearrangements of the mixed lineage leukaemia (*MLL*) gene through t(11q23) via pathway III in benzene-induced leukaemia is less convincing but may occur through an apoptotic pathway (Vaughan *et al.*, 2005).

AML can arise de novo via pathways VII and VIII without apparent chromosome abnormalities, but molecular analysis has revealed many genetic changes in these apparently normal leukemias, including mutations of *NPM1, AML1, FLT3, RAS* and *C/EBPα*. (Fig. 4.2; Cuneo *et al.*, 2002; Falini *et al.*, 2007; Mardis *et al.*, 2009). More work is needed to clarify the ability of benzene and its metabolites to produce mutations of the type found in these leukaemias, along with those found in Ph-negative MPDs such as Janus kinase 2 (*JAK2*), and somatic mutations in the ten-eleven translocation 2 (*TET2*) oncogene, which are found in about 15% of patients with various myeloid cancers (Delhommeau *et al.*, 2009). One potential mechanism for the induction of such mutations is through the generation of reactive oxygen species.

The ability of benzene and/or its metabolites to induce epigenetic changes related to the development of leukaemia, such as altered methylation status of *C/EBPα,* is unclear at this time. Bollati *et al.* (2007) reported that hypermethylation in *p15* (+0.35%; $P = 0.018$) and hypomethylation in the *MAGE-1* gene (encoding the human melanoma antigen) (–0.49%; $P = 0.049$) were associated with very low exposures to benzene (~22 ppb) in healthy subjects including gas-station attendants and traffic-police officers, although the corresponding effects on methylation were very low. Further study of the role epigenetics in the haematotoxicity and carcinogenicity of benzene is warranted, including studies of aberrant DNA methylation and altered microRNA expression.

While benzene and its metabolites are clearly capable of producing multiple forms of chromosomal mutation, including various translocations, deletions and aneuploidies, these are usually insufficient as a single event to explain the induction of leukaemia (Guo *et al.*, 2008; Lobato *et al.*, 2008). Other secondary events, such as specific gene mutations and/or other chromosome changes, are usually required (Guo *et al.*, 2008; Lobato *et al.*, 2008). Thus, benzene-induced leukaemia probably begins as a mutagenic event in the stem cell or progenitor cell and subsequent genomic instability allows for sufficient mutations to be acquired in a relatively short time. Studies have shown that the benzene metabolite hydroquinone is similar to ionizing radiation in that it induces genomic instability in the bone marrow of susceptible mice (Gowans *et al.*, 2005). Recent findings showing the importance of genes involved in DNA repair and maintenance – such as the *WRN* gene encoding the Werner syndrome protein – in determining genetic susceptibility to the toxicity of benzene also support this mechanism (Shen *et al.*, 2006; Lan *et al.*, 2009; Ren *et al.*, 2009).

Haematotoxic effects may also contribute to leukaemogenesis from benzene. Haematopoietic stem cells occupy an ordered environment in the bone marrow and interact with supportive stromal cells and mature lymphocytes. Haematotoxic damage to this ordered stem-cell microenvironment most likely allows for the clonal expansion of the leukaemic stem cells. This dual mode of action for benzene fits with the known ability of benzene metabolites to induce chromosomal mutations and genomic instability in blood stem cells and progenitor cells, and with the fact that haematotoxicity is associated with an increased risk for benzene-induced haematopoietic malignancies (Rothman *et al.*, 1997).

Thus, exposure to benzene can lead to multiple alterations that contribute to the leukaemogenic process. Benzene may act by causing chromosomal damage (aneuploidy, deletions and translocations) through inhibition of topoisomerase II, disruption of microtubules and other mechanisms; by generating oxygen radicals that lead to point mutations, strand breaks and oxidative stress; by causing immune system dysfunction that leads to decreased immunosurveillance (Cho, 2008; Li *et al.*, 2009); by altering stem-cell pool sizes through haematotoxic effects (Irons *et al.*, 1992); by inhibiting gap-junction intercellular communication (Rivedal & Witz, 2005); and by altering DNA methylation and perhaps specific microRNAs. This multimodal mechanism of action for benzene suggests that the effects of benzene on the leukaemogenic process are not singular and can occur throughout the process.

4.2.3 Potential mechanisms of benzene-induced acute lymphocytic leukaemia (ALL) development

Evidence of an association between exposure to benzene from air pollution and childhood leukaemia is growing. The most common form of childhood leukaemia is ALL, with AML being less common at around 15% of the incidence of ALL. The opposite is true for adults where the ratio is reversed, with AML being predominant. Reasons for this difference were suggested to be age-related defects in lymphopoiesis (Signer *et al.*, 2007). Studies with a murine model of chronic myeloid leukaemia – an adult-onset malignancy that arises from transformation of haematopoietic stem cells by the breakpoint cluster region-Ableson (*BCR-ABL*^{P210}) oncogene – demonstrated that young bone-marrow cells transformed with *BCR-ABL*^{P210} initiated both MPD and B-lymphoid leukaemia, whereas *BCR-ABL*^{P210}-transformed old bone-marrow cells recapitulated the human disease by inducing MPD with rare lymphoid involvement (Signer *et al.*, 2007). Thus, if benzene were to induce a leukaemia-related oncogenic mutation in young bone-marrow cells, it could produce either an MPD that transformed to AML, or a B-cell ALL, whereas exposure in an adult would have only a very limited chance of producing ALL.

The long-standing distinction between AML and ALL also has become somewhat blurred in recent years. Both forms of leukaemia arise in pluripotential stem cells or early progenitor cells in the bone marrow. Either disease can occur under conditions that formerly seemed restricted to AML. These include ALL occurring in the acute leukaemia seen in Down Syndrome (Kearney *et al.*, 2009); in secondary leukaemias related to chemotherapy (Lee *et al.*, 2009); and in the blast crisis of chronic myelogenous leukaemia (Calabretta & Perrotti, 2004). Similarly, the Philadelphia chromosome, long considered to be specific to chronic myelogenous leukaemia, is also the most common chromosome rearrangement in adult ALL (Ravandi & Kebriaei, 2009).

Since the genotoxic action of benzene metabolites on pluripotent precursor cells in the bone marrow appears promiscuous, producing multiple genetic abnormalities, it seems probable that exposure to benzene can initiate both AML and ALL by causing the chromosomal rearrangements

and mutations that are on the causal pathway to these malignancies. For childhood ALL and AML it has been shown that the disease is usually initiated *in utero*, since leukaemic translocations and other genetic changes have been detected in blood spots collected at birth (Wiemels *et al.*, 1999; Wiemels *et al.*, 2002; Greaves & Wiemels, 2003; McHale *et al.*, 2003). Thus, exposure of the mother, and perhaps even the father, to benzene could be just as important as exposure of the child in producing childhood AML and ALL, as has been suggested in several epidemiological studies (van Steensel-Moll *et al.*, 1985; McKinney *et al.*, 1991; Shu *et al.*, 1999; Scélo *et al.*, 2009). Supporting this hypothesis is an animal study demonstrating that *in utero* exposure to benzene increases the frequency of micronuclei and DNA recombination events in haematopoietic tissue of fetal and post-natal mice (Lau *et al.*, 2009). Another study showed that oxygen radicals play a key role in the development of *in utero*-initiated benzene toxicity through disruption of haematopoietic cell-signalling pathways (Badham & Winn, 2010). These studies support the idea that genotoxic and non-genotoxic events following exposure to benzene may be initiators of childhood leukaemia *in utero*.

4.2.4 Mechanisms of lymphoma development

(a) General

Lymphoma is a cancer of the immune system that includes over 40 malignant diseases originating from B- and T-lymphocytes and natural killer (NK) cells (Swerdlow *et al.*, 2008). It is therefore not surprising that functional disorders of immune-system cells are associated with a risk for malignant transformation. Immune deficiency is one of the strongest known risk factors for non-Hodgkin lymphoma (NHL) (Hartge & Smith, 2007). The risk for NHL increases with the degree of immune deficiency, and there is no evidence of a threshold (Grulich *et al.*, 2007). Thus, even modest immunosuppression, especially at the local level, may increase the risk for lymphoma.

It is well recognized that lymphomas, like other tumours, develop according to a multistep pathogenic process (Smith *et al.*, 2004). Clonal progression of an initiated cell to a clone of highly malignant cells is well documented. Natural selection of clones already present within oligoclonal expansions gives rise to true monoclonal lymphomas. Thus, it is possible to make generalizations about the type of molecular mechanism responsible for each of the stages involved in lymphomagenesis. For example, a cell may become initiated and genetically unstable through errors in recombination and DNA repair, which could be spontaneous or induced by an exogenous chemical agent. Other early molecular events often inhibit apoptosis and lead to the expansion of an intrinsically genetically unstable population of cells, which is at risk for additional genetic events and tumour progression. An example is the t(14;18) chromosome translocation associated with B-cell lymphoma 2 gene *BCL2* dysregulation, which inhibits apoptosis (Cimmino *et al.*, 2005; Thomadaki & Scorilas, 2006). Normally, one of the key protectors against the selection and progression of malignant clones of cells into full-blown lymphoma is local immunosurveillance in which activated T-cells kill the mutated clones. It is generally accepted that if this immunosurveillance is no longer intact, e.g. in immuno-suppressed individuals, then the malignant cells divide and grow rapidly, collecting more mutations to become aggressive, rapidly growing tumours.

(b) Mechanisms of benzene-induced lymphoma development

From the discussion above, there are at least two probable mechanisms by which exposure to benzene could enhance the incidence of lymphoma, i.e. by inducing chromosome rearrangements associated with NHL, and through

immunosuppression leading to decreased immunosurveillance.

Benzene is well known to produce multiple cytogenetic abnormalities in lymphocytes (Tough & Brown, 1965; Forni, 1971, 1979; Picciano, 1979; Smith & Zhang, 1998; Zhang *et al.*, 2002). Further, benzene induces specific chromosomal changes associated with NHL in human lymphocytes (Zhang *et al.*, 2007). Fluorescence in situ hybridization (FISH) analysis showed increased levels of t(14;18) and del(6q) in benzene-exposed workers, but the higher levels of t(14;18) could not be confirmed in a follow-up study by use of real time-PCR (polymerase chain reaction) (McHale *et al.*, 2008). This may be because the PCR method only detected 50% of t(14;18) translocations or that the FISH method detects non-functional as well as functional translocations. Reduced immunosurveillance is another potential mechanism of NHL induction by benzene. The importance of T-cell immunosurveillance in preventing B-cell neoplasia is well established and is carried out by activated cytotoxic T lymphocytes. The toxic effects of benzene on T-cells is well documented and there appears to be a selective effect on $CD4^+$ T-lymphocytes resulting in a lowering of the $CD4^+/CD8^+$ ratio (Lan *et al.*, 2004). This immunosuppressive pattern is similar to the early onset of acquired immuno-deficiency syndrome (AIDS), and although it is not as severe it may be associated with an increased risk for NHL (Grulich *et al.*, 2007). Thus, benzene, like other leukaemogens including alkylating agents, topoisomerase inhibitors, and ionizing radiation, may cause NHL through a combination of immunosuppression and DNA double-strand break induction that leads to illegitimate recombination and chromosome rearrangements in lymphoid cells.

Thus, the biological plausibility of benzene as a cause of lymphoproliferative disorders has been strengthened in recent years. There are additional studies demonstrating that benzene produces lymphomas in laboratory animals, and a recent study showing that it does so simultaneously with AML in *Tp53*-deficient mice (Kawasaki *et al.*, 2009). Multiple studies show that it produces genotoxicity in the lymphocytes of exposed humans. Accordingly, there is considerable support for the notion that it is biologically plausible for benzene to cause human lymphatic tumours.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of benzene. Benzene causes acute myeloid leukaemia/acute non-lymphocytic leukaemia.

Also, a positive association has been observed between exposure to benzene and acute lymphocytic leukaemia, chronic lymphocytic leukaemia, multiple myeloma, and non-Hodgkin lymphoma.

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

There is strong evidence that benzene metabolites, acting alone or in concert, produce multiple genotoxic effects at the level of the pluripotent haematopoietic stem cell resulting in chromosomal changes in humans consistent with those seen in haematopoietic cancer. In multiple studies in different occupational populations in many countries over more than three decades a variety of genotoxic changes, including chromosomal abnormalities, have been found in the lymphocytes of workers exposed to benzene.

Benzene is *carcinogenic to humans (Group 1).*

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BIS(CHLOROMETHYL) ETHER AND CHLOROMETHYL METHYL ETHER

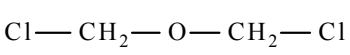
Bis(chloromethyl) ether (BCME) and chloromethyl methyl ether (CMME) were considered by previous IARC Working Groups in 1973 and 1987 (IARC, 1974, 1987a). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agents

1.1.1 Bis(chloromethyl) ether

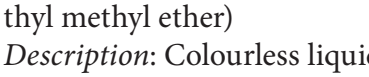
Chem. Abstr. Serv. Reg. No.: 542-88-1
Chem. Abstr. Serv. Name:
1,1′-Oxybis[1-chloromethane]
Formula: $C_2H_4Cl_2O$



Relative molecular mass: 115.0
Synonyms: BCME; Bis(chloromethyl) ether; chloromethyl ether (note: this name is sometimes used incorrectly for chloromethyl methyl ether)
Description: Colourless liquid, suffocating odour (O'Neil, 2006)
Boiling point: 106 °C (Lide, 2008)
Solubility: Miscible with ethanol and diethyl ether (Lide, 2008)

1.1.2 Chloromethyl methyl ether

Chem. Abstr. Serv. Reg. No.: 107-30-2
Chem. Abstr. Serv. Name:
Chloromethoxymethane
Formula: C_2H_5ClO



Relative molecular mass: 80.5
Synonyms: CMME; chloromethyl methyl ether; chloromethoxymethane; methyl chloromethyl ether; monochloromethyl ether; chlorodimethyl ether
Description: Colourless liquid (O'Neil, 2006)
Boiling point: 59.5 °C (Lide, 2008)
Solubility: Soluble in acetone, chloroform, diethyl ether, and ethanol (Lide, 2008)

1.2 Uses

BCME and CMME are used primarily as chemical intermediates and alkylating agents. BCME is used as a laboratory reagent in the manufacture of plastics, ion-exchange resins, and polymers (HSDB, 2003). Historical uses of

Table 1.1 Estimated numbers of workers exposed to BCME and CMME in the European Union

Industry, occupational activity	
Manufacture of industrial chemicals	1000
Manufacture of fabricated metal products, except machinery and equipment	350
Wholesale and retail trade, and restaurants and hotels	600
Sanitary and similar services	300
TOTAL	2250

From CAREX (1999)

BCME include crosslinking of cellulose, preparation of styrene and other polymers, surface treatment of vulcanized rubber to increase adhesion, and manufacture of flame-retardant fabrics (ATSDR, 1989). CMME is used as an alkylating agent and industrial solvent to manufacture dodecylbenzyl chloride, water repellants, ion-exchange resins, and polymers, and as a chloromethylating reagent (HSDB, 2003).

Exposure to these chemicals is strictly regulated in the United States of America (USA) and worldwide. Small quantities of BCME and CMME are currently produced, to be used only in enclosed systems for the synthesis of other chemicals (Brüske-Hohlfeld, 2009).

1.3 Human exposure

1.3.1 Occupational exposure

The primary route of occupational exposure to BCME or CMME is through inhalation of vapours; however, the potential for exposure nowadays is low because these chemicals are no longer produced or sold in large quantities and most industrial operations with these chemicals are conducted in closed containers. The most likely source of exposure to BCME is during the production or use of chemicals in which it may be present as a contaminant or be formed inadvertently (ATSDR, 1989).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the numbers of workers exposed to BCME and CMME in the EU by industry (CAREX, 1999)

From the US National Occupational Exposure Survey (1981–83) it was estimated that 14 workers (all laboratory personnel, including five women) were potentially exposed to BCME. No estimate of potential CMME exposure was reported (NIOSH, 1984).

1.3.2 Non-occupational exposure

The primary routes of potential human exposure to BCME and technical-grade CMME are inhalation and dermal contact. BCME is rapidly degraded in the environment and has not been detected in ambient air or water (ATSDR, 1989). According to the US Environmental Protection Agency's Toxics Release Inventory, almost all environmental releases of BCME and CMME have been into the air (US EPA, 2003).

2. Cancer in Humans

BCME and CMME were evaluated previously in *IARC Monograph* Volume 4 and in Supplement 7 (IARC, 1974, 1987a).

In a retrospective study of a small group of men exposed to BCME during the period 1956–1962, six cases of lung cancer were found among 18 workers in a testing laboratory. Five of these six men were moderate smokers, one was a non-smoker. Two further cases of lung cancer were seen in a group of 50 production workers. Five of these eight cases were oat-cell carcinomas. Duration of exposure had been six to nine years, while the period from first exposure to diagnosis was 8–16 years (Thiess *et al.*, 1973; IARC, 1974).

In a five-year observational study of 125 workers exposed to CMME, four cases of lung cancer were diagnosed, representing an eight-fold higher incidence than that in a control group (n = 2804) with similar smoking history. In a retrospective follow-up, a total of 14 cases were identified, all of whom had been working in the production of CMME. In the latter group, three men were non-smokers. Duration of exposure had been 3–14 years. Histological analysis revealed that 12 of the 14 cases were oat-cell carcinomas (Figueroa *et al.*, 1973; IARC, 1974). This cohort was further reported on (Weiss & Boucot, 1975; Weiss *et al.*, 1979; Weiss, 1982, Weiss & Nash, 1997) with confirmatory results (Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-20-Table2.1.pdf).

Several additional case-reports (Bettendorf, 1977; Reznik *et al.*, 1978; Roe, 1985; Nishimura *et al.*, 1990) and epidemiological studies from the USA (Collingwood *et al.*, 1987), the United Kingdom (McCallum *et al.*, 1983) and France (Gowers *et al.*, 1993) demonstrated that workers exposed to CMME and/or BCME have an increased risk for lung cancer. Among heavily exposed workers, the relative risks were ten-fold or more. An increase in risk was observed with duration of exposure and with cumulative exposure. Histological evaluation indicated that exposure resulted primarily in small-cell type lung cancer (Weiss & Boucot, 1975). The highest relative risks appeared to occur 15–19 years after first exposure (Weiss, 1982), and latency was shortened among workers with heavier exposure (Weiss & Figueroa, 1976; Pasternack & Shore, 1981).

3. Cancer in Experimental Animals

3.1 BCME

Studies on the carcinogenesis of BCME in rats, mice and hamsters after inhalation, skin application, and subcutaneous or intra-peritoneal injection have been reviewed in previous IARC Monographs (IARC, 1974, 1987b). The results of adequately conducted carcinogenicity studies are summarized in Tables 3.1, 3.2, 3.3, 3.4. There were no additional studies reported in the literature since *IARC Monographs* Supplement 7 (IARC, 1987b).

BCME was tested for carcinogenicity by inhalation exposure in five studies with rats, one study with mice and two studies with hamsters; by skin application in two studies with mice; by subcutaneous injection in one study with rats and three with mice; and by intra-peritoneal injection in one study with mice.

Exposure to BCME by inhalation caused an increased incidence of rare malignant tumours of the nose (esthesioneuroepitheliomas and squamous-cell carcinomas of the nasal mucosa) and squamous-cell carcinomas of the lung in male rats (Kuschner *et al.*, 1975; Leong *et al.*, 1981; Albert *et al.*, 1982; Sellakumar *et al.*, 1985) and of lung adenomas in male mice (Leong *et al.*, 1981). Skin application of BCME resulted in an increased incidence of skin papillomas in male and female mice (Van Duuren *et al.*, 1969; Zajdela *et al.*, 1980) and of squamous-cell carcinomas of the skin in female mice (Van Duuren *et al.*, 1969). Intra-peritoneal injection caused increased incidences of sarcomas at the site of injection in female mice (Van Duuren *et al.*, 1975). Subcutaneous injection of BCME caused strongly increased incidences of lung adenomas

Table 3.1 Carcinogenicity studies in experimental animals exposed to bis(chloromethyl) ether by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M) Lifetime Kuschner *et al.* (1975)	0.1 ppm, 6 h/d, 5 d/wk, 70 rats/group. In addition, rats received 10, 20, 40, 60, 80 or 100 6-h exposures, with 50, 50, 20, 20, 50, 30/group, respectively 240 rats served as controls	**Nose** Esthesioneuroepitheliomas: 0/50, 1/50, 2/20, 2/20, 9/50, 3/30 Malignant olfactory tumours: 0/50, 0/50, 0/20, 0/20, 0/50, 1/30 Ganglioneuroepitheliomas: 0/50, 0/50, 0/20, 0/20, 1/50, 0/30 Squamous cell carcinomas: 0/50, 0/50, 0/20, 0/20, 1/50, 0/30 Poorly differentiated epithelial tumours: 0/50, 1/50, 1/20, 0/20, 1/50, 1/30 Nasal cavity adenocarcinomas: 1/50,10/50, 0/20, 0/20, 1/50, 1/30 **Lung** Squamous cell carcinomas: 0/50, 0/50, 0/20, 2/20, 3/50, 8/30 Adenocarcinomas: 0/50, 0/50, 1/20, 0/20, 0/50, 0/30	NR	Purity NR Tumour incidence NR for controls
Rat, SPF Sprague-Dawley (M) Lifetime Leong *et al.* (1981)	0, 1, 10, 100 ppb 6 h/d, 5 d/wk for 6 mo 120/group	Esthesioneuroepitheliomas of the nose: 0/112, 0/113, 0/111, 96/111* Lung adenomas: 0/112, 0/113, 0/111, 4/111[$]	*$P < 0.05$ [$]$P = 0.059$	Purity NR Esthesioneuroepitheliomas were malignant tumours, several of which invaded the cribriform plate into the brain and metastasized to the regional lymph nodes and/or the lungs.
Rat, Sprague-Dawley (M) Lifetime Albert *et al.* (1982)	Premixed HCHO, 14.7 ppm + HCL, 10.6 ppm 6 h/d, 5 d/wk 99/group Air-sham controls, 50/group	Papillomas of the nasal mucosa: 0/50, 3/99 Squamous cell carcinomas of the nasal mucosa: 0/50, 25/99	[NS] [$P < 0.0001$]	Purity NR Weight gains in the exposed group lower than in the controls. All exposed animals had died by 100 wk.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M) Lifetime Sellakumar *et al.* (1985)	HCL alone, 10.0 ppm; HCHO alone, 14.8 ppm; premixed HCHO, 15.2 ppm + HCL, 9.9 ppm; non premixed HCHO, 14.9 ppm + HCL, 9.7 ppm 6 h/d, 5 d/wk 100/group	**Nasal cancers** Squamous cell carcinomas: 0/99, 38/100, 45/100, 27/100 Adenocarcinomas: 0/99, 0/100, 1/100, 2/100 Mixed carcinomas: 0/99, 1/100, 0/100, 0/100 Fibrosarcomas: 0/99, 1/100, 1/100, 0/100 Esthesioneuroepitheliomas: 0/99, 0/100 1/100, 0/100	**Nasal cancers** Premixed vs HCHO: $P < 0.025$ Non-premixed vs HCHO: NS	Purity NR Both combined exposures and HCHO exposure alone had a marked decreasing effect on body weight after 16 wk. Mortality was higher in the premixed group after 32 wk of exposure. The concentrations of BCME in the premixed HCl-HCHO chamber varied between 0.1 and 0.4 ppb. It was noted that alkylating agents other than BCME could have been formed by the interaction of HCHO and HCl and that, since the average amount of BCME in the exposure chamber of the premixed HCl-HCHO was less than 1 ppb, BCME may not have been the only agent responsible for the induction of tumours.
Mouse, Strain A/Heston (M) 27 wk Leong *et al.* (1971)	0 and 1.0 ppm (0.005 mg/L) 6 h/d, 5 d/wk; total of 82 exposures. Controls exposed to filtered air 50/group	Lung adenomas: 20/49, 26/47 Lung adenoma multiplicity: 2.2, 5.2	NS NR	Industrial grade (purity NR) Exposures resulted in loss of body weight and higher mortality
Mouse, Ha/ICR (M) Lifetime Leong *et al.* (1981)	0, 1, 10, 100 ppb 6 h/d, 5 d/wk for 6 mo 144–157/group	Lung adenomas: 6/157, 4/138, 2/143, 7/144 Lung adenocarcinomas: 4/157, 3/138, 1/143, 3/144 Lung adenomas in mice that survived beyond the initial 6 mo exposure period: 9/86, 5/45, 3/37, 8/27*	*$P < 0.05$	Purity NR There was an exposure concentration-related effect on cumulative mortality. Deaths began at approximately 1 mo of exposure, plateau-ing between 6 and 8 mo for exposed groups and 11 mo for the controls. Control and exposed mice developed an ascending urinary tract infection that was considered the direct cause of death.

d, day or days; h, hour or hours; HCl, hydrochloric acid; HCHO, formaldehyde vapours; M, male; mo, month or months; NR, not reported; NS, not significant; vs, versus; wk, week or weeks

Table 3.2 Carcinogenicity studies in mice exposed to bis(chloromethyl) ether by intra-peritoneal injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, ICR/Ha Swiss (F) 424–456 d Van Duuren *et al.* (1975)	0 (control) or 0.02 mg BCME in 0.05 mL nujol (purified paraffin oil) once weekly injection 50/group	Sarcoma (at injection site): 0/30, 4/30	$P < 0.05$, χ^2 test	Purity NR

d, day or days; F, female, NR, not reported

Table 3.3 Carcinogenicity studies in mice exposed to bis(chloromethyl) ether by skin application

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, ICR/Ha Swiss (F) 540 d Van Duuren *et al.* (1969)	**BCME as an initiator**			> 99% pure When BCME was used as a promoter or carcinogen, the experiment was terminated at 325 d due to ulcers, cancers and poor condition of the animals.
	Single application of 1.0 mg BCME in 0.1 mL benzene followed 14 d later by 0.025 mg PE in 0.1 mL acetone 3 × /wk. Controls received 0.025 mg PE in 0.1 mL acetone 3 × /wk. 20/group	Skin papillomas: 2/20, 5/20 Skin squamous cell carcinomas: 0/20, 2/20	[NS] [NS]	
	BCME as a promoter			
	Single application of 0.15 mg B[a]P in 0.1 benzene followed 14 d later by 2.0 mg BCME in 0.1 mg benzene 3 × /wk for 325 d. Controls received a single application of 0.15 mg B[a]P in 0.1 mL benzene. 20/group	Skin papillomas: 0/20, 13/20 Skin squamous cell carcinomas: 0/20, 12/20	[$P < 0.0001$] [$P < 0.0001$]	
	BCME as a carcinogen			
	2 mg BCME in 0.1 mL benzene 3x weekly. Controls received 0.1 mL benzene 3 × /wk. 20/group	Skin papillomas: 0/20, 13/20 Skin squamous cell carcinomas: 0/20, 12/20	[$P < 0.0001$] [$P < 0.0001$]	
Mouse, XVIInc./Z (M) 590 d Zajdela *et al.* (1980)	Single application of 1.0 mg BCME in 80 µl benzene followed by 2.0 µg TPA in 80 µl acetone 3 × /wk for 42 wk. Controls received 2.0 µg TPA in 80 µl acetone 3 × /wk for 42 wk 28/group	Skin: Papillomas: 4/28, 12/28 Carcinomas: 0/28, 3/28 Tumour multiplicity: 1.0, 1.3.	 [significant] NS NR	98.9% pure

B[a]P, benzo[a]pyrene; d, day or days; F, female; M, male; NR, not reported; NS, not significant; PE, phorbol ester; TPA, 12-*O*-tetradecanoylphorbol-13-acetate, wk, week or weeks

Table 3.4 Carcinogenicity studies in experimental animals exposed to bis(chloromethyl) ether by subcutaneous injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (F) 515 d Van Duuren *et al.* (1969)	3 mg BCME in 0.1 mL nujol (purified paraffin oil) once/wk for 114 d, reduced to 1 mg BCME in 0.1 mL nujol weekly for unspecified time and later reduced to 1 mg BCME in 0.1 mL nujol 3 × /mo. Vehicle control: 0.1 mL nujol weekly for 300 d 20/group	Skin fibromas: 0/20, 2/20 Skin fibros arcomas: 0/20, 5/20	[NS] [$P < 0.05$]	> 99% pure BCME dosage was reduced because of corrosive effects at injection site. By 300 d the treatment was discontinued because the animals were in poor condition with substantial weight loss and ulceration around the injection site.
Mouse, Newborn ICR Swiss (M, F) 6 mo Gargus *et al.* (1969)	Single injection of 50 µl of 0.05% BCME solution in peanut oil when 24–72 h old (dose, 12.5 µl/kg bw BCME) Vehicle controls: 50 µl of peanut oil (25 mL/kg bw) 50/treated groups, 20–30/control groups	Lung adenomas: 2/30, 25/50 (M); 5/20, 20/50 (F) Lung adenomas/animal: 0.07, 0.82 (M); 0.25, 0.46 (F)	[$P < 0.05$] (M) NR	Industrial grade (purity unspecified) One papilloma and one fibrosarcoma developed at the site of injection in two BCME-treated mice; such tumours did not occur in control mice.
Mouse, ICR/Ha Swiss (F) 371 d (BCME)–458 d (controls) Van Duuren *et al.* (1975)	0 (control) or 0.3 mg in 0.05 mL nujol Single injection, once/wk 50/group	Skin sarcomas: 1/50, 21/50	$P < 0.01$	Purity unspecified Animals sacrificed because of poor survival (median survival was 260 d in treated mice vs 443 d for vehicle controls)
Mouse, XVIInc./Z (M, F) 549 d Zajdela *et al.* (1980)	Vehicle control: 20 µl nujol (purified paraffin oil), 32 injections over 42 wk 30/group (M) BCME 0.3 mg in 20 µl nujol 32 injections over 42 wks 30/group/sex	Skin fibrosarcomas: 0/30, 12/27 (M); 10/24 (F)	$P < 0.0001$ $P < 0.0001$ (vs male control group)	98.9% pure No female control group

bw, body weight; d, day or days; h, hour or hours; F, female; M, male; mo, month or months; NR, not reported; NS, not significant, vs, versus; wk, week or weeks

Table 3.5 Carcinogenicity studies in mice exposed to chloromethyl methyl ether by skin application

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, ICR/Ha Swiss (F) 540 d Van Duuren *et al.* (1969)	**CMME as an initiator** 0.1 mg CMME in 0.1 mL benzene (low dose) or 1.0 mg CMME in 0.1 mL benzene (high dose) followed 14 d later by 0.025 mg PE in 0.1 mL acetone 3 × /wk Controls received 0.025 mg PE in 0.1 mL acetone 3 × /wk	Skin papillomas: 2/20 (control), 7/20 (low dose), 5/20 (high dose) Skin squamous cell carcinomas: 0/20, 4/20, 1/20	[$P = 0.06$, low dose] [$P = 0.053$, low dose]	> 99.5% pure Treatment with CMME was discontinued at 325 d but animals were maintained and observed for the entire duration of the experiment. CMME probable initiator of skin papillomas and carcinomas combined.
	CMME as a promoter Single application 0.15 mg B[a]P in 0.1 mL benzene followed 14 d later by 2 mg CMME in 0.1 mL of benzene 3 × /wk. Controls received a single application of 0.15 mg B[a]P in 0.1 mL benzene	Skin papillomas: 0/20, 1/20 Skin squamous cell carcinomas: 0/20, 0/20	[NS] [NS]	
	CMME as a carcinogen 2 mg CMME in 0.1 mL benzene 3x weekly. Controls received 0.1 mL benzene 3 × /wk	Skin papillomas: 0/20, 0/20 Skin squamous cell carcinomas: 0/20, 0/20	[NS] [NS]	

B[a]P, benzo(a)pyrene; d, day or days; F, female; PE, phorbol ester; wk, week or weeks

Table 3.6 Carcinogenicity studies in experimental animals exposed to chloromethyl methyl ether (CMME) by subcutaneous injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague Dawley (F) 515 d Van Duuren *et al.* (1969)	Vehicle control: nujol 0.1 mL/wk for 300 d Treated: 3 mg CMME in 0.1 mL Nujol/wk for 300 d 20/group	Skin fibrosarcomas: 0/20, 1/20 Skin fibromas: 0/20, 0/20	[NS] [NS]	Treatment discontinued at 300 d due to local reaction at injection site
Mouse, Newborn ICR Swiss (M, F) 6 mo Gargus *et al.* (1969)	Single injection when 24–72 h old Vehicle controls: 50 µl peanut oil (25 mL/kg bw) Treated: 50 µl of CMME solution (125 µl/kg bw CMME) in peanut oil 48–51/treated groups 20–30/control groups	Lung adenomas: 2/30, 9/51 (M); 5/20, 8/48 (F) Lung adenomas/animal: 0.07, 0.23 (M); 0.25, 0.18 (F)	[NS] NR	99.7% pure
Mouse, Newborn ICR/Ha Swiss (F) 685 d Van Duuren *et al.* (1972)	Vehicle controls: 0.05 mL nujol Treated: 300 µg CMME in 50 µl nujol once/wk for life 30/group	Skin sarcomas: 0/30, 10/30	[$P < 0.01$]	Technical grade (purity NR)

bw, body weight; d, day or days; F, female; h, hour or hours; M, male; mo, month or months; NS, not significant; NR, not reported; wk, week or weeks

in male mice (Gargus *et al.*, 1969), of sarcomas and fibrosarcomas at the site of injection in male and female mice, and of fibrosarcomas in female rats (Van Duuren *et al.*, 1969; Van Duuren *et al.*, 1975; Zajdela *et al.*, 1980).

3.2 CMME

Studies on the carcinogenesis of CMME administered to mice, rats and hamsters by inhalation, skin application and subcutaneous injection have been reviewed in previous *IARC Monographs* (IARC, 1974, 1987b). The results of adequately conducted carcinogenicity studies are summarized in Tables 3.5, 3.6. There were no additional studies reported in the literature since the previous *IARC Monograph* (IARC, 1987b).

CMME was tested for carcinogenicity by inhalation exposure in one study in rats, one in mice and one in hamsters; by skin application in one study in mice; and by subcutaneous injection in one study in rats and two in mice.

Technical grade CMME induced skin sarcomas in female mice following subcutaneous injection (Van Duuren *et al.*, 1972). In a skin-painting study in female mice, CMME was found to be a probable initiator of skin papillomas and carcinomas combined (Van Duuren *et al.*, 1969).

4. Other Relevant Data

4.1 Toxicokinetics and toxicity

BCME and CMME belong to the group of chloroalkyl ethers. In water and aqueous biological fluids these substances are rapidly hydrolysed to form hydrochloric acid, methanol and formaldehyde (Nichols & Merritt, 1973; NTP, 2005).

The toxic effects of BCME are restricted to the epithelial tissue where exposure occurs, and this is consistent with the short half-life of BCME in aqueous media (ATSDR, 1989).

4.2 Genetic and related effects

Studies on the genotoxicity and cytotoxicity of BCME and CMME are limited and yielded mixed results (IARC, 1987b). [These studies are generally poorly documented.]

Both BCME and CMME are powerful alkylating agents (Van Duuren *et al.*, 1968; Van Duuren & Van Duuren, 1988; Van Duuren, 1989) that are mutagenic in bacteria (Mukai & Hawryluk, 1973; Anderson & Styles, 1978; IARC, 1987b). [The Working Group noted that the test systems used may not be optimal for investigating effects of rapidly hydrolysing material. Specifically, since BCME and CMME are short-lived alkylating agents, tests that favour hydrolysis of the compound before it enters the cell may yield misleading results.]

In one study, reaction of BCME with DNA *in vitro* did not affect the melting temperature or the buoyant density of the DNA, nor did it yield isolatable products upon reaction with purines or DNA, as did other alkylating agents (Van Duuren *et al.*, 1972). In another study, BCME was shown to bind to calf-thymus DNA at guanine and adenine residues (Goldschmidt *et al.*, 1975).

In vitro, CMME enhanced virus-induced transformation of Syrian hamster embryo cells (Casto, 1983; IARC, 1987b) and elicited unscheduled DNA synthesis, reflecting its activity as a DNA-damaging agent, in cultured human lymphocytes (Perocco *et al.*, 1983).

BCME did not cause chromosomal aberrations in bone-marrow cells of rats exposed to vapours for six months (Leong *et al.*, 1981; IARC, 1987b) but it did induce unscheduled DNA synthesis (Agrelo & Severn, 1981; IARC, 1987b) and cell transformation (Kurian *et al.*, 1990) in cultured human fibroblasts.

A slight increase in the incidence of chromosomal aberrations was observed in blood

lymphocytes of workers exposed to BCME or CMME during the preparation of ion-exchange resins (Srám *et al.*, 1983; IARC, 1987b).

4.3 Mechanistic considerations

The limited experimental studies on BCME and CMME preclude a detailed understanding of a mechanism of action, but sufficient information is available to support a genotoxic mode of action. Bernucci *et al.* (1997) outlined some possible steps in the process by which BCME and CMME may contribute to carcinogenesis. Similar to other alkylating agents, the observed formation of DNA adducts and resultant mutations are likely key steps in their mechanism of carcinogenicity. However, very little is known regarding their covalent interaction with DNA.

The hydrolysis products of BCME are formaldehyde and hydrochloric acid (HCl). Since formaldehyde is carcinogenic in animals and humans (see the *Monograph* on Formaldehyde in this volume), at least some of the carcinogenic potential of BCME may be due to this degradation product. However, the difference in carcinogenic potency between the two compounds (BCME being much more potent than formaldehyde) would indicate that this cannot be the sole mechanism of carcinogenicity.

BCME, formaldehyde and HCl could interact synergistically within the cell. Exposure of rats to mixtures of formaldehyde and HCl by inhalation resulted in little change in the frequency of nasal tumours compared with exposure to formaldehyde alone. However, one animal developed an esthesioneuroepithelioma, a very rare tumour that is characteristic of BCME exposure (Albert *et al.*, 1982; Sellakumar *et al.*, 1985).

4.4 Synthesis

BCME is among the most potent animal and human carcinogens known. The fact that BCME and CMME are powerful alkylating agents provides moderate to strong evidence that they operate by a genotoxic mechanism of action. This mechanism is likely to be similar to that of other strong alkylating agents, involving modification of DNA and resultant mutations.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of bis(chloromethyl)ether and chloromethyl methyl ether (technical grade). Bis(chloromethyl)ether and chloromethyl methyl ether (technical grade) cause cancer of the lung.

There is *sufficient evidence* in experimental animals for the carcinogenicity of bis(chloromethyl)ether.

There is *limited evidence* in experimental animals for the carcinogenicity of chloromethyl methyl ether.

There is moderate to strong evidence that bis(chloromethyl)ether and chloromethyl methyl ether, powerful alkylating agents, operate by a genotoxic mechanism. This mechanism is likely to be similar to that of other strong alkylating agents, involving modification of DNA and resultant mutations.

Bis(chloromethyl)ether and chloromethyl methyl ether (technical grade) are *carcinogenic to humans (Group 1).*

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1,3-BUTADIENE

1,3-Butadiene was considered by previous IARC Working Groups in 1991, 1998, and 2007 (IARC, 1992, 1999, 2008). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 106-99-0
Chem. Abstr. Serv. Name: 1,3-Butadiene
IUPAC Systematic Name: 1,3-Butadiene

C_4H_6
Relative molecular mass: 54.09
Description: Colourless gas
Solubility: Sparingly soluble in water (1 g/L at 20 °C); slightly soluble in ethanol and methanol; soluble in benzene, carbon tetrachloride, and diethyl ether
Conversion factor: $mg/m^3 = 2.21 \times ppm$
From O'Neil (2006) and Lide (2008)

1.2 Use

Butadiene is used primarily in the production of synthetic rubbers and polymers, which are used in a wide variety of industrial and consumer products (e.g. automobiles, construction materials, appliance parts, computers and telecommunication equipment, protective clothing, packaging and household articles). The advantages of butadiene-based polymers include improved functionality, performance and safety, and lower costs. Synthetic rubbers that are produced from butadiene include styrene-butadiene rubber, poly-butadiene rubber, styrene-butadiene latex, chloroprene rubber and nitrile rubber. Important plastics that contain butadiene as a monomeric component are shock-resistant polystyrene, a two-phase system that consists of polystyrene and poly-butadiene; polymers that consist of acrylonitrile, butadiene and styrene; and a co-polymer of methyl methacrylate, butadiene and styrene, which is used as a modifier for polyvinyl chloride. Butadiene is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals (White, 2007).

1.3 Human exposure

1.3.1 Occupational exposure

The highest exposures to butadiene occur in occupational settings. The potential for exposure exists in several industrial activities, such as petroleum refining and related operations (production of C4 fractions containing butadiene, and production and distribution of gasoline), production of purified butadiene monomer,

Table 1.1 Estimated numbers of workers exposed to 1,3-butadiene in the European Union (top 10 industries)

Industry, occupational activity	
Manufacture of industrial chemicals	8300
Manufacture of rubber products	7100
Manufacture of plastic products not elsewhere classified	7000
Petroleum refineries	2200
Construction	1600
Manufacture of other chemical products	1300
Education services	700
Manufacture of transport equipment	700
Wholesale and retail trade and restaurants and hotels	600
Manufacture of machinery except electrical	500
TOTAL	31600

From CAREX (1999)

production of various butadiene-based rubber and plastic polymers and other derivatives, and manufacture of rubber and plastic products, such as tyres, hoses and a variety of moulded objects (IARC, 1999).

Estimates of the number of workers potentially exposed to 1,3-butadiene have been developed by CAREX (CARcinogen EXposure) in Europe. CAREX is an international information system that provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Based on occupational exposure to known and suspected carcinogens collected from 1990 to 1993, the CAREX database estimates that 31 600 workers were exposed to 1,3-butadiene in the European Union (EU). Table 1.1 presents the number of exposed workers for 1,3-butadiene (top 10 industries) in the EU by industry (CAREX, 1999).

From the US National Occupational Exposure Survey (1981–1983) it was estimated that approximately 52 000 workers (including approximately 1400 women) were potentially exposed to 1,3-butadiene (NIOSH, 1990).

No measurements of exposure in butadiene-monomer production before the 1970s are available, but exposure levels have decreased from up to 20 mg/m^3 to less than 2 mg/m^3 between the late 1970s and the early 2000s (IARC, 2008). In styrene-butadiene polymer production, the estimated median levels of exposure to butadiene in earlier decades varied in the range 8–20 mg/m^3, while current exposure measurements in modern facilities in North America and western Europe generally show values below 2 mg/m^3. Concentrations of butadiene-in-air reported from the People's Republic of China are somewhat higher (~4 mg/m^3) (IARC, 2008). Butadiene is not usually found in detectable concentrations in workplace air during the manufacture of finished rubber and plastic products (IARC, 1999). Regardless of the type of factory, production process, or country, some tasks are still characterized by very high exposures (~200 mg/m^3), which are typically short in duration (IARC, 2008). For a detailed description of studies on occupational exposure to butadiene and Tables with data summarizing the results, the reader is referred to *IARC Monographs* Volumes 71 and 97 (IARC, 1999, 2008).

The utility of haemoglobin adducts as biomarkers of human exposure to butadiene has been investigated in several molecular epidemiological studies that often included the measurement of urinary metabolites and personal-air

monitoring of butadiene, as well as genotoxicity end-points and metabolic phenotypes (IARC, 2008).

1.3.2 Non-occupational exposure

Butadiene has been widely detected in ambient air but at much lower levels (μg/m^3) than reported in some occupational settings (mg/m^3). Elevated concentrations may occur in the vicinity of point sources, such as municipal structural fires, wood and brush fires; cigarette smoking; vehicle emissions and gasoline volatilization (IARC, 2008). Studies on non-occupational exposures to 1,3-butadiene have been reviewed in previous *IARC Monographs* (IARC, 1999, 2008).

In a study conducted between 1990 and 1994, concentrations of butadiene were determined in 1611 samples of outdoor air from 25 sites within 14 cities, towns or rural locations in Ontario, Canada. The mean concentration in all samples was 0.1 μg/m^3 (maximum, 1.7 μg/m^3) (Health Canada, 2000).

Dollard *et al.* (2007) measured butadiene concentrations at rural, urban background (UB), urban industry-influenced (UI) and 'busy-road-traffic' (BR) locations in the United Kingdom from 1993 to 2004. Mean rural levels dropped from 0.39 to 0.02 μg/m^3 between 1995 and 2004; mean UB levels decreased from 0.64 to 0.15 μg/m^3 in 1993–2004; mean UI levels came down from 0.85 to 0.35 μg/m^3 in 1995–2000; and mean BR levels went from 3.3 to 0.57 μg/m^3 in the period 1997–2004.

2. Cancer in Humans

In *IARC Monograph* Volume 97 (IARC, 2008) three cohort studies of workers in the butadiene-monomer industry were reviewed (Ward *et al.*, 1995; Divine & Hartman, 2001; Tsai *et al.*, 2001), along with two cohort studies of workers in the styrene–butadiene rubber (SBR) industry (McMichael *et al.*, 1974, 1976; Meinhardt *et al.*, 1982; Matanoski & Schwartz, 1987; Matanoski *et al.*, 1990, 1993; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-21-Table2.1.pdf). The excess of mortality from leukaemia in one of the butadiene-monomer industry cohorts, which did not increase with duration of exposure or with cumulative exposure, was more pronounced among workers who had been exposed during the Second World War, when exposures to butadiene had probably been higher (Divine & Hartman, 2001). These cohorts were subsequently combined, although data from one styrene-butadiene plant were excluded because the information was incomplete (Delzell *et al.*, 1996; Macaluso *et al.*, 2004; Graff *et al.*, 2005; Sathiakumar *et al.*, 2005; Delzell *et al.*, 2006; Cheng *et al.*, 2007). A series of overlapping analyses examined the mortality of approximately 17 000 male workers from eight SBR-manufacturing facilities in the USA and Canada (see Table 2.1 online). A limiting factor was that diagnosis and classification of lymphatic and haematopoietic malignancies are extremely complex, and that these underwent several changes over the course of time. Although mortality from leukaemia was only slightly elevated in the most recent updates (Sathiakumar *et al.*, 2005; Delzell *et al.*, 2006; Cheng *et al.*, 2007), large excesses of mortality from leukaemia were seen in workers in the most highly exposed areas of the plants and among hourly-paid workers, especially those who had been hired in the early years and had been employed for more than ten years. These excesses were attributable to both chronic lymphocytic and chronic myelogenous leukaemia, with significant exposure–response relationships between cumulative exposure to butadiene and mortality from both leukaemia types. The most recent analyses showed that the exposure–response for butadiene and leukaemia was independent of exposures to benzene,

styrene and dimethyl-dithiocarbamate (Delzell *et al.*, 2006; Cheng *et al.*, 2007).

The strongest evidence of an association between exposure to butadiene and non-Hodgkin lymphoma comes from studies in the butadiene-monomer industry (Ward *et al.*, 1995, 1996; Divine & Hartman, 2001). Although this association did not become stronger with duration of exposure, it was more pronounced among workers who had been exposed during the Second World War, when exposures had presumably been higher.

Whitworth *et al.* (2008) conducted an ecological study in South-eastern Texas that assessed whether census tracts with the highest ambient-air concentrations of benzene or 1,3-butadiene have a higher incidence of lymphohaematopoietic cancer in children. The analysis included 977 cases of childhood lymphohaematopoietic cancer diagnosed from 1995–2004. Estimates of airborne concentrations of benzene and 1,3-butadiene were obtained for 886 census tracts by use of the models proposed in 1999 by the US Environmental Protection Agency. Poisson-regression models were used to explore the associations between pollutant levels and census-tract cancer rates. Adjustments were made for age, sex, race/ethnicity, and socioeconomic status at the community level. Census tracts with the highest 1,3-butadiene concentrations had rate ratios of 1.4 (95%CI: 1.1–1.8), 1.7 (95%CI: 0.8–3.4), and 1.3 (95%CI: 1.0–1.8) for all leukaemia, acute myeloid leukaemia and acute lymphocytic leukaemia, respectively. A statistically significant dose–response trend was noted for all leukaemia. No association was found between 1,3-butadiene concentrations and lymphoma incidence. An excess of leukaemia was also found in association with environmental exposure to benzene, but analyses that examined both exposures simultaneously indicated that the effects were independent. [The Working Group noted that environmental levels of butadiene are considerably lower than in industrial settings but children may be a more sensitive group. However, potential confounding factors have not been fully addressed and the findings need to be confirmed in future studies.]

The mortality of women in the styrene-butadiene cohort has also been evaluated (Sathiakumar & Delzell, 2007, 2009; see Table 2.1 online). No increased risks were found for leukaemia or lymphoma. Statistically significant positive SMRs were seen for cancers of the lung and bladder. [Unlike in the male cohorts, the female cases were not confirmed pathologically, the exposure level was low, most women were short-term workers (median duration of employment was approximately 1.7 years; 70% had worked during less than four years), and only 30% of women were exposed to butadiene and styrene.]

In a further analysis of this SBR cohort, the lung-cancer risk among men and women was evaluated. Among men there was no indication of an increased risk for lung cancer and no evidence for an internal dose–response. Among women there was evidence of an increased risk for lung cancer, although there was no evidence for an internal dose–response in the exposed group (Sathiakumar & Delzell, 2009).

Overall, the epidemiological evidence from the styrene-butadiene and the butadiene-monomer industries clearly indicates an increased risk for haematolymphatic malignancies. Studies from the styrene-butadiene industry show an excess of leukaemia, and a dose–response relationship with cumulative exposure to butadiene, while studies from the monomer industry show an excess of haematolymphatic malignancies in general, attributable both to leukaemia and malignant lymphoma. The evidence for an association between exposure to butadiene and cancer of the haematolymphatic organs has gained some support by findings of an association between environmental levels of butadiene and risk for leukaemia in children.

The epidemiological evidence for an association with specific subtypes of haematolymphatic malignancies is weaker, mainly since numbers are lower, giving imprecise risk estimates. However, when malignant lymphomas and leukaemias are distinguished, the evidence is strongest for leukaemia.

3. Cancer in Experimental Animals

3.1 1,3-Butadiene

Studies on the carcinogenesis of 1,3-butadiene in rats and mice have been reviewed in previous IARC *Monographs* (IARC, 1999, 2008) and by Grosse *et al.* (2007). The results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There were no additional studies reported in the published literature since *IARC Monograph* Volume 97 (IARC, 2008).

1,3-Butadiene was tested for carcinogenicity by inhalation exposure in one study in rats and four studies in mice.

Inhalation of 1,3-butadiene induced tumours in rats at exposure concentrations ranging from 1000 to 8000 ppm [2200–17650 mg/m^3], and in multiple organs in mice at exposure concentrations ranging from 6.25 to 1250 ppm [13.8–2760 mg/m^3]. In rats, 1,3-butadiene caused a significantly increased incidence of carcinomas of the Zymbal gland, sarcomas of the uterus, adenomas and carcinomas (combined) of the mammary gland, and follicular cell adenomas of the thyroid gland in females. In males, it caused malignant gliomas and adenomas of the pancreas and testes in males (Owen *et al.*, 1987; Owen & Glaister, 1990; Melnick *et al.*, 1993; Melnick & Huff, 1993). In mice of both sexes, 1,3-butadiene caused a significantly increased incidence of Harderian gland adenomas and carcinomas, heart haemangiosarcomas, lymphoid tissue neoplasms (lymphoma, histiocytic sarcoma), lung adenomas and carcinomas, hepatocellular adenomas and carcinomas, and fore-stomach papillomas and carcinomas. It caused mammary gland cancers, benign tumours and carcinomas of the ovary, and skin sarcomas in females. It also caused preputial gland carcinomas and kidney tubule adenomas in males (NTP, 1984, 1993; Huff *et al.*, 1985; Miller *et al.*, 1989; Melnick *et al.*, 1990a, b, 1993; Melnick & Huff, 1993; Hong *et al.*, 2000; Melnick & Sills, 2001; Kim *et al.*, 2005). No increased incidence of tumours was observed in one study in mice exposed once to 1,3-butadiene at concentrations up to 10 000 ppm [22000 mg/m^3] (Bucher *et al.*, 1993).

3.2 Diepoxybutane

Diepoxybutane, a metabolite of 1,3-butadiene, was tested for carcinogenicity by inhalation in one study in rats and one study in mice, by four skin-application studies in mice, by one subcutaneous injection study in rats and two such studies in mice, and by one gavage and one intraperitoneal injection study in mice (Tables 3.1, 3.2, 3.3, 3.4).

Diepoxybutane increased the incidence of adenomas of the Harderian gland in female mice, and of squamous cell carcinoma of the nose in female rats after inhalation exposure (Henderson *et al.*, 1999, 2000). Subcutaneous injection resulted in an increased incidence of fibrosarcomas in female rats and female mice. The gavage study in mice did not produce any tumours (Van Duuren *et al.*, 1966). Intra-peritoneal injection led to an increased incidence of lung tumours in strain A/J mice (Shimkin *et al.*, 1966). Two skin-application studies in mice resulted in an increased incidence of dermoid carcinomas (Van Duuren *et al.*, 1963, 1965).

Table 3.1 Carcinogenicity studies in experimental animals exposed to 1,3-butadiene and diepoxybutane by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
1,3-Butadiene				
Rat, Sprague-Dawley (M, F) killed at 52 wk, remainder killed when survival was approximately 20% (105 wk for F, 111 wk for M) Owen & Glaister (1990), Melnick *et al.* (1993), Melnick & Huff (1993)	0, 1 000, 8 000 ppm, 6 h/d, 5 d/wk 110/group	Pancreas (exocrine adenomas): 3/100, 1/100, 10/100 (M); 2/100, 0/100, 0/100 (F)	$P \leq 0.001$ (high-dose M) $P \leq 0.001$ (trend M)	99.2% pure 16 deaths occurred during the first yr. During the second yr mortality increased with increasing dosage. Increased mortality in females was due to mammary tumours and in males due to renal lesions. The incidence of uterine sarcomas and Zymbal-gland tumours were similar to the historical laboratory control. Zymbal-gland tumours were noted between 76 and 90 wk.
		Uterus (sarcomas): 1/100, 4/100, 5/100 (F)	$P \leq 0.005$ (trend F)	
		Zymbal gland (adenomas): 1/100, 1/100, 1/100(M); 0/100, 0/100, 0/100 (F) Zymbal gland (carcinomas): 0/100, 0/100, 1/100 (M); 0/100, 0/100, 4/100 (F)	Carcinoma: $P \leq 0.05$ (trend F)	
		Mammary gland (benign): 0/100, 2/100, 0/100 (M); 32/100, 64/100, 55/100 (F)	NS	
		Mammary gland (malignant): 1/100, 0/100, 0/100 (M); 18/100, 15/100, 26/100 (F)	NS	
		Mammary gland (total combined benign and malignant mammary tumours): 1/100, 2/100, 0/100 (M); 50/100, 79/100, 81/100 (F)	$P \leq 0.001$ (trend F)	
		Thyroid (follicular cell adenomas): 3/100, 5/100, 1/100 (M); 0/100, 2/100, 10/100 (F)	$P \leq 0.01$ (trend F)	
		Thyroid (carcinomas): 1/100, 0/100, 0/100 (M); 0/100, 2/100, 1/100 (F)	NS	
		Testis (leydig cell tumours): 0/100, 3/100, 8/100 (M)	$P \leq 0.001$ (trend M)	
		Brain (glial cell tumours (malignant)): 1/100, 4/100, 5/100 (M)	$P \leq 0.05$ (trend M)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 60–61 wk NTP (1984), Huff *et al.* (1985), Miller *et al.* (1989), Melnick *et al.* (1993), Melnick & Huff (1993), Hong *et al.* (2000), Melnick & Sills (2001), Kim *et al.* (2005)	0, 625, 1 250 ppm 6 h/d, 5 d/wk. 50/group	Lung: (alveolar/bronchiolar adenomas): 2/50, 12/49, 11/49 (M); 3/49, 9/48, 20/49 (F)	$P < 0.001$ (trend), $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	> 99% pure The survival of both dose-groups of mice of each sex was significantly less than that of the corresponding controls. The study was planned for 103 wk, but was terminated after 60 wk for males and 61 wk for females, because of poor survival ($P < 0.01$) in all exposed groups due to malignant tumours in multiple organs. Malignant lymphomas and haemangiosarcomas were considered the major cause of early death in these studies.
		Lung (alveolar/bronchiolar carcinomas): 0/50, 2/49, 5/49 (M); 0/49, 6/48, 8/49 (F)	$P < 0.001$, $P = 0.018$, $P < 0.001$ (M); $P = 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Lung (alveolar/bronchiolar adenomas or carcinomas): 2/50, 14/49, 15/49 (M); 3/49, 12/48, 23/49 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Lymphoma (all lymphomas): 0/50, 23/50, 29/50 (M); 1/50, 10/49, 10/49 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P = 0.006$, $P < 0.001$, $P = 0.003$ (F)	
		Heart (haemangiosarcomas): 0/50, 16/49, 7/49 (M); 0/50, 11/48, 18/49 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Fore-stomach (all papillomas): 0/49, 5/40, 0/44; (M) 0/49, 4/42, 10/49 (F)	$P = 0.036$, $P < 0.001$, – (M); $P < 0.001$, $P = 0.001$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell carcinomas): 0/49, 2/40, 1/44 (M); 0/49, 1/42, 1/49 (F)	NS	
		Fore-stomach (all papillomas or carcinomas): 0/49, 7/40, 1/44 (M); 0/49, 5/42, 10/49 (F)	$P = 0.006$, $P < 0.001$, $P = 0.248$ (M); $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Liver (hepatocellular adenomas): 0/50, 1/47, 4/49 (F)	$P = 0.015$, $P = 0.278$, $P = 0.030$ (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 60–61 wk NTP (1984), Huff *et al.* (1985), Miller *et al.* (1989), Melnick *et al.* (1993), Melnick & Huff (1993), Hong *et al.* (2000), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Liver (hepatocellular carcinomas): 0/50, 1/47, 1/49 (F)	NS	
		Liver (hepatocellular adenomas or carcinomas): 0/50, 2/47, 5/49 (F)	$P = 0.009$, $P = 0.048$, $P = 0.015$ (F)	
		Mammary gland (acinar cell carcinomas): 0/50, 2/49, 6/49 (F)	$P = 0.004$, $P = 0.048$, $P = 0.007$ (F)	
		Mammary gland (adenosquamous carcinomas): 0/50, 4/49, 0/49 (F)	$P = 0.575$, $P = 0.030$, – (F)	
		Ovary (benign granulosa cell tumours): 0/49, 6/45, 12/48 (F)	$P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Preputial gland (carcinomas): 0/50, 3/50, 2/50 (M)	NS	
		Brain (gliomas): 0/50, 2/50, 1/50 (M)	NS	
		Zymbal gland (carcinomas): 0/50, 0/50, 2/50 (M); 0/50, 0/49, 1/49 (F)	NS	
Mouse, B6C3F1 (M, F) 2 yr stop study Bucher *et al.* (1993)	0, 1 000, 5 000, 10 000 ppm for 2 h, then held for 2 yr 60/group	Malignant lymphomas: 7/59, 8/58, 8/58, 10/58 (M); 13/57, 19/56, 18/57, 13/58 (F)	NS	Purity NR
		Forestomach (squamous neoplasms): 0/59, 1/58, 1/58, 3/58 (M); 0/57, 1/56, 0/57, 0/58 (F)	NS	
		Mammary gland (acinar cell neoplasms): 0/59, 0/58, 0/58, 1/58 (M); 2/57, 1/56, 3/57, 4/58 (F)	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005)	0, 6.25, 20, 62.5, 200, 625 ppm, 6 h/d, 5 d/wk for 103 wk 70/group for dosages 0–200 ppm, 90/group for 625 ppm	Lymphoma (all lymphomas): 4/50, 2/50, 4/50, 6/50, 2/50, 51/73 (M); 6/50, 12/50, 11/50, 7/50, 9/50, 32/80 (F)	$P < 0.001$ (trend), $P = 0.302$N, $P = 0.528$, $P = 0.238$, $P = 0.627$, $P < 0.001$ (M); $P < 0.001$, $P = 0.068$, $P = 0.029$, $P = 0.055$, $P < 0.001$, $P < 0.001$(F)	> 99% pure Two-yr survival was decreased for M and F exposed to ≥ 20 ppm due to chemical-related tumours. No F exposed to 200 or 625 ppm or M exposed to 625 ppm survived till the end of the experiments.
		Lymphocytic lymphomas: 2/50, 0/50, 2/50, 4/50, 2/50, 49/73 (M); 1/50, 3/50, 6/50, 3/50, 8/50, 31/80 (F)	$P < 0.001$, $P = 0.227$N, $P = 0.671$, $P = 0.253$, $P = 0.529$, $P < 0.001$ (M); $P < 0.001$, $P = 0.278$, $P = 0.026$, $P = 0.160$, $P < 0.001$, $P < 0.001$ (F)	
		Histiocytic sarcomas: 0/50, 0/50, 4/50, 5/50, 7/50, 4/73 (M); 3/50, 2/50, 7/50, 4/50, 7/50, 4/80 (F)	$P < 0.001$, –, $P = 0.051$, $P = 0.021$, $P < 0.001$, $P = 0.043$ (M); $P < 0.001$, $P = 0.518$N, $P = 0.077$, $P = 0.195$, $P = 0.002$, $P = 0.038$ (F)	
		Heart (haemangiosarcomas): 0/50, 0/49, 1/50, 5/48, 20/48, 4/73 (M); 0/50, 0/50, 0/50, 1/49, 21/50, 23/80 (F)	$P < 0.001$, –, $P = 0.451$, $P = 0.011$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, –, –, $P = 0.392$, $P < 0.001$, $P < 0.001$ (F)	
		Lung (alveolar/bronchiolar adenomas): 18/50, 20/50, 10/50, 25/49, 21/50, 3/73 (M); 4/50, 11/50, 12/50, 17/50, 14/50, 17/78 (F)	$P < 0.001$, $P = 0.579$N, $P = 0.269$N, $P = 0.004$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.031$, $P = 0.003$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Lung (alveolar/bronchiolar adenocarcinomas or carcinomas): 5/50, 6/50, 11,50, 12/49, 22/50, 3/73 (M);0/50, 5/50, 11/50, 9/50, 19/50, 8/78 (F)	$P < 0.001$, $P = 0.577$, $P = 0.017$, $P = 0.006$, $P < 0.001$, $P < 0.001$ (M), $P < 0.001$, $P = 0.029$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Lung (combined alveolar/ bronchiolar adenomas, adenocarcinomas, or carcinomas): 21/50, 23/50, 19/50, 31/49, 35/50, 3/73 (M); 4/50, 15/50, 19/50, 24/50, 25/50, 22/78 (F)	$P < 0.001$, $P = 0.552$N, $P = 0.276$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.004$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell papillomas): 1/50, 0/50, 0/50, 1/50, 7/50, 2/73 (M); 0/50, 0/50, 2/50, 1/50, 3/50, 16/80 (F)	$P < 0.001$, $P = 0.481$N, $P = 0.545$N, $P = 0.679$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, –, $P = 0.149$, $P = 0.260$, $P = 0.004$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell carcinomas): 0/50, 0/50, 0/50, 0/50, 1/50, 2/73 (M); 0/50, 0/50, 1/50, 1/50, 1/50, 6/80 (F)	$P < 0.001$, –, –, –, $P = 0.325$, $P = 0.018$ (M); $P < 0.001$, –, $P = 0.414$, $P = 0.277$, $P = 0.374$, $P < 0.001$ (F)	
		Fore-stomach (squamous cell papillomas or squamous cell carcinomas): 1/50, 0/50, 0/50, 1/50, 8/50, 4/73 (M); 0/50, 0/50, 3/50, 2/50, 4/50, 22/80 (F)	$P < 0.001$, $P = 0.481$N, $P = 0.545$N, $P = 0.679$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$,–, $P = 0.056$, $P = 0.044$, $P = 0.001$, $P < 0.001$ (F)	
		Liver (hepatocellular adenomas): 13/50, 13/50, 19/50, 16/48, 24/48, 5/72 (M); 11/49, 10/49, 9/50, 14/50, 12/50, 1/80	$P < 0.001$, $P = 0.480$, $P = 0.036$, $P = 0.056$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.590$, $P = 0.419$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Liver (hepatocellular carcinomas): 11/50, 16/50, 16/50, 17/48, 26/48, 1/72 (M); 4/49, 6/49, 8/50, 9/50, 8/50, 1/80 (F)	$P < 0.001$, $P = 0.289$, $P = 0.071$, $P = 0.020$, $P < 0.001$, $P = 0.009$ (M); $P < 0.001$, $P = 0.330$, $P = 0.064$, $P = 0.003$, $P < 0.001$, $P = 0.150$ (F)	
		Liver (hepatocellular adenomas or carcinomas): 21/50, 23/50, 30/50, 25/48, 33/48, 5/72 (M); 15/49, 14/49, 15/50, 19/50, 16/50, 2/80 (F)	$P < 0.001$, $P = 0.562$N, $P = 0.011$, $P = 0.022$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.555$, $P = 0.162$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Harderian gland (adenomas): 6/50, 7/50, 8/50, 19/50, 30/50, 6/73 (M); 8/50, 10/50, 6/50, 15/50, 20/50, 9/80 (F)	$P < 0.001$, $P = 0.575$, $P = 0.218$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.304$, $P = 0.544$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Harderian gland (carcinomas): 0/50, 1/50, 1/50, 3/50, 2/50, 0/73 (M); 0/50, 1/50, 1/50, 0/50,1/50, 0/80 (F)	$P = 0.080$, $P = 0.522$, $P = 0.425$, $P = 0.067$, $P = 0.166$, – (M); logistic regression test $P = 0.873$N, $P = 0.493$, $P = 0.631$, –, $P = 0.085$, – (F)	
		Harderian gland (adenomas or carcinomas): 6/50, 7/50, 9/50, 20/50, 31/50, 6/73 (M); 8/50, 10/50, 7/50, 15/50, 20/50, 9/80 (F)	$P < 0.001$, $P = 0.575$, $P = 0.141$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M); $P < 0.001$, $P = 0.304$, $P = 0.426$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Preputial gland (carcinomas): 0/50, 0/50, 0/50, 0/50, 5/50, 0/73 (M)	$P < 0.001$, –, –, –, $P < 0.001$, – (M)	
		Ovary (benign granulosa cell tumours): 1/49, 0/49, 1/48, 6/50, 6/50,6/79	$P < 0.001$, $P = 0.517$N, $P = 0.680$, $P = 0.003$, $P < 0.001$, $P < 0.001$ (F)	
		Ovary (malignant granulosa cell tumours): 0/49, 0/49, 0/48, 3/50, 2/50, 0/79 (F)	$P < 0.001$, –, –, $P = 0.018$, $P = 0.003$, –, (F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 2 yr Miller *et al.* (1989), Melnick *et al.* (1990a, b, 1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Ovary (benign or malignant granulosa cell tumours): 1/49, 0/49, 1/48, 9/50, 8/50, 6/79 (F)	$P < 0.001$, $P = 0.517$N, $P = 0.680$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (F)	
		Mammary gland (adenoacanthomas): 0/50, 1/50, 2/50, 6/50, 4/50, 0/80 (F)	$P = 0.025$, $P = 0.489$, $P = 0.152$, $P < 0.001$, $P = 0.021$, $P =$ – (F)	
		Mammary gland (carcinomas): 0/50, 2/50, 2/50, 6/50, 11/50,12/80 (F)	$P < 0.001$, $P = 0.221$, $P = 0.192$, $P = 0.008$, $P < 0.001$, $P < 0.001$ (F)	
		Mammary gland (malignant mixed tumours): 0/50,0/50, 0/50,0/50,0/50, 4/80 (F)	$P =$ < 0.001, –, –, –, –, $P = 0.003$ (F)	
		Mammary gland (adenoacanthomas, carcinomas, or malignant mixed tumours): 0/50, 2/50, 4/50, 12/50, 15/50, 16/80 (F)	Logistic regression test: $P = 0.026$, $P = 0.228$, $P = 0.056$, $P < 0.001$, $P = 0.004$, $P < 0.001$ (F)	
		Kidney (renal tubule adenomas): 0/50, 1/50, 0/50, 3/48, 1/49, 0/73 (M); 0/49, 0/49, 0/48, 0/50, 2/50, 0/80 (F)	Logistic regression test: $P = 0.630$, $P = 0.522$, –, $P = 0.053$, $P = 0.580$, – (M); $P = 0.816$, –, –, –, $P = 0.276$, – (F)	
		Small intestine (adenomas or carcinomas): 0/50, 1/50, 1/50, 1/50, 2/50, 0/73 (M); 0/50, 3/50, 0/50, 1/50, 0/50, 0/80 (F)	$P = 1.000$, $P = 0.101$, –, $P = 0.375$, –, – (F)	
		Skin, subcutaneous tissue (neurofibrosarcomas or sarcomas): 1/50, 2/50, 3/50, 5/50, 3/50, 3/80 (F)	$P < 0.001$, $P = 0.476$, $P = 0.238$, $P = 0.017$, $P = 0.002$, $P = 0.013$ (F)	
		Zymbal gland (adenomas): 0/50, 0/50, 0/50, 0/50, 0/50, 1/80 (F)	NS	
		Zymbal gland: carcinoma: 0/50, 0/50, 0/50, 0/50, 0/50, 1/80 (F)	NS	
		Zymbal gland (adenomas or carcinomas): 0/50, 0/50, 0/50, 0/50, 2/80	NS	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M) 2 yr stop study Melnick *et al.* (1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005)	0, 200 ppm for 40 wk, 625 ppm for 13 wk, 312 ppm for 52 wk, or 625 ppm for 26 wk 50/group	Lymphoma (all lymphomas): 4/50, 8/50, 22/50, 8/50, 33/50 (M)	–, $P = 0.023$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	> 99% pure Survival of all stop-exposure groups was markedly lower than that of controls due to development of malignant tumours, particularly malignant lymphoma and haemangiosarcoma of the heart. Neoplasms were induced usually after only 13 wk of exposure.
		Lymphocytic lymphomas: 2/50, 6/50, 17/50, 4/50, 30/50 (M)	–, $P = 0.033$, $P < 0.001$, $P = 0.034$, $P < 0.001$ (M)	
		Histiocytic sarcomas: 0/50, 5/50, 2/50, 7/50, 2/50 (M)	–, $P = 0.006$, $P < 0.011$, $P < 0.001$, $P = 0.036$ (M)	
		Heart (haemangiosarcomas): 0/50, 15/50, 7/50, 33/50, 13/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Lung (alveolar/bronchiolar adenomas): 18/50, 24/50, 17/50, 26/50, 12/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, (M)	
		Lung (alveolar/bronchiolar adenocarcinomas or carcinomas): 5/50, 22/50, 18/50, 16/50, 11/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Lung (alveolar/bronchiolar adenomas, adenocarcinomas, or carcinomas): 21/50, 36/50, 28/50, 32/50, 17/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Liver (hepatocellular adenomas): 13/50, 27/49, 19/49, 19/50, 11/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Fore-stomach (squamous cell carcinomas): 0/50, 0/50, 4/50, 5/50, 6/50 (M)	–, –, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Harderian gland (adenomas): 6/50, 26/50, 20/50, 28/50, 13/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, (M)	
		Harderian gland (carcinomas): 0/50, 2/50, 4/50, 2/50, 0/50 (M)	–, $P = 0.182$, $P < 0.001$, $P = 0.028$, – (M)	
		Harderian gland (adenomas or carcinomas): 6/50, 27/50, 23/50, 30/50, 13/50 (M)	–, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$ (M)	
		Preputial gland (adenomas): 0/50, 0/50, 1/50, 0/50, 0/50 (M)	NS	
		Preputial gland (carcinomas): 0/50, 1/50, 4/50, 4/50, 3/50 (M)	–, $P = 0.247$, $P = 0.012$, $P < 0.001$, $P < 0.001$ (M)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M) 2 yr stop study Melnick *et al.* (1993), Melnick & Huff (1993), NTP (1993), Melnick & Sills (2001), Kim *et al.* (2005) Contd.		Preputial gland (adenomas or carcinomas): 0/50, 1/50, 5/50, 4/50, 3/50 (M)	–, $P = 0.247$, $P = 0.003$, $P < 0.001$, $P < 0.001$ (M)	
		Kidney: renal tubule adenoma: 0/50, 4/48, 1/50, 3/49, 1/50 (M)	–, $P = 0.016$, $P = 0.181$, $P = 0.007$, $P = 0.278$ (M)	
		Brain (malignant gliomas): 0/50, 0/50, 2/50, 0/50, 1/50 (M)	NS	
		Brain (neuroblastomas): 0/50, 0/50, 2/50, 0/50, 0/50 (M)	NS	
		Zymbal gland (adenomas): 1/50, 0/50, 0/50, 0/50, 0/50 (M)	NS	
		Zymbal gland (carcinomas): 0/50, 1/50, 2/50, 0/50, 2/50 (M)	NS	
		Zymbal gland (adenomas or carcinomas): 1/50, 1/50, 2/50, 0/50, 2/50	–. $P = 0.531$, $P = 0.178$, $P = 0.998$, $P = 0.009$ (M)	
Diepoxybutane				
Mouse, B6C3F1 (F) 18 mo Henderson *et al.* (1999, 2000)	0, 2.5, 5.0 ppm diepoxybutane 6 h/d, 5 d/wk for 6 wk 50/group	Harderian gland (adenomas): 0/40, 2/42, 5/36	$P < 0.05$ (high-dose F)	Purity > 99%
Rat, Sprague-Dawley (F) 18 mo Henderson *et al.* (1999, 2000)	0, 2.5, 5.0 ppm diepoxybutane 6 h/d, 5 d/wk for 6 wk 50/group	Nose (papillomas): 0/47, 0/48, 2/48 (F)	[NS]	Purity > 99%
		Nose (squamous cell carcinomas): 0/47, 11/48, 21/48 (F)	–, [$P < 0.001$], [$P < 0.001$]	
		Nose (adenocarcinomas): 0/47, 0/48, 2/48 (F)	[NS]	
		Nose (sarcomas): 0/47, 2/48, 2/48 (F)	[NS]	

d, day or days; F, female; h, hour or hours; M, male; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks; yr, year or years

Table 3.2 Carcinogenicity studies in experimental animals exposed to diepoxybutane by intra-peritoneal or subcutaneous injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Rat, Eastern Sprague-Dawley (F) 550 d Van Duuren *et al.* (1966)	**Subcutaneous injection** Tricaprylin vehicle (0.1 ml) control, untreated control, or 1 mg D,L-diepoxybutane injected into the axillary region once/wk 50/group	Fibrosarcomas at injection site: 0/50, 0/50, 9/50	[$P < 0.01$]	
		Adenocarcinomas at injection site: 1/50, 0/50/, 1/50	[NS]	
		Fibroadenomas: 0/50, 1/50, 0/50	[NS]	
Diepoxybutane Mouse, A/J (M, F) 39 wk Shimkin *et al.* (1966)	**Intraperitoneal injection** Total dose: 0, 19.4, 78.1, 314, 1 255, 2 232 µmol/kg bw in water, 12 injections given over a 4-wk period 165 M and 195 F vehicle control at start; 30, 30, 45, 30, 30 (total M, F)/group at start	Lung tumours (M, F combined): 107/339 (32%), 6/28 (21%), 12/30 (40%), 17/31 (55%), 18/28 (64%), 21/27 (78%)	NR	
	Total dose: 0, 34.8, 139, 558, 2 232 µmol/kg bw in tricaprylin, 12 injections given over a 4-wk period 60 M and 60 F tricaprylin vehicle control at start; 30, 30, 30, 30 (total M, F)/group at start	Lung tumours (M, F combined): 37/108 (34%), 12/30 (33%), 13/30 (43%), 12/25 (48%), 12/24 (50%)	NR	

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Mouse, ICR/Ha Swiss (F) 401 d Van Duuren *et al.* (1966)	**Subcutaneous injection** Tricaprylin vehicle (0.05 ml) control, untreated control, or subcutaneous injection of 1.1 mg D,L-diepoxy-butane into the axillary region once/wk 30/group	Fibrosarcomas at injection site 0/30, 0/30, 5/30 Adenocarcinomas at injection site: 0/30, 0/30, 0/30	[$P < 0.05$] [NS]	
D,L-Diepoxybutane Mouse, ICR/Ha Swiss (F) 589 d Van Duuren *et al.* (1966)	**Subcutaneous injection** Tricaprylin-vehicle (0.05 ml) control, untreated control, or subcutaneous injection of 0.1 mg D,L-diepoxy-butane into the axillary region once/wk 50/group	Fibrosarcomas at injection site: 0/50, 0/50, 5/50 Adenocarcinomas at injection site: 0/50, 0/50, 2/50	[NS] [NS]	

bw, body weight; d, day or days; F, female; M, male; NR, not reported; NS, not significant; wk, week or weeks

Table 3.3 Carcinogenicity study in rats exposed intragastrically to diepoxybutane

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Rat, Eastern Sprague-Dawley (F) 363 d Van Duuren *et al.* (1966)	Tricaprylin vehicle (0.5 ml) 0 or 5 mg D,L-diepoxybutane once/wk 5/group	0/5, 0/5	[NS]	

d, day or days; F, female; NS, not significant; wk, week or weeks

Table 3.4 Carcinogenicity studies in mice exposed to diepoxybutane by skin application

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
D,L-Diepoxybutane Mouse, Swiss-Millerton (M) 78 d (median survival, treated group) Van Duuren *et al.* (1963)	Acetone-vehicle control, untreated control, or 100 mg D,L-1,2,3,4-diepoxybutane in acetone per application on the back 3 × /wk 120 acetone-vehicle controls, 276 untreated controls, 30/group for treated	Skin papillomas: 8/120, 13/267, 1/30 Squamous dermoid carcinomas: 0/120, 1/267, 1/30	[NS] [NS]	
meso-Diepoxybutane Mouse, Swiss-Millerton (M) 154 d (median survival, treated group) Van Duuren *et al.* (1963)	Acetone-vehicle control, untreated Control, or 100 mg *meso*-diepoxybutane in acetone per application on the back 3 × /wk 120 acetone-vehicle controls, 276 untreated controls, 30/group for treated	Skin papillomas: 8/120, 13/267, 2/30 Squamous dermoid carcinomas: 0/120, 1/267, 4/30	[NS] [$P < 0.005$]	
D,L-Diepoxybutane Mouse, Swiss-Millerton (F) Lifetime Van Duuren *et al.* (1965)	Acetone-vehicle control, untreated control, 30 mg, or 100 mg D,L-diepoxybutane painted on the back 3 × /wk 120 acetone-vehicle controls, 60 untreated controls, 30/group for treated	Skin papillomas: 0/120, 0/60, 10/30, 1/30 Squamous dermoid carcinomas: 0/120, 0/60, 6/30, 0/30	[$P < 0.0001$, 30 mg-treated group] [$P < 0.0001$, 30 mg-treated group]	Median survival of 472, 441, 475 and 165 d, respectively
meso-Diepoxybutane Mouse, Swiss-Millerton (F) Lifetime Van Duuren *et al.* (1965)	Acetone-vehicle control, untreated control, 30 mg, or 100 mg *meso*-diepoxybutane painted on the back 3 × /wk 120 acetone-vehicle controls, 60 untreated controls, 30/group for treated	Skin papillomas: 0/120, 0/60, 1/30, 5/30 Squamous dermoid carcinomas: 0/120, 0/60, 0/30, 4/30	[$P < 0.0005$, 100 mg-treated group] [$P < 0.005$, 100 mg-treated group]	Median survival of 472, 441, 491 and 357 d, respectively

d, day or days; F, female; M, male; NS, not significant; wk, week or weeks

4. Other Relevant Data

Experimental studies on butadiene have been evaluated in previous *IARC Monographs* (IARC, 1999, 2008). There is an extensive body of data on the mechanism of butadiene-induced carcinogenicity, encompassing toxicokinetics, metabolism, biomarkers, genotoxicity, and molecular biology. The carcinogenicity of butadiene is mediated by its metabolites. This view is based largely on the observations that butadiene-induced mutagenicity requires metabolic activation (Jackson *et al.*, 2000) and that the DNA-reactive epoxides formed during butadiene bio-transformation are direct-acting mutagens (IARC, 1999, 2008). Thus, butadiene metabolism, formation of reactive epoxides, interaction of these epoxides with DNA, and resultant mutagenicity are likely key steps in the mechanism of carcinogenicity for this agent.

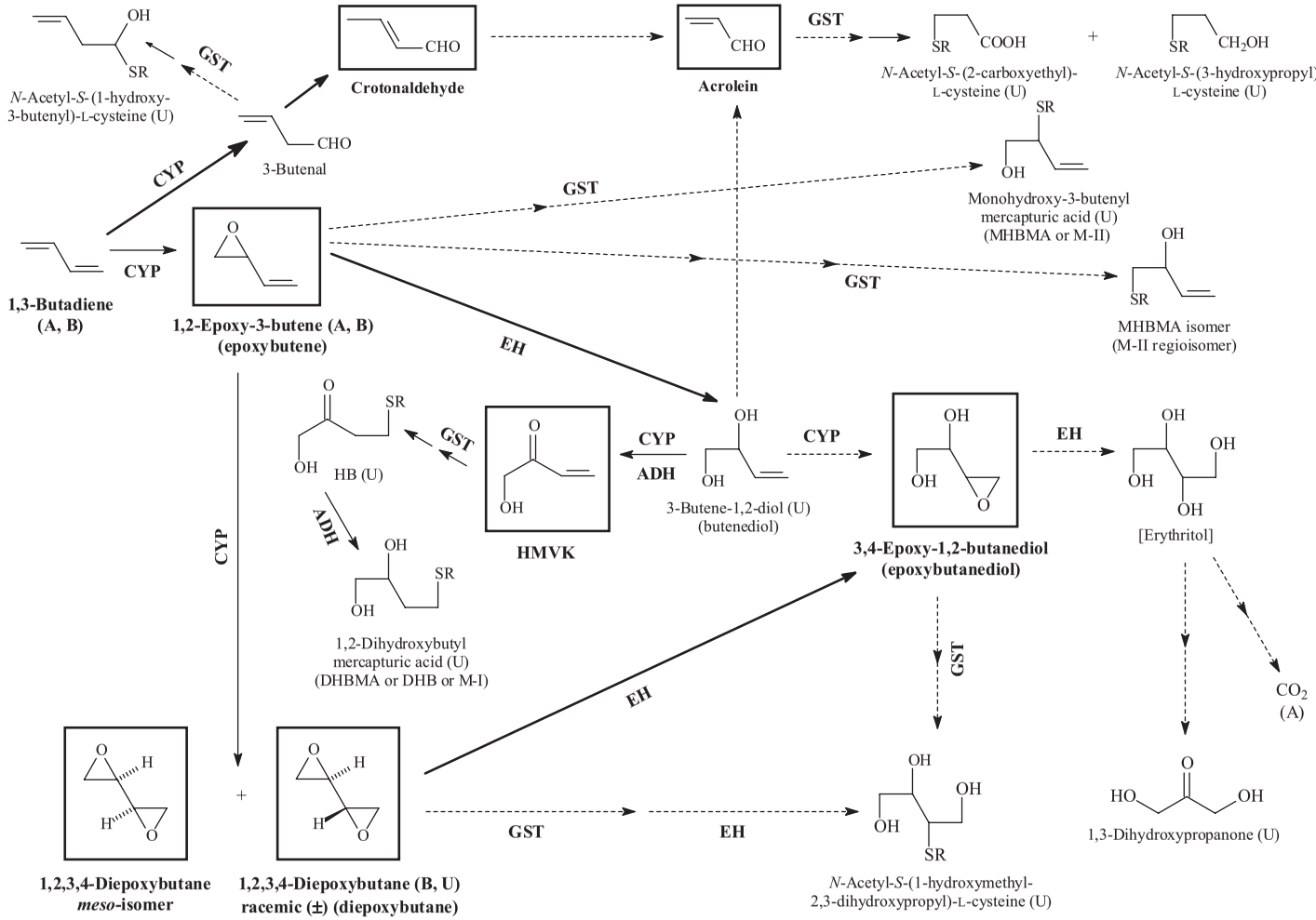
4.1 Metabolism of butadiene

The detailed pathways in the metabolism of butadiene have been described previously (Himmelstein *et al.*, 1997; IARC 1999, 2008) and are outlined in Fig. 4.1. Briefly, the first step in butadiene metabolism involves cytochrome P450 (CYP)-mediated oxidation to epoxybutene (Himmelstein *et al.*, 1997). At low concentrations of butadiene, metabolism via CYP2E1 predominates (IARC, 1999, 2008). Epoxybutene may be metabolized by conjugation with glutathione (GSH) mediated by glutathione *S*-transferase (GST), or by hydrolysis catalysed by epoxide hydrolase (EH) (Csanády *et al.*, 1992; Himmelstein *et al.*, 1997). Epoxybutene may also be oxidized to multiple diastereomers of diepoxybutane (Seaton *et al.*, 1995; Krause & Elfarra, 1997), while dihydroxybutene formed by hydrolysis of epoxybutene may be oxidized to epoxybutanediol. The latter epoxides are also detoxified by GST or EH (Boogaard *et al.*, 1996a, b). Partial hydrolysis of diepoxybutane also produces epoxybutanediol.

Each of the epoxide intermediates may contribute to the mutagenicity and carcinogenicity of butadiene. Factors that impact their relative contributions include concentration in tissues, reactivity with DNA, and repair of the ensuing DNA adducts. Variability in the expression of key enzymes involved in the biotransformation of butadiene may have an effect on metabolite concentrations in tissues, and on the subsequent mutagenic response (IARC, 2008). For example, genetically modified mice that are deficient in microsomal epoxide hydrolase (mEH) activity are more susceptible than wild-type mice to the mutagenic effects of butadiene and diepoxybutane, presumably because tissue concentrations of the epoxides are higher in the mEH-deficient mice (Wickliffe *et al.*, 2003). The detection of metabolites derived from hydroxymethylvinylketone and crotonaldehyde in the urine of rats or mice treated with butenediol suggests that these compounds may also be formed during the metabolism of butadiene (Sprague & Elfarra, 2003, 2004). The potential contribution of these DNA-alkylating agents (hydroxymethylvinylketone and crotonaldehyde) to the mutagenicity and carcinogenicity of butadiene is not known.

The enzymes that catalyse epoxide formation and elimination are polymorphic in human populations and some may be induced by a variety of environmental and pharmaceutical agents. While some reports indicate that genetic polymorphisms in GST and mEH affect the in-vitro mutagenicity of butadiene-derived epoxides or the in-vivo mutagenicity of butadiene in occupationally exposed workers (Wiencke *et al.*, 1995; Abdel-Rahman *et al.*, 2003), the extent to which these enzyme polymorphisms influence the carcinogenicity of butadiene is not known. Rates of butadiene metabolism have been reported for human tissues cultured *in vitro*. However, the range of observed rates is limited by the extent of the inter-individual variability in CYP, EH

Fig. 4.1 Metabolic pathways of butadiene deduced from findings in mammalian in-vitro systems and in mammals *in vivo*



A, B, U, metabolites in exhaled air, blood, urine, respectively; ADH, alcohol dehydrogenase; CYP, cytochrome P450; DHB, 4-(*N*-acetyl-L-cystein-*S*-yl)-1,2-dihydroxybutane; EH, epoxide hydrolase; GST, glutathione-*S*-transferase; HB, 4-(*N*-acetyl-L-cystein-*S*-yl)-1-hydroxy-2-butanone; HMVK, hydroxymethylvinyl ketone
Solid frame, electrophilic metabolites that can form DNA or haemoglobin adducts; dashed lines, assumed pathways
From IARC (2008)

and GST activities of the tissues sampled, and by the fact that only small numbers of human liver and lung samples were analysed (Csanády, *et al.*, 1992; Seaton, *et al.*, 1995; Boogaard, *et al.* 1996 a, b; Bolt *et al.*, 2003; Thier *et al.*, 2003; Norppa, 2004; Schlade-Bartusiak *et al.*, 2004). Thus, the actual kinetic range of the metabolism of butadiene in the human population is unknown.

The metabolism of butadiene in mice and rats shows linear elimination kinetics at exposures of up to about 1000 ppm [2210 mg/m^3] (Kreiling *et al.*, 1986b). Responses that increase proportionally above the levels of metabolic saturation probably represent effects of the parent compound. In the range of linear kinetics, mice metabolize butadiene about twice as rapidly as do rats. Although epoxybutene is formed primarily through CYP-mediated oxidation of butadiene, the formation of this alkylating agent by a myeloperoxidase-catalysed reaction in bone-marrow cells (Maniglier-Poulet *et al.*, 1995) may be relevant to the induction of haematopoietic cancers in mice and humans.

Data on urinary metabolites indicate that the elimination of epoxybutene in mice occurs to a greater extent by conjugation with GSH than by hydrolysis (IARC, 1999, 2008). Although no studies have been reported that characterize the full profile of urinary metabolites of butadiene in humans, the high ratio of 1,2-dihydroxybutyl-mercapturic acid (DHBMA) *vs* monohydroxy-3-butenyl-mercapturic acid (MHBMA) in exposed workers indicates that epoxybutene is preferentially metabolized by hydrolysis in humans (IARC, 2008). In rats, metabolic elimination of epoxybutene formed from butadiene occurs to a similar extent by hydrolysis or GSH conjugation. In molecular epidemiological studies of occupational exposure to butadiene, the ratio of MHBMA to MHBMA + DHBMA was lower in workers who were homozygous for *GSTM1*-null and *GSTT1*-null (Albertini *et al.*, 2001, 2003).

The formation of epoxybutanediol or diepoxybutane requires a second oxidation of either butenediol or epoxybutene, respectively. At increasing exposure concentrations of butadiene, competition between butadiene and butenediol or epoxybutene for CYP may limit the extent to which the second oxidation reaction may occur. Consequently, concentration of epoxybutanediol in blood is greater in rats exposed to 200 ppm [442 mg/m3] butadiene than in those exposed to 1000 ppm [2210 mg/m^3] or higher (Filser *et al.*, 2007). Competitive inhibition by butadiene of the second oxidation (Filser *et al.*, 2001) may account for the greater *Hprt* mutation efficiency in rats exposed to 62.5 ppm [138 mg/m^3] or mice exposed to 3 ppm [6.63 mg/m^3] compared with exposure of either species to 625 or 1250 ppm [1381 or 2762.5 mg/m^3] (Meng *et al.*, 2007). Thus, high-dose studies of butadiene (> 625 ppm) in animals may not adequately reveal the full mutagenic or carcinogenic potential of this substance at lower levels of exposure.

4.2 Haemoglobin adducts

While haemoglobin adducts are not causally related to mutagenic events, they offer an effective measure of exposure to reactive intermediates of chemicals. Haemoglobin adducts accumulate over the life-span of the erythrocyte, which is approximately 120 days in humans. Three adducts have been identified following reaction of butadiene epoxides with haemoglobin: *N*-(2-hydroxy-3-butenyl)valine (MHbVal), *N*,*N*-(2,3-dihydroxy-1,4-butadiyl) valine (PyrVal) and *N*-(2,3,4-trihydroxybutyl)valine (THbVal). These adducts are considered to reflect blood concentrations of epoxybutene, diepoxybutane and epoxybutanediol, respectively. Each of these adducts has been measured in rats and mice exposed to butadiene at concentrations as low as 3 ppm [6.63 mg/m3]. At equivalent exposures to butadiene, the levels of MHbVal and PyrVal were higher in mice than in rats, while levels of the major adduct, THbVal, were similar in these species (Boysen *et al.*, 2004, 2007). The formation

of each of these adducts in mice and rats was more efficient at 3 ppm than at higher exposure concentrations of butadiene.

MHbVal and THbVal have also been measured in workers exposed to butadiene (mean 8-hour TWA exposures, 0.3–0.8 ppm [0.66–1.76 mg/m^3]), while PyrVal could not be detected in workers exposed to mean concentrations of 0.37 ppm [0.82 mg/m^3]. In all samples, the amount of the PyrVal adduct was below the limit of quantification for the assay (Albertini *et al.*, 2003, 2007).

Species-specific differences in the amount of these haemoglobin adducts reflect differences in exposure to butadiene, blood concentrations of the epoxide intermediates, reactivity of the epoxide with the *N*-terminal valine and other reactive sites in haemoglobin, and the half-life of the red blood cell. When the amounts of adducts are normalized per gram of haemoglobin per ppm of butadiene, the levels of MHbVal adducts in workers are slightly lower than those in rats exposed to 3 ppm [6.63 mg/m^3] butadiene, while the levels of THbVal adducts are higher in workers than in rats or mice exposed to 3 ppm butadiene. These data demonstrate the systemic availability of epoxybutene and epoxybutanediol in workers exposed to occupational levels of butadiene. In these workers, the THbVal-adduct levels are influenced by the combined polymorphisms for *CYP2E1*, *GSTM1* and *GSTT1* genes (Fustinoni *et al.*, 2002).

4.3 DNA adducts

The major DNA adducts formed in the liver, lung and kidney of rats and mice exposed to butadiene are at the N7 position of guanine. These adducts are: N7-(2-hydroxy-3-butenyl) guanine (G1); N7-(1-(hydroxymethyl)-2-propenyl)guanine (G2); N7-(1-(hydroxymethyl)-2,3-dihydroxypropyl)guanine (G3); N7-(2,3,4-trihydroxybut-1-yl)guanine (G4). The G4 adducts are much more abundant than the G1 and G2 adducts, which are derived from epoxybutene (Koc *et al.*, 1999). The G4 adducts reach a plateau in rats after exposure to about 62 ppm [137 mg/m^3] butadiene, while G1 and G2 adducts increase nearly linearly with exposures to butadiene of up to 625 ppm [1381 mg/m^3]. Powley *et al.* (2005) have proposed that the similarity in the shape of the dose–response curves for THbVal-adduct formation in haemoglobin, G4-adduct formation in DNA, and *Hprt* mutation induction in splenic T-cells from mice and rats exposed to butenediol, suggests that epoxybutanediol may play a role in the mutagenicity and carcinogenicity of butadiene.

N7-Guanine adducts can undergo spontaneous depurination, which leaves an apurinic site in the DNA. Epoxide metabolites of butadiene can also react at sites involved in base-pairing and form adducts at N3 of cytosine, N1 of adenine, N^6 of adenine, N1 of guanine and N^2 of guanine (Selzer & Elfarra, 1996a, b, 1997; Zhao *et al.*, 1998; Zhang & Elfarra, 2004). An increase in N1-trihydroxybutyladenine adducts was detected in lymphocytes of workers exposed to butadiene (Zhao *et al.*, 2000). Alkylation of N1-adenine by epoxybutene followed by hydrolytic deamination under formation of deoxyinosine is a highly mutagenic event (Rodriguez *et al.*, 2001): deoxy-inosine forms a base-pair with cytosine during DNA replication, which leads to the generation of A→G mutations.

Diepoxybutane is a bi-functional alkylating agent that can form DNA–DNA crosslinks. Diepoxybutane first alkylates the N7 position of guanine in DNA and forms N7-(2′-hydroxy-3′,4′-epoxybut-1′-yl)-guanine mono-adducts (Tretyakova *et al.*, 1997). The epoxide group of this adduct can then undergo hydrolysis to yield N7-(2′,3′,4′-trihydroxybut-1′-yl)-guanine, or, less frequently, react with another site in DNA, such as the N7 of another guanine or the N1 of an adenine. The latter reactions result in formation of 1,4-bis-(guan-7-yl)-2,3-butanediol and 1-(guan-7-yl)-4-(aden-1-yl)-2,3-butanediol

crosslinks (Goggin *et al.*, 2009). These two diepoxybutane-specific DNA–DNA crosslinks have been identified in mice and rats exposed to 625 ppm butadiene, with much higher amounts of both crosslinks occurring in mice compared with rats (Goggin *et al.*, 2009). Depurination of these inter-strand or intra-strand lesions can induce point mutations and large deletion mutations. When diepoxybutane alkylates DNA at the N^6-position of adenine, an exocyclic adenine adduct is formed preferentially to DNA–DNA crosslinked products (Antsypovich *et al.*, 2007). Diepoxybutane is considered to be the most potent genotoxic metabolite of butadiene due to its strong genotoxicity and mutagenicity attributed to its ability to form DNA–DNA crosslinks.

4.4 Mutagenicity of butadiene and butadiene metabolites

Butadiene and its epoxide metabolites are genotoxic at multiple tissue sites in mice and rats, and in a variety of other test systems. In-vitro studies demonstrate that diepoxybutane is more potent than epoxybutene or epoxybutanediol in inducing micronuclei and gene mutations in mammalian cells. To investigate the role of two 1,3-butadiene (BD) metabolites, viz. 1,2-epoxybutene (EB) and 1,2,3,4-diepoxybutane (DEB) in the mutagenicity of the parent compound, in-vivo and in-vitro mutational spectra of BD, EB and DEB were analysed in *lac-i*-transgenic mice and in cultured human and rodent cells. A mutation that was consistently found across all biological systems examined was the AT→TA transversion, which was increased in the spleen and bone marrow of BD-exposed B6C3F1 *lac-i*-transgenic mice, in Rat2 *lac-i* cells exposed to EB, in the lungs of EB-exposed B6C3F1 *lac-i*-transgenic mice and at the *HPRT* locus in human TK6 lymphoblasts exposed to either EB or DEB (Recio *et al.*, 2001).

The mutation frequencies and mutation spectra induced by 3,4-epoxy-1,2-butanediol (EBD) and 1,2,3,4-diepoxybutane (DEB) were investigated at the *Hprt* locus in Chinese hamster ovary-K1 cells (CHO-K1). EBD was mutagenic at levels that were approximately 100 times higher than mutagenic concentrations of DEB. Among 41 EBD-induced mutants, there were 16 exon deletions, 11 GC→AT transitions, and five AT→GC transitions. Among 39 DEB-induced mutants, 15 exon deletions, 11 GC→AT transitions and five AT→TA transversions were found. In this study, the most common base substitution induced by both substances was the GC→AT transition. The sites of the single base substitutions that were induced by EBD and DEB were guanine and adenine, which is consistent with the DNA-adduct profiles (Lee *et al.*, 2002).

Fernandes & Lloyd (2007) have shown that replication of DNAs containing specific butadiene-derived 2′-deoxyuridine adducts in mammalian COS-7 cells resulted in predominantly C→T transitions. They also showed that replicative DNA polymerases were blocked by these lesions *in vitro*.

The genotoxic effects of butadiene can be modulated by alterations in key determinants of its metabolism, which suggests that markers of individual susceptibility can be identified. For example, mice that lack a functional *mEH* gene were more susceptible than wild-type mice to the mutagenic effects of butadiene or diepoxybutane (Wickliffe *et al.*, 2003). Epoxide hydrolase (EH) activity varies considerably among humans. Butadiene-exposed workers with the genotype for low-activity EH were reported to be more susceptible to butadiene-induced genotoxicity (assessed by *HPRT* mutant-variant frequency in lymphocytes) than individuals with the more common *EH* genotype (Abdel-Rahman *et al.*, 2001, 2003). No significant effects were observed for induction of *HPRT* mutations or sister chromatid exchange (SCE) in individuals with *GSTM1* or *GSTT1* polymorphisms (Abdel-Rahman *et al.*,

2001). These differences in response are consistent with the known important role of EH in the detoxification of butadiene epoxides in tissues in which these intermediates are produced.

In contrast, several other molecular epidemiological studies report no effect of butadiene – at occupational exposure levels – on *HPRT* mutation frequency or chromosomal changes, and no significant associations with genotype (Zhang *et al.*, 2004; Albertini *et al.*, 2001, 2007; Lovreglio *et al.*, 2006; Wickliffe *et al.*, 2009). Discrepancies among these studies may be related to differences in levels of exposure to butadiene at the workplace, the influence of exposures to butadiene or other genotoxic agents from other sources (e.g. cigarette smoke, automobile exhaust), the group size and the level of enzyme activity associated with a particular genotype.

The induction of SCE in human lymphocytes exposed *in vitro* to diepoxybutane was significantly higher in cells from *GSTT1*-null individuals than from *GSTT1*-positive individuals (Wiencke *et al.*, 1995), which indicates that the GST pathway may be important in the detoxification of diepoxybutane released into the blood. Epoxybutene can induce SCE and chromosomal aberrations in human peripheral lymphocytes treated *in vitro*; the lack of induction of these effects in G0 lymphocytes appears to be due to effective excision repair of DNA lesions (Kligerman *et al.*, 1999). Other studies also demonstrate the importance of DNA repair in the genotoxicity of butadiene-derived epoxides. For example, mice deficient in nucleotide excision-repair are more susceptible than wild-type mice to the mutagenic effects of butadiene and diepoxybutane (Wickliffe *et al.*, 2007).

The mechanistic link between animal and human neoplasia induced by butadiene is supported by the identification in mice of genetic alterations in butadiene-induced tumours that are frequently involved in the development of a variety of human cancers as well. The K-*Ras*, H-*Ras*, *p53*, *p16*/*p15* and *β-catenin* mutations detected in tumours in mice probably occurred as a result of the DNA-reactive properties and the genotoxic effects of butadiene-derived epoxides. A consistent pattern of K-*Ras* mutations (G→C transversion at codon 13) was observed in butadiene-induced cardiac haemangiosarcomas, neoplasms of the lung and fore-stomach, and lymphomas (Hong *et al.*, 2000; Sills *et al.*, 2001; Ton *et al.*, 2007). Alterations in the *p53* gene in mouse-brain tumours were mostly G→A transition mutations (Kim *et al.*, 2005). Inactivation of the tumour-suppressor genes *p16* and *p15* may also be important in the development of butadiene-induced lymphomas (Zhuang *et al.*, 2000). Mammary gland adenocarcinomas induced by butadiene in mice frequently had mutations in the *p53*, H-*Ras* and *β-catenin* genes (Zhuang *et al.*, 2002). These observations point to a genotoxic mechanism that underlies the development of butadiene-induced cancers. Although genotoxicity data indicate that diepoxybutane is the most genotoxic of the butadiene epoxides, the relative contribution of these metabolic intermediates to the mutagenicity and carcinogenicity of butadiene is not known. A comparison of the weight of evidence on metabolism, haemoglobin-adduct formation and genetic changes in rodents and humans exposed to butadiene is summarized in Table 4.1.

4.5 Synthesis

The numerous studies that have been conducted on butadiene toxicokinetics, metabolism, and genotoxicity provide strong evidence that the carcinogenicity of butadiene involves a genotoxic mechanism of action mediated by reactive epoxide metabolites. The metabolic pathways for butadiene in experimental animals have also been demonstrated in humans. This mechanism of action is based on the observations that butadiene-induced mutagenicity requires metabolic activation, and that the DNA-reactive epoxides formed during butadiene biotransformation

Table 4.1 Comparison of the degree of evidence on metabolism, haemoglobin-adduct formation and genetic changes in rodents and humans exposed to butadiene

Parameter	Rats	Mice	Humans
In-vitro metabolism of butadiene to epoxybutene	Strong	Strong	Strong
In-vitro metabolism of epoxybutene to diepoxybutane	Strong	Strong	Strong
In-vivo measure of epoxybutene in blood	Strong	Strong	NR
In-vivo measure of diepoxybutane in blood	Strong	Strong	NR
N-(2,3,4-Trihydroxybutyl)valine-haemoglobin adducts	Strong	Strong	Strong
N-(2-Hydroxy-3-butenyl)valine-haemoglobin adducts	Strong	Strong	Strong
N,*N*-(2,3-Dihydroxy-1,4-butadiyl)valine-haemoglobin adduct	Strong	Strong	Weak[a]
Urinary excretion of butadiene-derived mercapturic acid metabolites	Strong	Strong	Strong
DNA adducts	Strong	Strong	Strong
Mutations in reporter genes in somatic cells	Strong	Strong	Inconsistent[b]
Chromosomal aberrations or micronuclei	No evidence	Strong	Weak[a]

NR, not reported
[a] Possibly due to a lack of adequate studies
[b] One positive and three negative studies
From IARC (2008)

are direct-acting mutagens (IARC, 1999, 2008). Thus, butadiene metabolism, formation of reactive epoxides, interaction of these epoxides with DNA, and resultant mutagenicity are key steps in the mechanism that underlies the carcinogenicity of this agent.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of 1,3-butadiene. 1,3-Butadiene causes cancer of the haematolymphatic organs.

There is *sufficient evidence* for the carcinogenicity of 1,3-butadiene in experimental animals.

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

There is strong evidence that the carcinogenicity of 1,3-butadiene in humans operates by a genotoxic mechanism that involves formation of reactive epoxides, interaction of these direct-acting mutagenic epoxides with DNA, and resultant mutagenicity. The metabolic pathways for 1,3-butadiene in experimental animals have also been demonstrated in humans.

1,3-Butadiene is *carcinogenic to humans (Group 1).*

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2,3,7,8-TETRACHLORODIBENZO-*para*-DIOXIN, 2,3,4,7,8-PENTACHLORODIBENZOFURAN, AND 3,3′,4,4′,5-PENTACHLOROBIPHENYL

Two of these agents (2,3,7,8-tetrachlorodibenzo-*para*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran were specifically considered by previous IARC Working Groups in 1977, 1987, and 1997 (IARC, 1977, 1987, 1997). The Working Group in 1987 reviewed polychlorinated biphenyls, but did not specifically consider 3,3′,4,4′,5-pentachlorobiphenyl. Since the previous evaluations new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

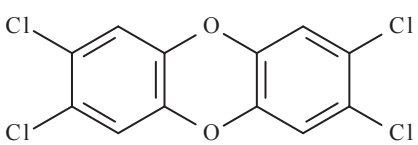
1. Exposure Data

1.1 Identification of the agents

From NTP (2006a, b, c)

1.1.1 2,3,7,8-Tetrachlorodibenzo-para-dioxin (2,3,7,8-TCDD, TCDD)

Chem. Abstr. Serv. Reg. No.: 1746-01-6
Chem. Abstr. Serv. Name: 2,3,7,8-Tetrachlorodibenzo[*b*,*e*][1,4]dioxin
Synonyms: 2,3,7,8-TCDD; TCDD; dioxin; tetradioxin

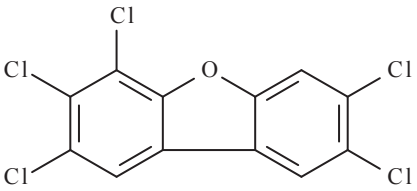


$C_{12}H_4Cl_4O_2$
Relative molecular mass: 321.98

Description: Colourless to white crystalline solid
Solubility: Insoluble in water; slightly soluble in n-octanol and methanol; and soluble in other organic solvents (e.g. dichlorobenzene, chlorobenzene, benzene, chloroform, and acetone)
Octanol/water partition coefficient: log K_{ow}, 6.80

1.1.2 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF)

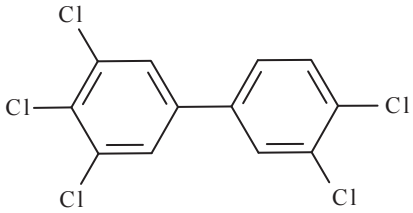
Chem. Abstr. Serv. Reg. No.: 57117-31-4
Chem. Abstr. Serv. Name: 2,3,4,7,8-Pentachlorodibenzofuran
Synonym: 2,3,4,7,8-PeCDF; 2,3,4,7,8-penta-CDF



$C_{12}H_3Cl_5O$
Relative molecular mass: 340.42
Description: Solid with a melting point of 195–196 °C (NTP Chemical Repository Information). It is stable under normal laboratory conditions.
Solubility in water: 2.36×10^{-4} mg/L at 22.7 °C
Octanol/water partition coefficient: log K_{ow}, 6.92

1.1.3 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126)

Chem. Abstr. Serv. Reg. No.: 57465-28-8
Chem. Abstr. Serv. Name: 3,3′,4,4′,5-Pentachlorobiphenyl
Synonym: PCB 126



$C_{12}H_5Cl_5$
Relative molecular mass: 326.42
Description: Solid with a melting point of 160–161 °C.
Solubility in water: 1.03×10^{-3} mg/L at 25 °C
Octanol/water partition coefficient: log K_{ow}, 6.89

1.2 Occurrence and use

2,3,7,8-Tetrachlorodibenzo-*para*-dioxin (TCDD) has no known commercial applications. It occurred as a contaminant in chlorophenoxy herbicides, including 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which were widely used in the 1960s and 1970s to control weeds (e.g. on pastureland and food crops) and as a defoliant during the Viet Nam war. It is used as a research chemical and was tested, but never used commercially, as a flame-proofing agent and as a pesticide against insects and wood-destroying fungi (NTP, 2004). TCDD may also be produced in thermal processes such as incineration, in metal-processing, and in the bleaching of paper pulp with free chlorine. The relative amounts of the TCDD congeners produced depend on the production or incineration process and vary widely (IARC, 1997).

Polychlorinated dibenzofurans (PCDFs) are not manufactured commercially other than for scientific research purposes. Release of PCDF into the environment is mainly from combustion and incineration. Based on congener-specific profiles, combustion sources all produce 2,3,7,8-substituted polychlorinated dibenzo-*para*-dioxins (PCDDs) and PCDFs, including 2,3,4,7,8-pentachlorodibenzofuran (PeCDF). The latter substance is the major congener emitted from cement kilns burning hazardous waste (approximately 20% of the total congener emission). Other major sources of PeCDF are metal smelting, refining, and processing; chemical manufacturing/processing (production of chlorophenols, PCBs, vinyl chloride); pulp bleaching; and existing reservoirs that reflect past releases (IARC, 1997; USEPA, 2000a).

Mixtures of polychlorobiphenyls (PCBs), including 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), were produced for commercial purposes during the period 1929–1977 for the electrical industry, to be used as dielectric insulating fluids for transformers and capacitors. PCBs

were also used in hydraulic fluids, plastics, and paints. The manufacture and use of PCBs in the United States was stopped in 1977 in view of increasing amounts of PCB residues in the environment that had accumulated during the previous decades. However, PCBs continue to be released into the environment through the use and disposal of products containing PCBs, as by-products during the manufacture of certain organic chemicals, and during combustion of some waste materials (USEPA, 2000a). Due to their lipophilic nature (log K_{ow}, 6.5–7.7) and resistance to biodegradation, specific PCBs bioconcentrate and bio-accumulate in the environment. PCBs are widespread in their distribution and are found in virtually all environmental compartments including air, soil, water, sediment, and biota (USEPA, 2000b).

1.3 Human exposure

PCDDs are ubiquitous in soil, sediments and air. Excluding occupational or accidental exposures, most human exposure to TCDD occurs as a result of eating meat, milk, eggs, fish and related products, as TCDD is persistent in the environment and accumulates in animal fat. Occupational exposures to TCDD at higher levels have occurred since the 1940s as a result of production and use of chlorophenols and chlorophenoxy herbicides. Even higher exposures have occurred sporadically in relation to accidents in these industries (IARC, 1997).

Mean background levels of TCDD in human tissues are in the range of 2–3 ng/kg (parts per trillion; ppt) fat [because PCDDs are stored in fat tissue, body burdens of PCDDs are often expressed as concentration in lipid, e.g. 100 ppt lipid]. Available data suggest that these levels have decreased three- to fivefold since the late 1970s, when the development of gas chromatography/mass spectrometry methodology permitted these extremely low concentrations of TCDD in tissues and in the environment to be measured accurately for the first time. Similarly, since the mid-1980s, mean tissue concentrations of total TCDD in the general population have decreased by two- to threefold. Human exposures related to occupation or accidents have led to tissue concentrations of TCDD up to several orders of magnitude higher than background levels (IARC, 1997).

1.3.1 Occupational exposure to dioxins

Because TCDD has never been intentionally manufactured for large-scale commercial use, estimates of the numbers of workers exposed in the past are not available. From the US National Occupational Exposure Survey (1981–1983), it was estimated that approximately 14 workers were potentially exposed to TCDD in the USA (NIOSH, 1990). Historical occupational exposures to TCDD have been reviewed (IARC, 1997).

In a series of studies, Collins *et al.* (2006, 2007, 2008) examined concentrations of TCDD in serum from 375 workers in Michigan who had been exposed in the past (26–62 years before) to trichlorophenol and/or pentachlorophenol. Workers exposed only to trichlorophenol had mean lipid-adjusted TCDD levels of 15.9 ppt, compared with 6.5 ppt in unexposed workers and 3.3 ppt in community controls. Those exposed to pentachlorophenol only had mean TCDD concentrations of 8.0 ppt; workers exposed to both chemicals had mean TCDD levels of 13.9 ppt; and tradesmen with plant-wide responsibilities had mean levels of 20.7 ppt. A follow-up study to evaluate the influence of various factors on TCDD concentrations in serum of 412 workers exposed to penta- and trichlorophenol, showed that age and body fat were important determinants, whereas diet and jobs-outside-the-plant had little impact (Burns *et al.*, 2008).

To estimate job-specific exposures over time at a facility in New Zealand that manufactured 2,4,5-T along with other products between 1962 and 1988, Aylward *et al.* (2010) integrated

available work records, TCDD pharmacokinetic data, and serum-sampling data from 346 workers. Estimated TCDD concentrations in serum were below 300 ppt lipid for all individuals in the cohort over the entire study period, i.e. lower than estimates for other 2,4,5-T worker populations.

McLean *et al.* (2009) measured dioxin concentrations in serum of 94 randomly selected former sawmill workers (71 exposed to pentachlorophenol, 23 non-exposed) in New Zealand, twenty years after the use of pentachlorophenol (PCP) had ceased. The authors compared age-adjusted dioxin levels in the exposed and non-exposed groups, examined the effect of duration and intensity of the exposure to PCP, and compared congener profiles with those found in the commercial-grade PCP used at the time. Mean levels in exposed and unexposed workers were 1.88 pg/g (range, 0.51–4.13) and 1.48 pg/g, respectively. The congener profiles in serum were consistent with those in PCP solutions, and dioxin levels increased with both employment duration and estimated exposure intensity.

In a study of Russian workers who manufactured phenoxy herbicides and related compounds in the 1960s, workers who handled 2,4,5-T (n = 34) had median blood-lipid TCDD concentrations of 165.7 ng/kg (parts per trillion), with a range from 35 to 1680 ng/kg. Workers who manufactured 2,4-dichlorophenoxyacetic acid (2,4-D; n = 6) had median levels of 68.9 ng/kg. Children of workers who handled 2,4,5,-T (n = 8) and administrative workers (n = 5) had higher median levels than two control groups drawn from the general population (n = 60): the median blood-lipid TCDD concentrations in the children and administrative staff were 39.5 and 31.0 ng/kg, respectively, compared with 12 and 62.4 ng/kg for the two control groups (Ryan & Schecter, 2000).

Several exposure-response analyses have been performed in the industrial cohorts that served as a basis for the previous *IARC Monographs* evaluation (Becher *et al.*, 1998; Flesch-Janys *et al.*, 1998; Hooiveld *et al.*, 1998; Steenland *et al.*, 1999, 2004; Crump *et al.*, 2003).

1.3.2 Non-occupational exposure to dioxins

PCDDs, including TCDD, have been measured in all environmental compartments including ambient air, surface water, groundwater, soil, and sediment. While the manufacture and use of chlorinated compounds – chlorophenols, chlorinated phenoxy herbicides – were important sources of PCDD-release into the environment in the past, the restricted manufacture of many of these compounds has substantially reduced their contribution to environmental pollution. It is now recognized that incineration/combustion processes are the most important sources of PCDDs to the environment (Zook & Rappe, 1994; ATSDR, 1998).

The identified sources of environmental release of TCDDs have been grouped into four major categories: incineration sources (municipal waste, hospital waste, hazardous waste, sewage sludge), combustion sources (cement kilns, wood-burning, diesel vehicles, coal-fired utilities, crematoria), industrial sources (pulp and paper mills, chemical manufacturing, metal industry), and reservoir sources (biochemical processes, photolytic processes, forest fires, accidental releases) (Kulkarni *et al.*, 2008).

Human exposure to all dioxin-like compounds is usually calculated in terms of toxic equivalence quotients (TEQs).

> Because the various polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and polychlorinated biphenyls have different activity levels, a toxic equivalence quotient (TEQ) is calculated by standardizing the individual congener levels detected in each sample, multiplying them with the appropriate toxic equivalency factor (TEF), and summing these normalized values. TEFs have been established by the World Health Organization and are calculated relative to 2,3,7,8-TCDD (Van den

Berg *et al.*, 2006; Charnley & Kimbrough, 2006; see also Section 4).

The US Environmental Protection Agency (USEPA) reported that emissions from quantified sources – waste incineration, pesticide manufacture, chlorine bleaching of pulp and paper – in the USA decreased from about 14 000 g TEQ/year in 1987 to approximately 1500 g TEQ/year in 2000 (a 90% reduction). This decline is expected to continue (Charnley & Kimbrough, 2006; USEPA, 2006).

People are exposed to PCDDs primarily through foods that are contaminated as a result of the accumulation of these substances in the food-chain and in high-fat foods, such as dairy products, eggs, animal fats, and some fish. Additional exposure sources include industrial accidents (Baccarelli *et al.*, 2002) and several miscellaneous exposures (Yoshimura, 2003; Kulkarni *et al.*, 2008). Because dioxins are fat-soluble, lowering the fat content in food can reduce the intake of dioxin. The average adult in the USA has a daily TEQ intake of approximately 1 pg/kg, lower than a decade ago, whereas a nursing infant has an average TEQ intake of 35–53 pg/kg bw/day (Schecter *et al.*, 1994; USEPA, 2004; Schecter *et al.*, 2006).

Data on TCDD concentrations in lipids have been collected over a 30-year period (1970–2000) among the general population in the USA, Canada, Germany, and France. Mean lipid-levels of TCDD steadily decreased nearly 10-fold over this time period, with lipid-adjusted TCDD concentratrions of about 2 ppt in the year 2000. On the basis of pharmacokinetic modelling, mean concentrations of TCDD in the general population are likely to decrease further to 0.5–1 ppt by 2015, even if intake levels do not decrease further (Aylward & Hays, 2002; Hays & Aylward, 2003).

There is evidence from the NHANES serum PCDD/F data (including TCDD) that concentrations of these compounds in the US population are declining. Median serum-lipid concentrations (population-weighted) of PCDDs/Fs were 13.46, 13.98 and 11.39 TEQ/g lipid for 1999–2000 (n = 1271), 2001–2002 (n = 1244), and 2003–2004 (n = 1290), respectively. When the temporal trends are examined by age, median levels appear to have declined in the younger part of the population and remained essentially constant (or have slightly increased) in the older part (LaKind *et al.*, 2009).

Several exposure studies have shown that some US Viet Nam veterans who were exposed to Agent Orange had serum TCDD levels up to 600 ppt in lipid many years after they had left Viet Nam, compared with values of approximately 1–2 ppt of TCDD for the general population (Kahn *et al.*, 1988; Schecter *et al.*, 1990, 1992; Michalek *et al.*, 1995). In Viet Nam, TCDD levels up to 1 000 000 ppt have been found in soil or sediment from Agent Orange-contaminated areas 3–4 decades after spraying. In addition, elevated concentrations have been measured in food and wildlife in Viet Nam (Olie *et al.*, 1989) as well as in Vietnamese people from contaminated areas (Schecter *et al.*, 2001, 2002, 2003; Dwernychuk *et al.*, 2002; Schecter *et al.*, 2006).

Bates *et al.* (2004) determined persistent organochlorines, including TCDD, in serum of the non-occupationally exposed New Zealand population in 1996–1997. The weighted mean concentration of TCDD in adult New Zealanders was 2.3 ng/kg lipid weight basis (range, < 1–7.0 ng/kg). The age group-specific data showed a trend towards higher concentrations in the older age groups.

Several recent studies have assessed and reviewed the exposure to and intake of dioxins, including TCDD, from dietary sources (Liem *et al.*, 2000; Tsutsumi *et al.*, 2001; Huwe, 2002; Parzefall, 2002; Baars *et al.*, 2004; Charnley & Doull, 2005; Nakatani *et al.*, 2005; Larsen, 2006; Sasamoto *et al.*, 2006; Gies *et al.*, 2007; Todaka *et al.*, 2008).

1.3.3 Human exposure to polychlorinated dibenzofurans, polychlorinated biphenyls and dioxin-like compounds

Due to high lipophilicity and low solubility in aqueous media, polychlorinated dibenzofurans (PCDFs) accumulate in the fat tissue of animals. The highest concentrations of PCDFs are found in fish, meat, eggs, and dairy products (Schecter *et al.*, 1994; USEPA, 2000b). This results in widespread exposure of the general population to PCDFs and related dioxin-like compounds, with an estimated 90% of human exposure due to ingestion of contaminated food, and a small fraction via inhalation and dermal absorption.

Human exposure to all dioxin-like compounds is usually calculated in terms of TEQs (see definition above). Adult daily intake of dioxin-like compounds including PCDDs, PCDFs and dioxin-like PCBs from all sources is estimated to be approximately 70pg TEQ/day, where TEQ reflects the potency-adjusted amount of all dioxin-like compounds. The intake from all sources of PCDDs and PCDFs is estimated to be 45pg TEQ/day and intake from dioxin-like PCBs is 25pg TEQ/day. Approximately 90% of the daily intake is from food sources (40pg TEQ/day for PCDDs and PCDFs and 22pg TEQ/day for dioxin-like PCBs). Intake of PeCDF from food is approximately 6.6 pg per day and represents 16% of the total intake of PCDDs and PCDFs on a TEQ basis. This level of exposure together with the long half-life of dioxin-like compounds in humans leads to persistent body burdens in humans in the range of 25 pg TEQ/g lipid (USEPA, 2000b). Depending upon dietary practice and proximity to specific sources of exposure, some populations may have higher exposure or body burdens. In contrast to the general population, several specific groups may have been exposed to much higher levels of PeCDF as a result of occupational exposure. In European tissue samples, PeCDF levels are on average 13 ppt TEQ (lipid-adjusted) and represent approximately 36% of the TEQ contributed by PCDDs and PCDFs. PeCDF is the highest contributor of the PCDF class of DLCs to the total TEQ, based on both intake levels and tissue concentrations.

The majority (90%) of ambient human exposure to dioxin-like compounds occurs through the ingestion of food containing PCB residues. Levels of PCB 126 in food range from 0.05 to 0.83 pg/g. Human exposure to all dioxin-like compounds is usually calculated in terms of toxic equivalents (TEQs). On a TEQ basis, it is estimated that humans are exposed via food to 22 pg TEQ/day (for a 70-kg person) from dioxin-like PCBs of which PCB 126 (13 pg/day) accounts for 60% of the TEQ intake. Bioaccumulation of PCBs results in persistent levels of these substances in human tissues. With an average concentration of 12 pg TEQ/g lipid, PCB 126 accounts for approximately half of the PCB TEQ in human tissues (USEPA, 2000b).

2. Cancer in Humans

Human exposures related to occupation or accidents have led to tissue concentrations of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) that are 10–100-fold higher than average background levels in the 1980s. The highest exposures occurred in industrial cohorts of workers producing phenoxy herbicides and chlorophenols, while exposure to professional sprayers of these compounds was considerably lower. It has been shown that TCDD levels in professional applicators increase considerably above background only after several years of spraying of TCDD-contaminated chemicals. For example, in the most heavily exposed applicators of 2,4,5-T in New Zealand, who applied this chemical for at least 180 months, the estimated mean serum concentration of TCDD at the time of blood drawing was 53 ng/kg (Smith *et al.*, 1992).

Occasional spraying is unlikely to lead to any measurable increase in TCDD level.

The evaluation of the evidence of carcinogenicity of TCDD was based on studies with direct measurements of TCDD and studies involving heavy exposure to herbicides likely to be contaminated with TCDD. There are numerous studies that assessed dioxins, furans and polychlorobiphenyls (PCBs) in workers, but these are not systematically reviewed here. These studies indicate that the highest exposure occurs in industrial settings producing 2,4,5-T. There are also numerous studies in workers evaluating a wide range of health effects. Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-22-Table2.1.pdf) shows estimated exposures to TCDD in industrial workers, in workers handling and spraying 2,4,5-T and in the population in Seveso, Italy. Average exposures in industrial workers are the highest. The effects of TCDD and those of the products in which it was found cannot be separated in most of the epidemiological studies; however, the focus here is on the contaminant. The most important studies for the evaluation of the carcinogenicity of TCDD are cohort studies of herbicide producers, one each in the United States (Fingerhut *et al.*, 1991; Steenland *et al.*, 1999, 2001), the Netherlands (Bueno de Mesquita *et al.*, 1993; Hooiveld *et al.*, 1998; Boers *et al.* 2010), two in Germany (Manz *et al.*, 1991; Ott *et al.*, 1993; Flesch-Janys *et al.*, 1995, 1998; Becher *et al.*, 1996), and one cohort of residents in a contaminated area from Seveso, Italy (Bertazzi *et al.*, 2001; Pesatori *et al.*, 2009). These studies involve the highest exposures to TCDD among all epidemiological studies, although the exposures at Seveso were lower and the follow-up was shorter than those in the industrial settings. In addition, the multicountry cohort study from IARC (Saracci *et al.*, 1991; Kogevinas *et al.*, 1995, 1997) is of special interest because it includes three of four high-exposure cohorts and other industrial cohorts, many of them not reported in separate publications, as well as information on professional applicators. Most of the industrial cohort studies include analyses of subcohorts considered to have the highest exposure and/or longest latency. These cohorts and their respective high-exposure subcohorts are the focus of this evaluation. In reporting the findings, preference has been given to the most updated follow-up, unless earlier publications presented evidence not included in a later publication, e.g. results by exposure classifications of interest. Results are presented in Table 2.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-22-Table2.2.pdf), Table 2.3 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-22-Table2.3.pdf), Table 2.4 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-22-Table2.4.pdf), and Table 2.5 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-22-Table2.5.pdf). Additional studies of herbicide applicators, both cohort and case–control studies, which have considerably lower exposures to TCDD, are not considered critical for the evaluation and are not reported in the tables. Among the studies not included, there are several that have been widely quoted and that have been important in responding to concerns in the community and in raising public awareness regarding potential effects of dioxin exposure, such as Ketchum & Aktar (1996), Ketchum *et al.* (1999), and Hardell & Sandström (1979).

2.1 Description of the most informative studies

2.1.1 US NIOSH cohort

The largest study of production workers in the US exposed to PCDDs was conducted by the National Institute for Occupational Safety and Health (NIOSH) and published by Fingerhut *et al.* (1991) and Steenland *et al.* (1999, 2001). This 12-plant cohort study included most workers in the USA likely to have been exposed to TCDD

in chemical manufacturing, comprising 5000 men with work records showing assignment to a production or maintenance job in a process involving TCDD contamination. Serum levels of TCDD in 253 cohort members at two plants measured in 1987 averaged 233 ng/g lipid, compared with 7 ng/g lipid in a group of 79 unexposed workers. Levels increased to 418 ng/kg for 119 workers exposed for more than one year. Estimates of TCDD exposure were based on occupational records, on an exposure matrix based on industrial hygiene measurements, and on modelling based on measured TCDD in serum samples. A series of publications on two separate plants were available (Zack & Suskind, 1980; Zack & Gaffey, 1983; Cook *et al.*, 1986; Ott *et al.*, 1987; Bond *et al.*, 1989; Collins *et al.*, 1993).

2.1.2 German accident cohort

In the 1953 accident at the 2,4,5-trichlorophenol (TCP) production unit of BASF at Ludwigshafen, Germany, the total number of employees identified as being involved directly or in the subsequent clean-up, repair or maintenance activities was 247 (243 men, 4 women). Analyses of adipose tissue and blood from groups of these workers were conducted. Part of the cohort was first studied by Thiess *et al.* (1982) and was completed by Ott & Zober (1996).

2.1.3 IARC multicountry study

An international cohort of workers exposed to phenoxy herbicides and chlorophenols was set up by the International Agency for Research on Cancer, France (Saracci *et al.*, 1991). The cohort included 16 863 men and 1527 women employed in production or spraying, distributed among 20 cohorts from ten countries. Exposure assessment was based on plant-production records collected in each factory through questionnaires and on detailed individual job histories. Two nested case–control studies of soft-tissue sarcoma and non-Hodgkin lymphoma were conducted by Kogevinas *et al.* (1995). The international cohort studied by Saracci *et al.* (1991) was updated and expanded with the data of Fingerhut *et al.* (1991), Becher *et al.* (1996), and Kogevinas *et al.* (1997). TCDD was measured in serum samples for workers in Germany, the Netherlands and the USA. Results from cohorts in specific countries have been published separately (Coggon *et al.*, 1991; Lynge, 1993).

2.1.4 German cohorts

Several reports have considered workers from a chemical plant operated by Boehringer-Ingelheim, Hamburg, Germany. This plant produced herbicides heavily contaminated with TCDD and other PCDDs/PCDFs (Manz *et al.*, 1991; Flesch-Janys *et al.*, 1995, 1998; Becher *et al.*, 1996). In the latter study, workers from three other German plants were also considered. TCDD analyses were done on serum samples from the workers in the Boehringer-Ingelheim cohort.

2.1.5 Dutch cohorts

The mortality of two cohorts of workers employed between 1955 and 1986 in the synthesis and formulation of phenoxy herbicides and chlorophenols in the Netherlands has been studied (Bueno de Mesquita *et al.*, 1993). In one of the plants (A), where the production was focused on 2,4,5-T and derivatives, an accident in 1963 caused a release of PCDDs, including TCDD. Serum samples have been analysed for the presence of TCDD. The study has been updated (Hooiveld *et al.*, 1998; Boers *et al.*, 2010)

2.1.6 Seveso population exposed during an industrial accident

On 10 July 1976, a runaway reaction led to a blow-out of a TCP-production reactor at the ICMESA plant at Seveso, Italy. The chemical cloud

that was released from the reactor contained a substantial amount of TCDD. The contaminated area was subdivided into Zones A and B, and Zone R in descending order of TCDD contamination in the soil. The mortality and cancer incidence in the population of Seveso exposed during this industrial accident were investigated. The exposed and referent populations were followed-up as if they belonged to a unique cohort, blind to the exposure status of the subjects. The follow-up after 20 years was > 99% successful (Bertazzi *et al.*, 2001; Pesatori *et al.*, 2009)

2.2 All cancers combined

An increased risk for all cancers combined was found in the industrial cohort studies cited above in the USA, Germany, and the Netherlands and to a lesser extent in the international cohort (see Table 2.2, online). The magnitude of the increase is generally small. It is higher in subcohorts considered to have the heaviest TCDD exposure, e.g. the chloracne subcohort in the NIOSH study. Furthermore, statistically significant positive dose–response trends for all cancers combined were present in the NIOSH cohort and in the largest and most heavily exposed German cohort. A positive trend ($P = 0.05$) was also seen in the smaller German cohort where an accident occurred with release of large amounts of TCDD. However, the positive trend in this cohort was limited to smokers. Cumulative dose in these trend analyses was estimated by combining data from TCDD concentrations in blood and information on job categories, work processes and calendar time of exposure. A metanalysis of data from three cohorts occupationally exposed to TCDD and related compounds (NIOSH, Boehringer-Ingelheim, Germany, and BASF, Germany) found a statistically significant ($P = 0.02$) trend in total cancer mortality with increasing exposure to dioxin (Crump *et al.*, 2003). The trend tests show an increase in total cancers at cumulative TEQ – a metric TCDD-like compounds that is defined here as the amount of TCDD that would produce the same toxicity as a mixture of TCDD-like compounds – serum levels that would result from lifetime intake of 7 pg TEQ/kg body weight/day. A linear dose–response provided a good fit to the combined data. There was no overall increase of cancer in the population in Seveso, with only minor increases observed for all-cancer mortality and incidence at 15 or more years since the accident in the most heavily exposed zones. [The overall increase identified in all industrial cohorts, and the positive trends with increased exposure that are based on internal comparisons, reinforce an overall positive association between all cancers combined and exposure to TCDD, making it less likely that the increase is explained by confounding, either by smoking or by other exposures to carcinogens in the industrial setting.]

2.3 Cancer of the lung

An increased risk for lung cancer was observed in the industrial cohort studies, especially in the more highly exposed subcohorts (see Table 2.3, online). The relative risk for lung cancer in the highly exposed subcohorts was around 1.5 in several studies. [It is possible that relative risks of this order for lung cancer could result from confounding by smoking, but this would only be the case if there were a pronounced difference in smoking habits between the exposed and the referent populations, a difference that seems unlikely. Therefore, confounding by smoking can probably not explain all the excess risk for lung cancer, although it could explain part of it. It is also possible that other occupational carcinogens, many of which would affect the lung, are causing some confounding.] In Seveso, increased mortality and cancer incidence for lung cancer was observed at more than 15 years since the accident.

2.4 Soft-tissue sarcoma

An association between soft-tissue sarcoma and spraying of phenoxy herbicides was first suggested by results from case–control studies in Umea, Sweden (Hardell & Sandström, 1979). Exposure to TCDD in these and other community-based case–control studies is, however, not accurately estimated. An excess risk for soft-tissue sarcoma, based on a small number of deaths, has been reported in the largest industrial cohorts, specifically those of NIOSH and IARC (see Table 2.4, online). In both, the mortality ratios (SMRs) tended to be higher among the most exposed subcohorts. Incidence data for soft-tissue sarcoma were generally not available. A dose–response relationship, with estimated exposure to TCDD, was found in a case–control study nested in the IARC cohort; however, strong positive trends were also found with exposure estimates for 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). In Seveso, there were no cases of soft-tissue sarcoma in the most heavily contaminated Zones A and B. [Soft-tissue sarcomas are subject to serious misclassification on death certificates. Although it is unlikely that this occurs differentially in the exposed and the referent populations, re-classification of a few cases would have important consequences on results based on small numbers.]

2.5 Non-Hodgkin lymphoma

An increased risk for non-Hodgkin lymphoma was found in most of the populations studied in the four industrial cohort studies and in the Seveso population, although the relative risks were mostly non-significant and below 2 (see Table 2.5, online). A case–control study nested in the IARC cohort provided weak evidence of a dose–response relationship with estimated exposure to TCDD. [Although it is plausible that other chemicals cause non-Hodgkin lymphoma, strong potential confounding factors are not known. The lack of complete consistency among the cohorts and the weak effect detected in most of the positive studies, however, caution against a causal interpretation of the findings.]

2.6 Other cancers

Increased risks for several other malignant neoplasms have been sporadically reported among workers exposed to TCDD, and at Seveso. Most notable are risks for breast and rectal cancers and myeloid leukaemia in Seveso, bladder cancer in the NIOSH and Dutch cohorts, multiple myeloma in the NIOSH cohort, cancers of the oral cavity and pharynx in the German cohorts, genital cancers in the Dutch cohort, and kidney cancer in the IARC cohort. [The available results are not fully consistent, and several studies have not reported the results for each individual cancer site.]

2.7 Synthesis

Overall, the strongest evidence for the carcinogenicity of TCDD is for all cancers combined, rather than for any specific site. The relative risk for all cancers combined in the most highly exposed and longer-latency subcohorts is around 1.4. In dose–response analyses, higher relative risks are observed for the groups with the highest measured and modelled exposure to TCDD. This relative risk for all neoplasms does not appear likely to be explained by confounding, particularly since dose–response was typically based on internal comparisons among workers of the same cohort. The evidence for specific cancers is strongest for lung cancer, soft-tissue sarcoma and non-Hodgkin lymphoma, but confounding cannot be ruled out for lung cancer, while the findings on soft-tissue sarcoma are based on small numbers. Several studies identified statistically significant increases in many cancers, but findings for other

cancers including major cancers are, overall, inconsistent between studies. It should be borne in mind that the general population is exposed to levels that are much lower than those experienced by the industrial populations.

The Working Group did not review the epidemiological evidence of other PCDDs, PCDFs or PCBs with a dioxin-like activity.

3. Cancer in Experimental Animals

3.1 2,3,7,8-Tetrachlorodibenzo-para-*dioxin*

Carcinogenicity studies with several strains of rats, mice and Syrian hamsters treated with 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) via the oral route (gavage or diet), by intra-peritoneal injection, or by skin application have been reviewed in *IARC Monograph* Volume 69 (IARC, 1997). At the time, the review of the available data led to the conclusion that there is *sufficient evidence* in experimental animals for the carcinogenicity of TCDD. The present *Monograph* also evaluates relevant carcinogenicity studies in TCDD-treated experimental animals that were published since 1997. The results of adequately conducted carcinogenicity studies are summarized below and in Table 3.1 and Table 3.2.

TCDD was tested for carcinogenicity by oral administration (gavage or dose feed) in four studies in mice and six studies in rats, by skin (topical) application in two studies in mice, by intraperitoneal injection in one study in mice, one study in rats and one study in hamsters and by subcutaneous injection in one study in hamsters. TCDD produced tumours in both sexes of mice and rats, and in multiple organs and tissues.

Oral administration of TCDD caused increased incidences of thyroid follicular adenomas and hepatocellular adenomas and carcinomas in male and female mice, of alveolar/bronchiolar adenomas and carcinomas in male mice, and of histiocytic lymphomas and subcutaneous fibrosarcomas in female mice. In rats, it caused increased incidences of hepatocellular adenomas in males and females, cholangiocarcinomas and hepatocellular carcinomas in females, lung cystic keratinizing epitheliomas and squamous-cell carcinomas in females, adrenal gland (cortex) adenomas and squamous-cell carcinomas of the hard palate/nasal turbinates in males and females, tongue squamous-cell carcinomas and thyroid follicular adenomas and carcinomas combined in males, subcutaneous fibromas in males and subcutaneous fibrosarcomas in females, and pituitary adenomas, uterine and oral mucosa (gingival) squamous-cell carcinomas and pancreatic adenomas and carcinomas combined in females (Van Miller *et al.*, 1977; Kociba *et al.*, 1978; Tóth *et al.*, 1979, NTP, 1982a, 2006a; Della Porta *et al.*, 1987; Goodman & Sauer, 1992; Hays *et al.*, 1997, Yoshizawa *et al.*, 2005). Skin application or gavage caused benign and malignant tumours of the skin in female mice including transgenic mice (NTP, 1982b; Wyde *et al.*, 2004). Hamsters that received TCDD by intraperitoneal or subcutaneous injection developed squamous-cell carcinomas of the facial skin (Rao *et al.*, 1988). Intraperitoneal injection caused increased incidence of hepatocellular adenomas and carcinomas in female mice and of lymphomas in male and female mice (Della Porta *et al.*, 1987).

Several studies in mice showed that administration of TCDD with known carcinogens enhanced the incidence of skin papillomas, lung adenomas, liver adenomas and hepatoblastomas. In female rats, TCDD co-administered with various nitrosamines enhanced the incidence of focal hepatic lesions. In one study, TCDD enhanced the incidence of lung carcinomas in ovariectomized female rats following administration of *N*-nitrosodiethylamine (NDEA) (IARC, 1997). In two more recent studies in female rats, TCDD given orally or subcutaneously enhanced

Table 3.1 Carcinogenicity studies in mice exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, Swiss-Webster (M, F) 99 or 104 wk NTP (1982b)	Skin application TCDD applied to the skin of male (0.001 µg/animal) or females (0.005 µg/animal) per application (equivalent to 0.15 µg/kg/wk and 0.75 µg/kg/wk respectively), 3 × / wk on alternate days in 0.1 ml acetone. Vehicle controls were given 0.1 ml acetone 3 × /wk. Groups of mice of either sex were also given a single application of 50 µg DMBA one week before TCDD applications. This study did not include a control group treated with DMBA alone Untreated controls: 15 mice/ group Vehicle-control: 45 animals/sex TCDD: 30 animals/sex TCDD + DMBA: 30 animals/sex	Data given for vehicle controls, TCDD (alone)- and TCDD+DMBA-treated groups. Skin: Fibrosarcomas– 3/42 (7%), 6/28 (21%), 5/30 (17%) (M); 2/41 (5%), 8/27 (30%), 8/29 (27%) (F) Myxomas– 0/41, 0/27, 1/29 (3%) (F)	 NS (M) TCDD: $P = 0.007$ *vs* vehicle control (F) TCDD+DMBA: $P = 0.01$ *vs* vehicle control (F) NS	Purity 99.4% In the DMBA-TCDD experiment, the lack of groups treated with DMBA alone, precluded interpretation of the results.
Mouse, Tg.AC (F) 26 wk Wyde *et al.* (2004)	Skin application 0, 5, 17, 36, 76, 121, 166, 355, or 760 ng/kg bw TCDD in acetone applied topically 3 × /wk for 26 wk (equivalent to 0, 2.1, 7.3, 15, 33, 52, 71, 152, 326 ng/kg/d) 20 animals/group	Skin (squamous cell papillomas): 0/20, 1/20 (5%), 3/20 (15%), 11/20 (55%), 10/20 (50%), 13/20 (65%), 17/20 (85%), 19/20 (95%), 20/20 (100%) Skin (squamous cell carcinomas): 0/20, 0/20, 1/20 (5%), 1/20 (5%), 3/20 (15%), 5/20 (25%), 8/20 (40%), 14/20 (70%), 16/20 (80%) Keratoacanthomas: 0/20, 0/20, 0/20, 0/20, 3/20 (15%), 3/20 (15%), 2/20 (10%), 3/20 (15%), 1/20 (5%)	$P < 0.01$ (15, 33, 52, 71, 152, and 326 ng/kg/d) $P < 0.01$ (52, 71, 152, 326 ng/kg/d) NS	Purity 99% Survival for TCDD-treated mice was similar as in the controls, except for lower survival rates observed in mice receiving 33 and 52 ng TCDD/kg (65 and 50%, respectively) *vs* controls (85%); however, this was thought not to be treatment-related because the survival rate of higher dose groups was similar to that of the control group. The response of TCDD-induced papilloma formation in the skin was greater in mice exposed to TCDD by the dermal route than by oral administration (see gavage study by Wyde *et al.* (2004), below)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, Swiss/H/Riop (M) Lifetime Tóth *et al.* (1979)	Gavage 0, 0.007, 0.7, 7.0 µg/kg bw in sunflower oil, once/wk for 1 yr. Controls received sunflower oil 45 animals/group	Liver (tumours): 7/38 (18%), 13/44 (29%), 21/44 (48%), 13/43 (30%)	$P < 0.01$ (0.7 µg/kg)	Purity NR Tumour type NR Average lifespan decreased considerably in the 7.0-µg/kg dose group (428 d) compared with controls (588 d). [Mortality-adjusted analysis was not performed and, therefore, the tumour incidence in the high-dose group may have been underestimated.]
Mouse, B6C3F1 (M, F) 104 wk NTP (1982a)	Gavage 0.01, 0.05, 0.5 µg/kg bw 2x/wk (M) or 0.04, 0.2, 2.0 µg/kg bw 2x/wk (F) as a suspension in 9:1 corn-oil/acetone at a volume of 0.05 ml/100 g bw, for 104 wk Vehicle controls: 75 mice/sex Untreated controls: 25 mice/sex TCDD: 50 mice/sex	Liver (hepatocellular adenomas): 7/73 (9%), 3/49 (6%), 5/49 (10%), 10/50 (20%) (M); 2/73 (3%), 4/50 (8%), 4/48 (8%), 5/47 (11%) (F) Liver (hepatocellular carcinomas): 8/73 (11%), 9/49 (18%), 8/49 (16%), 17/50 (34%) (M); 1/73 (1%), 2/50 (4%), 2/48 (4%), 6/47 (13%) (F) Liver (hepatocellular adenomas or carcinomas): 15/73 (20%), 12/49 (24%), 13/49 (26%), 27/50 (54%) (M); 3/73, (4%), 6/50 (12%), 3/48 (6%), 11/47 (23%) (F)	$P = 0.024$ (trend, M) $P = 0.002$ (M) $P = 0.002$ (trend, M) $P = 0.014$ (F) $P = 0.008$ (trend, F) $P < 0.001$ (high-dose M) $P < 0.001$ (trend, M) $P < 0.005$ (high-dose F) $P < 0.002$ (trend, F)	Purity 99.4% Toxic hepatitis: 1/73, 5/49, 3/49, 44/50 (M); 0/73, 1/50, 2/48, 34/47 (F)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, B6C3F1 (M, F) 104 wk NTP (1982a) Contd.		Thyroid (follicular adenomas):		
		0/69, 3/48 (6%), 0/48, 0/49 (M);	$P = 0.008$ (trend M)	
		0/69, 3/50 (6%), 1/47 (2%), 5/46 (11%) (F)	$P = 0.009$ (high-dose, F) $P = 0.016$ (trend, F)	
		Lung (alveolar/bronchiolar adenomas):		
		7/71 (10%), 2/48 (4%), 4/48 (8%), 11/50 (22%) (M)	$P = 0.006$ (trend, M)	
		Lung (alveolar/bronchiolar adenomas or carcinomas):		
		10/71 (14%), 2/48 (4%), 4/48 (8%), 13/50 (26%) (M)	$P = 0.004$ (trend, M)	
		Subcutaneous tissue (fibrosarcomas):		
		1/74 (1%), 1/50 (2%), 1/48 (2%), 5/47 (11%) (F)	$P = 0.032$ (high-dose, F) $P = 0.007$ (trend, F)	
		Lymphoid tissue (histiocytic lymphomas):		
		9/74 (12%), 4/50 (8%), 8/48 (17%), 14/47 (30%) (F)	$P = 0.016$ (high-dose, F) $P = 0.003$ (trend, F)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, C57BL/6J x C3Hf, (M, F) 110 wk Della Porta *et al.* (1987)	Gavage 0, 2.5, 5.0 µg/kg bw in 0.01 ml/kg bw corn-oil containing 1.2% acetone, once/wk for 52 wk, followed until 104 wk. Controls given corn-oil/acetone (~99:1) once/wk for 52 wk. At 31–39 wk, 41 M and 32 F in the 2.5-µg/kg bw group were erroneously treated once with a dose of 25 µg/kg TCDD. The treatment of these mice was interrupted for five wk and then continued until wk 57, as for the other treated mice. At the end of treatment, all groups were kept under observation until 110 wk. 45–55 animals/group	Hepatocellular adenomas: 10/43, 11/51, 10/50 (M); 2/49, 4/42, 11/48 (F) Hepatocellular carcinomas: 5/43 (12%), 15/51 (29%), 33/50 (66%) (M); 1/49 (2%), 12/42 (29%), 9/48 (19%) (F)	$P < 0.001$, for fatal tumour test (M); $P < 0.01$, for fatal tumour test (F); $P < 0.001$ for incidental tumour test (F) $P < 0.005$, for both fatal and incidental tumour test (M, F)	Laboratory grade; purity NR Treatment with both doses caused a marked depression in mean body weight (no significance given) and reduced survival ($P < 0.001$) in male and female mice. Male mice, treated once by mistake with a dose of 25 µg/kg TCDD had an increased mortality shortly after the 2.5-µg/kg treatment. The incidence of other tumour types were uniformly low in treated and control groups of both sexes, and were not treatment related.
Mouse, Tg.AC (F) 26 wk Wyde *et al.* (2004)	Gavage 0, 105, 450, or 1 250 ng TCDD/kg bw, 5 d/wk, for 26 wk (equivalent to 0, 75, 321, 893 ng/kg/d, 5 d/wk) 20 animals/group	Skin (squamous cell papillomas): 1/20 (5%), 2/20 (10%), 3/20 (15%), 11/20 (55%) Skin (squamous cell carcinomas): 0/20, 0/20, 1/20 (5%), 13/20 (65%) Keratoacanthomas: 0/20, 0/20, 0/20, 1/20 (5%)	$P < 0.01$ (high-dose) $P < 0.01$ (high-dose) NS	Purity 99%

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, immature B6C3 and B6C (M, F) 78 wk Della Porta *et al.* (1987)	Intraperitoneal injection 0, 1, 30, 60 µg/kg bw in 0.01 ml/kg bw corn-oil vehicle containing 1.2% acetone starting at 10 days of age, once/wk for 5 wk and observed until 78 wk of age. Controls given corn-oil/acetone (~99:1) B6C3: 151–186; 97 controls B6C: 89–138; 105 controls	*B6C3 mice*: Hepatocellular adenomas– 6/45 (13%), 5/55 (9%), 5/52 (10%), 11/43 (26%) (M); 0/42, 1/57 (2%), 1/48 (2%), 5/56 (9%) (F) Hepatocellular carcinomas– 3/45 (7%), 1/55 (2%), 9/52 (17%), 14/43 (32%) (M); 0/42, 0/57, 1/48 (2%), 1/57 (2%) (F) Thymic lymphomas– 0/45, 0/55, 1/52 (2%), 2/43 (5%) (M); 0/42, 0/57, 0/48, 5/57 (9%) (F) Non thymic lymphomas– 1/45 (2%), 2/55 (4%), 1/52 (2%), 1/43 (2%) (M); 0/42, 1/57 (2%), 8/48 (17%), 3/57 (5%) (F)	$P < 0.043$ (trend, M) $P < 0.014$ (trend, F) $P < 0.001$ (60 µg/kg, M) $P = 0.002$ (trend, M) $P < 0.05$ (60 µg/kg, F) $P < 0.05$ (30 µg/kg, F)	Treatment with 30 and 60 µg/kg bw TCDD increased mortality in both hybrids (no statistical analysis). Animals that died within 13 wk of age were excluded from histopathological evaluation and the statistical analyses. The 15 thymic lymphomas were found in animals dying between 16 and 41 wk of age and most of them (11 out of 15) were observed within 26 wk of age. Non-thymic lymphomas involved mesenteric lymph nodes, Peycr patches, and the spleen, with secondary involvement of multiple organs.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, immature B6C3 and B6C (M, F) 78 wk Della Porta *et al.* (1987) Contd.		*B6C mice*: Hepatocellular adenomas– 1/32 (3%), 2/54 (4%), 1/27 (4%), 0/30 (M) Thymic lymphomas– 0/32, 0/54, 2/27 (7%), 2/30 (7%) (M); 0/48, 0/57, 1/39 (3%), 2/38 (5%) (F) Non thymic lymphomas– 0/32, 0/54, 1/27 (4%), 2/30 (7%) (M); 1/48 (2%), 3/57 (5%), 5/39 (13%), 3/38 (8%) (F)	 NS $P < 0.05$ (trend, M, F) $P < 0.05$ (trend, M, F)	

bw, body weight; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; M, male; NR, not reported; NS, not significant; *vs*, versus; wk, week or weeks; yr, year or years

Table 3.2 Carcinogenicity studies in rats and hamsters exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rat, Osborne-Mendel (M, F) 104 wk NTP (1982a)	**Gavage** 0, 0.01, 0.05, 0.5 µg/kg bw twice/wk as a suspension in 9:1 corn-oil/acetone at a volume of 0.05 ml/100 g bw, for 104 wk Vehicle controls received 9:1 corn-oil/acetone Vehicle controls: 75 rats/sex Untreated controls: 25 rats/sex TCDD: 50 rats/sex	Liver neoplastic nodules (adenomas): 0/74, 0/50, 0/50, 3/50 (M); 5/75 (7%), 1/49 (2%), 3/50 (6%), 12/49 (24%) (F) Liver neoplastic nodules and hepatocellular carcinomas: 0/74, 0/50, 0/50, 3/50 (2%) (M); 5/75 (7%), 1/49 (2%), 3/50 (6%), 14/49 (29%) (F) Thyroid: Follicular adenomas: 1/69 (1%), 5/48 (10%), 6/50 (12%), 10/50 (20%) (M); 3/73 (4%), 2/45 (4%), 1/49 (2%), 6/47 (13%) (F) Follicular adenomas/carcinomas: 1/69 (1%), 5/48 (10%), 8/50 (16%), 11/50 (22%) (M); 5/73 (7%), 2/45 (4%), 1/49 (2%), 6/47 (13%) (F) Adrenal: Cortical adenomas– 6/72 (8%), 9/50 (18%), 12/49 (24%), 9/49 (18%) (M); 11/73 (15%), 8/49 (16%), 4/49 (8%), 14/46 (30%) (F) Subcutaneous tissue: Fibromas– 3/75 (4%), 1/50 (2%), 3/50 (6%), 7/50 (14%) (M) Fibrosarcomas– 0/75, 2/50 (4%), 3/50 (6%), 4/49 (8%) (F) Pituitary (adenomas): 1/66 (1%), 5/47 (8%), 2/44 (4%), 3/43 (7%) (F)	$P < 0.005$ (trend, M) $P = 0.006$ (high-dose F) $P < 0.001$ (trend, F) $P < 0.005$ (trend, M) $P = 0.001$ (high-dose F) $P < 0.001$ (trend, F) $P = 0.042$ (low-dose), 0.021 (mid-dose), 0.001 (high-dose M) $P < 0.006$ (trend, M) $P = 0.042$ (low-dose, M), $P = 0.004$ (mid-dose, M), $P < 0.001$ (high-dose M) $P < 0.005$ (trend, M) $P = 0.015$ (mid-dose M) $P = 0.019$ (trend, F) $P = 0.048$ (high-dose M) $P < 0.01$ (trend, M) $P = 0.023$ (high-dose F) $P = 0.044$ (low-dose F)	Purity 99.4% Increased incidence of hepatotoxicity occurred in the high-dose male (0/50, 0/74, 1/50, 0/50, 14/50 and female (0/49, 0/75, 0/49, 1/50, 32/49) rats. One high dose-treated male developed a hepatocellular carcinoma.

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rat, Harlan Sprague-Dawley (F) 105 wk NTP (2006a), Yoshizawa *et al.* (2005)	**Gavage** 0, 3, 10, 22, 46, or 100 ng/kg bw, 5 d/wk, for up to 105 wk Controls given corn-oil with acetone (91:1) A stop-exposure group of 50 females received 100 ng/kg TCDD in corn-oil/acetone (91:1) for 30 wk and then the vehicle for the remainder of the study 8–10 animals/group were evaluated at 14, 31 or 53 wk; 81–82 animals/group	Liver: Hepatocellular adenomas– 0/53, 0/54, 0/53, 0/53, 1/53 (2%), 13/53 (25%), 2/50 (4%) Cholangiocarcinomas– 0/53, 0/54, 0/53, 1/53, (2%) 4/53 (8%), 25/53 (47%), 2/50 (4%) Lung (cystic keratinizing epitheliomas): 0/53, 0/54, 0/53, 0/52, 0/53, 9/52 (17%), 0/50 Oral mucosa (gingiva) (squamous cell carcinomas): 1/53 (2%), 2/54 (4%), 1/53 (2%), 0/53, 4/53 (8%), 10/53 (19%), 5/50 (10%) Uterus (squamous cell carcinomas): 0/53, 0/54, 0/53, 0/53, 5/53 (9%), 0/53, 2/50 (4%) Pancreas (acinar adenomas or carcinomas): 0/51, 0/54, 0/52, 0/53, 0/52, 3/51 (6%)	$P < 0.001$ (100 ng/kg) $P < 0.001$ (trend) $P < 0.057$ (46 ng/kg). $P < 0.001$ (100 ng/kg) $P < 0.001$ (trend) $P = 0.002$ (100 ng/kg) $P < 0.001$ (trend) $P = 0.007$ (100 ng/kg) $P < 0.001$ (trend) $P = 0.032$ (46 ng/kg) $P < 0.001$ (trend)	Purity 98% Survival of dosed groups was similar to that of the vehicle-control group. Mean body weights of the 22-, 46-, 100-ng/kg core study and stop-exposure groups were less than those of the vehicle-control group

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rats, Sprague-Dawley (M) 95 wk Van Miller *et al.* (1977)	**Diet** Rats were placed on diet containing 0, 1, 5, 50, 500 ppt or 1, 5, 50, 500, or 1 000 ppb suspended in acetone and dissolved in corn oil, for 78 wk after which feeding with basal diet was continued. A control group was maintained on the basal diet. At 65 wk, laparotomies were performed on all animals and biopsies taken from all tumours observed. Surviving rats were sacrificed at 95 wk. 10 animals/group	Total tumours: Diet (ppt) 0/10, 0/10, 5/10 (50%)[a], 3/10 (30%), 4/10 (40%) Diet (ppb) 1 ppb–4/10 (40%) 5 ppb–7/10 (70%)[b] Neoplasms included 5 ppt: 1 ear duct carcinoma, 1 lymphocytic leukaemia, 1 renal adenocarcinoma, 1 malignant histiocytoma (peritoneal), 1 angiosarcoma (skin), 1 Leydig-cell adenoma (testes) 50 ppt: 1 fibrosarcoma (muscle), 1 squamous cell tumour (skin), 1 astrocytoma (brain) 500 ppt: 1 fibroma (striated muscle), 1 carcinoma (skin), 1 adenocarcinoma (kidney), 1 sclerosing seminoma (testes) 1 ppb–1 cholangiocarcinoma, 1 angiosarcoma (skin), 1 glioblastoma (brain), 2 malignant histiocytomas (peritoneal) 5 ppb–4 squamous cell tumours (lung), 4 neoplastic nodules (liver), 2 cholangiocarcinomas	[a][$P = 0.0325$] [b][$P = 0.0031$]	All animals given 50, 500 and 1 000 ppb died between the second and fourth wk on study. At the end of the study, 4/10, 8/10, 6/10, 6/10 and 5/10 rats were alive in the 0, 1, 5, 50 and 500 ppt treated rats, respectively. No animals were alive in the 1- and 5-ppb groups. Food intake in these groups was significantly less than that of the controls and the animals had acute toxicity. Weight gain was significantly different from that of the controls only in the 5-ppb group. Neoplasms of the liver occurred only in animals fed 1 or 5 ppb TCDD.

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rat, Sprague-Dawley (Spartan substrain) (M, F) 24 mo Kociba *et al.* (1978), Hays *et al.* (1997), Goodman & Sauer (1992)	**Diet feed** 0, 0.001, 0.01, 0.1 µg/kg bw/d (equivalent to 0, 22, 210, 2 200, ppt) in the diet. Control groups received a basal diet containing the vehicle acetone alone. Controls: 86 animals/group/sex Treated: 50 animals/group/sex	Liver: Hyperplastic nodules– 6/85 (7%), 0/50, 3/50 (6%), 2/50 (4%) (M); 8/86 (9%), 3/50 (6%), 18/50 (36%), 23/49 (47%) (F) Hepatocellular carcinomas– 2/85, 0/50, 0/50, 1/50 (2%) (M); 1/86 (1%), 0/50, 2/50 (4%), 11/49 (22%) (F) Hard palate/nasal turbinates (squamous cell carcinomas): 0/85, 0/50, 0/50, 4/50 (8%) (M); 0/86, 0/50, 1/50 (2%), 4/49 (8%) (F) Tongue (squamous cell carcinomas): 0/85, 1/50 (2%), 1/50 (2%), 3/50 (6%) (M); 1/86 (1%), 0/50, 0/50, 2/49 (4%) (F) Lung (keratinizing squamous cell carcinomas): 0/85, 0/50, 0/50, 1/50 (2%) (M); 0/86, 0/50, 0/50, 7/50 (14%) (F)	$P < 0.05$; 0.01 and 0.1µg/kg (F) $P < 0.05$; 0.1 µg/kg (F) $P < 0.05$; 0.1 µg/kg (M, F) $P < 0.05$; 0.1 and 0.01µg/kg (M) $P < 0.05$; 0.1 µg/kg (F)	Purity > 99% Reduced survivaI was observed in high-dose females and in mid- and high-dose males. Mean body weights of high-dose males and mid- and high-dose femaIes were less than those of the controls throughout the major portion of the study. Decreased incidence of tumours of the pituitary, uterus, mammary glands, pancreas, adrenal gland occurred in treated rats.

Table 3.2 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Results Incidence and/or multiplicity of tumours (%)	Significance	Comments
Hamster, Syrian Golden (M) 12–13 mo Rao *et al.* (1988)	**Intraperitoneal injection** 0, 100 µg/kg bw TCDD in dioxane as 6 injections at 4 wk intervals (once every 4 wk). An additional group received 2 injections of 100 µg/kg TCDD. Controls received corresponding amounts of dioxane alone. 12–24 animals/group	Skin (squamous-cell carcinomas): Dioxane alone–0/12 100 µg/kg TCDD, 6 doses–4/18 (21%) 100 µg/kg TCDD, 2 doses–0/20	[NS]	Purity NR 6/24 animals injected with 100 µg/kg died before completion of the 6-dose schedule of TCDD due to intestinal adhesions along with obstruction and peritonitis
	Subcutaneous injection 0, 50 or 100 µg/kg bw TCDD in dioxane as 6 injections at 4-wk intervals (once every 4 wk). Controls received corresponding amounts of dioxane alone. 10 or 17 animals/group	Skin (squamous cell carcinomas): Dioxane alone–0/10 50 µg/kg TCDD–0/10 100 µg/kg TCDD–3/14 (21%)	[NS]	Purity NR 4/17 injected with 100 µg/kg died before completion of the 6-dose schedule of TCDD due to marked pulmonary and hepatic congestion. Tumours were moderately differentiated squamous-cell carcinomas that developed exclusively in the facial skin

d, day or days; F, female; M, male; mo, month or months; NR, not reported; NS, not significant; ppb, part per billion; ppt, part per trillion; wk, week or weeks

Table 3.3 Studies on 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) administered to rats, in combination with known carcinogens or modifying factors

Species, strain (sex), duration, reference	Known carcinogen	Route of administration	Interval	Dose and frequency (times per week/number of weeks)	Route of administration	Tumour promotion
Liver tumour promotion						
Han/Wistar rats (F) Duration (NR) Viluksela *et al.* (2000)	30 mg/kg bw NDEA Starting 5 wk after PH	i.p.	35 d	170 µg/kg bw TCDD/weekly/20 wk Purity > 99%	s.c.	+
Mammary gland tumour promotion						
SD rats (F) Brown *et al.* (1998)	30 mg/kg bw DMBA in sesame oil (to 50-d-old offspring)	oral	N/A	1 µg/kg bw TCDD in sesame oil in pregnant F on Day 15 p.c.	oral	+
Ovarian tumour promotion						
SD rats (F) Davis *et al.* (2000)	175 mg/kg bw NDEA in saline	i.p.	14 d	1.75 µg/kg bw TCDD in corn oil, twice/wk for 60 wk	oral	+
	175 mg/kg bw NDEA in saline	i.p.	18 wk	1.75 µg/kg bw TCDD in corn oil, twice/wk for 30 wk followed by vehicle for 16 wk	oral	+

bw, body weight; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; i.p., intraperitoneal injection; M, male; mo, month or months; N/A, not applicable; NDEA, *N*-nitrosodiethylamine; NR, not reported; p.c., post-conception; PH, partial hepatectomy; s.c., subcutaneous injection; SD, Sprague-Dawley; wk, week or weeks

Table 3.4 Carcinogenicity and initiation-promotion studies in experimental animals exposed to 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and/or mutiplicity of tumours (%)	Significance	Comments
PeCDF				
Rats, Harlan (F) 105 wk NTP (2006b)	Groups of 81 female rats were given 0, 6, 20, 44, 92, or 200 ng PeCDF/kg bw in corn-oil/acetone (99:1) by gavage, 5 d per wk, for up to 105 wk. A stop-exposure group of 50 female rats was administered 200 ng/kg PeCDF in corn-oil/acetone (99:1) by gavage for 30 wk and then the vehicle for the remainder of the study. 8–10 rats per group were evaluated at 14, 31, and 53 wk. Controls received corn-oil.	Liver: Hepatocellular adenomas– 1/53 (2%), 0/53, 1/53 (2%), 0/52, 2/53 (4%), 4/53 (8%), 1/50 (2%) Cholangiocarcinomas– 0/53, 0/53, 0/53, 1/52 (2%), 1/53 (2%), 2/53 (4%), 0/50 Mammary gland (carcinomas): 5/53 (9%), *12/53 (23%), **13/53 (24%), 2/53 (4%), 5/53 (9%), 3/53 (6%)	 $P = 0.006$ (trend) $P = 0.036$ (trend) *$P = 0.036$ **$P = 0.021$ $P = 0.021$ (trend, negative)	Purity > 97%. Survival of dosed groups was similar to that of the vehicle-control group.
Mice, HRS/J hairless (F) 21 wk Hébert *et al.* (1990)	Skin application of 5 µmol MNNG in 50 µL acetone followed one wk later by application with 0 (control), 25, 50 or 100 ng PeCDF in 25 µL acetone twice a wk for 20 wk 20 animals/group	Skin (papillomas): 1/19 (5%); 9/19 (47%); 11/18 (61%); 8/18 (44%)	$P < 0.05$ (all PeCDF-treated groups)	

Table 3.4 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and/or mutiplicity of tumours (%)	Significance	Comments
Rats, Wistar (M) up to 24 wk Nishizumi & Masuda (1986)	50 mg/L NDEA in the drinking-water for 4 wk followed by weekly s.c. injections of olive oil (control), 10 or 100 µg PeCDF/kg bw until end of experimental wks 16, 20 or 24 12 animals/group	Liver hyperplastic nodules + HCC: *16 wk* high-dose, 3.3/rat; control, 0.3/rat *20 wk* high-dose, 3.3/rat; control, 0.5/rat *24 wk* low-dose, 17.0/rat; high-dose, 24.3/rat; control, 3.0/rat Liver tumours at 24 wk HCC: high-dose, 2.5/rat; control, 0.3/rat	 $P < 0.05$ $P < 0.05$ $P < 0.05$ (mid-dose); $P < 0.01$ (high-dose) $P < 0.05$	Purity > 99%. Tumour incidence was not given.
Rats, Sprague-Dawley (F) 25 wk Waern *et al.* (1991)	70% partial hepatectomy 30 mg/kg bw NDEA by intraperitoneal injection followed 5 wk later by 0 (control), 0.16, 0.64 or 2.6 µg PeCDF/kg bw (weekly s.c. injections) for 20 wk 10 animals/group	Gamma-GT positive focal hepatic lesions in the liver (as%): control, 0.15; low-dose, 0.25; mid-dose, 0.5; high-dose, 0.5 Number/liver: control, 2000; low-dose, 2 500; mid-dose, 3 500; high-dose, 4 000	$P < 0.05$ (mid-dose and high-dose) $P < 0.05$ (mid-dose and high-dose)	

Table 3.4 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and/or mutiplicity of tumours (%)	Significance	Comments
PCB 126				
Rats, Harlan (F) 104 wk NTP (2006c)	Groups of 81 female rats were given 0, 30, 100, 175, 300, 550, or 1 000 ng PCB 126/kg bw in corn-oil/acetone (99:1) by gavage, 5 d per wk, for up to 104 wk. A stop-exposure group of 50 female rats was administered 1 000 ng/kg PCB 126 in corn-oil/acetone (99:1) by gavage for 30 wk, then the vehicle for the remainder of the study. 8–10 rats per group were evaluated at 14, 31, or 53 wk.	Liver: Cholangiocarcinomas– 0/53, 0/55, 1/53, (2%) 0/53, 5/53 (9%), 6/51 (12%), 22/53*(41%), 2/50 (4%) Hepatocellular adenomas– 1/53 (2%), 2/55 (4%), 1/53 (2%), 0/53, 2/53 (4%), 4/51 (8%), 7/53* (13%), 0/50 Hepatocholangiomas– 0/53, 0/55, 0/53, 0/53, 0/53, 0/51, 3/53 (6%), 0/50 Lung: Cystic keratinizing epitheliomas– 0/53, 0/55, 0/53, 0/53, 1/53 (2%), 11/51*(22%), 35/51**(69%), 0/50 Squamous cell carcinomas– 0/53, 0/55, 0/53, 0/53, 0/53, 1/51 (2%), 2/51 (4%), 0/50 Oral Mucosa (gingival squamous cell carcinomas): 0/53, 1/55 (2%), 1/53 (2%), 1/53 (2%), 2/53 (4%), 2/53 (4%), 7/53* (13%), 2/50 (4%) Adrenal Cortex (adenomas or carcinomas): 0/52, 2/55 (4%), 1/53 (2%), 0/53, 1/53 (2%), 1/52 (2%), 4/53 (8%), 3/50 (6%)	$P < 0.01$* $P < 0.001$ (trend) $P < 0.05$* $P < 0.001$ (trend) $P < 0.001$ (trend) $P = 0.002$*, $P < 0.01$** $P < 0.001$ (trend) NS $P < 0.01$* $P < 0.001$ (trend) $P = 0.022$ (trend)	Purity 99% There was a significant change in the survival of 550 ng/kg and 1 000 ng/kg stop-exposure females, with an increase observed relative to the vehicle-control group.

bw, body weight; d, day or days; F, female; HCC, hepatocellular carcinoma; M, male; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; NDEA, *N*-nitrosodiethylamine; NS, not significant; s.c., subcutaneous injection; wk, week or weeks

the carcinogenicity of previously administered NDEA (Davis *et al.*, 2000; Viluksela *et al.*, 2000). In another study, the oral administration of TCDD to pregnant rats increased 7,12-dimethylbenz[*a*] anthracene-induced mammary-gland tumours in offspring (Brown *et al.*, 1998; see Table 3.3).

3.2 Dioxin-like compounds

3.2.1 2,3,4,7,8-Pentachlorodibenzofuran

Oral administration of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) resulted in significant dose-dependent trends for increased incidence of cholangiocarcinomas and hepatocellular adenomas (Walker *et al.*, 2005; NTP, 2006b). (see Table 3.4)

Skin application of PeCDF after a single dose of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine resulted in an increased incidence of skin papillomas in mice (Hébert *et al.*, 1990). Subcutaneous injections of PeCDF after oral treatment with NDEA resulted in an increased multiplicity of hepatocellular carcinomas and liver hyperplastic nodules in male rats (Nishizumi & Masuda, 1986). Subcutaneous injections of PeCDF after a single intraperitoneal injection of NDEA increased the number of focal hepatic lesions in female rats (Waern *et al.*, 1991).

3.2.2 3,3′,4,4′,5-Pentachlorobiphenyl

Oral administration of 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) resulted in significantly increased incidence of hepatocellular adenomas, cholangiocarcinomas, lung cystic keratinizing epitheliomas, and oral mucosa (gingiva) squamous-cell carcinomas in female rats (Walker *et al.*, 2005; NTP, 2006c).

4. Other Relevant Data

4.1 AhR activation

Most, if not all of the effects of TCDD are related to its binding to and activation of the aryl hydrocarbon receptor (AhR), a member of the basic helix–loop–helix/Per-Arnt-Sim family of transcription factors. This receptor was first identified in mouse liver (Poland *et al.*, 1976) where it showed high affinity towards TCDD. Further studies found that AhR is expressed in most mammalian tissues and that many other halogenated aromatic compounds can bind this receptor, including the coplanar polychlorinated biphenyls and the polychlorinated dibenzodioxins and dibenzofurans. It is generally proposed that the toxic and carcinogenic effects of dioxin and other halogenated compounds are due to their high affinity to AhR, and to the sustained pleiotropic response from a battery of genes – many of which encode drug-metabolizing enzymes – that follows the receptor-ligand complex formation (Mandal, 2005; Walker, 2007). Much of the research in the three decades since the discovery of the AhR has focused on dissecting this pleiotropic response to fully understand the mechanisms involved in dioxin-mediated toxicity.

The free AhR resides in the cytoplasm as an inactive complex containing a heat-shock protein dimer, Hsp90, XAP2 and p23 (Meyer *et al.*, 1998). When the AhR binds to a ligand, XAP2 is released and, through a conformational change, the complex is moved to the nucleus where the Hsp90 dimer dissociates and the AhR-nuclear-translocator (ARNT) binds to the PAS domains of the receptor. The activated AhR/ARNT complex forms a heterodimer that is then capable of binding to the 5′-regulatory region of dioxin-responsive genes (Mimura & Fujii-Kuriyama, 2003). The primary targets following activation of AhR include genes encoding many phase-I and phase-II metabolic enzymes (e.g. *CYP1A1*,

CYP1A2, *CYP1B1*, *NQO1*, *UGT1A2*, *GSTA1* and *ALDH3A1*) (Nebert *et al.*, 2000; Schwarz & Appel, 2005). However, through direct and indirect pathways, TCDD is able to alter the expressions of a much larger number of genes (Martinez *et al.*, 2002; Dere *et al.*, 2006; Pastorelli *et al.*, 2006; Schwanekamp *et al.*, 2006). In addition, there is cross-talk with several other receptor-mediated systems including the estrogen receptor (Safe & Wormke, 2003) and the retinoic-acid receptor β (Lu *et al.*, 1994; Berkers *et al.*, 1995; Toyoshiba *et al.*, 2004).

Despite the strong conservation of the AhR across species, gene polymorphisms, differences in co-activators and differences in downstream signalling following activation are all likely to modulate TCDD carcinogenicity (Ema *et al.*, 1994; Tuomisto *et al.*, 1999). These factors could explain the interindividual differences observed in the magnitude of the carcinogenic response after exposure to TCDD. For example, different AhR polymorphisms triggered a threefold difference in EROD activity in human lymphocytes (Smart & Daly, 2000).

4.2 Mechanisms of carcinogenicity

TCDD is not directly genotoxic and the tumorigenic activity is likely to be due to a fairly long half-life, especially in humans, resulting in a sustained activation of the AhR. TCDD half-life in the human body is estimated at 7.2 years (Milbrath *et al.*, 2009); long half-life in the environment and the ability to bio-accumulate in the food-chain are also reported (IARC, 1997). The sustained downstream signalling may trigger an adaptive biochemical and physiological response in the cell that can promote carcinogenesis (Biegel & Safe, 1990; Lu *et al.*, 1994; Berkers *et al.*, 1995; Schwarz & Appel, 2005), also by inducing mutations (Stohs *et al.*, 1990; Tritscher *et al.*, 1996; Shertzer *et al.*, 1998; Yoshida & Ogawa, 2000; Thornton *et al.*, 2001; Nebert *et al.*, 2004; Knerr *et al.*, 2006; Schlezinger *et al.*, 2006; Lin *et al.*, 2007; Green *et al.*, 2008). TCDD may also enhance – although it sometimes inhibits – the progression and invasiveness of initiated tumours (Marlowe & Puga, 2005; Peng *et al.*, 2009), but this topic will not be discussed in detail here.

The primary mechanism by which TCDD is thought to cause cancer is by altering the cellular ability to proliferate, migrate, apoptose, senesce and terminally differentiate (Safe, 2001; Marlowe & Puga, 2005; Ray & Swanson, 2009) in a multistep process focused on the accumulation of mutations and/or heritable epigenetic changes. Chemicals that inhibit apoptosis and increase proliferation usually increase cancer risk as well. TCDD has been shown to increase cellular proliferation both *in vivo* and *in vitro* in several tissues (Maronpot *et al.*, 1993; Barrett, 1995; Dere *et al.*, 2006) possibly through interactions with protein-kinase C signalling (Barrett, 1995), inhibition of senescence (Ray & Swanson, 2009) or activation of growth-signalling factors (Kohn, 1995). In initiation-promotion models, TCDD expanded the populations of preneoplastic foci in rat liver (Dragan *et al.*, 1992; Maronpot *et al.*, 1993; Tritscher *et al.*, 1995) and promoted carcinogenesis in liver, skin and lung in rodents (DiGiovanni *et al.*, 1977; Hébert *et al.*, 1990; Lucier *et al.*, 1991; Dragan *et al.*, 1992; Beebe *et al.*, 1995; Tritscher *et al.*, 1995; Tritscher *et al.*, 2000).

Finally, TCDD may upregulate drug-metabolizing enzymes, thus increasing the presence of highly reactive intermediates that form during metabolic activation and/or transformation of several key hormones. For example, CYP1A1, CYP1A2 and CYP1B1 induction is a major source of reactive oxygen species (ROS) formation in hepatocytes and this has been linked to the decoupling of the P450 catalytic cycle (Nebert *et al.*, 2004; Knerr *et al.*, 2006; Schlezinger *et al.*, 2006; Green *et al.*, 2008). A hormonal linkage with estrogen has been demonstrated through the increase in 8-oxo-deoxyguanosine (a marker of oxidative stress) in the liver of intact female

rats compared with rats that are ovariectomized before exposure to TCDD (Tritscher *et al.*, 1996). TCDD may induce prolonged oxidative stress, and consequent DNA damage and mutations, also in several other strains of rats and mice, and in cell lines (Stohs *et al.*, 1990; Shertzer *et al.*, 1998; Yoshida & Ogawa, 2000; Thornton *et al.*, 2001; Wyde *et al.*, 2001; Lin *et al.*, 2007). TCDD is a complete carcinogen in mice and rats in multiple strains (IARC, 1997; NTP, 2006a); see Section 3).

TCDD thus may both promote and initiate carcinogenesis through indirect oxidative stress, leading some to refer to dioxin as an activator of carcinogenesis and to adopt initiation-promotion models to better explain the toxicity of TCDD and better fit pre-neoplastic and neoplastic data (Portier & Kohn, 1996; Portier *et al.*, 1996; Luebeck *et al.*, 2000).

4.3 Dioxin-like compounds

As noted above, the carcinogenicity of TCDD is tied to a sustained, pleiotropic response following exposure to dioxin. The 17 laterally-substituted (2,3,7,8-substituted) polychlorinated dibenzodioxins (PCDD-Ls), the coplanar polychlorinated biphenyls (CP-PCBs) and the 17 laterally-substituted polychlorinated dibenzofurans (PCDF-Ls) are all structurally and toxicologically inter-related halogenated aromatic hydrocarbons referred to as dioxin-like compounds (DLCs). They potentially induce pleiotropic responses in cells very similar to those induced by TCDD (IARC, 1978, 1987, 1997; Vezina *et al.*, 2004) as they are all able to bind to the AhR. Binding affinity is different among DLCs, with some of them having so little binding affinity that there is almost no information on their biological impact. We focus here on the 28 DLCs (DLC-28) listed in Table 4.1 that produce a TCDD-like response in human cell lines (Endo *et al.*, 2003), in animal and primary human cells, and in animal and human tissue (Vezina *et al.*, 2004; Silkworth *et al.*, 2005; Kopec *et al.*, 2008; N'Jai *et al.*, 2008).

DLCs co-occur in virtually every environmental compartment where they are found, and assessment of their potential effect on human risk can be complicated. Public health authorities (in particular WHO expert panels) have developed the toxicity equivalence factor (TEF) methodology for measuring the potency of DLCs, with TCDD as the index chemical. Exposures are calculated as a simple weighted sum of the individual amounts multiplied by their individual TEFs to yield the equivalent dose in units of TCDD exposure. Only the compounds of the DLC-28 series have TEF values > 0 (Table 4.1), all of the remaining congeners have TEF = 0.

WHO recently evaluated TEFs (Van den Berg *et al.*, 2006) thus reviewing much of the literature on DLCs (Haws *et al.*, 2006), namely 189 studies with over 1000 targets used for analysis. For every compound, there is at least one *in vitro* study showing AhR binding and one showing 7-ethoxyresorufin-*O*-dethylase activity, which is associated with an increase in CYP1A1 activity. Congeners 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF, and PCB-77, -126, and -169 have a very broad spectrum of information both in animal and in human cells, demonstrating activity consistent with the mechanisms described earlier. In addition, for PCB-81 there are nine studies in human cell lines showing the same alterations in gene expression and enzyme activity as reported for TCDD.

Congeners 1,2,3,7,8-pentaCDD, 1,2,3,4,6,7,8-heptaCDD, a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexaCDD, and a mixture of PCDDs have been studied in two-stage experimental models (IARC, 1978) and shown to enhance the carcinogenic potential of known carcinogens just like TCDD. There are some human studies on the compounds in the DLC-28 series, but they have either poor exposure characterization or also contain TCDD, making

Table 4.1 Toxicity Equivalence Factors (TEFs) and half-lives of the dioxin-like compounds

Congener	TEF[a]	Half-life[b] (years)
1,2,3,7,8-PeCDD	1	11.2
1,2,3,4,7,8-HxCDD	0.1	9.8
1,2,3,6,7,8-HxCDD	0.1	13.1
1,2,3,7,8,9-HxCDD	0.1	5.1
1,2,3,4,6,7,8-HpCDD	0.01	4.9
OCDD	0.0003	6.7
2,3,7,8-TCDF	0.1	2.1
1,2,3,7,8-PeCDF	0.03	3.5
2,3,4,7,8-PeCDF	0.3	7.0
1,2,3,4,7,8-HxCDF	0.1	6.4
1,2,3,6,7,8-HxCDF	0.1	7.2
1,2,3,7,8,9-HxCDF	0.1	7.2
2,3,4,6,7,8-HxCDF	0.1	2.8
1,2,3,4,6,7,8-HpCDF	0.01	3.1
1,2,3,4,7,8,9-HpCDF	0.01	4.6
OCDF	0.0003	1.4
PCB 77	0.0001	0.1
PCB 81	0.0003	0.7
PCB 126	0.1	1.6
PCB 169	0.03	7.3
PCB 105	0.00003	2.4
PCB 114	0.00003	10.0
PCB 118	0.00003	3.8
PCB 123	0.00003	7.4
PCB 156	0.00003	16.0
PCB 157	0.00003	18.0
PCB 167	0.00003	12.0
PCB 189	0.00003	22.0

[a] Toxicity equivalence factors from Van den Berg *et al.* (2006)
[b] Half-life of congeners in humans based on Milbrath *et al.* (2009)

it difficult to interpret their individual effects (IARC, 1978).

While the carcinogenicity of TCDD has been clearly established in rodents, for the remaining compounds in the DLC-28 series bioassays on two-year chronic exposure are lacking. A two-year chronic study on 2,3,4,7,8-PeCDF in rodents (NTP, 2006b) demonstrated tumour effects consistent with those seen for TCDD (hepatocellular adenomas, cholangiocarcinomas, gingival squamous cell carcinomas, and an equivocal finding of lung cystic keratonizing epitheliomas). When compared for potency, the result from this study agreed with the TEF concept (Walker *et al.*, 2005). 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF were also enhanced tumorigenesis in two-stage studies of cancer (IARC, 1997). Follow-up of populations in Taiwan, China (Tsai *et al.*, 2007) and Japan (Onozuka *et al.*, 2009) accidentally exposed to rice-oil containing PCDFs and PCBs, shows a significantly increased risk of mortality from chronic liver disease in men and a non-significant increase from liver cancer in men and women in Taiwan, China while in Japan all cancer mortality, and liver and lung cancer-mortalities were increased in men.

The carcinogenicity of mixtures of PCBs in rodents has also been clearly established through studies of various Aroclors (IARC, 1978; Mayes *et al.*, 1998; NTP, 2006c) yielding predominantly liver cancers (Cogliano, 1998). Two-year chronic exposure studies done by the US National Toxicology Program (NTP) on PCB 126 (NTP, 2006d) and PCB 118 (NTP, 2009), demonstrated tumour effects consistent with those seen for TCDD (hepatocellular adenomas, cholangiocarcinomas, gingival squamous cell carcinomas, and lung cystic keratonizing epitheliomas). Moreover, when equivalent TCDD doses where applied with the current TEF, a carcinogenic response equivalent to that predicted for TCDD from the NTP study (Walker *et al.*, 2005) was observed.

The set of DLC-28 (IARC, 1978, 1997; Milbrath *et al.*, 2009) have a long half-life similar to that of TCDD (estimated at 7.2 years in the human body) (Table 4.1). Many congeners have similar or longer half-lives (1,2,3,7,8-PeCDD, 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDD, 1,2,3,6,7,8- and 1.2.3.7.8.9-HxCDF, and PCBs 169, 114, 123, 156, 167 and 189) while most of the remaining half-lives are in excess of 1.4 years. Several authors report the presence of these compounds in human blood in the general population (Costopoulou *et al.*, 2006; Scott *et al.*, 2008; Zubero *et al.*, 2009) indicating a sustained, long-term exposure that, when coupled with the analyses for common pleiotropic response, argues in favour of the notion that all of the DLC-28s have the same carcinogenic potential in humans.

Experimental data on mechanism of carcinogenesis induced by DLC-28 are available for 2,3,4,7,8-PeCDF and PCB 126, in particular (Table 4.2), Both have been shown to bind to the AhR in humans and animals (IARC, 1978; Safe, 2001), to translocate into the nucleus and activate numerous metabolic enzymes *in vitro* (human and non-human cell lines) and *in vivo* in experimental animals (IARC, 1997; Safe, 2001; Vezina *et al.*, 2004; Haws *et al.*, 2006), to trigger changes in growth factors and signalling pathways related to cellular replication in rodents (Hemming *et al.*, 1995; Vondrácek *et al.*, 2005; N'Jai *et al.*, 2008). 2,3,4,7,8-PeCDF potential effect on cell replication is suggested in the NTP study (Walker *et al.*, 2007), and promotion in skin, liver and lung tissues is reported in initiation-promotion studies (Hébert *et al.*, 1990; Anderson *et al.*, 1991; Waern *et al.*, 1991). PCB 126 acts as a promoter of liver cancer in initiation-promotion studies (Hemming *et al.*, 1995; Haag-Grönlund *et al.*, 1998) with measured increases in cell-replication rate in the populations of initiated cells (Vondrácek *et al.*, 2005). PCB 126 and 2,3,4,7,8-PeCDF induce oxidative stress, the latter in a dose-dependent manner in brain and liver of rats (Hassoun *et al.*, 2002; Hennig *et al.*, 2002). These two compounds are carcinogenic in mixtures with TCDD (IARC, 1978; Hassoun *et al.*, 2001; NTP, 2006d) and by themselves in the NTP chronic bioassays in rats, where they increase hepatocellular adenomas, cholangiocarcinomas, gingival squamous-cell carcinomas, and, possibly, lung cystic keratonizing epitheliomas (NTP, 2006b, c, d).

4.4 Synthesis

There is strong evidence to support a receptor-mediated mechanism of action for TCDD-associated carcinogenesis in humans where the primary mechanism is the promotion of tumour development through the activation of cellular replication and the alteration in cellular senescence and apoptosis. Dioxin, through activation of an array of metabolic enzymes also increases the risk for oxidative stress, which serves as an indirect initiator of carcinogenesis. These events make dioxin a complete carcinogen. The conservation of the AhR and the related signalling pathways across species strongly support this mechanism in humans.

The receptor-mediated mechanism of action for TCDD-associated carcinogenesis in humans

Table 4.2 Experimental evidence on the mechanisms of carcinogenesis for TCDD and the dioxin-like compounds 2,3,4,7,8-PeCDF and PCB 126 (positive, non-determined, and indirect)

Response	TCDD	2,3,4,7,8-PeCDF	PCB 126
Binding to AhR – human and non-human	+	+	+
CYP1A1 &1A2 activation – human and non-human cells	+	+	+
CYP1A1 &1A2 activation – *in vivo*	+	+	+
Multiple other phase 1 and 2 enzymes – human and non-human cells	+	+	+
Multiple other phase 1 and 2 enzymes – *in vivo*	+	+	+
Increased replication – human cells	+	Non-determined	Non-determined
Increased replication – non-human cells	+	Non-determined	+
Increased replication – *in vivo*	+	Indirect[a]	+
Other cellular function – human and non-human cells	+	+	+
Other cellular function – *in vivo*	+	+	+
Oxidative stress biomarker – *in vivo* or *in vitro*	+	+	+
Pre-neoplastic lesions – *in vivo*	+	+	+
Promoter studies – *in vivo*	+	+	+
Four specific tumours in female Sprague-Dawley rats	+	+	+
Other species/strains complete carcinogen	+	Non-determined	Non-determined[b]

[a] Gene expression changes associated with cellular replication and metaplasia in rat lung
[b] PCB mixtures, including PCB 126 are positive in rodent studies

is strongly suggested as the mechanism of action that would result in 2,3,4,7,8-PeCDF and PCB 126 causing cancer in humans. The primary mechanism is the promotion of carcinogenesis through the activation of cellular replication and the alteration in cellular senescence and apoptosis through the aryl-hydrocarbon receptor (AhR). These congeners, through activation of an array of metabolic enzymes, increase the risk for oxidative stress as an indirect initiator of carcinogenesis, which makes these congeners complete carcinogens. The conservation of the AhR and the related signalling pathways across species strongly support this mechanism of action in humans.

There is compelling evidence that the mechanism of action for TCDD-associated carcinogenesis in humans operates as the mechanism of action for carcinogenesis in humans for 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF and PCBs 77, 81, 105, 114, 118, 123, 156, 157, 167, 169, and 189. These compounds all bind to the AhR in human cells and demonstrate changes in gene expression consistent with those seen for TCDD, 2,3,4,7,8-PeCDF and PCB 126. Where examined, data have been collected for these compounds supporting some, but not all, aspects of the mechanisms outlined for TCDD, 2,3,4,7,8-PeCDF and PCB 126 that relate to activation of cell replication, alterations in cellular senescence and apoptosis, and increases in oxidative stress.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. The strongest evidence in humans for the carcinogenicity of

2,3,7,8-tetrachlorodibenzo-*para*-dioxin is for all cancers combined.

Also, a positive association has been observed between exposure to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin and soft-tissue sarcoma, non-Hodgkin lymphoma and cancer of the lung.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,3,4,7,8-pentachlorodibenzofuran.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3,3′,4,4′,5-pentachlorobiphenyl.

There is strong evidence to support a receptor-mediated mechanism that operates in humans for carcinogenesis associated with 2,3,7,8-tetrachlorodibenzo-*para*-dioxin, where the primary mechanism is the promotion of tumour development through modification of cell replication and apoptosis, with a secondary mechanism related to increases of oxidative stress causing DNA damage. The conservation of the aryl hydrocarbon receptor and the related signalling pathways and responses across species, including humans, add additional strength to the notion that this mechanism is active in humans.

2,3,7,8-Tetrachlorodibenzo-*para*-dioxin is *carcinogenic to humans (Group 1)*.

2,3,4,7,8-Pentachlorodibenzofuran is *carcinogenic to humans (Group 1)*.

3,3′,4,4′,5-Pentachlorobiphenyl is *carcinogenic to humans (Group 1)*.

In making the second and third overall evaluations, the Working Group considered the following mechanistic arguments:

There is strong evidence to support a receptor-mediated mechanism for 2,3,4,7,8-pentachlorodibenzofuran- and 3,3′,4,4′,5-pentachlorobiphenyl-associated carcinogenesis in humans based upon evidence of carcinogenicity in experimental animals and upon extensive evidence showing activity identical to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) for every step of the mechanism described for TCDD-associated carcinogenesis in humans including receptor binding, gene expression, protein-activity changes, cellular replication, oxidative stress, promotion in initiation-promotion studies and complete carcinogenesis in laboratory animals.

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ETHYLENE OXIDE

Ethylene oxide was considered by previous IARC Working Groups in 1976, 1984, 1987, 1994, and 2007 (IARC, 1976, 1985, 1987, 1994, 2008). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

From IARC (2008), unless indicated otherwise
Chem. Abstr. Serv. Reg. No.: 75-21-8
Chem. Abstr. Serv. Name: Oxirane
Synonyms: 1,2-Epoxyethane

C_2H_4O
Relative molecular mass: 44.06
Description: Colourless, flammable gas (O'Neill, 2006)
Boiling-point: 10.6 °C (Lide, 2008)
Solubility: Soluble in water, acetone, benzene, diethyl ether, and ethanol (Lide, 2008)
Conversion factor: $mg/m^3 = 1.80 \times ppm$; calculated from: mg/m^3 = (relative molecular weight/24.45) × ppm, assuming standard temperature (25 °C) and pressure (101.3 kPa).

1.2 Uses

Ethylene oxide is an important raw material used in the manufacture of chemical derivatives that are the basis for major consumer goods in virtually all industrialized countries. More than half of the ethylene oxide produced worldwide is used in the manufacture of mono-ethylene glycol. Conversion of ethylene oxide to ethylene glycols represents a major use for ethylene oxide in most regions: North America (65%), western Europe (44%), Japan (63%), China (68%), Other Asia (94%), and the Middle East (99%). Important derivatives of ethylene oxide include di-ethylene glycol, tri-ethylene glycol, poly(ethylene) glycols, ethylene glycol ethers, ethanol-amines, and ethoxylation products of fatty alcohols, fatty amines, alkyl phenols, cellulose and poly(propylene) glycol (Occupational Safety and Health Administration, 2005; Devanney, 2010).

A very small proportion (0.05%) of the annual production of ethylene oxide is used directly in the gaseous form as a sterilizing agent, fumigant and insecticide, either alone or in non-explosive mixtures with nitrogen, carbon dioxide or dichlorofluoromethane (Dever *et al.*, 2004). It is used to sterilize drugs, hospital equipment, disposable and reusable medical items, packaging

materials, foods, books, museum artefacts, scientific equipment, clothing, furs, railcars, aircraft, beehives and other items (Lacson, 2003).

1.3 Human exposure

1.3.1 Occupational exposure

Most of the data on occupational exposure are related to the production of ethylene oxide and its use in industrial and hospital sterilization. Data were not available on exposures that are incurred outside North America and Europe, where almost half of the global amount of ethylene oxide is produced (IARC, 2008).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, with data collected from 1990 to 1993 in the European Union (EU). The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for ethylene oxide for the top-10 industries in the EU (CAREX, 1999). From the US National Occupational Exposure Survey (1981–1983), it was estimated that approximately 270 000 workers (including approximately 120 000 women) in the USA were potentially exposed to ethylene oxide (NIOSH, 1990).

More recent data on employment in the industrial sectors that use ethylene oxide have been published by the US Occupational Safety and Health Administration (2005). Estimated employment figures were: 1100 ethylene oxide-production workers, 4000 ethoxylators, who use ethylene oxide to make chemical derivatives, and 40 000 workers using ethylene oxide as a sterilant or fumigant in hospitals. In addition, approximately 2700 workers were employed in commercial sterilization by manufacturers of medical and pharmaceutical products and producers of food spices, as contract sterilizers, and in other sterilization and fumigation facilities (IARC, 2008).

Table 1.1 Estimated numbers of workers exposed to ethylene oxide in the European Union (top 10 industries)

Industry, occupational activity	
Medical, dental, other health and veterinary services	22300
Manufacture of other chemical products	5100
Construction	3000
Printing, publishing and allied industries	2400
Manufacture of industrial chemicals	1700
Manufacture of rubber products	1500
Crude petroleum and natural gas production	1100
Manufacture of plastic products, not elsewhere classified	1100
Agriculture and hunting	1000
Manufacture of furniture and fixtures, except primary of	1000
TOTAL	46900

From CAREX (1999)

(a) Production of ethylene oxide and its derivatives

The *IARC Monographs* Volumes 60 and 97 provide detailed descriptions of studies on historical occupational exposures to ethylene oxide (IARC, 1994, 2008).

Table 1.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-23-Table1.2.pdf) summarizes reported exposure levels in industries where ethylene oxide and its derivatives are manufactured. Exposures vary with job category: workers involved in loading and distribution of ethylene oxide have the highest exposure. Where comparisons over time are possible, exposures appear to have decreased, presumably as control measures have been improved, with the most recent time-weighted average (TWA) values in the range of 1 ppm or less. Exposure to a large variety of chemicals other than ethylene oxide may occur, depending on the types of industrial process and job. These other chemicals include unsaturated aliphatic

hydrocarbons (e.g. ethylene, propylene), other epoxides (e.g. propylene oxide), chlorohydrins (e.g. epichlorohydrin and ethylene chlorohydrin), chlorinated aliphatic hydrocarbons (e.g. dichloromethane, dichloroethane), glycols and ethers (e.g. ethylene glycol, glycol ethers, bis(2-chloroethyl)ether), aldehydes (e.g. formaldehyde), amines (e.g. aniline), aromatic hydrocarbons (e.g. benzene, styrene), alkyl sulfates and other compounds (Shore *et al.*, 1993).

(b) Use of ethylene oxide for industrial sterilization

Industrial workers may be exposed to ethylene oxide during sterilization of a variety of items such as medical equipment and products (e.g. surgical instruments, single-use medical devices), disposable health-care products, pharmaceutical and veterinary products, food spices and animal feed (see Table 1.3, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-23-Table1.3.pdf). Short-term exposures may be high for some workers. Despite recent reductions in exposure, in some countries and for some job categories high exposures to ethylene oxide may still occur.

Workers involved in the sterilization of medical products may also be exposed to gases that are present with ethylene oxide in the sterilizing mixture, such as chlorofluorocarbons and carbon dioxide (Heiden Associates, 1988), and – in the past – to methyl formate, as reported in a study from Sweden (Hagmar *et al.*, 1991).

(c) Use of ethylene oxide in hospitals

Ethylene oxide is widely used in hospitals as a gaseous sterilant for heat-sensitive medical items, surgical instruments and other objects and fluids that come in contact with biological tissues. Large sterilizers are found in central supply areas of most hospitals, and smaller sterilizers are found in clinics, operating rooms, tissue banks and research facilities (Glaser, 1979).

The *IARC Monograph* Volume 97 (IARC, 2008) summarized levels of exposure to ethylene oxide in hospitals. The more recent studies from Japan and France suggest that the 8-hour TWA concentrations are often below 1 mg/m^3 in hospitals.

Exposure to ethylene oxide appears to result mainly from peak emissions during operations such as opening the door of the sterilizer and unloading and transferring sterilized material. Proper engineering controls and work practices result in full-shift exposure levels of less than 0.1 ppm [0.18 mg/m^3] and short-term exposure concentration of less than 2 ppm [3.6 mg/m^3] (Mortimer & Kercher, 1989). In a survey of 125 hospitals in the USA, however, use of personal protective equipment was found to be limited to wearing gloves while transferring sterilized items, but respirators were not used (Elliott *et al.*, 1988).

As in industrial sterilization facilities, sterilizer operators in hospitals may also be exposed to other gases present in the sterilizing mixture, e.g. chlorofluorocarbons – banned by the Montreal Protocol in 1989 – and carbon dioxide (Wolfs *et al.*, 1983; Deschamps *et al.*, 1989). Some operating-room personnel handling ethylene oxide may also be exposed to anaesthetic gases and X-rays (Sarto *et al.*, 1984a; Chessor *et al.*, 2005), and some may have occasional exposure to low concentrations of formaldehyde (Gardner *et al.*, 1989).

1.3.2 Non-occupational exposure

Most ethylene oxide is released into the atmosphere (WHO, 2003). Ethylene oxide degrades in the atmosphere by reaction with photochemically produced hydroxyl radicals. The half-life of ethylene oxide in the atmosphere, assuming ambient concentrations of 5×10^5 hydroxyl radicals/cm^3, was reported to be 211 days. Neither rain nor absorption into aqueous aerosols is capable

of removing ethylene oxide from the atmosphere (National Library of Medicine, 2005).

Mainstream tobacco smoke contains 7 mg/cigarette ethylene oxide (IARC, 2004). With the possible exception of cigarette smoke, other non-occupational sources of exposure to ethylene oxide (e.g. residues in spices and other food products (Jensen, 1988; Fowles *et al.*, 2001) and in skin-care products (Kreuzer, 1992) are expected to be minor. Ethylene oxide is formed during the combustion of fossil fuel, but the amount is expected to be negligible (WHO, 2003). Hospital patients may be exposed during dialysis when the equipment has been sterilized with ethylene oxide (IPCS-CEC, 2001).

2. Cancer in Humans

Epidemiological evidence of the risk for human cancer from ethylene oxide derives principally from the follow-up of 14 cohorts of exposed workers, either in chemical plants where ethylene oxide was produced or converted into derivatives, or in facilities where it was used as a sterilant. Many of the workers employed at chemical factories were also exposed to other chemicals. The *IARC Monograph* Volume 97 (IARC, 2008) concluded that there is *limited evidence* in humans for the carcinogenicity of ethylene oxide.

The most informative epidemiological investigation of ethylene oxide and cancer risk was a study by NIOSH of more than 18 000 employees at 14 industrial facilities where ethylene oxide was used to sterilize medical supplies or food spices, or to test the sterilizing equipment (Steenland *et al.*, 1991; Stayner *et al.*, 1993). This investigation benefited from greater statistical power than did other studies, as a consequence of its large sample size. In addition, there was a lower potential for confounding by concomitant exposure to other chemicals, while detailed quantitative assessments were made of individual exposures to ethylene oxide. For these reasons, the Working Group gave greatest weight to the findings of this study when assessing the balance of epidemiological evidence on ethylene oxide, although findings from other studies were also taken into account.

2.1 Lympho-haematopoietic malignancies

Steenland *et al.* (1991) reported on the initial mortality results for the NIOSH ethylene-oxide cohort. There were 343 deaths from cancer (380.3 expected; SMR, 0.90; 95%CI: 0.81–1.00). SMRs were not statistically significantly increased for lymphatic and haematopoietic cancers combined (SMR, 1.06; 95%CI: 0.75–1.47), for lymphosarcoma-reticulosarcoma [ICD-9 200] (SMR, 1.52; 95%CI: 0.65–3.00), Hodgkin lymphoma (SMR, 1.14; 95%CI: 0.31–2.92), leukaemia (SMR, 0.97; 95%CI: 0.52–1.67), non-Hodgkin lymphoma [ICD-9 202] (SMR, 1.20; 95%CI: 0.57–2.37) or myeloma (SMR, 0.59; 95%CI: 0.12–1.73). No significant trend in mortality was observed in relation to duration of exposure, but the SMR for leukaemia (1.79, based on five deaths) and non-Hodgkin lymphoma (1.92, based on five deaths) were higher after allowance for a latency of more than 20 years. Among the sterilizer operators, mortality ratios were 2.78 (two deaths observed) for leukaemia and 6.68 (two deaths) for lymphosarcoma/reticulosarcoma. In a further analysis of the same study (Stayner *et al.*, 1993), an exposure–response analysis was conducted with the use of previously derived quantitative estimates of individual exposure to ethylene oxide (Greife *et al.*, 1988). Analysis was limited to 13 of the facilities studied, since exposure information at one facility was inadequate. Mortality from lymphatic and haematopoietic cancer was greatest in the group with the highest category of cumulative exposure to ethylene

oxide (> 8500 ppm–days) (13 deaths; SMR, 1.24; 95%CI: 0.66–2.13), but the trend across three categories of cumulative exposure was weak (χ^2, 0.97; $P = 0.32$). A similar pattern was observed for non-Hodgkin lymphoma, but not for leukaemia. In addition, a Cox proportional-hazard model was used to examine risk in relation to cumulative exposure (ppm–days), average exposure (ppm), maximal exposure (ppm) and duration of exposure (days) to ethylene oxide. A significant positive trend in risk with increasing cumulative exposure to ethylene oxide was observed for all neoplasms of the lymphatic and haematopoietic tissues [$P < 0.05$, two-tailed]. Moreover, this trend was strengthened [$P < 0.01$] when the analysis was restricted to neoplasms of lymphoid-cell origin (lymphocytic leukaemia, ICD-9 204; non-Hodgkin lymphoma, ICD-9 200, 202). The exposure–response relationship between cumulative exposure to ethylene oxide and leukaemia was positive but non-significant [$P = 0.23$]. The regression coefficients for neoplasms of the lymphatic and haematopoietic tissues for duration of exposure, average exposure, and maximal exposure were either weakly positive or negative.

Extending the mortality assessments through 1998, an updated life-table analysis of cancer mortality in the cohort was carried out (Steenland *et al.*, 2004) (see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-23-Table2.1.pdf). There were 860 deaths from cancer overall (SMR, 0.98; 95%CI: 0.92–1.03), and a statistically significant excess was found only for cancer of the bone (SMR, 2.82; 95%CI: 1.23–5.56, based on six observed deaths). In an internal analysis (excluding one small plant for which exposure data were not available), mortality from lymphatic and haematopoietic cancer was associated with log-cumulative exposures to ethylene oxide lagged by 15 years in men (p for trend = 0.02), but not in women. However, duration of exposure, peak exposure, and average or cumulative exposure did not predict mortality from lymphatic and haematopoietic cancer. A similar pattern was observed for lymphoid-cell tumours specifically (including non-Hodgkin lymphoma, myeloma and lymphocytic leukaemia), with risks of 3.76 (95%CI: 1.03–13.64; p for trend = 0.13). [The Working Group noted that the exposure assessment in the Steenland *et al.* (2004) update was limited by the assumption that exposure levels did not change during extended follow-up for about 25% of workers who were exposed at the time of the last actual exposure-data collection. Follow-up taking place long after the period of highest exposure may have attenuated results in the most recent publication.]

A cohort of ethylene oxide-production workers in the Kanawha Valley Union Carbide facility (UC) in West Virginia (USA) was studied by Greenberg *et al.* (1990), Teta *et al.* (1993), and most recently by Swaen *et al.* (2009). The latter report updated the cohort to include 2063 men employed between 1940 and 1988, and updated mortality information through 2003. No indications were found for excess cancer risks from exposure to ethylene oxide, including risks for lympho-haematopoietic malignancies, by SMR analysis. Combining primary data from the NIOSH and UC cohorts Valdez-Flores *et al.* (2010) also reported no excess cancer risks by SMR-based analysis for these cohorts followed respectively to 1998 (Steenland *et al.*, 2004) and 2003 (Swaen *et al.*, 2009). Other cohort studies did not consistently point at an increased risk for specific haematolymphoproliferative malignancies, although moderate elevations of risk were reported in some investigations (see Table 2.1 online).

A meta-analysis on the data available on nearly 33 000 workers from Germany, Italy, Sweden, the United Kingdom and the USA was performed by Teta *et al.* (1999). The meta-SMR for all leukaemia was 1.08 (95%CI: 0.61–1.93, based on 35 deaths); for non-Hodgkin lymphoma it was 1.34 (95%CI: 0.96–1.89, based on 33 deaths).

[The Working Group noted that evaluation of the possible risks for lymphatic and haematopoietic cancer was hampered by inconsistencies in the histopathological classification of diagnoses over time. The interpretation of results for these malignancies was constrained by the diagnostic groupings that had been used by researchers when the studies were conducted.]

2.2 Cancer of the breast

Studies from four cohorts of workers exposed to ethylene oxide provided useful information on the association between this exposure and breast cancer (Gardner *et al.*, 1989; Hagmar *et al.*, 1991, 1995; Norman *et al.*, 1995; Steenland *et al.*, 2003, 2004; Coggon *et al.*, 2004; see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-23-Table2.2.pdf). The NIOSH study (Steenland *et al.*, 2004) and a cohort study of hospital-based sterilization workers in the United Kingdom (Gardner *et al.*, 1989; Coggon *et al.*, 2004) examined mortality from breast cancer and found no overall excess risk. Three studies examined the incidence of breast cancer: the NIOSH study (Steenland *et al.*, 2003) and a cohort study from Sweden (Hagmar *et al.*, 1991, 1995) found no overall excess risk, while another cohort study from New York State, USA, found an excess risk of about 60%, which was borderline significant (Norman *et al.*, 1995). Internal analyses with inclusion of questionnaire data were carried out in the NIOSH study (Steenland *et al.*, 2003) showing increased relative risks for breast cancer at the highest level of cumulative exposure to ethylene oxide (> 11620 ppm–days, 15-year lag, OR = 1.87, 95%CI: 1.12–3.10), with a significant exposure–response relationship [*P* for trend = 0.002), after controlling for parity and history of breast cancer in a first-degree relative.

2.3 Other cancers

Several cohort studies provided data on exposure to ethylene oxide and mortality from other cancers (stomach, brain, pancreas; see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-23-Table2.2.pdf). There was no consistent evidence of an association of these cancers with exposure to ethylene oxide.

2.4 Synthesis

The Working Group found some epidemiological evidence for associations between exposure to ethylene oxide and lymphatic and haematopoietic cancers, and specifically lymphoid tumours (i.e. non-Hodgkin lymphoma, multiple myeloma and chronic lymphocytic leukaemia).

3. Cancer in Experimental Animals

Carcinogenicity studies with mice and rats exposed to ethylene oxide by inhalation, oral gavage, and subcutaneous injection were previously reviewed (IARC, 1994, 2008). Results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There have been no additional carcinogenicity studies in animals reported since the previous evaluation in *IARC Monograph* Volume 97 (IARC, 2008).

3.1 Inhalation exposure

In two inhalation studies in mice, there was an increased incidence of alveolar bronchiolar carcinomas and combined adenomas and carcinomas in male and female B6C3F$_1$ mice (NTP, 1987) and of lung adenomas in strain A/J female mice (Adkins *et al.*, 1986). Treatment-related increases in lymphomas, Harderian gland

Table 3.1 Carcinogenicity studies in experimental animals exposed to ethylene oxide by inhalation, oral gavage and subcutaneous injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 2 yr Lynch *et al.* (1984)	Inhalation 0, 50, 100 ppm 7 h/d, 5 d/wk 80/group	Brain[a]: 0/76, 2/77, 5/79 Mononuclear-cell leukaemia 24/77, 38/79, 30/76 Peritoneal mesotheliomas 3/78, 9/79, 21/79	$P < 0.05$ (high dose) $P = 0.03$ (low dose) $P = 0.002$ (high dose)	99.7% purity
Rat, F344 (M) 2 yr Snellings *et al.* (1984), Garman *et al.* (1985, 1986)	Inhalation 0 (control I), 0 (control II), 10, 33, 100 ppm 6 h/d, 5 d/wk 120/group	Brain[b]: 1/181, 0/92, 3/85, 6/87 Mononuclear-cell leukaemia 13/97, 9/51, 12/39, 9/30 Peritoneal mesotheliomas 2/97, 2/51, 4/39, 4/30 Subcutaneous fibromas 3/97, 9/51, 1/39, 11/30	$P < 0.01$ (trend); $P < 0.05$ (high dose) $P < 0.05$ (trend) $P < 0.005$ (trend) $P < 0.001$ (high dose)	> 99.9% purity Two control groups combined. Interim sacrifices at 6 (10 rats), 12 (10 rats), and 18 mo (20 rats). Increased mortality due to viral sialodacryoadenitis at 15 mo. No increases in tumour incidence up to 18 mo. Incidence for all sites other than brain are for rats that died or were sacrificed after 18 mo.
Rat, F344 (F) 2 yr Snellings *et al.* (1984), Garman *et al.* (1985, 1986)	Inhalation 0 (control I), 0 (control II), 10, 33, 100 ppm 6 h/d, 5 d/wk 120/group	Brain[b] 0/187, 1/94, 2/90, 2/78 Mononuclear-cell leukaemia 11/116, 11/54, 14/48, 15/26	$P < 0.05$ (trend) $P < 0.005$ (trend); $P < 0.001$ (high dose)	> 99.9% purity Two control groups combined. Interim sacrifices at 6 (10 rats), 12 (10 rats), and 18 mo (20 rats). Increased mortality due to viral sialodacryoadenitis at 15 mo. No increases in tumour incidence up to 18 mo. Incidence for all sites other than brain are for rats that died or were sacrificed after 18 mo
Mouse, A/J (F) 6 mo Adkins *et al.* (1986)	Inhalation 0, 70, 200 ppm 6 h/d, 5d/wk 0, 200 ppm 6 h/d, 5 d/wk 30/group	Lung adenomas 8/30, 16/28, 25/29 Lung adenomas 8/29, 12/28	[$P < 0.001$, trend & high dose] NS	≥ 99.7% purity Two independent experiments; tumour multiplicities increased for high-dose *vs* control in both ($P < 0.05$).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 102 wk NTP (1987)	Inhalation 0, 50, 100 ppm 6 h/d, 5 d/wk 50/group	Lung (alveolar/bronchiolar carcinomas): 6/50, 10/50, 16/50 Lung (alveolar/bronchiolar adenomas and carcinomas combined): 11/50, 19/50, 26/50 Harderian gland cystadenomas 1/43, 9/44, 8/42[c]	$P = 0.032$ (trend), $P = 0.048$ (high dose) $P = 0.010$ (trend), $P < 0.05$ (high dose) $P < 0.03$ (trend), $P < 0.05$ (low and high dose)	> 99% purity
Mouse, B6C3F$_1$ (F) 102 wk NTP (1987), Picut *et al.* (2003)	Inhalation 0, 50, 100 ppm 6 h/d, 5 d/wk 50/group	Lung (alveolar/bronchiolar carcinomas): 0/49, 1/48, 7/49 Lung (alveolar/bronchiolar adenomas and carcinomas combined): 2/49, 5/48, 22/49 Harderian gland cystadenomas 1/46, 6/46[c], 8/47 Lymphoma: 9/49, 6/48, 22/49 Uterine adenocarcinomas 0/49, 2/47, 5/49 Mammary gland adenocarcinomas or adenosquamous carcinomas 1/49, 8/48, 6/49	$P = 0.005$ (trend), $P < 0.05$ (high dose) $P < 0.001$ (trend, high dose) $P < 0.05$ (trend, high dose) $P = 0.023$ (trend), $P < 0.05$ (high dose) $P < 0.03$ (trend) $P \leq 0.02$ (low dose)	> 99% purity
Rat, SD (F) 150 wk Dunkelberg (1982)	Gavage 0 (untreated), 0 (vehicle, salad oil), 7.5, 30.5 mg/kg bw 2x/wk 50/group	Fore-stomach squamous cell carcinomas 0/50, 0/50, 8/50, 29/50	[$P < 0.01$, low and high dose]	99.7% purity Many of the fore-stomach tumours in the high-dose group metastasized or were locally invasive to other organs.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, NMRI (F) 95 wk Dunkelberg (1981)	Subcutaneous injection 0 (untreated), 0 (vehicle, tricaprylin), 0.1, 0.3, 1.0 mg/injection once/wk 200/group (controls), 100/group	Sarcomas at the injection site: 0/200, 4/200, 5/100, 8/100, 11/100	[$P < 0.001$, trend]	99.7% purity

[a] Brain tumours were gliomas; focal proliferation of glial cells (termed 'gliosis') also observed in two low-dose and four high-dose treated rats
[b] Brain tumours included in Table are only gliomas
[c] One cystadenocarcinoma in an animal with cystadenoma
bw, body weight; d, day or days; F, female; h, hour or hours; M, male; mo, month or months; NS, not significant; wk, week or weeks; yr, year or years

cystadenoma, mammary gland carcinomas and uterine adenocarcinomas were also seen in $B6C3F_1$ mice (NTP, 1987; Picut *et al.*, 2003).

In two inhalation studies in F344 rats (Lynch *et al.*, 1984; Snellings *et al.*, 1984; Garman, *et al.*, 1985, 1986), there was an increased incidence in gliomas [not further specified], mononuclear cell leukaemia and peritoneal mesotheliomas. A treatment-related increase in subcutaneous fibromas also occurred in male rats (Snellings *et al.*, 1984).

3.2 Other routes of exposure

In one study, subcutaneous injection of ethylene oxide in female NMRI mice resulted in a dose-related increase in the incidence of sarcomas at the injection site (Dunkelberg, 1981).

In one study with female Sprague-Dawley rats that received ethylene oxide by gavage, there was a treatment-related increase in fore-stomach squamous-cell carcinomas (Dunkelberg, 1982).

4. Other Relevant Data

Experimental studies on ethylene oxide have been evaluated previously in *IARC Monograph* Volumes 60 and 97 (IARC, 1994, 2008). There is an extensive body of data on the mechanism of ethylene oxide-induced carcinogenicity encompassing toxicokinetics, DNA-adduct formation, biomarkers, genotoxicity, and molecular biology. Ethylene oxide is a direct alkylating agent that reacts with nucleophiles without the need for metabolic transformation. It has been shown to have genotoxic and mutagenic activity in numerous assays in both somatic and germ cells, and prokaryotic and eukaryotic organisms (IARC, 1994, 2008). Ethylene oxide is active in a wide range of *in vitro* and *in vivo* systems. Increases in both gene mutations and chromosomal alterations, two general classes of cancer-related genetic changes, have been observed. The direct reaction of ethylene oxide with DNA is thought to initiate the cascade of genetic and related events that lead to cancer (Swenberg *et al.*, 1990). Thus, formation of DNA adducts and resultant mutations are key steps in the mechanism of carcinogenicity for this agent.

4.1 Absorption, distribution, metabolism, and excretion

Ethylene oxide is readily taken up by the lungs and is absorbed relatively efficiently into the blood. A study of workers exposed to ethylene oxide revealed an alveolar retention of 75–80%, calculated from hourly measurements of ethylene oxide in ambient air, which ranged from 0.2 to 24.1 mg/m^3 [0.11–13.2 ppm], and in alveolar air, which ranged from 0.05 to 6 mg/m^3 [0.03–3.3 ppm] (Brugnone *et al.*, 1985, 1986). At steady-state, therefore, 20–25% of inhaled ethylene oxide that reached the alveolar space was exhaled as the unchanged compound and 75–80% was taken up by the body and metabolized. Blood samples taken from workers at four hours after the work-shift gave venous blood/alveolar air coefficients of 12–17 and venous blood/environmental air coefficients of 2.5–3.3.

The mammalian metabolic pathways of ethylene oxide are shown in Fig. 4.1 and can be summarized as follows: Ethylene oxide is converted (*a*) by enzymatic and non-enzymatic hydrolysis to ethylene glycol, which is partly excreted as such and partly metabolized further via glycolaldehyde, glycolic acid and glyoxalic acid to oxalic acid, formic acid and carbon dioxide; and (*b*) by conjugation with glutathione (GSH) followed by further metabolism to *S*-(2-hydroxyethyl)cysteine, *S*-(2-carboxymethyl) cysteine and *N*-acetylated derivatives (*N*-acetyl-*S*-(2-hydroxyethyl)cysteine (also known as *S*-(2-hydroxyethyl)mercapturic acid or HEMA) and *N*-acetyl-*S*-(2-carboxymethyl)cysteine) (Wolfs

et al., 1983; Popp *et al.*, 1994), which are partly converted to thio-diacetic acid (Scheick *et al.*, 1997).

Concentrations of ethylene glycol were determined at the end of day 3 of a normal working week in blood samples from sterilization personnel exposed to ethylene oxide. TWA concentrations of ethylene oxide determined over eight hours ranged from 0.3 to 52 ppm [0.55–95.2 mg/m^3] (overall mean, 4.2 ppm [7.7 mg/m^3]). The mean concentrations of ethylene glycol in the blood of exposed subjects were twice as high (90 mg/L) as those in controls (45 mg/L) (Wolfs *et al.*, 1983).

The concentration of thioethers excreted in urine collected at the end of sterilization processes was found to be twice as high in non-smoking personnel (10.2 mmol/mol creatinine) exposed to peak concentrations of 1–200 ppm [1.83–366 mg/m^3] ethylene oxide as the thioether concentration in unexposed workers (5.46 mmol/mol creatinine). The concentration of ethylene oxide in air was not monitored routinely (Burgaz *et al.*, 1992).

The glutathione-*S*-transferase (GST) activity towards ethylene oxide in cytosolic fractions from human livers was low (too low to determine the Michaelis-Menten constant [Km] value). The maximum velocity (V_{max}) varied from 7.6 to 10.6 nmol/min/mg protein. Epoxide-hydrolase (EH) activity in the microsomal fraction of human liver averaged 1.8 nmol/min/mg protein. The Km for hydrolysis was estimated to be approximately 0.2 mM, but non-enzymatic hydrolysis was considerable and precluded accurate measurement (Fennell & Brown, 2001).

Metabolism of ethylene oxide to the GSH conjugate and ethylene glycol is generally considered to be the major pathway for the elimination of DNA-reactive ethylene oxide. However, strongly suggestive evidence *in vitro* was presented by Hengstler *et al.* (1994) that glycolaldehyde is formed by further metabolism of ethylene glycol and that this derivative leads to DNA–protein crosslinks and DNA strand-breaks (as measured with the alkaline elution assay) after in-vitro incubation with human mononuclear peripheral blood cells.

4.2 Genetic and related effects

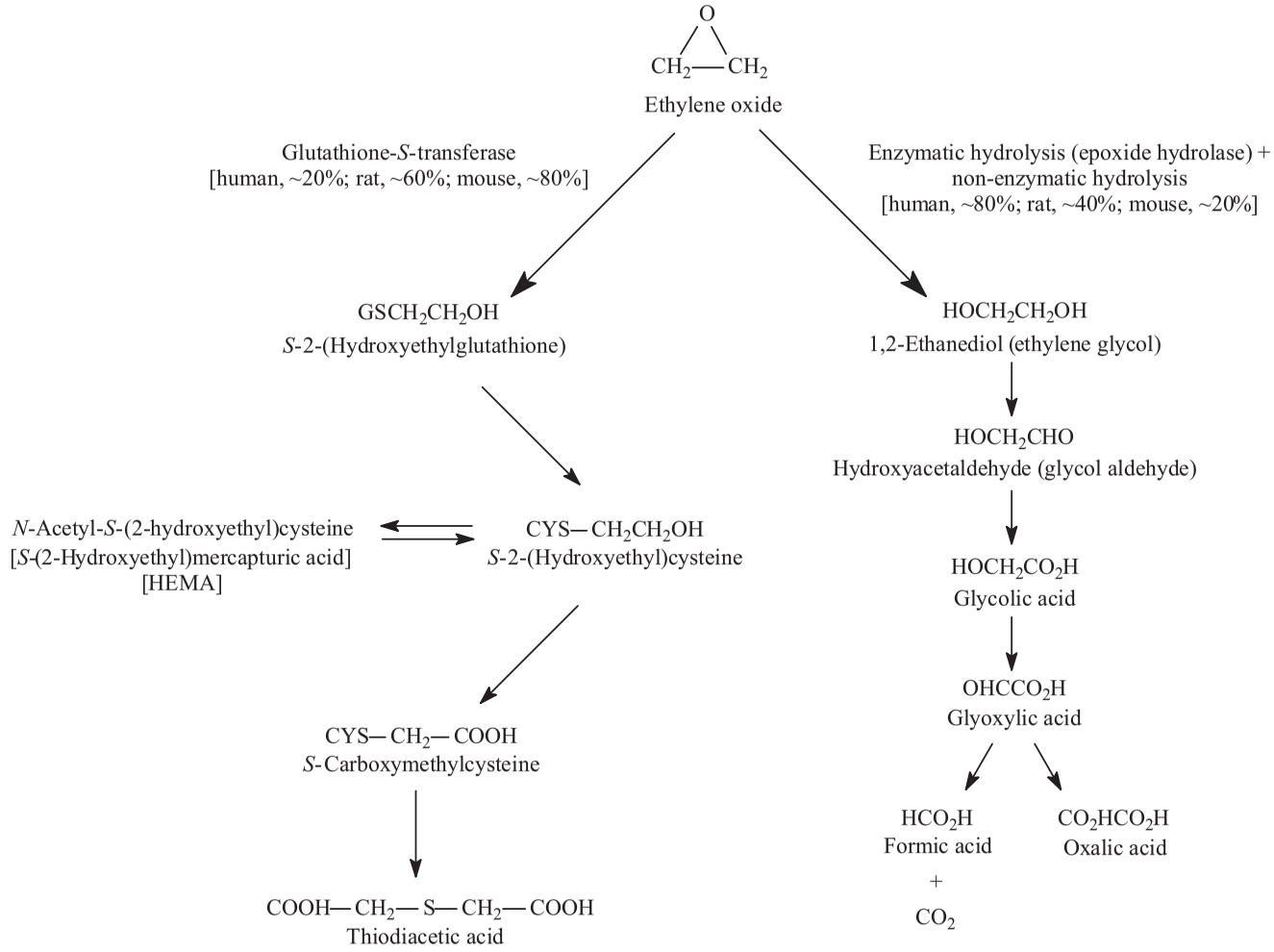
4.2.1 GST polymorphisms

Ethylene oxide is a substrate of the GST iso-enzyme T1 (Hayes *et al.*, 2005). This detoxifying enzyme is polymorphic and a relatively large proportion of the population (about 20% of Caucasians, almost 50% of Asians) has a homozygous deletion (*GSTT1*-null genotype) (Bolt & Thier, 2006). As expected, these individuals show a significantly higher amount of hydroxyethyl valine in their haemoglobin due to the presence of endogenous ethylene oxide (Thier *et al.*, 2001). Nevertheless, the influence of this genetic trait on the formation of this type of adduct as a result of exposure to exogenous ethylene oxide at the workplace is much less clear, as discussed below.

In the cytoplasm of erythrocytes obtained from 36 individuals, ethylene oxide was eliminated three to six times faster in samples from so-called conjugators (defined by a standardized conjugation reaction of methyl bromide and GSH; 75% of the population) than in those from the remaining 25% (who lack this GST-specific activity). In the latter samples, the rate of disappearance did not differ from that of controls. In this experiment, the disappearance of ethylene oxide was investigated in the gas phase, in closed vials that contained GSH and cytoplasm of erythrocytes (Hallier *et al.*, 1993).

Studies on the genotoxicity of ethylene oxide were reviewed in detail in *IARC Monograph* Volume 97 (IARC, 2008). Studies with peripheral blood of exposed workers have shown that exposure to ethylene oxide is associated with an elevated number of chromosomal aberrations including breaks, gaps, exchanges, and supernumerary chromosomes. An increased frequency

Fig. 4.1 Metabolism of ethylene oxide



Adapted from Wolfs *et al.* (1983), Scheick *et al.* (1997)

of sister chromatid exchange (SCE) in the peripheral lymphocytes of workers handling ethylene oxide was also reported.

4.2.2 DNA-adduct formation

In-vitro and in-vivo studies have shown that ethylene oxide can bind to cellular macromolecules, which results in a variety of DNA, RNA and protein adducts. The major DNA adduct recovered *in vivo* is *N*7-(2-hydroxyethyl) guanine (7-HEG), while some minor adducts such as *N*3-(2-hydroxyethyl)adenine (3-HEA) and O^6-(2-hydroxyethyl)guanine (O^6-HEG), are detected at much lower levels (Walker *et al.*, 1992). In-vitro studies indicate that other minor adducts can also be formed from the reaction of ethylene oxide with the *N*1 and N^6 positions of adenine and the *N*3 position of cytosine, uracil and thymine (IARC, 1994; Tates *et al.*, 1999; Kolman *et al.*, 2002).

Tompkins *et al.* (2009) suggested that the mutagenicity and carcinogenicity of ethylene oxide could be attributed to formation of multiple 2-hydroxyethyl (HE) DNA adducts such as 3-HEA and O^6-HEG. Boysen *et al.* (2009) argued that there is little evidence that 7-HEG adducts cause mutations since – unlike the *N*1, N^2, or O^6 positions of guanine – they do not participate in hydrogen bonding in the DNA double-helix and easily de-purinate. These authors conclude that the formation of *N*7-guanine adducts cannot be used in isolation as a quantitative biomarker for pro-mutagenic DNA lesions or mutagenic response. Marsden *et al.* (2009) used a dual-isotope approach to distinguish between endogenously formed background levels of 7-HEG and exogenously formed 7-HEG adducts in rats following exposure to [^{14}C]-labelled ethylene oxide. By combining liquid chromatography-tandem mass spectrometry and high-performance liquid chromatography/accelerator mass spectrometry analysis, both the endogenous and exogenous *N*7-HEG adducts were quantified in tissues of [^{14}C]ethylene oxide-treated rats. Levels of [^{14}C]-7-HEG induced in spleen, liver, and stomach DNA were insignificant compared with the measured background levels of *N*7-HEG naturally present.

The exact mechanism by which the other ethylene oxide-induced DNA adducts such as 3-HEA and O^6-HEG may lead to mutation is unknown. Several mechanisms could be involved, including the mispairing of altered bases or the formation of apurinic/apyrimidinic sites via DNA repair or chemical depurination/depyrimidination combined with the insertion of another base, which would typically be an adenine opposite an apurinic site (Tates *et al.*, 1999; Houle *et al.*, 2006). These lesions can also lead to the formation of DNA single-strand breaks and, subsequently, to chromosomal breakage. In addition, the putative ethylene oxide metabolite, glycolaldehyde, has been shown to form DNA–protein crosslinks and DNA single-strand breaks (Hengstler *et al.*, 1994).

4.2.3 Cytogenetic alterations and mutations

Studies of human exposure to ethylene oxide have focused on individuals employed in the operation of hospital- or factory-based sterilization units, and on workers who were involved in manufacturing or processing of ethylene oxide. The studies show that exposure to ethylene oxide results in chromosomal alterations that are related to both the level and duration of exposure, while a single study suggested that exposure to ethylene oxide causes gene mutations.

(a) Sister chromatid exchange

The induction of increased frequencies of sister chromatid exchange (SCE) has been found to be a sensitive indicator of genotoxic exposure to ethylene oxide in humans (Tates *et al.*, 1991). In several studies, significant differences were found in SCE frequencies in individuals and/or groups exposed to levels of ethylene oxide higher

than the designated low-exposure group from the same or a similar environments (Yager *et al.*, 1983; Sarto *et al.*, 1984a; Stolley *et al.*, 1984; Tates *et al.*, 1991; Schulte *et al.*, 1992). These findings support the observation that SCE frequencies varied with level and frequency of exposure to ethylene oxide. In two studies SCE frequencies were investigated over time: they remained elevated for at least six months even when exposures diminished or ceased after the first assessment (Sarto *et al.*, 1984b; Stolley *et al.*, 1984).

(b) Chromosomal aberrations

Chromosomal aberration frequencies correlate with exposure concentrations of ethylene oxide and/or duration of the exposure (Clare *et al.*, 1985; Galloway *et al.*, 1986; Tates *et al.*, 1991; Lerda & Rizzi, 1992). As reported for SCE, the validity of these comparisons is supported by the observation that some investigators found significant increases in chromosomal aberrations in high-dose groups but not in low-dose groups exposed in the same or similar environments (Sarto *et al.*, 1984b; Galloway *et al.*, 1986). In workers exposed to a range of concentrations of ethylene oxide [0.01–200 ppm; 0.02–366 mg/m^3] the presence of chromosomal aberrations was evaluated; in most of the workers significant increases in chromosomal aberrations were found (Pero *et al.*, 1981; Högstedt *et al.*, 1983, 1990; Sarto *et al.*, 1984b; Richmond *et al.*, 1985; Galloway *et al.*, 1986; Karelová *et al.*, 1987; Tates *et al.*, 1991; Lerda & Rizzi, 1992; Ribeiro *et al.*, 1994; Major *et al.*, 1996). In one study, such increases were found in individuals exposed to concentrations of ethylene oxide of approximately 1 ppm [1.83 mg/m^3] and even lower (Högstedt *et al.*, 1983). However, in other studies of workers exposed to these low concentrations of ethylene oxide, evidence of increased chromosomal aberrations was not found (Van Sittert *et al.*, 1985; Mayer *et al.*, 1991).

(c) Micronuclei

Few investigators have evaluated the impact of exposure to ethylene oxide on the frequency of micronucleated cells, and the available studies reported positive or no effects. Högstedt *et al.* (1990) and Ribeiro *et al.* (1994) found an increased frequency of micronucleated lymphocytes in workers, while Tates *et al.* (1991) found significant increases in micronucleus frequency in workers exposed to high, but not to low doses. Exposure concentrations in all these studies varied widely, ranging from < 1 ppm to 400 ppm [1.83–732 mg/m^3] ethylene oxide. Studies that evaluated micronucleus formation in individuals exposed to ethylene oxide at concentrations ≤ 1 ppm were negative (Högstedt *et al.*, 1983; Sarto *et al.*, 1990, 1991; Tates *et al.*, 1995).

In two studies micronucleus formation was determined in cells other than lymphocytes. Ribeiro *et al.* (1994) evaluated both peripheral blood lymphocytes and exfoliated buccal cells in individuals exposed to 2–5 ppm [3.66–9.15 mg/m^3] ethylene oxide (TWA); micronucleus frequencies in buccal cells were not associated with the exposure, while those in lymphocytes showed a significant positive association. Sarto *et al.* (1990) found a significant increase in micronucleus frequency in nasal mucosal cells, but not in exfoliated buccal cells of workers exposed to ethylene oxide at concentrations below 0.38 ppm [0.7 mg/m^3] (TWA).

(d) Gene mutations

The question whether occupational exposure to ethylene oxide is associated with the induction of gene mutations has been addressed in three reports. In the first study, the T-cell cloning assay was used to measure *HPRT* mutant frequencies in peripheral blood lymphocytes from nine ethylene oxide-exposed hospital workers and 15 ethylene oxide-exposed factory workers (Tates *et al.*, 1991). Hospital workers included nurses and technicians involved in the sterilization of medical

equipment and exposed to ethylene oxide once or twice a week for about 10 minutes. The concentrations of ethylene oxide ranged from 20 to 25 ppm [36.6–45.8 mg/m^3] in the sterilization room and from 22 to 72 ppm [40.3–131.8 mg/m^3] in front of the sterilizer immediately after opening. The hospital workers were matched for age, sex and smoking habits with a control group of eight unexposed administrative workers. The factory workers were employed at a plant that was involved in the production of ethylene oxide-sterilized disposable medical equipment, and were similarly matched with a group of 15 unexposed controls in the same factory. During a four-month monitoring period (equivalent to the lifespan of erythrocytes in humans), five workers were engaged in 'daily' sterilization activities, two workers were involved in 'daily' sterilization except for leave periods of 7 or 11 days, and the eight remaining workers were 'occasionally' exposed to ethylene oxide during exposure control, packing and quality control of sterilized products. Before the collection of samples in early 1990, the mean duration of exposure of factory workers to ethylene oxide had been 12 years (range, 3–27 years), with average ambient exposure levels from 1989 onwards that were estimated at about 17 ppm [~31 mg/m^3]. Based on measurements of *N*-(2-hydroxyethy)valine–haemoglobin adducts, which integrate exposure over time, average exposures to ethylene oxide in the four months before blood sampling were estimated at a 40-hour TWA of 0.025 ppm [0.046 mg/m^3] for hospital workers and 5 ppm [9.15 mg/m^3] for factory workers (Tates *et al.*, 1991). The average *HPRT* mutant frequencies in hospital workers ($12.4 \pm 9.9 \times 10^{-6}$) and factory workers ($13.8 \pm 4.4 \times 10^{-6}$) were remarkably similar and showed increases of 55% and 60%, respectively, above the background frequency in their respective control groups ($8.0 \pm 3.6 \times 10^{-6}$ and $8.6 \pm 4.4 \times 10^{-6}$); however, the mutagenic response was significantly elevated only in the factory workers, which was probably due to the higher exposure concentrations and tissue doses of ethylene oxide in these workers.

In a follow-up study of workers in an ethylene oxide-production plant, Tates *et al.* (1995) used the T-cell cloning assay to measure *HPRT* mutant frequencies in three exposed groups and one unexposed group (seven subjects per group). Group-I workers were incidentally exposed to acute high concentrations of ethylene oxide, while workers in Groups II and III had been chronically exposed to low concentrations of ethylene oxide for < 5 years and > 15 years, respectively. No significant differences in mutant frequencies were observed between any combination of worker or control groups, which implies that incidental exposure to high levels of ethylene oxide (28–429 ppm; 52–785 mg/m^3) or chronic exposure to low concentrations of ethylene oxide (< 0.005–0.02 ppm; < 0.01–0.04 mg/m^3) did not cause any measurable permanent gene mutation in lymphocytes at this locus.

In-vivo mutation-induction studies with reporter genes such as *Hprt* or the *LacI*-transgene have shown that ethylene oxide can significantly increase the frequency of mutations in both mice and rats (Walker & Skopek, 1993; Sisk *et al.*, 1997; Walker *et al.*, 1997a, b; Tates *et al.*, 1999; Recio *et al.*, 2004). The type of mutation that is recovered appears to be influenced by the assay system used. In mouse splenic and/or thymic T lymphocytes, mutations in *Hprt* could be detected after shorter exposures (a 4-week inhalation exposure or multiple intra-peritoneal injections over the course of one week) and appeared to consist of larger deletion mutations as well as base-pair substitutions and frame-shift mutations (Walker & Skopek, 1993; Walker *et al.*, 1997a, b). The latter point mutations appeared to originate primarily from either altered G or A nucleobases (Walker & Skopek, 1993; IARC, 1994). In the inhalation study, no significant increases in *LacI* mutations were seen in the spleen, bone marrow or germ cells of mice after four weeks of exposure to ethylene oxide (Sisk

et al., 1997). A modest but significant increase in *LacI* mutants was seen in the lungs of mice exposed to 200 ppm [366 mg/m^3] ethylene oxide. In a follow-up study with prolonged exposure (up to 48 weeks), significant increases in *LacI* mutants were seen in the bone marrow and testes of the ethylene oxide-exposed transgenic mice (Recio *et al.*, 2004). DNA-sequence analysis of mutants obtained from the bone marrow showed that only AT→TA transversions were recovered at a significantly increased frequency in the exposed mice. A unique mutational spectrum was not seen in the testes.

An elevated frequency of mutations or a change in mutational spectra has been seen in the tumours of ethylene oxide-treated mice (Houle *et al.*, 2006; Hong *et al.*, 2007). In the study by Hong *et al.* (2007), K-*Ras* mutations were detected in 100% (23/23) of ethylene oxide-induced lung tumours compared with 25% (27/108) of spontaneous tumours. Codon-12 G→T transversions occurred frequently in the ethylene oxide-induced lung neoplasms (21/23) but infrequently in spontaneous lung neoplasms (1/108). Similarly, K-*Ras* mutations were found in 86% (18/21) of Harderian gland tumours from ethylene oxide-treated animals, but were seen in only 7% (2/27) of the spontaneous tumours in this organ. Codon-13 G→C and codon-12 G→T transversions were common in the ethylene oxide-induced Harderian gland tumours, but they were absent in the spontaneous tumours in this organ (0/27). K-*Ras* mutations were also seen in 83% (5/6) of ethylene oxide-induced uterine tumours, all of which showed a G→C transition in codon 13. The incidence in spontaneous uterine tumours was not reported. A similar study by Houle *et al.* (2006) provided evidence of the involvement of H-*Ras* and *p53* mutations in mammary gland tumours induced by ethylene oxide in mice. The mutation frequency was only slightly elevated for H-*Ras* (33% in treated *vs* 26% in controls) or *p53* (67% in the ethylene oxide-treated versus 58% in the control animals), but the mutational spectra in tumours obtained from control and treated animals differed significantly. The mutational spectra were generally consistent with a targeting of G and A bases by ethylene oxide (Houle *et al.*, 2006; Hong *et al.*, 2007). The high frequency of mutation in these genes, particularly mutations in the critical codons of K-*Ras* and inactivation of *p53*, indicate that mutations are induced in the tumours of ethylene oxide-treated mice and that the changes probably play an important role in ethylene oxide-induced tumour development in these tissues.

Acute myelogenous leukaemia in patients previously treated with alkylating agents frequently shows specific characteristics that allow it to be distinguished from acute myelogenous leukaemia induced by other agents (such as topoisomerase II-inhibitors) or occurring spontaneously (Pedersen-Bjergaard & Rowley, 1994; Pedersen-Bjergaard *et al.*, 2006). One of the hallmarks of leukaemias induced by alkylating agents is that they frequently show loss of chromosomes 5 or 7 (–5, –7) or loss of part of the long arms of these chromosomes (5q-, 7q-). In addition, mutations in *p53* are frequently seen in leukaemias with the –5/5q- karyotype, and mutations in *p53* and *Ras* are seen in a subset of those that exhibit the –7/7q- karyotype (Christiansen *et al.*, 2001; Pedersen-Bjergaard *et al.*, 2006). Although ethylene oxide has not been investigated specifically for its ability to induce losses of chromosomes 5 or 7, or deletions of the long arms of these chromosomes (5q- or 7q-), it has been reported to induce similar types of chromosomal alteration and deletions in a variety of experimental models and/or in the lymphocytes of exposed workers (IARC, 1994; Major *et al.*, 1996, 1999). The detection of elevated levels of chromosomal aberrations and micronuclei in the peripheral blood lymphocytes of ethylene oxide-exposed workers is of particular interest, as individuals with increased levels of chromosomal aberrations or micronuclei in these cells are at an increased risk for cancer (Hagmar *et al.*,

Table 4.1 Comparison of the evidence for key events – cytogenetic, genetic, and related changes – induced by ethylene oxide in humans, human cells, and experimental animals

End-point	In-vivo exposure		In-vitro exposure
	Animals	Humans	Human cells
Haemoglobin-adduct formation	Strong	Strong	Strong
DNA-adduct formation	Strong	Weak[a]	Strong
Mutations in reporter genes in somatic cells	Strong	Weak[a]	Strong
Mutations in cancer-related genes in tumours	Strong	NR	not applicable
Increased levels of cancer-related proteins in tumours	Strong	NR	not applicable
Cytogenetic alterations in somatic cells			
Sister chromatid exchange	Strong	Strong	Strong
Structural chromosomal aberrations	Strong[b]	Strong	Moderate
Micronucleus formation	Strong[b]	Strong	NR

[a] Possibly due to a lack of adequate studies
[b] Positive responses were seen only at exposure concentrations above those used in the rodent cancer-bioassays
NR, not reported
From IARC (2008)

1998; Liou *et al.*, 1999; Smerhovsky *et al.*, 2001; Hagmar *et al.*, 2004; Boffetta *et al.*, 2007; Bonassi *et al.*, 2007).

A comparison of the evidence for ethylene oxide-induced genetic and related changes in experimental animals and humans is summarized in Table 4.1.

In conclusion, the numerous studies on ethylene oxide that focused on toxicokinetics, DNA-adduct formation, biomarkers, genotoxicity, and molecular biology provide strong evidence that the carcinogenicity of ethylene oxide, a direct-acting alkylating agent, involves a genotoxic mechanism of action. The direct reaction of ethylene oxide with DNA is thought to initiate the cascade of genetic and related events that lead to cancer. Ethylene oxide induces a dose-related increase in the frequency of ethylene oxide-derived haemoglobin adducts in exposed humans and rodents, induces a dose-related increase in the frequency of ethylene oxide-derived DNA adducts in exposed rodents, consistently acts as a mutagen and clastogen at all phylogenetic levels, induces heritable translocations in the germ cells of exposed rodents, and induces a dose-related increase in the frequency of sister chromatid exchange, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers.

5. Evaluation

There is *limited evidence* in humans for a causal association of ethylene oxide with lymphatic and haematopoietic cancers (specifically lymphoid tumours, i.e. non-Hodgkin lymphoma, multiple myeloma and chronic lymphocytic leukaemia), and breast cancer.

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

There is strong evidence that the carcinogenicity of ethylene oxide, a direct-acting alkylating agent, operates by a genotoxic mechanism. A dose-related increase in the frequency of ethylene oxide-derived haemoglobin adducts has been observed in exposed humans and rodents, and a dose-related increase in the frequency of ethylene oxide-derived DNA adducts has been demonstrated in exposed rodents. Ethylene oxide consistently acts as a mutagen and clastogen at all phylogenetic levels, it induces heritable

translocations in the germ cells of exposed rodents, and a dose-related increase in the frequency of sister chromatid exchange, chromosomal aberrations and micronucleus formation in the lymphocytes of exposed workers.

Ethylene oxide is *carcinogenic to humans (Group 1)*.

In making the overall evaluation, the Working Group considered that there is *sufficient evidence* for the carcinogenicity of ethylene oxide in experimental animals, and relied heavily on the compelling data in support of the genotoxic mechanism described above.

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FORMALDEHYDE

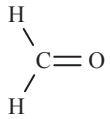
Formaldehyde was considered by previous IARC Working Groups in 1981, 1987, 1994, and 2004 (IARC, 1982, 1987, 1995, 2006). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1 Exposure Data

1.1 Identification of the agent

From (IARC, 2006).

Chem. Abstr. Serv. Reg. No.: 50-00-0
Chem. Abstr. Name: Formaldehyde
IUPAC Systematic Name: Methanal
Synonyms: Formaldehyde gas; formic aldehyde; methaldehyde; methyl aldehyde; methylene oxide; oxomethane; oxymethylene



CH_2O
Relative molecular mass: 30.03
Description: Colourless gas with a pungent odour
Conversion factor: $mg/m^3 = 1.23 \times ppm$; calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming standard temperature (25 °C) and pressure (103.5 kPa).

1.2 Use

Formaldehyde is produced worldwide on a large scale by catalytic, vapour-phase oxidation of methanol. Formaldehyde is used mainly in the production of various types of resin. Phenolic, urea, and melamine resins have wide uses as adhesives and binders in the wood-production, pulp-and-paper, and the synthetic vitreous-fibre industries, in the production of plastics and coatings, and in textile finishing. Polyacetal resins are widely used in the production of plastics. Formaldehyde is also used extensively as an intermediate in the manufacture of industrial chemicals, such as 1,4-butanediol, 4,4′-methylenediphenyl diisocyanate, penta-erythritol, and hexamethylenetetramine. Formaldehyde is used directly in aqueous solution (known as formalin) as a disinfectant and preservative in many applications (IARC, 2006).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence and exposure

Formaldehyde is found as a natural product in most living systems and in the environment. It occurs naturally in fruits and some foods, and it is formed endogenously in mammals, including humans, as a consequence of oxidative metabolism. In addition to these natural sources, common non-occupational sources of exposure to formaldehyde include combustion processes, e.g. through emissions from motor vehicles, power plants, incinerators, refineries, wood stoves, and kerosene heaters. Formaldehyde may be released from particle boards and similar building materials, carpets, paints and varnishes, during cooking of some foods, and during its use as a disinfectant. It is also present in tobacco smoke. An indirect source of exposure to formaldehyde is its formation via photochemical oxidation of hydrocarbons, such as methane, and other precursors emitted from combustion processes (NTP, 2005; IARC, 2006). Formaldehyde has a short half-life in the environment, because it is removed from the air by photochemical processes and by precipitation and biodegradation (NTP, 2005).

Concentrations of formaldehyde in outdoor air are generally below 0.001 mg/m^3 in remote areas and below 0.02 mg/m^3 in urban settings. The levels of formaldehyde in indoor air of houses are typically 0.02–0.06 mg/m^3; indoor combustion sources can significantly increase these levels. Cigarettes may contribute as much as 10–25% of the indoor exposure. Average concentrations of 0.5 mg/m^3 or more have been measured in 'mobile homes', but these have declined since the late 1980s as a result of standards that require that building materials – e.g. particle boards – emit lower concentrations of formaldehyde. A recent study of emissions from mosquito coils found the average concentration of formaldehyde exceeded 100 µg/m^3 (IARC, 2006, 2010; Lee & Wang, 2006). Data on formaldehyde concentrations in outdoor air in residential and public settings, and information on exposure to formaldehyde associated with household use of solid fuels and high-temperature frying, have been reviewed in *IARC Monograph* Volumes 88 and 95 (IARC, 2006, 2010).

Automobile exhaust is a major source of formaldehyde in ambient air. Recent reports suggest that formaldehyde emissions may be higher from vehicles powered by compressed natural gas compared with those running on ethanol or gasohol (Corrêa & Arbilla, 2005), and that these emissions may be decreased by substitution of an ethanol-biodiesel-diesel blend for diesel fuel (Shi *et al.*, 2006). In addition, formaldehyde can be absorbed through the skin from cosmetics or via contact with other consumer products containing formaldehyde, such as unwashed permanent-press fabrics treated with formaldehyde-releasing resins (NTP, 2005).

1.3.2 Occupational exposure

Occupational exposure to formaldehyde occurs in a wide variety of occupations and industries. CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the results for formaldehyde in the EU by industry (CAREX, 1999).

In *IARC Monograph* Volume 88 (IARC, 2006) data were reviewed on occupational exposure to formaldehyde by type of industry. The highest continuous exposures (2–5 ppm; 2.5–6.1 mg/m^3) were measured in the past during varnishing of furniture and wooden floors, in the finishing of

Table 1.1 Estimated numbers of workers exposed to formaldehyde above background levels in the European Union

Industry, occupational activity	
Manufacture of furniture and fixtures, except primarily of metal	179000
Medical, dental, and other health and veterinary services	174000
Manufacture of wearing apparel, except footwear	94000
Manufacture of wood and wood and cork products, except furniture	70000
Personal and household services	62000
Construction	60000
Manufacture of textiles	37000
Iron and steel basic industries	29000
Manufacture of fabricated metal products, except machinery	29000
Manufacture of other non-metallic mineral products	23000
Manufacture of machinery, except electrical	20000
Manufacture of industrial chemicals	17000
Manufacture of other chemical products	17000
Manufacture of plastic products not classified elsewhere	16000
Agriculture and hunting	16000
Manufacture of paper and paper products	13000
Printing, publishing and allied industries	13000
Wholesale and retail trade and restaurants and hotels	13000
Manufacture of transport equipment	11000
Manufacture of electrical machinery, apparatus and appliances	10000
Manufacture of footwear	9000
Manufacture of glass and glass products	8000
Research and scientific institutes	7000
Non-ferrous metal basic industries	6000
Manufacture of leather and products of leather or of its substitutes	6000
Beverage industries	4000
Manufacture of instruments, photographic and optical	4000
Other manufacturing industries	3000
Food manufacturing	3000
Crude petroleum and natural gas production	2000
Manufacture of rubber products	4000
Financing, insurance, real estate and business services	3000
Education services	2000
Sanitary and similar services	2000
Services allied to transport	2000
Manufacture of miscellaneous products of petroleum and coal	1000
Other industries	2000
Total (all industries)	971000

From Kauppinen *et al.* (2000), CAREX (1999)

textiles, in the garment industry, in the treatment of fur, and in certain jobs within manufactured board mills and foundries. Short-term exposures to high levels (3 ppm and higher; ≥ 3.7 mg/m^3) have been reported for embalmers, pathologists, and paper workers. Lower concentrations have usually been encountered during the manufacture of man-made vitreous fibres, abrasives and rubber, and in formaldehyde-production industries. A very wide range of exposure levels has been observed in the production of resins and plastic products. The development of resins that release less formaldehyde, and improved ventilation have resulted in lower exposure levels in many industrial settings in recent decades (IARC, 2006).

2. Cancer in Humans

2.1 Cancer of the nasopharynx

In *IARC Monograph* Volume 88 (IARC, 2006) it was concluded that there was *sufficient evidence* for the carcinogenicity of formaldehyde, based primarily on its association with nasopharyngeal cancer. There have been relatively few new studies published on this association since that time, although there have been several re-evaluations and meta-analyses.

2.1.1 Cohort studies

In the most recent follow-up of the largest cohort study from the USA of industrial workers exposed to formaldehyde, a statistically significant excess of deaths from nasopharyngeal cancer was observed in comparison with the US national population, with statistically significant exposure–response relationships for peak exposure and cumulative exposure (Hauptmann *et al.*, 2004; see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.1.pdf). Based on eight cases, a significant excess mortality from nasopharyngeal cancer was observed among formaldehyde-exposed workers (SMR, 2.10; 95%CI: 1.05–4.21). A highly statistically significant (P_{trend} < 0.001) exposure–response relationship was observed between peak-exposure to formaldehyde and risk for nasopharyngeal cancer in a Poisson regression-analysis. All exposed cases were in the highest category of peak-exposure, and the relative risk was 1.83. This analysis excluded one case which, according to cancer registry data, had been misclassified as nasopharyngeal cancer. Weaker exposure–response relationships were observed between nasopharyngeal cancer and average or cumulative exposure, and duration of exposure (P_{trend} = 0.07, 0.03 and 0.15, respectively).

In the two other large cohort studies of industrial workers, cases of nasopharyngeal cancer were fewer than expected, but the power of these studies to detect an effect on nasopharyngeal cancer was low and the deficits were small. In the first study, of British chemical workers, one death was observed when 2.0 were expected (Coggon *et al.*, 2003); in the second study, no deaths were observed among US garment-manufacturers, where 0.96 were expected (Pinkerton *et al.*, 2004).

An excess of deaths from nasopharyngeal cancer was observed in a proportionate mortality analysis of the largest US cohort of embalmers (Hayes *et al.*, 1990) and in a Danish study of proportionate cancer incidence among workers at companies that used or manufactured formaldehyde (Hansen & Olsen, 1995; see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.2.pdf).

Marsh *et al.* (1996) conducted a cohort study in one of the plants considered in the NCI study (where five of the nine cases of nasopharyngeal cancer occurred). The cohort included earlier year of entry and was enumerated independently. Significantly increased mortality due to nasopharyngeal cancer was observed among formaldehyde-exposed workers compared with US and regional populations (Connecticut State and

local county). In a recent follow-up through 2003, Marsh *et al.* (2007a) showed elevated SMRs when both national and local county rates were used. In addition, when conducting a case–control study nested within the cohort and including seven deaths from nasopharyngeal cancer, the authors obtained information on employment outside the formaldehyde industry and showed that five of these workers had been employed as a silversmith. However, while there was some evidence of effect modification by activities as a silversmith (based on small numbers), confounding alone did not explain the relatively high number of deaths from nasopharyngeal cancer in this plant (Marsh *et al.*, 2007a).

Two analyses have been conducted to re-analyse the data from the most recent update of the NCI cohort, with a focus on solid tumours (Hauptmann *et al.*, 2004). The first included an analysis of exposure category and SMR, as well as an analysis of Plant 1, where five of nine deaths from nasopharyngeal cancer occurred, compared with all other plants in the cohort (Marsh & Youk, 2005). Using their own cut-points of exposure, the authors concluded that their analysis lent uncertainty to the findings from the NCI cohort. In another re-analysis, the authors further controlled for the effect of plant for the peak-exposure metric and performed sensitivity analyses by imputing additional cases, which showed instability in the risk estimates (Marsh *et al.*, 2007b). The authors concluded that an interaction between plant group and exposure makes generalization beyond Plant 1 difficult.

2.1.2 Case–control studies

The relationship between nasopharyngeal cancer and exposure to formaldehyde has also been investigated in seven case–control studies, five of which found elevated risks for overall exposure to formaldehyde or in higher exposure categories, although not all were statistically significant (see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.3.pdf; Vaughan *et al.*, 1986b; Roush *et al.*, 1987; West *et al.*, 1993; Vaughan *et al.*, 2000; Hildesheim *et al.*, 2001). One study found an elevation among women, but not men (Olsen *et al.*, 1984) and one found no evidence of an association (Armstrong *et al.*, 2000). Two case–control studies were considered as the most informative because of their size, their exposure assessment, and the evaluation of potential confounders. The first, a population-based case–control study in the USA, showed a significant association for the workers whose exposure duration had been the longest (OR = 2.1; 95%CI: 1.0–4.5, P_{trend} = 0.07), but not for maximum exposure (P_{trend} = 0.57) (Vaughan *et al.*, 2000). When the analysis was limited to differentiated squamous-cell and epithelial NOS, there was a significant association in the highest exposure category for both duration and cumulative exposure with significant exposure-response trends (P_{trend} = 0.014 and 0.033, respectively). In the other study, conducted in Taiwan, China, an OR of 1.6 (95%CI: 0.91–2.9, P_{trend} = 0.08) was found in the category with the longest duration of exposure (Hildesheim *et al.*, 2001). For cumulative exposure, there was a non-significant elevation in the highest exposure category and the trend test was not significant (P = 0.10). In subanalyses that were restricted to cases and controls who were seropositive for antibodies against Epstein-Barr virus, the association between exposure to formaldehyde and nasopharyngeal cancer appeared to be stronger, with an OR for ever exposure of 2.7 (95%CI: 1.2–6.2). However, no clear dose–response pattern was observed with increasing duration of exposure, or with estimated cumulative exposure.

2.1.3 Meta-analyses

A meta-analysis published in 1997 included some but not all of the above studies, and found an overall meta-relative risk for nasopharyngeal

cancer of 1.3 (95%CI: 1.2–1.5) (Collins *et al.*, 1997). From a pooled analysis including the three recently updated industrial cohorts (Coggon *et al.*, 2003; Hauptmann *et al.*, 2004; Pinkerton *et al.*, 2004), Bosetti *et al.* (2008) reported an overall SMR of 1.33 (95%CI: 0.61–2.53). A recently published meta-analysis included both case–control studies (n = 6) and cohort studies (n = 7) (Bachand *et al.*, 2010). For the case–control studies, the overall OR was 1.22 (95%CI: 1.00–1.50), with the meta-regression OR no longer significant when limited to studies that included adjustment for socioeconomic status, smoking or location. The risk estimate for cohort studies was 0.72 (95%CI: 0.40–1.29), including seven studies (Bachand *et al.*, 2010). For the cohort studies, the authors used a re-analysis of the NCI cohort study from which Plant 1 was left out (Marsh & Youk, 2005).

2.2 Leukaemia

In *IARC Monograph* Volume 88 (IARC, 2006) it was concluded that there was strong, but not sufficient evidence for the leukaemogenic effects of formaldehyde. Since that time, an update to the NCI cohort and a nested case–control study of workers in the funeral industry have been published (Beane Freeman *et al.*, 2009; Hauptmann *et al.*, 2009), as well as three meta-analyses (Bosetti *et al.*, 2008; Zhang *et al.*, 2009; Bachand *et al.*, 2010; see Table 2.1 online, and Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.5.pdf).

2.2.1 Cohort studies

Excess mortality from leukaemia has been observed relatively consistently in studies of professional workers (i.e. embalmers, funeral parlour workers, pathologists and anatomists), with six mortality studies showing positive associations (Walrath & Fraumeni, 1983, 1984; Levine *et al.*, 1984; Stroup *et al.*, 1986; Hayes *et al.*, 1990; Hall *et al.*, 1991) and one not (Logue *et al.*, 1986; see Table 2.2 online).

A weakness of the proportionate mortality studies among professionals has been the lack of exposure assessment. A recently published nested case–control study conducted among professionals in the funeral industry examined lifetime work practices and exposure in the funeral industry to develop metrics of exposure among this group, which included duration of jobs held while embalming, number of embalmings, average intensity of embalming and peak exposure (Hauptmann *et al.*, 2009). Details of work practices were obtained by interviews with next of kin and co-workers. Positive associations were seen – at many levels of exposure and for multiple exposure metrics – for deaths from lymphohaematopoietic malignancies of non-lymphoid origin (n = 48). For myeloid leukaemia (n = 34) the OR was 13.6 (95%CI: 1.6–119.7; P_{trend} = 0.020) for the longest duration of work in jobs with embalming. Because only one case was reported to have never embalmed, additional analyses were conducted in which those who reported to have embalmed ≤ 500 times were taken as the referent group, to provide a more stable estimate. Results were attenuated, but still significant (OR = 3.9; 95%CI: 1.2–12.5). [There was a considerable amount of missing data that required imputation for analyses.]

The findings for leukaemia in studies of professional workers appeared to be contradicted by the lack of such findings among industrial workers. However, some evidence for an excess of deaths from leukaemia has been reported in the recent updates of two of the three major cohort studies of industrial workers. Since the previous evaluation (IARC, 2006), the NCI cohort of industrial workers in the USA has been updated with an additional ten years of mortality data resulting in 123 deaths from leukaemia, including 48 from myeloid leukaemia (Beane Freeman *et al.*, 2009). This update extended the mortality follow-up

through 2004 and included additional deaths before 1994 that had not been previously considered. Risk estimates from follow-up through 2004 were diminished for leukaemia and myeloid leukaemia compared with the follow-up through 1994 (Hauptmann *et al.*, 2003), when both conditions had been significantly associated with increasing peak-exposure and average intensity of exposure to formaldehyde. As in the previous analysis of leukaemia, the association in the most recent update was stronger for myeloid leukaemia and peak exposure than for lymphatic leukaemia and for other metrics of exposure (Beane Freeman *et al.*, 2009). However, because the last known exposure occurred in 1980 and median follow-up was over 40 years, the authors not only examined risks at the end of follow-up in 2004, but also assessed associations over time by extending follow-up in yearly increments. Risks appeared to be highest before 1980, but only achieved statistical significance in the mid-1990s, when a sufficient number of deaths had accrued. Additional analyses with time since first exposure and time since first high peak-exposure indicated that risks were highest during the first twenty-five years. Patterns were similar, but attenuated, for average intensity of exposure; no association was observed with cumulative exposure.

Mortality from leukaemia was also found to be in excess in an update of the study of US garment workers exposed to formaldehyde (Pinkerton *et al.*, 2004). A small and statistically non-significant excess was observed for the entire cohort in comparison with rates among the general population (SMR = 1.09; 95%CI: 0.7–1.63). This excess was somewhat stronger for myeloid leukaemia (SMR = 1.44; 95%CI: 0.80–2.37), which is consistent with the findings from the study of industrial workers in the USA and several of the studies of medical professionals and embalmers. The excess was also stronger among workers with a longer duration of exposure and longer follow-up, and among those who had been employed early in the study period when exposures to formaldehyde were believed to be highest. The positive associations observed in the subgroup analyses presented in the study of US garment workers were based on a relatively small number of deaths, and were thus not statistically stable.

The updated study of British industrial workers found no excess mortality for leukaemia among all workers exposed to formaldehyde (SMR = 0.91; 95%CI: 0.62–1.29) or among those with the highest exposure (SMR = 0.71; 95%CI: 0.31–1.39) (Coggon *et al.*, 2003). The lack of positive findings in this study is difficult to reconcile with the findings from the studies of garment workers and industrial workers in the USA, and with the results of studies on professionals exposed to formaldehyde. This British study is a relatively large, high-quality study with sufficiently long follow-up to have had a reasonable chance to detect an excess of deaths from leukaemia. It did not examine specifically the risk for myeloid leukaemia, which represented the strongest finding in the studies of garment workers and industrial workers in the USA and in several of the studies of medical professionals and funeral workers.

2.2.2 Case–control studies

Three case–control studies evaluated exposure to formaldehyde and risk for leukaemia (Linos *et al.*, 1990; Partanen *et al.*, 1993; Blair *et al.*, 2001; Table 2.5 online). However, the numbers of exposed cases were few, and no significant elevations of risk were found.

2.2.3 Meta-analyses

A meta-analysis published in 2004 for 'ever exposure' to formaldehyde and leukaemia included eighteen studies and presented separate analyses by type of job: for industrial workers, the mRR was 0.9 (95%CI: 0.8–1.0); for

embalmers 1.6 (95%CI: 1.2–2.0); and for pathologists and anatomists 1.4 (95%CI: 1.0–1.9), with an overall mRR of 1.1 (95%CI: 1.0–1.2) (Collins & Lineker, 2004). In another meta-analysis, analysis was restricted to 13 cohort or proportionate mortality studies and similar results were found, with a pooled RR based on the weighted average of the SMRs for leukaemia among industrial workers of 0.9 (95%CI: 0.75–1.07), based on 122 deaths, and of 1.39 (95%CI: 1.15–1.68) among professionals, based on 106 deaths (Bosetti *et al.*, 2008). A further meta-analysis differed from these two previous ones by excluding all proportionate mortality studies and including the most recent update of the NCI cohort (Bachand *et al.*, 2010). For leukaemia overall, a risk estimate of 1.05 (95%CI: 0.93–1.20) was calculated for 'ever exposure', based on 15 studies with the use of a fixed-effects model. For myeloid leukaemia, the calculated mRR was 1.09 (95%CI: 0.84–1.40, based on three studies) and for lymphatic leukaemia the mRR was 1.11 (95%CI: 0.81–1.52, based on two studies).

Zhang *et al.* (2009) published a meta-analysis that included 15 cohort or case–control studies. The authors selected only studies where it was clear that the workers had been exposed to formaldehyde. In contrast to the other meta-analyses, this one used one exposure metric from each study and considered the highest exposure category for calculating the mRR. For leukaemia, the mRR was 1.54 (95%CI: 1.18–2.00). In addition, a separate analysis of myeloid leukaemia – for the six studies that reported it – found an mRR of 1.90 (95%CI: 1.31–2.76).

2.3 Cancer of the nasal sinuses

2.3.1 Cohort studies

An analysis of proportionate cancer incidence among industrial workers in Denmark showed an increased risk for squamous-cell carcinomas (Hansen & Olsen, 1995, 1996). No excess of mortality from sinonasal cancer was observed in the three recently updated studies of industrial and garment workers in the USA, and of chemical workers in the United Kingdom (see Table 2.1 online; Coggon *et al.*, 2003; Hauptmann *et al.*, 2004; Pinkerton *et al.*, 2004).

2.3.2 Case–control studies

The association between exposure to formaldehyde and the risk for sinonasal cancer has been evaluated in six case–control studies that primarily focused on formaldehyde (see Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.4.pdf; Olsen *et al.*, 1984; Hayes *et al.*, 1986; Olsen & Asnaes, 1986; Vaughan *et al.*, 1986a; Roush *et al.*, 1987; Luce *et al.*, 1993; Pesch *et al.*, 2008). Four of these six studies reported an increased risk (Olsen *et al.*, 1984; Hayes *et al.*, 1986; Vaughan *et al.*, 1986a; Luce *et al.*, 1993).

2.3.3 Pooled analysis

Four of the cohort studies contributed to a pooled analysis that collated occupational data from 12 case–control investigations (Luce *et al.*, 2002). After adjustment for known occupational confounders, this analysis showed an increased risk for adenocarcinoma associated with high exposure (> 1 ppm) to formaldehyde in both men (OR, 3.0; 95%CI: 1.5–5.7) and women (OR, 6.3; 95%CI: 2.0–19.7). An exposure–response trend was observed in relation to an index of cumulative exposure. There was some evidence of an association with squamous-cell carcinoma.

[Most epidemiological studies of sinonasal cancer have not distinguished between tumours that arise in the nose and those that develop in the nasal sinuses. Thus, any effect on the risk for nasal cancer specifically would tend to be diluted if there were no corresponding effect on the risk for cancer in the sinuses and could mask its detection, particularly in cohort studies that

have relatively low statistical power. However, the apparent discrepancy between the results of the case–control as compared with the cohort studies might also reflect residual confounding by wood dust in the former. Almost all of the formaldehyde-exposed cases in the case–control studies were also exposed to wood dust, which resulted in a high relative risk, particularly for adenocarcinomas.]

2.4 Other cancers

Several studies have identified statistically significant positive associations between exposure to formaldehyde and cancer at other sites, including the oral cavity, oro-and hypopharynx, larynx, lung, brain, pancreas, Hodgkin lymphoma, and multiple myeloma. However, the results are inconsistent (see Tables 2.4 and 2.5 online; Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.6.pdf, and Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-24-Table2.7.pdf).

2.5 Synthesis

The Working Group noted one industrial cohort study with both a strong overall association between exposure to formaldehyde and nasopharyngeal cancer, and the most elevated risks in the highest exposure category. Positive associations were also observed in many of the case–control studies, in particular those of larger size and higher-quality exposure assessment. While there was no association observed in the two other large industrial cohort studies, the expected number of cases in those studies was quite small. It is concluded that occupational exposure to formaldehyde causes nasopharyngeal cancer in humans. The Working Group noted that it was unlikely that confounding or bias could explain the observed association.

Elevated risks of leukaemia have been consistently observed in proportionate mortality studies of professionals exposed to formaldehyde (i.e. embalmers, workers in the funeral industry, pathologists and anatomists). Results from a nested case–control study of workers in the funeral industry show elevated risks for many measures of exposure, which are strongest for myeloid leukaemia. In two of the three large industrial cohort studies positive associations were observed for leukaemia, which were somewhat stronger for myeloid leukaemia. It is difficult to reconcile the lack of association observed in the third industrial cohort study with the overall positive associations in the others. However, there seems to be no strong evidence that confounding or bias explains the positive associations seen in multiple settings. On balance, the Working Group concluded that the epidemiologic evidence shows that occupational exposure to formaldehyde causes leukaemia.

Many case–control studies show positive associations for exposure to formaldehyde and sinonasal cancer, some with evidence of an exposure–response pattern. However, many of these cases were also exposed to wood dust, which was strongly associated with sinonasal cancer in these studies. The industrial cohort studies show no such association, which may be due to lack of statistical power, or could indicate that uncontrolled confounding to wood dust partially explains the observed associations in the case–control studies. The Working Group could not rule out the possibility of residual confounding in the case–control studies and noted the discordant results between the cohort and case–control studies.

3. Cancer in Experimental Animals

Carcinogenicity studies with mice, rats and hamsters exposed to formaldehyde by inhalation, via the drinking-water, or through the skin were reviewed in *IARC Monograph* Volume 88 (IARC, 2006). Results of adequately conducted carcinogenicity studies are summarized in Table 3.1. There have been no additional carcinogenicity studies in experimental animals reported since the previous review.

3.1 Inhalation

In one inhalation study in B6C3F1 mice, formaldehyde marginally increased the incidence of squamous cell carcinomas of the nasal cavity in males. The incidence of lymphoma in females exposed to 14.3 ppm (27/121) was also marginally increased ($P = 0.06$) when compared (pair-wise) with controls (19/121) (CIIT, 1981; Kerns *et al.*, 1983a, b; Gibson, 1984).

In six studies (Swenberg *et al.*, 1980; CIIT, 1981; Albert *et al.*, 1982; Kerns *et al.*, 1983a, b; Gibson, 1984; Sellakumar *et al.*, 1985; Feron, *et al.*, 1988; Woutersen *et al.*, 1989; Monticello *et al.*, 1996; Kamata *et al.*, 1997) in different strains of rats (F344, Wistar, and Sprague-Dawley), there were treatment-related increases in tumours of the nasal cavity (primarily squamous-cell carcinomas but also squamous-cell papillomas, polypoid adenomas, carcinomas, rhabdomyosarcomas, adenocarcinomas, and mixed/combined tumours). In one study (CIIT, 1981), the incidences of undifferentiated leukaemia [Fischer rat leukaemia, as indicated in the report] were 12/120 (control), 17/120 (2 ppm), 16/120 (5.6 ppm) and 7/120 (14.3 ppm) in females; there was a marked decrease in survival in the animals exposed to the high dose. Based on a survival-adjusted analysis, the incidence of leukaemia in females exposed to 14.3 ppm was increased compared with controls ($P = 0.0056$; Tarone-extension of the Cox test; level of significance, $P < 0.0167$). [The Working Group noted that this type of leukaemia is a very common, spontaneously occurring neoplasm in the F344 rat strain].

3.2 Oral administration (drinking-water)

In one drinking-water study in male Wistar rats, there was a treatment-related increase in fore-stomach squamous-cell papillomas (Takahashi *et al.*, 1986). Another study in male and female Wistar rats did not report any increase in tumours (Til *et al.*, 1989).

In a study with life-long exposure to formaldehyde, beginning *in utero* (transplacentally), there was an increased incidence of smooth-muscle tumours of the small intestine (leiomyosarcoma) in female offspring (Soffritti *et al.*, 1989). In another study in male and female rats, increased incidences of total malignant tumours, haematopoietic tumours, and interstitial-cell adenomas were observed in males (Soffritti *et al.*, 1989, 2002). [The Working Group reaffirmed the concerns of the previous Working Group (IARC, 2006) regarding the pooling of all 'leukaemias' diagnosed as lymphoblastic leukaemias and lymphosarcomas, immunoblastic lymphosarcomas, and "other types" of leukaemia and haemolymphoreticular sarcomas. Also noted were the lack of reporting of non-neoplastic lesions and historical control data, and the numerous discrepancies in tumour incidence between the first (Soffritti *et al.*, 1989) and second report (Soffritti *et al.*, 2002) of the results of this study].

3.3 Skin application

In one study in male and female hairless Oslo mice, topical application of 10% formaldehyde in water reduced the latency of 7,12-dimethylbenz[*a*]anthracene-induced skin tumours (Iversen, 1986).

Table 3.1 Carcinogenicity studies in experimental animals exposed to formaldehyde

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Inhalation studies				
C3H mouse (unspecified) 35 wk (some for 64 wk) Horton *et al.* (1963)	0, 50, 100, 200 mg/m^3 1 h/d, 3 d/wk 42–60/group	No pulmonary tumours	NS	USP grade Due to severe toxicity, exposure to 200 mg/m^3 was discontinued after the 11th exposure. Thirty-six mice exposed to 50 mg/m^3 were exposed to 150 mg/m^3 for 29 additional wk. Basal-cell hyperplasia, squamous metaplasia and atypical hyperplasia were observed in trachea and bronchi of many exposed mice. Nasal tissues were not examined. Short period of exposure and short duration of study.
B6C3F1 mouse (M) 30 mo Kerns *et al.* (1983a, b), Gibson (1984)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 119–120/group	No increased tumour incidence Nasal cavity (malignant)[a]: 2/17 (14.3 ppm) vs 0/21 (controls) at 24 mo	NS	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 0–1 at 18 mo; 17–21 at 24 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of mice exposed to 14.3 ppm.
B6C3F1 mouse (F) 30 mo Kerns *et al.* (1983a, b), Gibson (1984), CIIT (1981)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 120–121/group	No increased tumour incidence Lymphoma: 27/121 (14.3 ppm) vs 19/121 (controls)	NS (P = 0.06)	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 19–20 at 18 mo; 26–41 at 24 mo; 9–16 at 27 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of mice exposed to 14.3 ppm.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 rat (M) 30 mo Swenberg *et al.* (1980), Kerns *et al.* (1983a, b), Gibson (1984)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 119–120/group	Nasal cavity (malignant)[a]: 0/118, 0/118, 1/119, 51/117*	*$P < 0.001$	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 20 at 18 mo; 13–54 at 24 mo; 5–10 at 27 mo; 0–6 at 30 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of rats exposed to 14.3 ppm.
		Nasal cavity (malignant)[b]: 0/118, 0/118, 0/119, 4/117	NS	
		Nasal cavity (benign)[c]: 1/118, 4/118, 6/119, 4/117	NS	
F344 rat (F) 30 mo Swenberg *et al.* (1980), Kerns *et al.* (1983a, b), Gibson (1984), CIIT (1981)	0, 2, 5.6, 14.3 ppm (0, 2.5, 6.9, 17.6 mg/m^3) 6 h/d, 5 d/wk for 24 mo 120/group	Nasal cavity (malignant)[a]: 0/114, 0/118, 1/116, 52/115*	*$P < 0.001$	> 97.5% purity Interim sacrifices (10/group) at 6 and 12 mo; 19–20 at 18 mo; 14–47 at 24 mo; 0–10 at 27 mo; 0–5 at 30 mo. Squamous-cell hyperplasia, metaplasia and dysplasia were commonly present in nasal passages of rats exposed to 14.3 ppm.
		Nasal cavity (malignant)[b]: 0/114, 0/118, 0/116, 1/115	NS	
		Nasal cavity (benign)[c]: 0/114, 4/118, 0/116, 1/115	NS	
		Haematopoietic tissue (spleen, F344 rat leukaemia diagnosed as undifferentiated leukaemia): 12/120, 17/120, 16/120, 7/120	$P = 0.0056$; Tarone-extension of the Cox test (adjustment for mortality), level of significance is $P < 0.0167$	
Sprague-Dawley rat (M) Lifetime Albert *et al.* (1982); Sellakumar *et al.* (1985)	0, 14.3 ppm (0, 17.6 mg/m^3) 6 h/d, 5 d/wk 99–100/group	Nasal cavity (malignant)[a]: 0/99, 38/100	$P \leq 0.001$	A mixed carcinoma and fibrosarcoma of the nasal cavity was also present in the formaldehyde-treated group.
		Nasal cavity (benign): 0/99, 10/100	$P \leq 0.001$	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar rat (M) 126 wk Feron *et al.* (1988)	0, 10, 20 ppm (0, 12.3 or 25 mg/m^3) 6 h/d, 5 d/wk for 4, 8 or 13 wk 45/group/interval	Nasal cavity: 2/134, 2/132, 10/132 (denominator combines all intervals of exposure for control and treated groups); the authors considered 6/10 tumours[d] in the high-dose group as treatment-related.	NR	Purity NR Hyperplasia and metaplasia of nasal epithelium were observed in all rats exposed to formaldehyde. Authors considered most nasal-cavity tumours in the high-dose group to be related to the treatment.
Wistar rat (M) 28 mo Woutersen *et al.* (1989)	0, 0.1, 1, 10 ppm (0, 0.123, 1.23, and 12.3 mg/m^3) 6 h/d, 5 d/wk for 3 or 28 mo 30/group (U: undamaged) or 60/group (D: damaged)	Nasal cavity (malignant)[e]: 28 mo exposure U: 0/26, 1/26, 1/26, 1/26 D: 1/54, 1/58, 0/56, 17/58* 3 mo exposure U: 0/26, 0/30, 0/29 2/26 D: 0/57, 2/57, 2/53, 2/54	*[$P < 0.001$; Fisher's exact test]	Purity NR Mucosa severely damaged by electro-coagulation during the first wk. Eight squamous-cell carcinomas from the nasolacrimal duct were excluded by the authors.
Sprague Dawley rat (F) 104 wk Holmström *et al.* (1989)	0, 12.4 ppm (0, 15.3 mg/m^3) 6 h/d, 5 d/wk 16/group	Nasal cavity: 0/16, 1/16[a]	NS	Purity NR Pronounced squamous-cell metaplasia and/or dysplasia in 10/16 rats exposed to formaldehyde vs 0/15 controls. Small group-size noted.
F344 rat (M) 24 mo Monticello *et al.* (1996)	0, 0.69, 2.05, 6.01, 9.93, 14.96 ppm (0, 0.84, 2.4, 7.2, 12, 19 mg/m^3) 6 h/d, 5 d/wk 90 or 147 (high dose group only)/group	Nasal cavity (malignant)[a]: 0/90, 0/90, 0/90, 1/90, 20/90, 69/147* Nasal cavity (benign)[c]: 0/90, 0/90, 0/90, 0/90, 5/90, 14/147[$] One nasal rhabdomyosarcoma and nasal adenocarcinoma each present in groups given 9.93 and 14.96 ppm	NR,*[$P < 0.001$] NR,[$][$P < 0.02$]	Formaldehyde vapour of paraformaldehyde Interim sacrifices at 3, 6, 12 and 18 mo (6/group).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 rat (M) 28 mo Kamata *et al.* (1997)	0, 0, 0.3, 2.17, 14.85 ppm (0, 0, 0.36, 2.6, 17.8 mg/m^3) 6 h/d, 5 d/wk 32/group (one room-air control and one methanol-exposed control group)	Nasal cavity (malignant)[a]: 0/32, 0/32, 0/32, 0/32, 13/32* Nasal cavity (benign)[f]: 0/32, 0/32, 0/32, 0/32, 3/32	*$P < 0.01$ NR	Formaldehyde vapour of 37% aqueous formaldehyde solution with 10% methanol (4.2 ppm) Interim sacrifices at 12, 18 and 24 mo (5/group).
Rat (strain not specified) (F) Lifetime Yanysheva *et al.* (1998)	0, 0.003, 0.03, 0.3 mg/m^3, 7 h/d, 5 d/wk for 12 mo Intratracheal injection of a total dose of 0, 0.02, 0.1 or 5.0 mg B[*a*]P/animal over 20 wk 50/group	Lung tumours: 24/35 (68.6%, 5.0 mg B[a]P + 0.3 mg/m^3 formaldehyde) vs 8/28 (28.1%, 5.0 mg B[a]P)	$P < 0.01$	Purity NR Promotion effect
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 10 ppm (0, 12.3 mg/m^3) 5 h/d, 5 d/wk 88/group (132 controls)	No tumours	-	Purity NR Hyper- and metaplastic areas were each observed in the nasal epithelium of 5% of exposed animals
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 30 ppm (0, 36.9 mg/m^3) 5 h/d, 5 d/wk 50/group	No tumours	-	Purity NR
Hamster, Syrian golden (M) Lifetime Dalbey (1982)	0, 30 ppm (0, 36.9 mg/m^3) 5 h/d, 5 d/wk Both groups subcutaneously injected wkly with 0.5 mg NDEA for 10 wk 50/group	Tracheal tumours: [~2.8 tumours/tumour-bearing animal (NDEA + formaldehyde) vs ~1.7 tumours/tumour-bearing animal (NDEA)]	$P < 0.05$	Purity NR Promotion effect
Drinking-water studies				
Wistar rat (M) 40 wk Takahashi *et al.* (1986)	Experiment #1 0 or 0.5% formaldehyde 10/group	Fore-stomach squamous-cell papillomas: 8/10 vs 0/10	$P < 0.01$	Purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar rat (M) 40 wk Takahashi *et al.* (1986)	Experiment #2 0 and 0.5% formaldehyde. MNNG administered in the drinking-water to both groups for first 8 wk with a diet containing 10% NaCl 30 (control) and 17/group	Adenocarcinoma of the pylorus: 4/17 vs 1/30	$P < 0.05$	Purity NR Weak promotion of tumour incidence in the glandular stomach. Incidence of fore-stomach papillomas was also increased vs controls, but was similar to that in the group treated with formaldehyde only (experiment #1)
Wistar rat (M) 105 wk Til *et al.* (1989)	0, 1.2, 15, 82 mg/kg bw/d 70/group	No increase in tumour incidence	NS	Formaldehyde generated from 95% pure paraformaldehyde. Interim sacrifices of 10 rats after 53 and 79 wk.
Wistar rat (F) 105 wk Til *et al.* (1989)	0, 1.8, 21, 109 mg/kg bw/d 70/group	No increase in tumour incidence	NS	Formaldehyde generated from 95% pure paraformaldehyde. Interim sacrifices of 10 rats after 53 and 79 wk.
Sprague Dawley (M, F) Lifetime Soffritti *et al.* (1989)	0 and 2 500 ppm Exposure of breeders (18–20/group/sex) and offspring (36–59/group/sex) for 104 wk. Exposure of offspring began on d 13 of gestation.	Leukaemias: Breeders (M): 2/18 (11.1%) vs 0/20 (0%) Breeders (F): 2/18 (11.1%) vs 1/20 (5%) Offspring (M): 4/36 (11.1%) vs 3/59 (5.1%) Offspring (F): 0/37 (0%) vs 3/49 (6.1%) Small intestine leiomyosarcoma: Offspring (F): 6/37 (16.2%) vs 0/49 (0%)	 NS NS NS NS $P < 0.01$	Previous and current Working Group and other authors (Feron *et al.*, 1990; IARC, 2006) noted concern about study design, significance of tumour findings and laboratory-control incidences. The Working Group also noted that leukaemias included three types, diagnosed as lymphoblastic leukaemias and lymphosarcomas, immunoblastic lymphosarcomas, and other types of leukaemias and haemolymphoreticular sarcomas. Increased incidence of a variety of benign and malignant stomach and intestinal tumours was also reported. The stomach tumours in treated groups represented single occurrences of neoplasm of different sites/cell type fore-stomach/squamous, smooth muscle and glandular stomach epithelium.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Sprague Dawley (M, F) Lifetime Soffritti *et al.* (1989, 2002)	0 (control), 0 (15 mg/L methanol control), 10, 50, 100, 500, 1 000, 1 500 mg/L for 104 wk 50/group/sex (100/control group)	Testicular interstitial-cell adenomas: 24/50 (1 000 mg/L) vs 6/50 (methanol control)	[$P < 0.01$]	Purity of 99% with 0.3% methanol as stabilizer. Concerns by the Working Group about pooling of several types of lymphohaematopoietic tumours. Concern also about interpretation of the results for lymphohaematopoietic tumours due to the numerous and extensive discrepancies in tumour incidences between the first (Soffritti *et al.*, 1989) and second report (Soffritti *et al.*, 2002) on results for this study. Increased incidence of total malignant tumours in males treated with 1 500 mg/L [$P < 0.01$].
		Lymphohaematopoietic tumours: Males 8/100, 20/50, 8/50, 20/50, 26/50, 24/50, 22/50, 46/50*	[$P < 0.01$, trend], *[$P < 0.01$, vs methanol control]	

[a] Nasal cavity tumours were squamous-cell carcinomas.
[b] Nasal cavity tumours (combined) included carcinomas (2), undifferentiated carcinomas or sarcomas (2) or carcinosarcomas (1).
[c] Nasal cavity tumours were polypoid adenomas.
[d] Three squamous-cell carcinomas, 2 polypoid adenomas, and 1 carcinoma in situ.
[e] Nasal cavity tumours were squamous-cell carcinomas except for one adenosquamous carcinoma and one adenocarcinoma at 10 ppm (D) with 28 months of exposure and one carcinoma in situ at 10 ppm (D) and one polypoid adenoma at 10 ppm (U) with 3 mo of exposure.
[f] Nasal cavity tumours were squamous-cell papillomas.
B[*a*]P, benzo[*a*]pyrene; d, day or days; h, hour or hours; F, female; M, male; mo, month or months; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; vs, versus; wk, week or weeks

4. Other Relevant Data

In *IARC Monograph* Volume 88 (IARC, 2006) mechanistic considerations supported a role for cytotoxicity and genotoxicity in formaldehyde-induced nasal tissue carcinogenesis. With regards to leukaemia, it was unclear to the Working Group at the time how this reactive compound could penetrate to the bone marrow, and no animal model of formaldehyde-induced leukaemia was available.

The discussion below focuses on mechanistic issues related to the potential causal association between formaldehyde inhalation and haematological cancers, and includes considerations on the mechanism underlying nasal carcinogenesis in laboratory animals and humans. A more detailed review can be found in Volume 88 (IARC, 2006).

4.1 Absorption, distribution, metabolism, and excretion

One-carbon metabolism is central to many biological processes, including the biosynthesis of purines and thymidine – essential components of nucleic acids –, the biosynthesis of certain amino acids, and the demethylation of a variety of important biological compounds that are central to cell function and survival. Formaldehyde is an intermediate in the one-carbon pool and is present in measurable concentrations in all metabolically active cells and tissues (Heck *et al.*, 1982, 1985; Casanova *et al.*, 1988). In aqueous solution, formaldehyde is rapidly converted to its diol form, methanediol (formaldehyde hydrate, $CH_2(OH)_2$, methylene glycol), which enters in a dynamic equilibrium with formaldehyde. The concentration of the diol and that of formaldehyde depend on the precise conditions (temperature, pH, formaldehyde concentration) under which the reaction occurs (Walker, 1964). Importantly, methanediol, with a molecular weight of only 48, can readily penetrate into tissue (Fox *et al.*, 1985). Thus, formaldehyde may reach the bone-marrow through the blood as methanediol, where it equilibrates again to reactive formaldehyde. Further investigation of this equilibrium in living biological systems is warranted.

The absorption of formaldehyde occurs readily in the upper respiratory tract (Casanova *et al.*, 1991; Kimbell *et al.*, 2001a, b). Once inhaled, formaldehyde can react directly with mucus or with macromolecular cellular components including proteins and nucleic acids; it can be incorporated into biological molecules through folate-dependent enzymatic processes; it can be oxidized to formic acid or to carbon dioxide through enzymatic processes dependent on formaldehyde dehydrogenase, aldehyde dehydrogenase and, in limited situations, catalase (Hedberg *et al.*, 2002), or itcan be exhaled. It has been estimated that as much as 22–42% of inhaled formaldehyde may be removed by mucus flow (Schlosser, 1999).

Formaldehyde reacts readily and reversibly with amino groups to form Schiff bases, and with sulfhydryl groups resulting in the formation of *S*-hydroxymethylglutathione, which is oxidized by alcohol dehydrogenase-3 (ADH3) to *S*-formylgluthahione. The latter is further metabolized by *S*-formylgluthione hydrolase to generate formate and gluthione. The formate can also be formed non-enzymatically (Hedberg *et al.*, 2002). Incubation of 0.1–5.0 mM formaldehyde with reduced glutathione in solution followed by addition to deoxyguanosine or to calf-thymus DNA leads to the formation of the relatively stable adduct *S*-[1-(N^2-deoxyguanosinyl) methyl]glutathione (Lu *et al.*, 2009). This adduct may form endogenously, as both formaldehyde and reduced glutathione are present in reasonably high concentrations within cells. It may also serve as a biomarker to study the penetration of inhaled radio-labelled formaldehyde, to distinguish endogenous from exogenous

formaldehyde-derived adducts. Whether this could also be a mechanism by which inhaled formaldehyde could lead to bone-marrow toxicity has not been studied.

Red blood cells have relatively high levels of enzymes that rapidly metabolize formaldehyde. The concentration of formaldehyde in the blood of six human volunteers did not change immediately after exposure to 1.9 ppm [2.34 mg/m^3] formaldehyde for 40 minute (Heck *et al.*, 1985), and no change in formic acid concentration was observed in the urine of medical students over a three-week period during which they were exposed to air concentrations < 0.5 ppm [0.62 mg/m^3] (Gottschling *et al.*, 1984). No statistically significant change in the concentration of formaldehyde in blood was found after inhalation of this substance at 1.9 ppm [2.34 mg/m^3] for 40 minute by six human volunteers; at 14.4 ppm [17.8 mg/m^3] for two hours in rats (Heck *et al.*, 1985); and at 6 ppm [7.4 mg/m^3] for six hours/day, five days per week, for four weeks in Rhesus monkeys (Casanova *et al.*, 1988). Blood was drawn approximately 7 min and 45 hours after the end of the exposure period, from monkeys whose blood levels were 1.84 ± 0.15 μg/g and 2.04 ± 0.40 μg/g, respectively. However, there are methodological concerns with these studies. In the monkey study three animals were used to determine control levels and three others were exposed to formaldehyde. The mean levels were then compared. It would have been better if the monkeys had served as their own control. A similar lack of change in formaldehyde levels was reported in rats (2.25 ± 0.07 *vs* 2.24 ± 0.07 μg/g blood) and humans (2.77 ± 0.28 *vs* 2.61 ± 0.14 μg/g blood; approximately 0.1 mM) exposed for short periods to 14.4 and 1.9 ppm, respectively (Heck *et al.*, 1985; Casanova *et al.*, 1988; IARC, 2006). [The Working Group noted that, given the short half-life for formaldehyde observed in rodents, the time from end-of-exposure to sampling in monkeys and in humans was likely too long; this could not be evaluated for rats].

Studies of the uptake of radio-labelled formaldehyde by inhalation, ingestion and through the skin do not provide information that would help to determine whether unreacted formaldehyde reaches the bone marrow, because it is rapidly taken up in the one-carbon pool and incorporated in macromolecules. There was no evidence of the formation of formaldehyde-specific DNA–protein crosslinks in the bone marrow of Rhesus monkeys exposed for six hours to 0.7, 2.0 and 6.0 ppm formaldehyde (Heck & Casanova, 2004), or in rats, including glutathione (GSH)-depleted rats, exposed to concentrations of formaldehyde up to 10 ppm (Casanova-Schmitz *et al.*, 1984; Casanova & Heck, 1987). The formation of formaldehyde-DNA adducts was demonstrated in lymphocytes of smokers (Wang *et al.*, 2009). In this study, liquid chromatography-electrospray ionization-tandem mass spectrometry was used to quantify the adduct N^6-hydroxymethyldeoxyadenosine (N^6-HOMe-dAdo) in leukocyte-DNA samples from 32 smokers (≥ 10 cigarettes per day) and 30 non-smokers. This adduct would be expected to be formed upon exposure to formaldehyde. N^6-HOMe-dAdo was detected in 29 of the 32 samples from smokers, but in only 7 of the 30 samples from non-smokers ($P < 0.001$). These findings would support a role for inhaled formaldehyde in causing the DNA adducts that may ultimately lead to smoking-associated leukaemia. The authors caution that the observed adducts may result from cigarette-smoke components other than formaldehyde – such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or nicotine, which may be metabolized to formaldehyde within the cell – or from other sources, rather than reflecting the actual penetration of inhaled formaldehyde to lymphocytes.

Rietbrock (1965) reported that the half-life in plasma of formaldehyde injected intravenously in the rat was about one minute. [Assuming a similar half-life of formaldehyde in humans, this would be sufficient time for inhaled formaldehyde

to reach the blood and circulate to the bone marrow in humans].

4.2 Toxic effects

Formaldehyde produces irritation of the nose and pharynx in humans and laboratory animals under a variety of circumstances. There appears to be a large inter-individual variation in the human response to the irritating effects of formaldehyde. Under controlled exposure conditions, symptoms of irritation were noted by healthy individuals exposed to formaldehyde concentrations of 2–3 ppm during periods that varied between 40 minute and three hours (for details, see Table 30 in *IARC Monograph* Volume 88 (IARC, 2006)).

Formaldehyde is a known cause of allergic contact dermatitis and, somewhat more controversial, of occupational asthma. Nasal biopsies of workers chronically exposed to formaldehyde showed chronic inflammation, loss of cilia, mild dysplasia, hyperplasia and squamous metaplasia, although the latter finding has been inconsistent and may have been confounded by other exposures, such as to wood dust (IARC, 2006).

The cytotoxicity of formaldehyde has been confirmed in numerous in-vitro systems. Irritation of the nasal and upper respiratory tract is also noted in animal studies. Dose-dependent pathological findings include inflammation, hyperplasia, degenerative changes, necrosis and squamous metaplasia.

Recently, a finding relevant to the possible involvement of formaldehyde in leukaemogenesis was reported by Zhang *et al.* (2010). Their study showed that colony formation by colony-forming unit-granulocyte-macrophage (CFU-GM) progenitor cells is inhibited in cell cultures exposed to formaldehyde at toxicologically relevant concentrations. Furthermore, colony formation by the more primitive CFU-granulocyte- erythrocyte-monocyte-megakaryocyte (CFU-GEMM) progenitors, which give rise to formation of all myeloid cells, showed a linear negative dose–response when treated with formaldehyde. These effects were observed at formaldehyde concentrations of 100–200 μM [3–6 μg/mL], which are toxicologically relevant since background levels of formaldehyde in human blood have been reported to be 50–100 μM [1.5–3 μg/mL] (Heck *et al.*, 1985; Casanova *et al.*, 1988). Because the CFU-GEMM multipotent myeloid progenitor cells and the pluripotent stem cells are the target cells for leukaemogenesis and are converted to leukaemic stem cells in acute myeloid leukaemia, the finding that formaldehyde damages these cells *in vitro* adds some weight to the notion that it may be associated with myeloid leukaemia.

4.3 Genetic and related effects

The genotoxicity of formaldehyde was thoroughly reviewed in *IARC Monograph* Volume 88 (IARC, 2006). Genotoxicity has been observed *in vitro* in many systems with multiple endpoints.

4.3.1 Humans

Micronucleus formation has been repeatedly reported to occur in cells of the nasal and oral mucosa of formaldehyde-exposed humans. The outcome of studies on induction of micronuclei, sister chromatid exchange and chromosomal aberrations in the lymphocytes of exposed humans – which is pertinent to the question concerning the potential of formaldehyde to cause lympho-haematopoietic cancer – has been less consistent (see Table 4.1).

DNA–protein crosslinks in circulating white blood cells were found to be higher in 12 workers exposed to formaldehyde in an anatomy department and a pathology institute than in eight controls (P = 0.03) (Shaham *et al.*, 1996). The number of crosslinks tended to be higher in workers who had been exposed longer (exposure duration, 2–31 years). Smoking had no effect. In a

Table 4.1 Cytogenetic studies on formaldehyde–exposed humans

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Costa *et al.* (2008), Portugal	Workers in an anatomy-pathology laboratory (n = 30) Controls non-exposed (n = 30)	0.44 ± 0.08 ppm (range 0.04–1.58)	Micronucleus formation in lymphocytes (MN/1000 cells)	5.47 ± 0.76	3.27 ± 0.69	P = 0.003
			Sister-chromatid exchange(SCE)	6.13 ± 0.29	4.49 ± 0.16	P < 0.05
			DNA-breakage (comet tail length, µm) in lymphocytes	60.00 ± 2.31	41.85 ± 1.97	P < 0.05
Pala *et al.* (2008), Italy	Laboratory workers (n = 36) in a cancer research institute	Exposure range: 4.9–268.7 µg/m^3 Low: < 26 µg/m^3 High: ≥ 26 µg/m^3		High:	Low:	
			Micronucleus formation (MN)	MN: 0.31 ± 0.17	MN: 0.26 ± 0.24	
			Chromosomal aberrations (CA)	CA: 2.22 ± 1.27	CA: 2.95 ± 1.79	
			SCE in lymphocytes	SCE: 5.06 ± 0.76	SCE: 6.57 ± 1.38	
Iarmarcovai *et al.* (2007), France	Pathologists/anatomists (n = 18) Controls (n = 18)	2.3 ppm (range 0.4–7 ppm)	MN in lymphocytes (MN/1000 cells)	21.0 ± 12.6	14.4 ± 8.1	P < 0.01
Yu *et al.* (2005), the People's Republic of China	Workers (n = 151; low-exposed, 62; high-exposed, 89) in two plywood factories; 112 non-exposed controls.	TWA: 0.10–7.88 mg/m^3 (< 0.01 mg/m^3 in controls)	MN in lymphocytes (MN/1000 cells)	High exp. 6.5 ± 3.6 Low exp. 4.1 ± 2.5	2.7 ± 1.3	P < 0.05
			DNA-breakage (comet tail length, µm) in lymphocytes	High exp., 12.6 (95% CI: 11.8–13.4) Low exp., 11.3 (95% CI: 10.1–12.5)	6.8 (95%CI: 6.1–7.6)	P < 0.05
Ye *et al.* (2005), the People's Republic of China	Workers (n = 18; all non-smokers) in a formaldehyde factory (mean exposure duration, 8.5 yrs), and waiters (n = 16; all non-smokers) exposed to formaldehyde for 12 wk in a newly fitted ballroom. Controls were 23 students (non-smokers)	Factory: 8-h TWA, 0.99 ± 0.29 mg/m^3, with peak exposure 1.69 mg/m^3. Ballroom: 5-h TWA 0.11 ± 0.07 mg/m^3, with peak exposure 0.3 mg/m^3 Control: 0.01 ± 0.003 mg/m^3, with peak 0.015 mg/m^3	MN in nasal mucosa (MN/1000 cells) of factory workers	2.70 ± 1.50	1.25 ± 0.65	P < 0.05
			SCE in lymphocytes of factory workers	8.24 ± 0.89	6.38 ± 0.41	P < 0.05
			No effects in ballroom waiters			

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Shaham *et al.* (2002), Israel	Workers (n = 90) in hospital pathology departments; controls were 52 unexposed workers from the same hospitals. Average duration of exposure 15.4 yrs (range, 1–39)	Low: 0.4 ppm (0.04–1.7) High: 2.24 ppm (0.72–5.6)	SCE in lymphocytes (mean number of SCE per chromosome)	0.27 ± 0.003	0.19 ± 0.002	$P < 0.01$
Burgaz *et al.* (2002), Turkey	a) Workers (n = 22) in a shoe factory	n-hexane, toluene, methyl ethyl ketone	MN in buccal cells (MN/1000 cells)	a) 0.62 ± 0.45		$P < 0.05$
	b) Pathologists (n = 28), anatomy laboratories	2–4 ppm formaldehyde		b) 0.71 ± 0.56		$P < 0.05$
	c) Controls (n = 18): university staff	none			c) 0.33 ± 0.30	
Burgaz *et al.* (2001), Turkey	Workers (n = 23) in pathology and anatomy laboratories, and 25 non-exposed controls	Air concentration of formaldehyde in the breathing zone 2–4 ppm.	MN in nasal mucosa (MN/1000 cells; mean ± SD).	1.01 ± 0.62	0.61 ± 0.27	$P < 0.01$
Suruda *et al.* (1993), USA	Students (n = 29) taking 85-d course in embalming; average number of embalmings, 6.9; total number 144.	Average air concentration of formaldehyde: 1.4 ppm, range 0.15–4.3 mean duration of embalming 125 minute with peak exposures > 4 ppm.	MN per 1 000 cells:	MN after course	MN before course	
			- buccal cells	0.60 ± 1.27	0.046 ± 0.17	$P < 0.05$
			- nasal cavity	0.50 ± 0.67	0.41 ± 0.52	NS
			- lymphocytes	6.36 ± 2.03	4.95 ± 1.72	$P < 0.05$
			SCE in lymphocytes	7.14 ± 0.89	7.72 ± 1.26	$P = 0.01$ (decrease in SCE)
Ying *et al.* (1999), China	Students (n = 23; non-smokers) in 8-wk (3h x 3 times weekly) anatomy class.	TWA 0.51 ± 0.30 mg/m^3, with peak exposure 1.28 Background level in dormitories 0.012 ± 0.0025 mg/m^3		SCE after course	SCE before course	
			SCE in lymphocytes	6.61 ± 0.79	6.38 ± 0.41	NS

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
He *et al.* (1998), China	Students (n = 13; non-smokers) in 12-wk (10h per wk) anatomy class. Ten other students (non-smokers) served as non-exposed controls.	Average air concentration of formaldehyde: 2.37 ppm (3.17 mg/m^3)	MN per 1 000 cells in lymphocytes	6.38 ± 2.50	3.15 ± 1.46	$P < 0.01$
			CA in lymphocytes	5.92 ± 2.40	3.40 ± 1.57	$P < 0.01$
			SCE in lymphocytes	5.91 ± 0.71	5.26 ± 0.51	$P < 0.05$
Ying *et al.* (1997), China	Students (n = 25; non-smokers) in 8-wk (3h x 3 times weekly) anatomy class.	TWA 0.51 ± 0.30 mg/m^3, with peak exposure 1.28 Background level in dormitories 0.012 ± 0.0025 mg/m^3	MN per 1 000 cells in			
			- nasal exfoliated cells	3.84 ± 1.48	1.20 ± 0.67	$P < 0.001$
			- oral mucosa cells	0.86 ± 0.56	0.57 ± 0.32	$P < 0.01$
			- lymphocytes	1.11 ± 0.54	0.91 ± 0.39	n.s.
Titenko-Holland *et al.* (1996), USA	Students (n = 28) taking a 90-d embalming class; average number of embalmings, 6.9; total number 144.	Accumulated dose over 90 d: 4.3–26.4 ppm Maximum peak exposure: 2.86 ppm	MN per 1 000 cells with centromere-specific probes (FISH) in:	MN after class	MN before class	[between parentheses: values for centromere-negative MN]
			- buccal cells	2.0 ± 2.0 (0.9 ± 1.1)	0.6 ± 0.5 (0.1 ± 0.2)	$P = 0.007$ ($P = 0.005$)
			- nasal cells	2.5 ± 1.3 (1.0 ± 0.6)	2.0 ± 1.3 (0.5 ± 0.5)	$P = 0.20$ ($P = 0.03$)

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Dobiás *et al.* (1988), Czechoslovakia	- Schoolchildren (n = 20 children) in school building constructed with wood-particle boards - Control group (n = 17 children) in brick school building in the same area - Control group in brick school building elsewhere	Formaldehyde concentration in air: 1984: 317.0 µg/m^3 1985: 130.0 µg/m^3 1986: 36.5 µg/m^3	CA in lymphocytes (per 100 cells) in lymphocytes	1984: 7 1985: 4.2 1986: NR	1984: 4.2 NR NR The frequency of chromosomal aberrations is stated 'normal' in 1986 but the value is not given	
		Building was cleaned in '85, '86	Percentage of aberrant cells	1984: 4.71 ± 2.09 1985: 2.82 ± 1.64 1986: 2.06 ± 1.51	1984: 1.37 ± 0.87 1985: 1.4 ± 0.79 NR	P = 0.005
Bauchinger & Schmid (1985), Germany	Workers (n = 20; 6 smokers) in a paper factory, where formaldehyde was used for impregnating the paper. Duration of exposure 2–30 yrs, average 14.5 ± 7.2 yrs. Controls were 20 non-exposed workers (13 smokers) elsewhere in the factory	Formaldehyde exposure, < 0.2 ppm Peak during repair and cleaning, 3 ppm	CA in lymphocytes (dicentrics, rings) per 100 cells SCE/cell ± SE	0.13 ± 0.05 8.87 ± 0.24	0.05 ± 0.02 9.53 ± 0.35	 NS

CA, chromosomal abberations; d, day or days; h, hour or hours; min, minute or minutes; MN, micronuclei; NR, not reported; NS, not significant; SCE, sister chromatid exchange; TWA, time-weighted average concentration

subsequent study, Shaham *et al.* (2003) reported an increase in the number of DNA–protein crosslinks in lymphocytes and in serum concentrations of the p53 protein in hospital pathology-department workers. The exposed subjects were assigned to a high-exposure subgroup (mean formaldehyde concentration in air, 2.24 ppm) and a low-exposure subgroup (mean, 0.4 ppm), based on personal sampling and field sampling for 15-minute periods on typical working days. The control group consisted of personnel of the administrative sections in the hospital. The amount of protein–cross-linked DNA was statistically significantly higher in the exposed group than in the controls (0.20 *vs* 0.14; these values are the ratios between protein-bound DNA – precipitable with sodium dodecyl sulfate – and total DNA) after controlling for age, smoking and other factors. Very little difference in DNA–protein crosslink levels was observed between the high- and low-exposure groups (0.20 *vs* 0.19); or between workers with > 16 years or < 16 years of exposure (0.20 *vs* 0.19). The percentage of formaldehyde-exposed male workers who had pantropic p53-protein (wild-type plus mutant p53) concentrations in serum higher than 150 pg/ml was statistically significantly greater than in the control group (54.8% *vs* 36.5%, $P < 0.05$; this difference was not seen in female workers). Formaldehyde-exposed workers with DNA–protein crosslink levels above the median had a significantly greater likelihood of having p53 concentrations in serum above 150 pg/ml. [The Working Group noted that the rationale for using a p53-protein level of 150 pg/ml as a cut-point was based upon previous experience with this assay; no reason is given for using 16 years as the cut-point between longer/shorter exposure. Questions have also been raised about the persistence of DNA–protein crosslinks, which are thought to be rapidly repaired within the cell (Schmid & Speit, 2007)]. In an earlier study, Casanova-Schmitz *et al.* (1984) failed to observe DNA–protein crosslinks in bone marrow of Fischer-344 rats exposed to [^{14}C]- and [^{3}H]-formaldehyde.

Compared with matched controls, pathology/anatomy workers from five hospitals, who were exposed to mean formaldehyde concentrations of 2.0 ppm (range, < 0.1 to 20.4 ppm) during 15 minute, or to 0.1 ppm (range < 0.1 to 0.7 ppm) during 8 hours, showed a statistically significant increase in bi-nucleated cells and in mono-centromeric micronucleus formation in a cytokinesis-blocked micronucleus assay combined with fluorescence in situ hybridization (FISH) (Orsière *et al.*, 2006).

A higher frequency of micronuclei was found in exfoliated nasal and oral cells of students with short-term exposure (3 hours per day; 3 days per week, for 8 weeks) to an average of 0.508 ± 0.299 mg/m^3 formaldehyde, compared with controls (Ying *et al.*, 1997, 1999). No increase in micronucleus formation or in the level of sister chromatid exchange (SCE) was observed in lymphocytes. Ye *et al.* (2005) reported a comparative analysis of 18 workers involved for various periods (mean, 8.5 years; range, 1–15 years) in a formaldehyde-manufacturing process, 16 waiters exposed for 12 weeks to an indoor source of formaldehyde during interior renovations, and a control group of 23 students; all were non-smokers. Average formaldehyde exposure-concentrations were 0.011 mg/m^3 for the student controls; 0.107 mg/m^3 for the waiters; and 0.99 mg/m^3 for the formaldehyde-plant workers. There was a statistically significantly higher frequency of micronuclei in nasal mucosal cells and of SCE in peripheral lymphocytes in the workers at the formaldehyde-manufacturing plant, but not in the waiters, although both groups were exposed to formaldehyde at comparable concentrations (Table 4.1). The result is in line with the much longer exposure duration for the plant workers. The same authors (Ye *et al.*, 2005) reported an increase in B-cells and changes in the ratios between lymphocyte subsets, similar to those reported by Madison *et al.* (1991) in an

Alaskan community subject to acute formaldehyde exposure (estimated at 2–5 ppm) for a few days. Blood analyses were done three years after the accident. Total white blood cell counts and total lymphocyte counts did not differ from those in the control community in Alaska, also measured three years later (Madison *et al.*, 1991).

An increase in SCE and other genotoxic effects were observed in the lymphocytes of workers with long-term exposure to formaldehyde (Costa *et al.*, 2008). Thirty workers from four pathology/anatomy hospital units and 30 matched controls were included in the study. Compared with the control group, statistically significant effects were seen in SCE (6.13 ± 0.29 *vs* 4.49 ± 0.16 SCE/cell, $P < 0.05$), micronucleus frequency (in 1000 bi-nucleated cells: 5.47 ± 0.76 ‰ *vs* 3.27 ± 0.69 ‰, $P = 0.003$) and tail length in the comet assay (60.0 ± 2.31 µm *vs* 41.85 ± 1.97 µm, $P < 0.05$). A statistically significant positive correlation was found between formaldehyde exposure levels and micronucleus frequency and tail length. The mean formaldehyde exposure was 0.44 ppm (range, 0.04–1.58 ppm). None of the observed effects were related to the duration of exposure.

No genotoxic effects were observed in a study of 36 laboratory workers at a cancer-research institute who were exposed to 4.9–268.7 µg/m^3 formaldehyde. There was a direct relationship between formaldehyde exposure levels and the presence of a formaldehyde human serum-albumin (FA-HSA) conjugate. The genotoxic endpoints measured were SCE, micronuclei and chromosome aberrations, but these did not show significantly elevated levels (Pala *et al.*, 2008). Although a small study, its strength is its linkage to a biological marker of formaldehyde exposure.

Hayes *et al.* (1997) evaluated O^6-Alkylguanine-DNA–alkyltransferase (AGT) activity as a measure of DNA-repair capacity in blood lymphocytes of 23 science students in a mortuary, before and after a nine-week period of classroom exposure to approximately 1.5 ppm formaldehyde. A statistically significant finding was that more students had a reduction in AGT activity than an increase. There was no clear link between the extent of exposure to formaldehyde and AGT activity.

Zhang *et al.* (2010) cultured myeloid progenitor cells from the peripheral blood of formaldehyde-exposed workers and controls and measured leukaemia-specific chromosomal changes. In a subset of ten of the most highly exposed subjects in their study, monosomy (loss) of chromosome 7 and trisomy (gain) of chromosome 8 were significantly elevated in the myeloid progenitor cells of formaldehyde-exposed workers compared with the same phenomena in 12 unexposed controls. The loss of chromosome 7 and gain of chromosome 8 were examined because they are among the most frequent cytogenetic changes observed in myeloid leukaemia and myelodysplastic syndromes; these events have been shown to be affected by exposure to the established human leukemogen, benzene. [The Working Group noted that the study is small and needs to be replicated].

4.3.2 Experimental systems

(a) In-vivo studies (laboratory animals)

Studies on a variety of genotoxic endpoints in laboratory animals inhaling formaldehyde have generally shown effects in the nasal tissues of these animals (IARC, 2006). Much less consistent have been the findings of genotoxic effects in the blood lymphocytes from exposed animals. Among the recent studies, Im *et al.* (2006) reported genotoxicity based on a positive result in the comet assay in the lymphocytes of rats inhaling 5 or 10 ppm formaldehyde for two weeks, six hours/day, five days/week. In contrast, in a review of their own work and of the literature, Speit *et al.* (2009) concluded that there was no evidence of systemic genotoxic effects in laboratory animals inhaling formaldehyde. Their own negative studies in this review focused on

lymphocyte genotoxicity, measured as micronuclei, SCE, and DNA-breakage – the latter determined with a sensitive form of the comet assay – in rats exposed for four weeks (six hours/day, five days/week) to formaldehyde concentrations of 0.5, 1, 2, 6, 10 and 15 ppm.

More recently, DNA strand-breaks were induced by formaldehyde *in vivo*, in mouse liver (maternal and fetal), and in lung cells in the rat (Wang & Liu, 2006; Sul *et al.*, 2007).

(b) In-vitro studies

The spectrum of mutations related to exposure to formaldehyde *in vitro* and *in vivo* was presented and discussed in *IARC Monograph* Volume 88 (IARC, 2006). In-vitro studies since then have expanded the wide range of potential mutagenic mechanisms, to include the hydroxymethylation of DNA and DNA-microsatellite instability (Zhong & Que Hee, 2004; Wang *et al.*, 2007).

The evidence of formaldehyde-induced mutations in various experimental systems is consistent, encompassing both clastogenic effects and direct DNA mutation. Formaldehyde showed mutagenic potential in several bacterial systems, both with and without S9 activation. Formaldehyde induced deletions, point mutations, insertions, and cell transformation in in-vitro assays with mammalian cells (IARC, 2006).

Formaldehyde-induced DNA strand-breaks (SSB) have been demonstrated in several mammalian cell systems, including hepatocytes, lymphosarcoma cells, and epithelial cells from the rat, leukaemia L1210 cells from the mouse, and lung/bronchial epithelial cells, skin fibroblasts, keratinocytes, and peripheral blood lymphocytes from humans (IARC, 2006).

Chromosomal aberrations, micronuclei and SCE were all increased *in vitro* in numerous rodent and human primary cells and cell lines treated with formaldehyde (IARC, 2006). Consistent with these findings, more recent data show increased numbers of chromosomal aberrations in Syrian hamster embryo cells, and chromosomal aberrations and SCE in Chinese hamster ovary and embryo cells (Hikiba *et al.*, 2005, Hagiwara *et al.*, 2006; Lorenti Garcia *et al.*, 2009).

Further evidence of formaldehyde-induced micronucleus formation was obtained in studies with human lymphocytes isolated from whole-blood cultures exposed *in vitro* to formaldehyde, 44 hours after the start of the culture (Schmid & Speit, 2007). Both micronuclei and SCE were induced upon in-vitro treatment of Chinese hamster V79 lung epithelial cells with formaldehyde (Speit *et al.*, 2007). In a recent study, SCE was induced in A549 human lung cells and V79 Chinese hamster cells following incubation with 0.1 mM [3 µg/mL] or higher concentrations of formaldehyde. One hour after the addition of the agent to the A549 cells, the culture medium still retained the capacity to produce SCE in non-exposed V79 cells, suggesting that genotoxicity persists despite the high reactivity of formaldehyde with macromolecules in the culture medium (Neuss & Speit, 2008). When the formaldehyde-exposed A549 cells were washed and then suspended in fresh culture medium containing the V79 cells, SCE formation was not observed in the latter. [The authors present no evidence that it is formaldehyde itself that persists, rather than a formaldehyde product that is responsible for genotoxicity. The Working Group noted that there is no reason to preclude the transfer of formaldehyde from cell to cell].

Formaldehyde has also been reported to interfere with DNA repair. A recent finding that chicken DT40 cells deficient in the FANC/BRCA (Fanconi's anaemia complementation groups/breast cancer A) pathway are hypersensitive to formaldehyde in plasma, is consistent with a role for this pathway in repairing DNA–protein cross-links caused by formaldehyde. The DT40 mutants were also more sensitive to acetaldehyde, but not to acrolein and other aldehydes (Ridpath *et al.*, 2007). Endogenous formaldehyde may be

important in producing leukaemia in patients with Fanconi's anaemia, a genetic disorder that is characterized by progressive pancytopenia. DT40 cells with deficient repair mechanisms have also been shown to be more sensitive to other cross-linking agents such as cisplatin, a myelotoxic chemotherapeutic agent that leads to pancytopenia and acute myelogenous leukaemia (AML) (Nojima *et al.*, 2005).

4.4 Mechanistic considerations

4.4.1 Cancer of the nasopharynx and nasal sinuses

Mechanistic evidence supporting a causal relation between inhalation of formaldehyde and induction of cancer of the nasopharynx and nasal sinuses is based on the chemical reactivity of formaldehyde in producing DNA–protein cross-links, and its genotoxicity *in vitro* and *in vivo*, including in the nasal cells of exposed humans. Computational fluid-dynamic models of formaldehyde in the nasal passages of rats, monkeys and humans have generally been accurate in predicting the area in the nose with the highest number of DNA–protein crosslinks (Georgieva *et al.*, 2003). Local effects in the nasal passages, genotoxicity, and cell-proliferation rate appear to be the major determinants of nasal carcinogenicity after exposure to formaldehyde.

4.4.2 Leukaemia

The findings reviewed in *IARC Monograph* Volume 88 (IARC, 2006) pertaining to a potential mechanism for formaldehyde-induced leukaemogenesis were summarized as follows: "Based on the data available at this time, it was not possible to identify a mechanism for the induction of myeloid leukaemia in humans." The Working Group further stated that "It is possible that formaldehyde itself can reach the bone marrow following inhalation, although the evidence is inconsistent." Since that time, Zhang *et al.* (2009), reviewed potential pathways by which formaldehyde could act as a leukaemogen. Three mechanisms were suggested:

- by damaging stem cells in the bone marrow directly, as most other leukaemogens do;
- by damaging haematopoietic stem/progenitor cells circulating in the peripheral blood and
- by damaging the primitive pluri-potent stem cells present within the nasal turbinates and/or olfactory mucosa.

This subject was reviewed by Heck & Casanova (2004), Pyatt *et al.* (2008), and Goldstein (2011).

(a) Studies in animals

Studies of bone marrow cells in formaldehyde-exposed animals have been inconsistent. Kitaeva *et al.* (1990) described clastogenic and cytogenetic effects in the bone marrow of rats inhaling 0.5 mg/m^3 or 1.5 mg/m^3 of formaldehyde during four hours/day for four months. In contrast, Dallas *et al.* (1992) found no evidence of cytogenetic abnormalities in the bone marrow of rats exposed to 0.5, 3 or 15 ppm [0.62, 3.7 or 18.45 mg/m^3] formaldehyde for six hours/day, five days per week, for one or eight weeks. Mice that received up to 25 mg/kg bw formaldehyde in two intra-peritoneal injections within 24 hours showed no increase in chromosomal aberrations or micronuclei in the femoral bone marrow (Natarajan *et al.*, 1983). As described in section 4.1 above, no increase in formaldehyde-specific DNA–protein cross-links was observed in the bone marrow of Rhesus monkeys or rats under various experimental conditions (Heck & Casanova, 2004).

(b) Considerations of formaldehyde as a leukaemogen in relation to other known myeloleukaemogens

Known myeloid leukaemogens in humans include benzene, ionizing radiation and a variety of chemotherapeutic anti-neoplastic agents, all of which give rise to pancytopenia. There is evidence that for each of these myeloleukaemogens, pancytopenia is caused by genotoxic damage leading to destruction of primitive progenitor cells in the bone marrow. These cells are responsible for the formation of red blood cells, white blood cells and platelets, and they are the same progenitor cells in which mutations and clonal expansion leads to myeloid leukaemia.

In view of the wide variety of genotoxic mechanisms shown by the diverse agents that have pancytopenia and myeloleukaemogenesis in common, it could be anticipated that genotoxic effects of formaldehyde on myeloid progenitor cells would also result in pancytopenia.

Pancytopenia has not been among the haematological findings in experiments with laboratory animals exposed to relatively high doses of formaldehyde, including classic long-term safety assessment studies. An increase in haemoglobin and monocytes and a decrease in lymphocytes were observed in rats receiving 0, 20, 40, or 80 mg/kg bw formaldehyde by gastric intubation on five days/week for four weeks. Lymph-node weights were increased but no change in lymph-node cellularity was observed (Vargová *et al.*, 1993). In one long-term study there was actually a statistically significant increase in bone-marrow hyperplasia in rats exposed to formaldehyde at 15 ppm (Batelle, 1981), the opposite of what would be expected for an agent that has effects similar to those of other known myeloleukaemogens.

In contrast to the findings in laboratory animals, there has been some evidence suggesting a mild pancytopenic effect in humans. A study of 50 haemodialysis nurses exposed to formaldehyde compared with 71 non-exposed ward nurses from five different hospitals comprised measurements of formaldehyde and two different blood counts recorded one a year apart. Both personal and ambient measurements of formaldehyde varied widely, from non-detectable up to 2.8 ppm. Average duration of employment was three years for both groups. Symptoms attributable to formaldehyde were reported in the exposed group. For the second blood count, but not the first, there was a statistically significant inverse correlation ($P < 0.05$) between white blood-cell count and formaldehyde concentration, as well as between white blood-cell count and symptom score. No statistically significant correlation was observed between formaldehyde concentrations or symptoms and platelet or red blood-cell counts. The exposed group had a lower white blood-cell count than the control group (Kuo *et al.*, 1997). [The Working Group noted that absolute data for blood counts were not given, nor was the statistical methodology described]

An increase in B-lymphocytes and changes in ratios of lymphocyte subsets were noted in formaldehyde-plant workers exposed to an average of 0.99 mg/m^3 formaldehyde for a mean duration of 8.5 years (Ye *et al.*, 2005). Differences in the ratios of lymphocyte subsets were also observed in an Alaskan community with an acute formaldehyde exposure (2–5 ppm for a few days), but no differences with the control community were seen in total white blood-cell counts or lymphocyte counts (Madison *et al.*, 1991). Likewise, no significant differences in blood counts were found in a comparative study of students of two schools, in one of which there were elevated concentrations of formaldehyde and toluene (Vozenílková *et al.*, 1991).

In a review of formaldehyde exposure in the People's Republic of China, Tang *et al.* (2009) mentioned eight studies on formaldehyde-exposed individuals. Lower white blood-cell counts were observed in the six studies that provided information on this point, four of which were statistically significant; platelet

counts were decreased in all three studies where this was measured, two of which were statistically significant; and haemoglobin was lower in one of the three studies for which data were reported. The one study that found lower than normal values for each blood count – consistent with a pancytopenic effect – was specifically the study with the lowest exposure to formaldehyde (0.022–0.044 mg/m^3), although the largest cohort. [From the Table in the Tang *et al.* (2009) paper, it is not clear whether these are the same individuals or separate individuals who have each of the lower counts, i.e. how many were pancytopenic. There also is no information about the usual confounders, including gender and age].

The finding of statistically significant, moderately lower blood counts in formaldehyde-exposed Chinese workers as compared to a matched control group would be consistent with formaldehyde-induced damage to either circulating haematopoietic precursor cells, or with a direct effect on such cells within the bone marrow (Zhang *et al.*, 2010). In this study the 43 exposed workers at a formaldehyde-melamine producing factory or a factory in which formaldehyde-melamine resins were used to produce utensils, were exposed to a median of 1.28 ppm formaldehyde (10–90%, range 0.63–2.51 ppm; 8-hour time-weighted average), compared with a median level, in a matched control group of 51 individuals, of 0.026 ppm (10–90%, range 0.0085–0.026 ppm). Absolute blood counts were only given for total white blood-cell counts: in controls, mean (SD) 6269 (1452) cells per µl blood; and in exposed: mean 5422 (1529) cells per µl blood, $P = 0.0016$. Data for the other blood counts are presented in a bar chart, and for red blood cells, platelets, granulocytes and lymphocytes there are small but statistically significant decreases that appear to fall within the clinical range of normal. Also of note is a statistically significant increase in the mean corpuscular volume (MCV) of red blood cells. The MCV tends to be increased in myelodysplastic conditions. The study appeared to have adequately taken into account possible confounders such as alcoholism and nutritional issues that might cause pancytopenia and an increased MCV.

(c) Leukaemogenesis on the basis of reactions with myeloid stem cells within the nose

As indicated above, Zhang *et al.* (2009) have suggested that one mechanism of formaldehyde-induced leukaemogenesis might involve reaction of formaldehyde or a reactive formaldehyde derivative with myeloid precursors present within the nose. This has been questioned on two indirect grounds (Goldstein, 2011). Nasal tissue does not seem to have been reported as a location for chloromas, which are isolated collections of myeloid leukaemia cells, despite the presence of chloromas in virtually all other tissues. Second, known nasal carcinogens, including cross-linking agents such as nickel and chromium, are not reported to cause an increase in acute myelogenous leukaemia. The one possible exception is sulfur mustard, a nasal carcinogen for which an increase in leukaemia (13 deaths observed; 8.51 expected; not statistically significant) was reported by Easton *et al.* (1988) in workers producing sulfur mustard gas during World War II. However, this agent also produces pancytopenia, an outcome that led to the development of nitrogen mustard as a chemotherapeutic compound. None of the other known human nasal carcinogens has been reported to cause pancytopenia.

(d) Formaldehyde and lymphoid cancers

Genotoxicity studies on blood lymphocytes from laboratory animals that inhaled formaldehyde have tended to be negative, although not consistently so. In comparison, somewhat more studies with the lymphocytes of humans exposed to formaldehyde have reported genotoxicity, although the findings are also inconsistent. Genotoxicity in circulating lymphocytes would be consistent with the possibility that

formaldehyde is a cause of lymphatic tumours. Particularly at risk would be mucosa-associated lymphatic tissue in the nasal area.

The evolution of our understanding of lymphohaematopoitetic cancers has led to ongoing reclassification of these tumours. There is also recognition of their inter-relatedness through a common stem cell, and the fact that there is a risk for malignant transformation during various stages of the differentiation and maturation process of the precursor cells. Recent evidence suggests that an underlying cytogenetic abnormality in an early precursor cell predisposes to subsequent mutations leading to a specific haematological cancer. The possibility of a mutagenic effect of formaldehyde on circulating lymphocytes or local lymphatic tissue cannot be excluded.

4.5 Synthesis

The current data strongly indicate that genotoxicity plays an important role in the carcinogenicity of formaldehyde in nasal tissues in humans, and that cellular replication in response to formaldehyde-induced cytotoxicity promotes the carcinogenic response. Three possible mechanisms, all focused around genotoxicity, are moderately supported as the underlying mechanism for induction of haematological malignancies in humans. Further research is needed to decide which of the mechanisms is the most important.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of formaldehyde. Formaldehyde causes cancer of the nasopharynx and leukaemia.

Also, a positive association has been observed between exposure to formaldehyde and sinonasal cancer.

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

The Working Group was not in full agreement on the evaluation of formaldehyde causing leukaemias in humans, with a small majority viewing the evidence as sufficient of carcinogenicity and the minority viewing the evidence as limited. Particularly relevant to the discussions regarding sufficient evidence was a recent study accepted for publication which, for the first time, reported aneuploidy in blood of exposed workers characteristic of myeloid leukaemia and myelodysplastic syndromes, with supporting information suggesting a decrease in the major circulating blood-cell types and in circulating haematological precursor cells. The authors and Working Group felt that this study needed to be replicated.

Formaldehyde is *carcinogenic to humans (Group 1)*.

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SULFUR MUSTARD

Sulfur mustard, also known as mustard gas, was considered by previous IARC Working Groups in 1975 and 1987 (IARC, 1975, 1987a). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 505-60-2
Chem. Abstr. Serv. Name:
1,1′-Thiobis(2-chloroethane)
Synonyms: Sulfur mustard, mustard gas
Description: Colourless, oily liquid; forms prisms on cooling (O'Neill, 2006)
Melting-point: 13–14 °C (O'Neill, 2006)
Vapour pressure: 0.90 mm Hg at 30 °C (O'Neill, 2006)
Solubility: Very sparingly soluble in water; soluble in fat solvents and other common organic solvents; high lipid solubility (O'Neill, 2006)
Octanol-water partition coefficient: log K_{ow}, 2.41 (HSDB, 2009)

$$Cl—CH_2—CH_2—S—CH_2—CH_2—Cl$$

$C_4H_8Cl_2S$
Relative molecular mass: 159.1

1.2 Uses

Vesicants or blistering agents were among the first chemicals that were applied as lethal/tactical weapons during World War I. Mustard agents, also known as sulfur mustard or mustard gas, were the most widely used (WHO, 1970).

Mustard gas was first used during World War I during the battle of Flanders, near Ypres, Belgium, in July 1917 (the French name for mustard gas is Ypérite). It was then used in 1918 and again in Ethiopia in 1936. During World War II, mustard gas was the major chemical warfare agent; it was produced and stockpiled by many countries and is probably still the most distributed chemical warfare agent in the world (Szinicz, 2005). Mustard gas has more recently been used in the Egypt-Yemen conflict (1963–67) and in the war between Iraq and the Islamic Republic of Iran in 1984 (ATSDR, 2003; WHO, 2004).

On April 29, 1997, the Chemical Weapons Convention took effect. This Convention banned the development, production, acquisition, stockpiling, and transfer (direct or indirect), of chemical weapons. It prohibits the use of chemical weapons, the engagement in any military preparations aimed at using chemical weapons

and the encouragement, induction, or assistance with such activities. Each participating/signing state is commited to take measures to destroy their own chemical weapons and production facilities and to not use riot-control agents as a method of warfare. To oversee compliance with the Chemical Weapons Convention, the Organization for Prohibition of Chemical Weapons was created. It is based in The Hague, the Netherlands (Szinicz, 2005).

Sulfur mustard has been used as an antineoplastic agent without success, because of its high toxicity. A similar product, nitrogen mustard, has been successfully employed as an anticancer agent (IARC, 1975; Saladi *et al.*, 2005). Mustard gas/sulfur mustard has provided a useful model in biological studies on the mode of action of alkylating agents (IARC, 1975). It has also been used medicinally to control hyper-proliferation of psoriatic keratinocytes (ATSDR, 2003).

1.3 Human exposure

1.3.1 Occupational exposure

Occupational exposure to mustard gas may occur in the following activities or industrial sectors: storage and destruction of mustard gas; construction work on military bases where mustard gas was previously released and remained as a contaminant in the soil or in excavated munitions dumps; activities in research laboratories where workers do not take the necessary precautions to prevent exposure; during fishing, when lumps of mustard gas are inadvertently caught in areas where it was historically dumped in the sea; and during armed conflicts, when it is used as a chemical warfare agent (ATSDR, 2003).

Methods currently available for detection of exposure to several chemical warfare agents, including mustard gas, have been reviewed (Noort *et al.*, 2002; Riches *et al.*, 2007; Black, 2008). These include analyses of metabolites in urine and blood, DNA adducts, and protein adducts.

1.3.2 Non-occupational exposure

Non-occupational exposure to mustard gas may occur around sites where the agent was released during warfare (e.g. Belgium, Morocco, Ethiopia, China, Iraq, and the Islamic Republic of Iran), where munitions are buried or where contaminated soils containing mustard gas are disturbed during excavation activities (ATSDR, 2003). The average and maximum atmospheric concentrations that are likely to have occurred under war conditions in areas where mustard gas-containing grenades or artillery shells were dropped, have been estimated at 3 and 5 ppm, respectively (Thorpe, 1974).

Environmental exposure may result from mustard gas/sulfur mustard vapour being carried over long distances by the wind and from local contamination of water (WHO, 2004). Although mustard gas/sulfur mustard is a reactive substance that hydrolyses rapidly upon contact with water, the oily liquid may persist in the environment for many years, or even decades. For example, there are sites where mustard gas originating from the First and Second World Wars still poses a threat to human health and the environment. The environmental fate of mustard gas/sulfur mustard has been discussed (Munro *et al.*, 1999; Ashmore & Nathanail, 2008).

In this *Monograph* the term mustard gas will be used in connection with its military use. In other cases, the agent will be termed sulfur mustard.

2. Cancer in Humans

The carcinogenic hazards of mustard gas were previously evaluated in *IARC Monograph* Volume 9 and in Supplement 7 (IARC, 1975, 1987a). Mustard gas causes respiratory cancers. Human data on the health effects of mustard gas are from battlefield exposures and accidents (single exposures), and from long-term exposures

in chemical factories. Epidemiological studies in humans point at a causal association between exposure to mustard gas and an excess risk for respiratory cancers.

In an early study, the 1930–52 mortality records of 1267 war pensioners who had suffered from mustard gas-poisoning during World War I in the years 1917–18 were analysed and compared with records of 1421 pensioners who had chronic bronchitis but were never exposed to mustard gas, and with those of 1114 pensioners who were wounded in the war but not exposed to mustard gas (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-25-Table2.1.pdf). Mortality from cancer of the lung and pleura was increased in the first two groups (in both, 29 observed deaths, 14 expected), but not in the third (13 deaths observed, 16 expected). There were no significant differences with respect to cancers at other sites. Almost all mustard gas-exposed subjects also had chronic bronchitis (Case & Lea, 1955).

In a similar study, mortality records (1919–55) were examined of 2718 American soldiers exposed to mustard gas during 1917–18, of 1855 soldiers who had pneumonia but were not exposed to mustard gas, and of 2578 wounded soldiers without mustard gas-poisoning or pneumonia. Differences in mortality were seen only in the second decade (1930–39) of the follow-up. Deaths from all respiratory cancers (observed/expected), calculated from US mortality rates, showed a ratio of 39/26 (1.47) for the mustard gas-exposed soldiers (Beebe, 1960). A further study added another ten years of follow-up, but did not alter the initial conclusion: the relative risk of death from lung cancer among the exposed was 1.3 compared with the controls (95%CI: 0.9–1.9) (Norman, 1975).

In a Japanese factory producing mustard gas in the period 1929–45 – with large-scale production of 450 tonnes/month during 1937–44 – concentrations at the workplace were 50–70 mg/m^3. The first report of a cancer case in this plant appeared in 1952: a death from bronchial cancer of a 30-year old man who had been occupationally exposed to mustard gas for 16 months from 1941 (Yamada *et al.*, 1953). Further expansion and follow-up of the plant cohort were reported during the following decade (Yamada *et al.*, 1957; Yamada, 1963). In an extended study over the period 1952–67, observed numbers of deaths were compared with those expected on the basis of mortality rates in the Japanese population (Wada *et al.*, 1968). Of 495 workers who had manufactured mustard gas, 33 had died from cancers of the respiratory tract, compared with 0.9 expected. Of 960 male employees not engaged in the production, only three were known to have died since 1952 from respiratory tract cancers, compared with 1.8 expected. Although there was evidence of preferential reporting of deaths in the mustard gas-exposed group, the excess of respiratory tract cancers was substantial. There was evidence of a dose–response relationship between exposure to mustard gas and subsequent development of respiratory cancer (Nishimoto *et al.*, 1983, 1988; Yamakido *et al.*, 1996).

Another study considered workers in Germany engaged in production, testing and destruction of mustard gas and nitrogen mustard, mainly during the period 1935–45. The factory employed 878 workers, of whom 402 had worked in close contact with mustard gas, nitrogen mustard or with a mixture of the two. In addition, there had been limited exposure in the factory to bromoacetone, phosgene, chloropicrine and organic arsenicals. Among 271 workers exposed to mustard gas or nitrogen mustard and followed-up for compensation of occupational disease and mortality during 1951–74 there were 85 deaths, 32 of which were due to cancer. Twenty-six were lost to follow-up. Compared with Lower-Saxony mortality rates, a significant excess was found for bronchial carcinomas (11 deaths observed, five expected) (Weiss & Weiss, 1975).

In a follow-up of British workers involved in mustard gas-production during World War II, a

statistically significant increase in risk for cancer of the lung and pleura (RR 1.6, $0.05 < P < 0.10$) and of the larynx and trachea (three deaths, RR 7.5, $P < 0.02$) were identified among 502 individuals (Manning *et al.*, 1981).

From a cohort of 2498 men and 1032 women who had been involved in the manufacture of mustard gas in Cheshire, United Kingdom, during World War II, 3354 workers (95%) were traced for mortality until the end of 1984. Between April 1938 and November 1944 the factory had produced 24000 tonnes of mustard gas (none of this material was in fact used). Gas escaped on several occasions and several hundred individuals, mainly in the processing plants, had suffered blistering on the arms and acute effects on the eyes and respiratory tract caused by small amounts of mustard gas. Compared with national death rates for lung cancer, a highly significant excess was observed (200 obs., 138.4 exp. $P < 0.001$). In addition, large and highly significant excesses were reported for deaths from cancers of the larynx (11 observed, four expected, $P = 0.003$), pharynx (15 obs., 2.73 exp, $P < 0.001$), and all other buccal cavity and upper respiratory sites combined (lip, tongue, salivary gland, mouth, nose) (12 obs., 4.29 exp., $P = 0.002$). The risks for cancers of the lung and pharynx were significantly related to duration of employment. Significant excess mortality was also observed for cancers of the oesophagus (20 obs., 10.72 exp.) and stomach (70 obs., 49.6 exp.), but these excesses showed no consistent relation with time since first exposure, or with duration of exposure (Easton *et al.*, 1988).

A retrospective mortality follow-up study was conducted among 1545 Navy recruits who were stationed in Bainbridge, Maryland, USA. During 1944–45 they had voluntarily participated in mustard gas-chamber tests, to assess the quality of protective clothing and masks. Controls were 2663 Navy recruits who were stationed at the same location at the same time as the exposed, but had not participated in the tests. These groups were followed-up until 31 December 1995. Cause-specific mortality risks associated with mustard gas-exposure and the extent or duration of the exposure were examined by use of adjusted and unadjusted relative risk estimates. There was no excess of any cause-specific mortality associated with different levels of mustard gas-exposure among the veterans, although the concentrations had been sufficient to cause skin reactions, such as erythema and ulceration (Bullman & Kang, 2000). [The Working Group noted that levels of exposure were probably substantially lower than those in studies of production workers and World War I veterans.]

Several studies have consistently shown an increased risk for lung cancer among workers in mustard gas-production and among World War I veterans who had been exposed to mustard gas. Two studies among workers in mustard gas-production showed evidence of an exposure–response relationship with duration of employment. Two studies, both based on small numbers, reported an excess risk for laryngeal cancer. However, neither of these studies adjusted for potential confounders, such as tobacco smoking and alcoholic beverage consumption.

3. Cancer in Experimental Animals

Studies with experimental animals exposed to sulfur mustard were reviewed in *IARC Monograph* Volume 9 and in Supplement 7 (IARC, 1975, 1987a). It was concluded that there was *limited evidence* in experimental animals for the carcinogenicity of mustard gas (sulfur mustard). Furthermore, it was noted that some routes of administration, e.g. subcutaneous or intravascular injection, may have little relevance to common human exposures.

In an inhalation study with male and female strain-A mice, an increased incidence in lung tumours [not further specified] was observed in

Table 3.1 Carcinogenicity studies in experimental animals exposed to sulfur mustard

Species, strain (sex) Duration, Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, strain A (M, F) 4–11 mo Heston (1953a)	*Inhalation* (single 15-min exposure) in 8-L dessicator containing 0 (controls) or 0.01 mL sulfur mustard on absorbent paper. 40/group/sex	Lung tumours (in M+F combined): 4 mo after exposure: 6/32 (controls), 9/30 11 mo after exposure: 10/25 (controls), 20/29 4–11 mo after exposure: 21/77 (27%, controls), 33/67* (49%)	*$P < 0.01$	Purity NR Mice were 2–3 mo of age at start Lung tumours not further specified. Three exposed mice and no controls developed lymphocytic leukaemias, which the authors considered unrelated to exposure.
Mouse, C3H, C3Hf, and strain A (M, F) Animals held until dead, moribund, or the appearance of tumours Heston (1953b)	*Subcutaneous injection* of 0.5 mL of 0.05% sulfur mustard in olive oil, once/wk C3H: 32 M, 8 F (six injections) C3Hf: 40 M, 10 F (six injections) strain A: 16 M, 14 F (five injections) Controls: C3H: 32 M, 8 F (untreated) C3Hf: 40 M (untreated) strain A: 16 M, 14 F (olive oil, five injections)	Fibrosarcomas at injection site: 1/8 C3H (M), 0/8 C3H (F), 2/38 C3Hf (M), 2/9 C3Hf (F), 1/14 strain A (M), 0/12 strain A (F) Rhabdomyosarcoma: 1/24 C3H (M) No subcutaneous sarcomas occurred in controls. Mammary tumours: *Exposed*: 2/9 C3Hf (F), 8/8 C3H (F), 1/12 strain A (F) *Controls*: 2/100 C3Hf (F, see comments), 7/8 C3H (F), 0/14 strain A (F)	NR, [NS] (see comments)	Purity NR Authors noted that 2/9 C3Hf female mice with mammary tumours is a significant incidence, compared with 2/100 untreated female C3Hf mice from another study [$P < 0.05$]. [The Working Group considered that untreated mice are inadequate controls for subcutaneous injection.]
Mouse, strain A (M, F) 4 mo Heston (1950)	*Intravenous injection* (4 × , on alternate d) of 0.25 mL 1:10 saturated solution of sulfur mustard in water (0.06–0.07%). Study 1: 15/group/sex Study 2: 24/group/sex	Pulmonary tumours (in M+F combined): Exposed, study 1: 93% [14/15]* Controls, study 1: 61% [15/28] Exposed, study 2: 68% [32/47]** Controls, study 2: 13% [6/46]	NR *[$P < 0.05$] **[$P < 0.0001$]	Purity NR Mice were 2 mo of age at start Lung tumours not further specified. The authors stated that preparation of dosing solutions differed, resulting in a slightly lower dose for study 2.
Rat, Sprague-Dawley (M, F) 42 wk Sasser *et al.* (1996)	*Oral* (*gavage*) 0, 0.03, 0.1, 0.4 mg/kg bw sulfur mustard, 5 d/wk (for 13 wk before mating and throughout gestation, parturition, lactation, in a 42-wk two-generation study) 27 F/group/generation 20 M/group/generation	Fore-stomach papillomas: F_0 (M) 0/20, 0/20, 1/20, 2/20 F_0 (F) 0/27, 0/27, 3/27, 3/27 F_1 (M) 0/20, 0/20, 2/20, 2/20 F_1 (F) 0/27, 0/27, 2/27, 3/27	NR, [NS]	Purity, 97.3% Two-generation study

bw, body weight; d, day or days; F, female; M, male; min, minute or minutes; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks

49% of the animals exposed to sulfur mustard, compared with 27% in controls (Heston, 1953a). Intravenous injection of sulfur mustard also increased the incidence in lung tumours [not further specified] in male and female strain A mice (Heston, 1950). When administered by subcutaneous injection to mice, sulfur mustard induced a few fibrosarcomas and one rhabdomyosarcoma at the injection site in males and females, and mammary tumours in females (Heston, 1953b). Oral administration of sulfur mustard induced fore-stomach papillomas in male and female rats (Sasser *et al.*, 1996; Table 3.1).

4. Other Relevant Data

Since its first use in 1917, there have been nearly 400 000 casualties among the victims of mustard gas-poisoning (Rall & Pechura, 1993). After a lethal dose, death usually occurs within 2–3 days of exposure and is related to respiratory tract injuries, in particular secondary bronchopneumonia (Papirmeister *et al.*, 1991).

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Sulfur mustard can be absorbed after inhalation or through dermal exposure from air and soil. It is a lipophilic substance that easily penetrates into the skin and mucosal surfaces (Drasch *et al.*, 1987; Somani & Babu, 1989), resulting in a high degree of bio-availability.

About 80% of non-occluded, topically applied sulfur mustard evaporates from human skin, while 20% penetrates the skin within ten min (Renshaw, 1946; Kehe *et al.*, 2000). A comparable result was found in studies of human foreskin grafted onto a-thymic mice (Papirmeister *et al.*, 1984a, b). Of the dose that penetrates the skin, 60% is bound in the epidermal and dermal tissue, mostly in the cornified layer, while 40% – i.e. 8% of the initially applied amount – passes rapidly into the blood stream (Cullumbine, 1946, 1947; Nagy *et al.*, 1946; Renshaw, 1946). The penetration rate of sulfur mustard into human skin was estimated to be 1–4 mg/cm^2/min (i.e. 6–25 µmol/cm^2/min), dependent on the temperature (Nagy *et al.*, 1946; Renshaw, 1946).

Elevated concentrations of thio-diglycol, the major hydrolysis product of mustard gas, were detected in human urine after exposure to mustard gas vapour and aerosol (Jakubowski *et al.*, 2000). Thio-diglycol was also found in the urine of people exposed to airborne mustard gas during the war between Iraq and the Islamic Republic of Iran (Wils *et al.*, 1985, 1988). A mustard gas-specific DNA adduct, viz. *N*7-(2-hydroxyethylthioethyl)-2′-deoxyguanosine, as well as adducts to albumin and haemoglobin have been detected in the blood of two victims of mustard gas-poisoning during the war between Iraq and the Islamic Republic of Iran (Benschop *et al.*, 1997; Noort *et al.*, 1999). Autopsy samples from an Iranian soldier who died seven days after inhalation and/or dermal exposure to mustard gas indicated the following organ-distribution pattern: brain > kidney > liver > spleen > lung (Drasch *et al.*, 1987).

4.1.2 Experimental animals

Analysis of blood samples from hairless guinea-pigs exposed nose-only to 300 mg/m^3 (46 ppm) sulfur mustard during eight min, showed that a peak concentration was reached within five min after exposure (Langenberg *et al.*, 1998). In rabbits and monkeys that had undergone tracheal cannulation and were then exposed to nominal chamber concentrations of 40, 100, and 500 mg/m^3 sulfur mustard, only 15% of the dose was recovered, indicating that 85% was absorbed through the nasal mucous membrane (Cameron *et al.*, 1946). The absorption of sulfur mustard through the cornea was demonstrated

in guinea-pigs (Klain *et al.*, 1991). Thirty min after a 5-µL single topical application of radio-labelled sulfur mustard to the cornea of guinea-pigs, radioactivity was detected in kidney, liver, lung, adipose tissue, adrenals, blood plasma, and muscle.

After six hours of cutaneous exposure with occlusion, > 90% of a topically applied dose of sulfur mustard was absorbed into rat skin (Hambrook *et al.*, 1993). Within 60 minutes of the application, the initial rate of uptake had increased linearly with the applied dose in the range of 3–605 µg/cm^2 (0.02–3.6 µmol/cm^2) and reached a maximum of approximately 7 µg/cm^2/min (0.042 µmol/cm^2/min) at a dosage of 955 µg/cm^2 (6 µmol/cm^2). The fraction of sulfur mustard retained in the skin ranged from 10–50% in different studies (Renshaw, 1946; Cullumbine, 1947; Hambrook *et al.*, 1992), while the remainder is absorbed systemically.

Exposure of experimental animals to sulfur mustard by intravenous or intra-peritoneal injection has been reviewed (ATSDR, 2003). These studies provide evidence about routes of exposure other than those involving he skin, the lung or the eyes. The concentration of radio-labelled sulfur mustard in rats four days after intravenous injection indicated the following distribution-pattern: kidney > lung > liver > spleen > brain (Maisonneuve *et al.*, 1994). The difference with the distribution in humans (see above) may be due to different measurement methods, inter-species differences, or variations in post-exposure time, but the route of exposure appears to be an important toxicokinetic factor as well.

The reactivity of sulfur mustard with a wide variety of cellular macromolecules is well documented (IARC, 1975, 1987b; ATSDR, 2003). The presence of two chlorine atoms makes it a strong bi-functional alkylating agent with a high chemical reactivity (Dacre & Goldman, 1996). The chlorine atom is typically released under formation of a carbonium ion, which then undergoes intra-molecular cyclization to create a highly reactive compound. Formation of the carbonium ion is facilitated in aqueous solution (Somani & Babu, 1989), which explains the sensitivity of mucosal tissues, such as the eye, to its effect (Solberg *et al.*, 1997).

The cyclic intermediate mentioned above reacts with and alkylates a variety of electron-rich structures in the cell, such as the guanine moieties in DNA (Dacre & Goldman, 1996) and the sulfhydryl (-SH) and amino (-NH2) groups of proteins and nucleic acids (Solberg *et al.*, 1997). Evidence of covalent binding to cellular DNA, RNA and proteins *in vivo* was obtained in mice injected intra-peritoneally with [^{35}S]-labelled sulfur mustard (IARC, 1987b). DNA is the most functionally sensitive cellular target of sulfur mustard (Crathorn & Roberts, 1966).

Sulfur mustard-specific DNA adducts have been found in the nasal epithelium, naso-pharynx, larynx, carina, lung, spleen, and bone marrow of guinea-pigs after nose-only exposure (Langenberg *et al.*, 1998). The evidence of sulfur mustard-induced DNA adducts in tissues (Somani & Babu, 1989; Fidder *et al.*, 1994, 1996a; van der Schans *et al.*, 1994; Niu *et al.*, 1996) and of sulfur mustard-derived metabolites in urine (Wils *et al.*, 1985, 1988; Jakubowski *et al.*, 2000) suggests the existence of other metabolic pathways, which may include direct alkylation reactions, reaction with glutathione, hydrolysis and oxidation.

4.2 Genetic and related effects

Exposure to sulfur mustard has long been known to produce DNA interstrand cross-links (Roberts *et al.*, 1971a, b; Shahin *et al.*, 2001), which were first noted in *E. coli* (Lawley & Brookes, 1965). When sulfur mustard reacts with DNA, one of the products comprises two guanines linked by a mustard molecule (Walker, 1971). This cross-link can arise from a pair of guanines in opposite strands of the DNA molecule: this interstrand cross-link inhibits cell division (Papirmeister,

1993). However, the cross-link can also arise in significant amounts between two neighbouring guanines in the same strand (Walker, 1971). Transcription, translation, enzyme catalysis and other cellular activities that are dependent on biological entities of much lower molecular size than chromosomal DNA are much less sensitive to sulfur mustard.

Sulfur mustard induced dose-related inter-strand cross-links in the DNA of rat epidermal keratinocytes in primary mono-layer culture (Lin *et al.*, 1996a), affecting cell cycle and DNA synthesis (Lin *et al.*, 1996b). Similar results were seen in HeLa cells (Ball & Roberts, 1972) and in rat cutaneous keratinocytes (Ribeiro *et al.*, 1991). Sulfur mustard has also been shown to affect DNA mismatch-repair in African green monkey kidney cells (Fan & Bernstein, 1991).

Sulfur mustard has been shown to form DNA adducts *in vitro* (van der Schans *et al.*, 1994; Niu *et al.*, 1996; ATSDR, 2003). Upon incubation of double-stranded calf-thymus DNA or human blood with [^{35}S]-labelled sulfur mustard, the following adducts were identified: *N*7-[2-[(2-hydroxyethyl)thio]ethyl]-guanine, bis[2-(guanin-7-yl)ethyl]sulfide, *N*3-[2-[(2-hydroxyethyl)thio]ethyl]-adenine, and O^6-[2-[(2-hydroxyethyl)thio]ethyl]-guanine and its 2′-deoxyguanosine derivative (Fidder *et al.*, 1994). The primary site of DNA-alkylation by sulfur mustard is the *N*7 position of deoxy-guanosine (Balali-Mood & Hefazi, 2005). Upon depurination of the resulting *N*7-(2-hydroxyethyl)-2′-deoxyguanosine, the base adduct *N*7-(2-hydroxyethylthioethyl)-guanine (*N*7-HETE-Gua) is released. The toxic effects of sulfur mustard have been attributed to DNA adducts such as *N*7-hydroxyethylthioethyl-guanine, 3-hydroxyethylthioethyl adenine, and the cross-link, di-(2-guanin-7-yl-ethyl) sulphide (Saladi *et al.*, 2006). DNA extracted from human leukocytes and exposed to [^{14}C]-labelled sulfur mustard *in vitro* was shown to contain the adduct *N*7-(2-hydroxyethylthioethyl)guanine (Ludlum *et al.*, 1994). It has been demonstrated that alkyltransferase is inefficient in repairing O^6-ethylthioethylguanine, and the persistence of this adduct could have serious consequences (Ludlum *et al.*, 1986). Alkylation by sulfur mustard also affects transcriptional processes and may lead to truncated transcripts by impairing RNA polymerase via an alkylated promoter (Masta *et al.*, 1996). Analysis of truncated transcripts revealed that sulfur mustard preferentially alkylates the DNA-template strand at 5′-AA and 5′-GG sequences. Low doses of sulfur mustard can also inhibit cell division by cross-linking of complementary DNA strands, or cause mutagenesis by inducing errors in replication or repair (Papirmeister, 1993; ATSDR, 2003). It has been noted that cells in late G1-phase (post-mitotic) or early S-phase (DNA synthesis) are particularly sensitive to the effects of alkylation (Somani & Babu, 1989).

The ability of sulfur mustard to induce mutations has been demonstrated in numerous experimental systems (Fox & Scott, 1980). *TP53* mutations – predominantly G→A transitions – were detected in tumours of individuals exposed to mustard gas (Hosseini-Khalili *et al.*, 2009). Sulfur mustard has been shown to induce mutations in specific DNA regions (r-RNA-coding locus) (Fahmy & Fahmy, 1971; IARC, 1975).

Fishermen who were exposed to mustard gas from leaking shells picked up during fishing showed an increased incidence of sister chromatid exchange in the lymphocytes (Wulf *et al.*, 1985). Sulfur mustard induces chromosomal aberrations and DNA damage in rodent cells *in vitro* and mutations in mouse-lymphoma cells *in vitro* and *in vivo* (IARC, 1987b). *In vivo,* sulfur mustard has been shown to induce micronuclei in mouse bone-marrow (Ashby *et al.*, 1991). It also induced chromosome aberrations in cultured rat lymphosarcoma cell lines (Scott *et al.*, 1974). In a host-mediated assay in male BDF1 mice, with a murine leukaemia cell line (L_{517}8Y/Asn) as an indicator, sulfur mustard induced both

chromosome aberrations and reversed mutations to asparagine-186 independence, after single subcutaneous doses of 100 mg/kg bw. Similar results were obtained with the same cell line tested *in vitro* (Capizzi *et al.*, 1973). Dominant lethal mutations in adult male rats were induced after exposure to sulfur mustard at 0.1 mg/m^3 for 52 weeks (Rozmiarek *et al.*, 1973). Aneuploidy, heritable translocations, dominant lethal mutations and sex-linked recessive lethal mutations have been observed in *Drosophila* exposed to sulfur mustard. The substance is mutagenic to fungi and induces DNA damage in bacteria and yeast (Kircher & Brendel, 1983).

Sulfur mustard appears to preferentially damage the cells that are the most actively regenerating after injury, such as basal cells located above the dermal papillae in the skin (Papirmeister *et al.*, 1991), and epithelial secretory cells in the trachea (Calvet *et al.*, 1996). In the cell, DNA and proteins are the main targets of alkylation by sulfur mustard; it is not unexpected, therefore, that the most severe lesions affect cells with the strongest proliferative and metabolic capacity. Impairment of the DNA-polymerase function has also been proposed. In particular, impairment of the replicative fidelity of DNA during the S-phase could contribute to mitotic and chromosomal effects (Bignold, 2006). Recently, both base-excision repair and nucleotide-excision repair were identified as repair pathways that are activated after exposure of human lymphoblastoid cell lines to the sulfur-mustard surrogate 2-chloroethyl-ethylsulphide (Jowsey *et al.*, 2009)

Several studies have shown that sulfur mustard applied topically on the skin can diffuse and produce biochemical alterations consistent with free-radical-mediated oxidative stress, including increased lipid peroxidation and antioxidant enzyme activities, depletion of glutathione content in the eye, kidney, brain, lungs, and liver of rats and mice (Arroyo *et al.*, 2000). Sulfur mustard undergoes nucleophilic substitution reactions to form a sulfonium ring (Yang *et al.*, 1992) that, in the presence of oxygen, first generates a non-toxic, reactive sulfoxide intermediate. Extensive oxidation leads to toxic sulfone species (Arroyo *et al.*, 2000).

Besides genotoxic mechanisms responsible for the acute and delayed effects of sulfur mustard, other mechanisms may be responsible for sulfur mustard-induced vesication, since acute skin injury develops much earlier than would be expected from genotoxic effects alone. Also, tissue injury does not develop when low, therapeutically effective doses of sulfur mustard are used to control the hyper-proliferation of psoriatic keratinocytes. While the mechanisms underlying the toxicity of sulfur mustard are currently not fully understood, one hypothesis to explain its cytotoxicity involves poly(ADP-ribose) polymerase (PARP). It has been proposed that sulfur mustard alkylates DNA, which causes DNA strand breaks whose accumulation can cause activation of the nuclear repair-enzyme PARP. This causes cellular depletion of nicotinamide adenine dinucleotide, which decreases glycolysis and leads to protease release and cellular injury. Dermal-epidermal separation and blister formation may involve the fragmentation of anchoring filaments by protease released from moribund or dead cells (Papirmeister, 1993). Treatment of HeLa cells with sulfur mustard produces a rapid stimulation of PARP activity, followed by a decline in nicotinamide-adenine-dinucleotide levels two hours later (Clark & Smith, 1993). The hypothesis is almost fully confirmed in a study in which PARP inhibitors prevent the sulfur mustard-induced losses of adenosine triphosphate, nicotinamide-adenine-dinucleotide and viability in human peripheral blood cells (Meier & Kelly, 1993). Several other studies provide partial support for this hypothesis and suggest that additional pathways may be involved.

Sulfur mustard was found to inhibit antioxidant enzyme activities in blood cells and other tissues of rats, after topical application; the treatment could impair cyto-protective

defence mechanisms (Husain *et al.*, 1996). Enzyme activities were measured 24 hours after dermal treatment with 98 mg/mg (0.5 LD50) of sulfur mustard. Superoxide dismutase activity decreased significantly in white blood cells (70%), in platelets (65%), in spleen (72%) and in brain (29%) while it was not significantly altered in red blood cells, liver, and kidney. Catalase activity decreased significantly in white (54%) and red blood (23%) cells and in spleen (51%), while the activity in platelets, liver, kidney, and brain was not significantly altered. Glutathione peroxidase activity, as a consequence of glutathione and nicotinamide-adenine-dinucleotide-phosphate depletion, decreased significantly in white blood cells (42%), spleen (43%), and liver (22%). Glutathione levels in red blood cells, platelets, kidney, and brain were within 10% of control values.

4.3 Synthesis

Data from a variety of sources all strongly support a genotoxic mechanism underlying the carcinogenic action of mustard gas/sulfur mustard, mainly based on the observation that this chemical is a bi-functional alkylating agent (IARC, 1987b). It was the first chemical reported to induce mutations and chromosome rearrangements in *Drosophila melanogaster* (Auerbach & Robson, 1947; ATSDR, 2003). The direct reaction of this substance with DNA likely initiates a cascade of genetic events that lead to cancer. There is evidence to support DNA-alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutation, and induction of chromosome-type and chromatid-type aberrations (ATSDR, 2003). Some of these changes are observed in nasal tissue, which is consistent with the nasal tissue being a target organ for this chemical. In addition, production of reactive oxygen species and cytotoxicity, other reported contributors to the mechanism of action, could act complementary to DNA alkylation.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of mustard gas. Mustard gas causes cancer of the lung.

Also, a positive association has been observed between mustard gas and cancer of the larynx,

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

There is *strong evidence* that the carcinogenicity of sulfur mustard operates by a genotoxic mechanism of action that involves DNA alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutations, and induction of chromosome-type and chromatid-type aberrations.

Sulfur mustard is *carcinogenic to humans (Group 1)*.

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VINYL CHLORIDE

Vinyl chloride was considered by previous IARC Working Groups in 1974, 1978, 1987, and 2007 (IARC, 1974, 1979, 1987, 2008). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

From IARC (2008) and Lide (2008)
Chem. Abstr. Serv. Reg. No.: 75-01-4
Chem. Abstr. Serv. Name: Chloroethene

$H_2C{=}CH{-}Cl$

C_2H_3Cl
Relative molecular mass: 62.5
Description: Colourless gas, with a mild, sweet odour
Boiling-point: -13.4 to -13.8 °C
Solubility: Slightly soluble in water (1.1 g/L at 25 °C); soluble in ethanol; very soluble in diethyl ether, carbon tetrachloride and benzene
Conversion factor: 1 ppm = 2.6 mg/m^3

1.2 Uses

Vinyl chloride is used primarily (> 95%) in the manufacture of polyvinyl chloride (PVC), which comprises about 12% of the total use of plastic worldwide (WHO, 1999). The largest use of PVC is in the production of plastic piping. Other important uses are in floor coverings, consumer goods, electrical applications and in the transport sector. About 1% of PVC is used to produce vinyl chloride/vinyl acetate copolymer. Minor uses of vinyl chloride (monomer) include the manufacture of chlorinated solvents (primarily 10000 tonnes per year of 1,1,1-trichloroethane) and the production of ethylene diamine for the manufacture of resins (WHO, 1999; European Commission, 2003).

Vinyl chloride has been used in the past as a refrigerant, as an extraction solvent for heat-sensitive materials, in the production of chloroacetaldehyde, as an aerosol propellant and in drugs and cosmetic products; these uses were banned in the United States of America (USA) by the Environmental Protection Agency in 1974 (IARC, 2008).

1.3 Human exposure

1.3.1 Occupational exposure

The main route of occupational exposure to vinyl chloride is by inhalation, which occurs primarily in vinyl chloride/PVC plants and in PVC-processing plants. Only few exposure

measurements have been reported, but estimates from the chemical industry indicate that exposure to vinyl chloride monomer (VCM) amounted to several thousands of milligrams per cubic metre in the 1940s and 1950s, and were several hundreds of milligrams per cubic metre in the 1960s and early 1970s. After its recognition as a human carcinogen (IARC, 1974), occupational exposure standards were set at approximately 13–26 mg/m^3 [5–10 ppm] in most countries in the 1970s (Fleig & Thiess, 1974; NTP, 2005; IARC, 2008).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 presents the estimated numbers of workers exposed to vinyl chloride in the EU for the top-10 industries (CAREX, 1999).

From the US National Occupational Exposure Survey (1981–83) it was estimated that approximately 81 300 workers (including approximately 28 400 women) were potentially exposed to vinyl chloride (NIOSH, 1990).

A report from the Centers for Disease Control and Prevention (CDC) in the USA concluded that the development and acceptance by the PVC-manufacturing industry of a closed-loop polymerization process in the late 1970s "almost completely eliminated worker exposures" (CDC, 1997). Even after the late 1970s, however, high concentrations may still be encountered and were in fact reported in some countries (IARC, 2008).

(a) Production of vinyl chloride and its derivatives

In vinyl-chloride production, workers may be exposed to ethylene dichloride and to catalysts such as iron(III)chloride. In PVC production, concurrent exposure to PVC-dust may occur (Casula *et al.*, 1977).

Measurements of VCM concentrations in indoor air of vinyl chloride/PVC production plants have been summarized in *IARC Monograph* Volume 97 (IARC, 2008). In a study on occupational exposure to vinyl chloride that was not included in the previous *Monograph*, Zhu *et al.* (2005) reported the exposure to VCM of workers in a plant in the People's Republic of China. Concentrations in air of VCM at different worksites in the plant ranged from 0.3 to 17.8 ppm [0.8–48.4 mg/m^3]; the geometric mean concentration was 2.6 ppm [7.1 mg/m^3].

(b) PVC processing

Measured concentrations of VCM in PVC-processing plants were considerably lower than those in plants where vinyl chloride and PVC were produced (IARC, 2008). Improvements in PVC production in the 1970s resulted in a much lower content of residual VCM in PVC resin. The lower monomer content led automatically to reduced concentrations of vinyl chloride in the workplace air of PVC-processing factories, reaching values of < 0.1 ppm [0.26 mg/m^3] (Holm *et al.*, 1982).

In PVC processing, the polymer may be mixed with antioxidants (such as *p*-nonylphenol), stabilizers (such as organic tin compounds), plasticizers (phthalates) and colouring agents (pigments) (Summers, 2006) and occupational exposure to these compounds, as well as to PVC-dust, may occur (Boraiko & Batt, 2005).

(c) Hairdressers and barbers

Infante *et al.* (2009) presented two case reports of hairdressers and barbers who used hairsprays containing vinyl chloride over a period of 4–5 years between 1966 and 1973, and developed angiosarcoma of the liver (ASL). The ranges of exposure were estimated at 129–1234 ppm (peak concentration), and as 70–1037 ppm (average concentration).

Table 1.1 Estimated numbers of workers exposed to vinyl chloride in the European Union (top-10 industries)

Industry, occupational activity	
Manufacture of industrial chemicals	10400
Manufacture of plastic products, not elsewhere classified	9100
Manufacture of other chemical products	7600
Manufacture of fabricated metal products, except machinery and equipment	2900
Manufacture of machinery, except electrical	2400
Services allied to transport	1300
Manufacture of electrical machinery, apparatus, appliances	980
Education services	870
Construction	800
Petroleum refineries	570
TOTAL	39600

From CAREX (1999)

1.3.2 Non-occupational exposure

The general population is potentially exposed to vinyl chloride through inhalation of contaminated air, ingestion of contaminated drinking-water and foods, or dermal contact with consumer products. However, the exposure levels for the majority of the population are very low (NTP, 2005).

(a) Ambient air

Vinyl chloride is released into the environment in emissions and effluents from the plastics industry. Atmospheric concentrations of VCM in ambient air are low (usually < 3 μg/m^3) and ambient air samples in rural and urban areas of the USA typically do not contain detectable levels of vinyl chloride (NTP, 2005). Vinyl chloride has been reported in landfill gas and groundwater, as a degradation product of chlorinated solvents that were deposited in landfills (WHO, 1999).

Populations living near emission sources (e.g. emissions and effluents from the plastics industry) may be exposed to relatively high concentrations of airborne vinyl chloride. Measured concentrations ranged from trace levels to over 2600 μg/m^3, and the average daily intake of vinyl chloride by residents living near such emission sources ranged from trace amounts up to 2100 μg (NTP, 2005). A monitoring programme in the 1970s around VCM- and PVC-production plants found some relatively high concentrations of vinyl chloride in the ambient air. Maximum 24-hour average concentrations ranged from 0.32 to 10.6 ppm [0.8–28 mg/m^3]. Levels of VCM were much lower in the vicinity of plants where PVC products were manufactured than near VCM- and PVC-production plants (Dimmick, 1981).

(b) Accidental releases

In June 1996, ten of 18 tank wagons filled with vinyl chloride derailed on the Magdeburg-Halle railway line just outside the Schönebeck train station in Germany. Vinyl chloride concentrations of 0.06–8 ppm [0.16–20.8 mg/m^3] were measured in surrounding residential areas. Nearly 300 urine samples were taken from rescue workers, residents and a control group, and analysed for the presence of the vinyl-chloride metabolite thiodiacetic acid. The measured values appeared to be in the range of those of non-exposed people (Thriene *et al.*, 2000).

(c) Residues in PVC resin and products

PVC products may contain VCM as a residue from production and release it in the air. In a German survey (1976–77), the following articles released VCM at levels > 0.05 ppm [0.13 mg/m^3] by off-gassing in the air: bathroom tiles, piping, plastic bottles for table oil, and kitchen wrapping-film. The highest concentrations were observed to come from vinyl music records, with values of 20–50 ppm measured for nine of 14 records sampled, but even higher in some of the others. The VCM concentrations released by toys, kitchen utensils, food wrappings, wall-paper, and car interiors were < 0.05 ppm (German Environmental Office, 1978). The introduction of improved manufacturing practices has considerably reduced the residual content of VCM in PVC products (WHO, 1999).

(d) Other sources of exposure

VCM is present in mainstream smoke of cigarettes (1.3–16 ng/cigarette) and cigars (14–27 ng/cigar). The measured concentrations correlated with the inorganic chloride content of the tobacco (Hoffmann *et al.*, 1976; IARC, 2004).

Exposure to vinyl chloride in drinking-water is unlikely for the majority of the general population. In a US survey of 945 ground-water supplies and 11202 public water supplies that used surface waters as their primary source, less than 1% of the samples contained detectable levels of vinyl chloride (detection limit, 1 ppb [1 µg/L]). The US Environmental Protection Agency estimated that approximately 0.9% of the US population is exposed to vinyl chloride in drinking-water at concentrations of 1.0 µg/L or higher, and that 0.3% is exposed to concentrations higher than 5 µg/L (NTP, 2005).

2. Cancer in Humans

Vinyl chloride was evaluated in previous *IARC Monographs* (IARC, 1979, 1987, 2008) and was classified in Group 1 based on increased risks for ASL and hepatocellular carcinoma (HCC).

A report of three cases of ASL in men who had been employed in the manufacture of PVC resins provided the first evidence of an association between vinyl chloride and cancer in humans (Creech & Johnson, 1974). The case report was particularly informative because of the extreme rarity of this tumour in the general population. The Working Group in 1974 already considered this observation to provide evidence of a causal relationship.

Epidemiological evidence for the carcinogenicity of vinyl chloride in humans derives principally from two large, multicentre cohort studies, one of which was carried out in the USA and the other in Europe. These investigations focused on plants that manufactured vinyl chloride monomer, polyvinyl chloride or polyvinyl chloride products. In addition to reports that pertained to these cohorts in their entirety, several studies reported findings from individual subcohorts. Results for subcohorts are given in the Tables, but only when they provide important information that is not available in analyses of the full cohorts. Results on six cohort studies have also been reported, in addition to and separate from the two multicentre investigations.

The first published report of the North-American multicentre cohort study (Cooper, 1981) included 10173 workers from 37 plants. Among the 37 plants included in the study, 11 plants with 1214 workers produced only VCM, 18 plants with 6848 workers produced only PVC, three plants with 935 workers produced both VCM and PVC and five plants with 1176 workers produced homopolymers and copolymers. To be eligible for inclusion into the cohort, male employees at the 37 participating plants

were required to have been exposed to VCM for at least one year before 31 December 1972 and to have been employed in or after 1942. A second major update of this cohort was published by Wong *et al.* (1991). A third major follow-up included 10 109 subjects and provided an update of the vital status through to 31 December 1995 (Mundt *et al.*, 2000).

The European cohort study was conducted in four countries (Italy, Norway, Sweden and the United Kingdom). It included workers from 19 factories: 11 of these produced VCM/PVC, two produced VCM only, five produced PVC only and one was a PVC-processing plant. Male workers who had been employed for at least one year in 1942–1972 in jobs that entailed exposure to VCM were included (Simonato *et al.*, 1991). An update of the study (Ward *et al.*, 2001) analysed incidence and mortality through to the latest year for which data were available in each country, which ranged between 1993 and 1997.

2.1 Angiosarcoma of the liver

In both multicentre cohort studies (Mundt *et al.*, 2000; Ward *et al.*, 2001) a substantial excess of ASL in exposed workers was found (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.1.pdf). This tumour is extremely rare in the general population and it is not possible to calculate an SMR or SIR, because age- and calendar time-specific reference rates are not available. In the study from the US, 33 of the 80 deaths from cancer of the liver and biliary tract were identified from the death certificate as due to ASL. A total of 48 deaths due to ASL were identified by combining information from death certificates with that from a registry of ASL-cases that were related to exposure to VCM. This registry is maintained and updated by the Association of Plastics Manufacturers of Europe.

In the European study there were 53 deaths from primary liver cancer and 18 incident cases of liver cancer. This total of 71 cases comprised 37 ASL, 10 HCC, 7 cases of other known histology, and 17 cases of an unspecified type of liver cancer. [The Working Group noted that the authors searched for the best evidence for diagnosis of liver cancers by reviewing all available documentation, including death certificates, cancer-registry records, medical records, and listings of ASL from two registries.]

In both studies, the risk for ASL increased strongly with duration of exposure to vinyl chloride. In the European study, there was also a clear trend of higher risk with increasing cumulative exposure. Multiple cases of ASL were also reported in one smaller cohort study (Thériault & Allard, 1981). Two cases of ASL were reported among hairdressers and barbers who had been exposed to vinyl chloride for 4–5-year periods in the late 1960s and early 1970s, when it was used as a propellant in hairspray (Infante *et al.*, 2009).

2.2 Hepatocellular carcinoma

The assessment of vinyl chloride as a cause of HCC is complicated because many studies do not have histological or other definitive clinical information to discriminate HCC from ASL and/or secondary neoplasms (see Table 2.1 online). In the US multicentre study, mortality from cancers of the liver and biliary tract (ICD9-code, 155–156) was increased (SMR 3.6, 95%CI: 2.8–4.5; 80 deaths). Of the 80 deaths, 48 were identified as ASL. The diagnosis of HCC among the remaining deaths was not verified.

In an internal analysis of the European multicentre cohort (Ward *et al.*, 2001) based on 10 verified cases of HCC, the risk increased significantly and substantially with duration of employment and with cumulative exposure to vinyl chloride. The relative risk for workers with the longest duration of employment (> 26 years) was 35 (95%CI: 3.3–377) compared with workers with < 10 years of employment. An analysis of a single Italian plant with extended follow-up – that

was included in the European study – indicated 12 confirmed cases of HCC (Pirastu *et al.*, 2003). The maximal overlap between these two analyses was four cases, since only four HCC from Italy were included in the multicentre cohort. In this subcohort, the incidence of HCC again increased significantly with cumulative exposure to vinyl chloride. There was suggestive evidence that the risk for HCC from vinyl chloride is substantially higher among workers who are infected with hepatitis B virus (Wong *et al.*, 2003), or who report high levels of alcoholic beverage consumption (Mastrangelo *et al.*, 2004).

A meta-analysis of cohort studies of vinyl choride-exposed workers published up to 2002 (Boffetta *et al.*, 2003) was based on eight independent studies, i.e. two multicentric investigations (Mundt *et al.*, 2000; Ward *et al.*, 2001) and six additional, smaller studies (Thériault & Allard, 1981; Weber *et al.*, 1981; Smulevich *et al.*, 1988; Laplanche *et al.*, 1992; Huang, 1996; Wong *et al.*, 2002) (*P*-value for the test for heterogeneity was ≥ 0.01). Six of these eight studies reported results for liver cancer, but these were considered to be too heterogeneous to be included in a meta-analysis because for 'liver cancer overall' and for 'liver cancer other than ASL', the *P*-value for heterogeneity was < 0.001. For the two multicentre studies (Mundt *et al.*, 2000; Ward *et al.*, 2001), the lack of heterogeneity allowed calculation of summary estimates for liver cancer overall (meta-SMR, 2.96; 95%CI: 2.00–4.39; random effects model; *P*-value for heterogeneity = 0.03) and for liver cancer other than ASL (meta-SMR, 1.35; 95%CI: 1.04–4.39; random effects model; *P*-value for heterogeneity = 0.7).

[The Working Group noted that the meta-analysis did not evaluate the quality of the studies and that some heterogeneity between studies may have resulted from variable quality of the data. Excluding one study from the People's Republic of China, other studies reported SMRs that ranged from 1.78 (95%CI: 1.15–2.62) to 57.1 (95%CI: 24.6–113) for liver cancer overall and from 1.27 (95%CI: 0.84–1.83) to 10.1 (95%CI: 4.37–20.0) for liver cancer other than ASL.]

2.3 Cancer of the lung

Among workers exposed to vinyl chloride, there was no overall evidence of an increased risk for lung cancer (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.2.pdf). However, in PVC-packers and -baggers, the risk for lung cancer increased significantly with cumulative exposure to vinyl chloride (Ward *et al.*, 2001). [These workers are known to have had concomitant exposure to PVC-dust; the study did not allow attribution of the association to a specific agent or combination of agents.]

2.4 Malignant neoplasms of connective and soft tissue

Suggestive evidence was found for malignant neoplasms of connective and soft tissue (ICD9-code, 171). This derived from the multicentre study in the USA (Mundt *et al.*, 2000), in which a nearly threefold statistically significant overall increase in mortality from these neoplasms was observed (SMR 2.7, 95%CI: 1.4–4.7; 12 observed, 4.4 expected). The risk was higher for workers with longer duration of employment (i.e. 10–19 *vs* > 20 years) and for those first employed before 1960. Four of the 12 observed deaths were from angiosarcomas for which the site was unknown. The increased mortality from neoplasms of connective and soft tissue persisted even after exclusion of these four angiosarcomas. [This presumes that the malignant neoplasms of connective and soft tissue were mis-classified deaths from angiosarcoma of the liver.]

The findings mentioned above were not supported by results from the European multicentre study, in which the number of deaths from connective-tissue neoplasms was too small

for an evaluation of exposure–response (Ward *et al.*, 2001): there were six observed deaths from neoplasms of connective and soft tissue (SMR = 1.9, 95%CI: 0.7–4.1), but in a re-evaluation of the diagnoses three of the six deaths coded as tumours of the connective tissue were found to be ASL. [The Working Group noted that, although a statistically significant increase in mortality from neoplasms of connective and soft tissue was found in the US study, the discrepant results with the European study and the difficulties in arriving at a correct diagnosis and coding of the tumour site for this type of neoplasm, complicate an evaluation of these findings.]

2.5 Other cancers

The Working Group did not find strong evidence for associations of exposure to vinyl chloride with cancers of the brain or the lymphatic and haematopoeitic tissues, with melanoma of the skin (see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.3.pdf, Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.4.pdf, and Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-26-Table2.5.pdf). Although the associations found for these cancers in specific studies may reflect true increases in risk, the findings were inconsistent between studies, no clear exposure–response relationships were found in the European multicentre study (Ward *et al.*, 2001), and, for several of the sites, the numbers of observed/expected cases were small.

No conclusion could be reached for breast cancer since the available studies included too few women.

2.6 Synthesis

There is compelling evidence that exposure to vinyl chloride is associated with angiosarcoma of the liver, and strong evidence that it is associated with hepatocellular carcinoma. Together with the observation that vinyl chloride increases the risk for liver cirrhosis, which is a known risk factor for hepatocellular carcinoma, the findings from two large multicentre cohort studies provide convincing evidence that vinyl chloride causes hepatocellular carcinoma as well as angiosarcoma of the liver. There is contradictory evidence that exposure to vinyl chloride is associated with malignant neoplasms of connective and soft tissue, and inconsistent or scanty evidence that it is associated with cancers of the lung, brain, lymphohaematopoietic system, and breast, or with melanoma of the skin.

3. Cancer in Experimental Animals

The carcinogenicity of vinyl chloride has been studied intensively and repeatedly in experimental animals, with a wide range of concentrations, spanning orders of magnitude. The many studies consistently showed hepatic and extrahepatic angiosarcomas in mice and rats. Various other malignant neoplasms also occurred at several anatomical sites. However, the reporting of the results has often been incomplete, and the outcomes of many studies are available only from summary tables in the published literature, in which technical details are given in footnotes.

Studies of the carcinogenicity of vinyl chloride in experimental animals after oral administration, inhalation, subcutaneous injection, intraperitoneal injection, and transplacental and perinatal exposure have been reviewed in previous *IARC Monographs* (IARC, 1974, 1979, 1987, 2008). No studies have been published since the most recent evaluation (IARC, 2008). The

following is a summary of the available data (see also Table 3.1).

3.1 Inhalation exposure

Vinyl chloride was tested by inhalation exposure in several studies in mice (Holmberg *et al.*, 1976; Lee *et al.*, 1978; Hong *et al.*, 1981; Maltoni *et al.*, 1981; Drew *et al.*, 1983; Suzuki, 1983), in several studies in rats (Lee *et al.*, 1978; Feron *et al.*, 1979; Feron & Kroes, 1979; Groth *et al.*, 1981; Kurliandskiĭ *et al.*, 1981; Maltoni *et al.*, 1981; Drew *et al.*, 1983), and in two studies in hamsters (Maltoni *et al.*, 1981; Drew *et al.*, 1983). Male and female animals of all three species were included, although some experiments were carried out only in one sex. Vinyl chloride induced hepatic angiosarcomas in three experiments in mice and in eight experiments in rats; a dose–response was observed for hepatic angiosarcomas in both species over a wide range of exposures. Extrahepatic angiosarcomas related to treatment with vinyl chloride were observed in three studies in mice and one study in rats. Vinyl chloride increased the incidence of malignant mammary tumours in seven experiments in mice, in two experiments in one study in rats, and in one study in hamsters. Exposure to vinyl chloride increased the incidence of skin epitheliomas in one study in rats and one study in hamsters, and of skin carcinomas in another study in hamsters. It increased the incidence of Zymbal gland carcinomas in three experiments in rats, with a dose–response pattern in one experiment. In mice, vinyl chloride increased the incidence of benign lung tumours in six experiments, and of lung carcinomas in two experiments. It also increased the incidence of nasal cavity carcinomas in one study in rats, of hepatocellular carcinomas in two experiments in rats, of glandular adenomas in one study in hamsters, and of benign fore-stomach tumours in another study in hamsters.

In one study in rats, combined oral administration of ethanol and inhalation exposure to vinyl chloride increased the incidence of hepatic angiosarcomas compared with exposure to vinyl chloride alone (Radike *et al.*, 1981).

3.2 Oral administration

Vinyl chloride was tested by oral administration in four experiments in male and female rats (Feron *et al.*, 1981; Maltoni *et al.*, 1981; Til *et al.*, 1991). It induced hepatic angiosarcomas in two experiments, lung angiosarcomas in one experiment and hepatocellular adenomas and hepatocellular carcinomas in two experiments.

3.3 Subcutaneous and intraperitoneal injection

When vinyl chloride was tested in rats by subcutaneous injection and by intraperitoneal injection in single studies, no increase in tumour incidence was observed (Maltoni *et al.*, 1981).

3.4 Transplacental administration and perinatal exposure

The transplacental carcinogenicity of vinyl chloride was evaluated in one study in the offspring of rats exposed by inhalation on days 12–18 of pregnancy. A low incidence of tumours was observed in prenatally exposed offspring at several sites including the kidney (nephroblastomas) and the Zymbal gland (carcinomas). However, no angiosarcomas or hepatomas developed in the offspring (Maltoni *et al.*, 1981).

Vinyl chloride was tested by perinatal inhalation exposure in two studies in rats. In one study, rats were exposed transplacentally, as neonates, and during adulthood. Treatment with vinyl chloride induced hepatic angiosarcomas and hepatocellular carcinomas. The rats also showed

Table 3.1 Carcinogenicity studies in experimental animals exposed to vinyl chloride and chloroethylene oxide

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, NMRI (M, F) 26 or 52 wk Holmberg *et al.* (1976)	Inhalation, 0, 50, 500 ppm [0, 130, 1 300 mg/m^3] 6 h/d, 5 d/wk, for 26 wk (0, 500 ppm) or 52 wk, (0, 50 ppm) 12/group/sex	Extrahepatic angiosarcomas: (M) 0/24, 6/12*, 3/12*; (F) 0/24, 8/12*, 5/12* Lung adenomas: (M) 0/24, 9/12*, 12/12*; (F) 0/24, 4/12*, 12/12* Mammary carcinomas: (F) 1/24, 1/12, 4/12	NR, *[$P < 0.05$]	Purity NR The animals exposed to 500 ppm were sacrificed after 26 wk because of poor survival. Control groups were combined.
Mouse, CD-1 (M, F) 52 wk Lee *et al.* (1978)	Inhalation 0, 50, 250, 1000 ppm [0, 130, 650, 2 600 mg/m^3] 6 h/d, 5 d/wk, 52 wk 36/group/sex, 4 animals/group were sacrificed at 1, 2, 3, 6 or 9 mo	Liver angiosarcomas: (M) 0/26, 3/29, 7/29*, 13/33*; (F) 0/36, 0/34, 16/34*, 18/36* Extrahepatic angiosarcomas: (M): 0/26, 5/29*, 2/29, 0/33; (F) 0/36, 1/34, 3/34, 9/36* Lung adenomas: (M) 1/26, 8/29**, 10/29**, 22/23**; (F): 0/36, 4/34, 12/34**, 26/36** Mammary gland tumours (malignant): (F) 0/36, 9/34**, 3/34, 13/36**	*$P < 0.05$ NR, **[$P < 0.05$]	99.8% pure Mammary gland tumours were adenocarcinomas and carcinomas
Mouse, CD-1 (M, F) up to 18 mo Hong *et al.* (1981)	Inhalation 0, 340, 1690, 6760 ppm [0, 130, 650, 2600 mg/m^3] 6 h/d, 5 d/wk for 1, 3 or 6 mo and observed for additional 12 mo 8–28/group/sex	Cumulative incidence Liver haemangiosarcomas: (M) 0/60, 1/40, 8/44*, 6/38*; (F) 1/60, 1/40, 5/40*, 12/38* Bronchioloalveolar tumours: (M) 8/60, 12/40, 29/44*, 27/38*; (F) 8/60, 6/40, 23/40*, 23/38* Mammary gland tumours (malignant): (F) 4/60, 10/40, 13/40*, 6/38*	*$P < 0.05$	Mammary gland tumours were adenocarcinomas and carcinomas; bronchioalveolar tumours were not further described.
Mouse, Swiss (M, F) 81 wk Maltoni *et al.* (1981)	Inhalation 0, 50, 250, 500, 2500, 6000, 10000 ppm [0, 130, 650, 1300, 6500, 15600, 26000 mg/m^3] 4 h/d, 5 d/wk, 30 wk 30/group/sex, 150 controls	Liver angiosarcomas: 0/150, 1/60, 18/60, 14/60, 16/59, 13/60, 10/56 Extrahepatic angiosarcomas: 1/150, 1/60, 3/60, 7/60, 8/59, 1/60, 1/56 Lung tumours: 15/150, 6/60, 41/60, 50/60, 40/59, 47/60, 46/56 Mammary gland carcinomas: 1/150, 12/60, 12/60, 8/60, 8/59, 8/60, 13/56	NR [$P < 0.05$, many exposed group], angiosarcomas (all sites including the liver), lung tumours and mammary gland carcinomas	99.97% pure Data reported for both sexes combined. A low incidence of skin tumours was also reported; lung tumours were not further described.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, CD-1 (F) Lifetime Drew *et al.* (1983)	Inhalation Study 1: 8–9 wk old mice exposed to 0 or 50 ppm [0, 130 mg/m^3] 6 h/d, 5 d/wk, for 6, 12, or 18 mo Initial group size NR	Study 1, incidence for controls and incidences for animals exposed for 6, 12, 18 mo Haemangiosarcomas (all sites): 1/71, 29/67, 30/47, 20/45 Lung carcinomas: 9/71, 18/65, 15/47, 11/45 Mammary gland carcinomas: 2/71, 33/67, 22/47, 22/45	Study 1, $P < 0.01$ (all vinyl chloride-exposed group)	Commercial grade, purity NR
	Study 2: 8 or 14 mo old mice exposed to 0 or 50 ppm 6 h/d, 5 d/wk, for 6 or 12 mo Initial group size NR	Study 2, incidence for controls and incidences for animals exposed for 6 mo (8 mo old at start), 6 mo (14 mo old), 12 mo (8 mo old), 12 mo (14 mo old) Haemangiosarcomas(all sites): 1/71, 11/49**, 5/53, 17/46**, 3/50 Lung carcinomas: 9/71, 13/49*, 7/53, 9/46*, 3/50 Mammary gland carcinomas: 2/71, 13/49**, 2/53, 8/45**, 0/50	Study 2, *$P < 0.05$ **$P < 0.01$	
Mouse, B6C3F1 (F) Lifetime Drew *et al.* (1983)	Inhalation Study 1: 8–9 wk old mice exposed to 0 or 50 ppm [0, 130 mg/m3] 6 h/d, 5 d/wk, for 6 or 12 mo Initial group size NR	Study 1: incidence for controls and incidences for animals exposed for 6, 12 mo Haemangiosarcomas (all sites): 4/69, 46/67**, 69/90** Mammary gland carcinomas: 3/69, 29/67**, 37/90**	*$P < 0.05$ **$P < 0.01$	Commercial grade, purity NR
	Study 2: 8 or 14 mo old mice exposed to 0 or 50 ppm 6 h/d, 5 d/wk, for 6 or 12 mo Initial group size NR	Study 2: incidence for controls and incidence for animals exposed for 6 mo (8 mo old at start), 6 mo (14 mo old), 12 mo (8 mo old), or 12 mo (14 mo old) Haemangiosarcomas (all sites): 4/69, 27/42**, 30/51**, 30/84**, 29/48** Mammary gland carcinomas: 3/69, 13/42**, 4/51*, 9/48**, 4/48**		

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse CD-1 (M) up to 44–45 wk Suzuki (1983)	Inhalation 0, 1, 10, 100, 300, 600 ppm [0, 2.6, 26, 260, 780, 1560 mg/m^3] 6 h/d, 5 d/wk, 4 wk 30/group except 60/control group and 40/600 ppm-treated group	Benign pulmonary tumours: 12 wk after exposure: 0/18, 0/10, 0/9, 0/6, 6/9*, 8/9* 40–41 wk after exposure: 0/17, 1/9, 3/9, 6/9*, 5/7*, 6/7*	NR *[$P < 0.05$]	Purity NR
Rat, CD (M, F) 52 wk Lee *et al.* (1978)	Inhalation 0, 50, 250, 1000 ppm [0, 130, 650, 2600 mg/m^3] 6 h/d, 5 d/wk 36/group/sex, 4 animals/group were sacrificed at 1, 2, 3, 6 and 9 mo	Liver angiosarcomas: (M) 0/35, 0/36, 2/36, 6/34; (F) 0/35, 0/36, 10/34*, 15/36* Extrahepatic angiosarcomas: (M) 0/35, 1/36, 2/36, 4/34; (F) 0/35, 1/36, 3/34, 10/36*	*$P < 0.05$	99.8% pure
Rat, Wistar (M, F) 52 wk Feron & Kroes (1979) Feron *et al.* (1979)	Inhalation 0, 5000 ppm [0, 13000 mg/m^3] 7 h/d, 5 d/wk 62/group/sex, 10 animals/group/sex were sacrificed at 4, 13, 26 or 52 wk	At 52 wk Liver angiosarcomas: (M) 3/9; (F): 6/10* Zymbal gland squamous-cell carcinomas: (M) 3/9; (F): 2/10 Nasal cavity carcinomas: (M) 2/9; (F) 5/10* No tumours observed in controls: (M) 0/10; (F) 0/10	NR *[$P < 0.05$]	99.97% pure Information on survival NR
Rat, Sprague-Dawley (M, F) 43 wk Groth *et al.* (1981)	Inhalation 0, 940 ppm [0, 2465 mg/m^3] 7 h/d, 5 d/wk, 24.5 wk to rats 6, 18, 32, 52 wk of age 110–128/group/sex	Angiosarcomas (mostly in the liver): In rats 6, 18, 32, 52 wk of age at start: (M) 0/37, 0/44, 3/45, 13/55; (F) 2/38, 7/47, 23/49*, 11/54 In controls 32 wk of age at start: (M) 1/86; (F) 0/85	NR *[$P < 0.0001$]	Purity NR Epidemic of pneumonia during the 28th wk prematurely ended the study.
Rat, random bred white (M) 126 wk Kurliandskiĭ *et al.* (1981)	Inhalation 0, 5.4, 9.6, 102, 1420 ppm [0, 14, 25, 266, 3690 mg/m^3] 4.5 h/d, 5 d/wk, 52 wk 50–58/group, 93 controls	Angiosarcomas (all sites): 0% in controls, 9.3–15.7% in the two higher dose-treated group Liver angiosarcomas: 0% in controls, 9.3–11.8% in the two higher dose-treated group	NR	Purity NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague Dawley (M, F) 156 wk Maltoni *et al.* (1981)	Inhalation 0, 50, 250, 500, 2500, 6000, 10000 ppm [130, 650, 1300, 6500, 15600, 26000 mg/m^3] 4 h/d, 5 d/wk, 17 wk 30/group/sex, 190 controls	Liver angiosarcomas: 0/190, 0/58, 0/59, 1/60, 1/60, 1/60, 0/58 Hepatomas: 0/190, 0/58, 0/59, 0/60, 2/60, 1/60, 1/58 Zymbal gland carcinomas: 2/190, 0/58, 1/59, 1/60, 7/60, 9/60, 9/58 Skin epitheliomas: 1/190, 1/58, 0/59, 0/60, 2/60, 5/60, 5/58	NR [$P < 0.05$, some exposed group], Zymbal gland carcinoma, skin epithelioma	99.97% purity Data reported for both sexes combined
Rat, Sprague Dawley (M, F) 154 wk Maltoni *et al.* (1981)	Inhalation 0 (control), 6000 (group I, III, V), 10 000 (group II, IV, VI) ppm [0, 15 600, 26 000 mg/m^3] group I, II: 4 h/d, 5 d/wk, 5 wk; group III, IV: 1 h/d, 4 d/wk, 25 wk; group V, VI: 4 h/d, 1/wk, 25 wk 60/group/sex, 240 controls	controls, I, II, III, IV, V, VI Liver angiosarcomas: 0/227, 1/118, 0/120, 1/119, 3/118, 1/119, 1/120 Extrahepatic angiosarcomas: 0/227, 0/118, 0/120, 0/119, 2/118, 0/119, 1/120 Zymbal gland carcinomas: 0/227, 9/118, 9/120, 9/119, 5/118, 8/119. 9/120 Mammary gland tumours (malignant): 17/227, 13/118, 13/120, 16/119, 11/118, 20/119, 12/120	NR [$P < 0.05$, all exposed group], Zymbal gland carcinoma	99.97% purity Data reported for both sexes combined
Rat, Sprague Dawley (M, F) up to 147 wk Maltoni *et al.* (1981)	Inhalation 0, 1, 5, 10, 25, 50, 50, 100, 150, 200, 250, 500, 2500, 6000, 10000, 30000 ppm [0, 2.6, 13, 26, 65, 130, 130, 260, 390, 520, 650, 1300, 6500, 15600, 26000, 78000 mg/m^3] 4 h/d, 5 d/wk, 52 wk 60–300 M+F/group, 461 controls	Liver angiosarcomas: 0/461, 0/118, 0/119, 1/119, 5/120, 1/60, 14/294, 1/120, 6/119, 12/120, 3/59, 6/60, 13/60, 13/59, 7/60, 18/60 Zymbal gland carcinomas: 4/461, 1/118, 1/119, 2/119, 4/120, 0/60, 9/294, 1/120, 4/119, 4/120, 0/59, 4/60, 2/60, 7/59, 16/60, 35/60	NR [$P < 0.05$, many exposed group] [$P < 0.05$, some exposed group]	99.97% purity Five studies combined to construct a dose–response table. Rats exposed to highest concentration were observed for 68 wk, others for 135–147 wk. There was no dose–response
Rat, Wistar (M) up to 165 wk Maltoni *et al.* (1981)	Inhalation 0, 1, 50, 250, 500, 2500, 6000, 10000 ppm [0, 2.6, 130, 650, 1300, 6500, 15600, 26000 mg/m^3] 4 h/d, 5 d/wk, 52 wk 30–130/group	Liver angiosarcomas: 0/132, 0/99, 0/28, 1/27, 3/28, 3/25, 3/26, 8/27 Extrahepatic angiosarcomas: 1/132, 3/99, 0/28, 1/27, 0/28, 1/25, 1/26, 0/27 Hepatomas: 0/132, 1/99, 0/28, 0/27, 0/28, 1/25, 2/26, 0/27 Zymbal gland carcinomas: 3/132, 2/99, 0/28, 0/27, 0/28, 0/25, 2/26, 2/27	NR [$P < 0.05$, some exposed group], liver angiosarcoma	99.97% purity

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Fischer (F) Lifetime Drew *et al.* (1983)	Inhalation 0, 100 ppm [0, 260 mg/m^3] 6 h/d, 5 d/wk, for 6, 12, 18 or 24 mo 55–112/group	Tumour incidence after 24 mo of exposure: Liver haemangiosarcomas: 1/112, 19/55* Haemangiosarcomas (all sites): 2/112, 24/55* Mammary gland adenocarcinomas: 5/112, 5/55* Hepatocellular carcinomas: 1/112, 9/55*	*$P < 0.01$	Purity NR Rats necropsied when moribund or dead
Rat, Fischer (F) Lifetime Drew *et al.* (1983)	Inhalation 0 (control), 100 ppm [0, 260 mg/m^3] 6 h/d, 5 d/wk for 6 mo (rats 2, 8, 14 or 20 mo old at start) or 12 mo (rats 2, 8 or 14 mo old at start) 51–112/group	Liver haemangiosarcomas: 1/112, 4/76*, 2/52, 0/51, 0/53, 11/55*, 5/54*, 2/49 Haemangiosarcomas (all sites): 2/112, 4/76, 2/53, 0/53, 0/53, 12/56*, 5/55*, 2/50 Mammary gland adenocarcinomas: 5/112, 6/76, 2/53, 3/53, 2/53, 11/56*, 4/55, 0/50 Hepatocellular carcinomas: 1/112, 3/75, 6/52*, 0/51, 1/53, 4/56*, 1/54, 0/49	*$P < 0.01$	Rats necropsied when moribund or dead. Statistically significant increases were also observed for mammary gland fibroadenomas and hepatocellular adenomas.
Rat, Sprague Dawley (M, F) 136 wk Maltoni *et al.* (1981)	Oral (gastric intubation) 0, 3.3, 17, 50 mg/kg bw, 4–5 × / wk, 52 wk 40/group/sex	Liver angiosarcomas: 0/80, 0/80, 10/80*, 17/80* Extrahepatic angiosarcomas: 0/80, 2/80, 0/80, 2/80 Nephroblastomas: 0/80, 0/80, 3/80, 2/80	NR *[$P < 0.05$]	99.97% purity Information on survival NR Data reported for both sexes combined
Rat, Sprague Dawley (M, F) 136 wk Maltoni *et al.* (1981)	Oral (gastric intubation) 0, 0.03, 0.3, 1 mg/kg bw, 4–5 × /wk, 52–59 wk 75/group/sex	Liver angiosarcomas: 0/150, 0/150, 0/148, 3/149. Extrahepatic angiosarcomas: 0/150, 0/150, 0/148, 1/149 Hepatomas: 0/150, 0/150, 1/148, 1/149 Zymbal gland carcinomas: 1/150, 0/150, 0/148, 5/149 Mammary gland tumours (malignant): 7/150, 14/150, 4/148, 12/149	NR, [NS]	99.7% purity Data reported for both sexes combined
Rat, Wistar (M, F) 135 wk (M), 144 wk (F) Feron *et al.* (1981)	Oral (feed) 0, 1, 3, 10% of 4000 ppm vinyl chloride in a PVC powder [resulting in a daily dose of 0, 1.7, 5.0, 14 mg vinyl chloride/kg bw] 4 h/d, 7 d/wk 60–80/group/sex	Liver haemangiosarcomas: (M) 0/55, 0/58, 6/56*, 27/59*; (F) 0/57, 0/58, 2/59, 9/57* Hepatocellular carcinomas: (M) 0/55, 1/58, 2/56, 8/59*; (F) 0/57, 4/58, 19/59*, 29/57* Hepatocellular adenomas: (M) 0/55, 1/58, 7/56*, 23/59*; (F) 2/57, 26/58, 39/59*, 44/57* Lung angiosarcomas: (M) 0/55, 0/58, 4/56*, 19/59*; (F) 0/57, 0/58, 1/59, 5/57*	*$P < 0.05$	99.7% purity

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Wistar (M, F) 149 wk (M), 150 wk (F) Til *et al.* (1991)	Oral (feed) PVC powder in food that resulted in dose of 0, 0.014, 0.13, 1.3 mg vinyl chloride/kg bw 4–6 h/d, 7 d/wk 50–100/group/sex	Hepatocellular carcinomas: (M) 0/99, 0/99, 0/99, 3/49*; (F) 1/98, 0/100, 1/96, 3/49 Hepatocellular adenomas: (M) 0/99, 0/99, 0/99, 1/49; (F) 0/98, 1/100, 1/96, 9/49* Liver haemangiosarcomas: (M) 0/99, 0/99, 0/99, 1/49; (F) 0/98, 0/100, 0/96, 2/49	*$P < 0.05$	99.7% purity
Rat, Sprague-Dawley (M, F) 145 wk Maltoni *et al.* (1981)	Subcutaneous injection Single injection of 0, 4.25 mg in 1 ml olive oil, 35 M/group, 40 F/group	Mammary gland tumours (malignant): 3/75, 1/75. Nephroblastomas: 0/75, 1/75	NR, [NS]	99.97% purity No other tumour types observed Data reported for both sexes combined
Rat, Sprague-Dawley (M, F) 144 wk Maltoni *et al.* (1981)	Intraperitoneal injection 0 (olive oil, once); 4.25 mg/kg bw, once, twice, three, or four times at 2 mo intervals 30/group/sex	Extrahepatic angiosarcomas: 0/55, 0/55, 1/56, 1/53, 0/56 Mammary gland tumours (malignant): 0/55, 2/55, 3/56, 1/53, 1/56	NR, [NS]	99.97% purity No liver angiosarcomas observed Data reported for both sexes combined
Rat, Sprague-Dawley (M, F) 143 wk Maltoni *et al.* (1981)	Transplacental Pregnant females exposed by inhalation to 6000, 10000 ppm [15600, 26000 mg/m^3] on D 12–18 of pregnancy 30–54 dams/group	In offspring Extrahepatic angiomas: 1/32, 0/51 Nephroblastomas: 0/32, 3/51 Zymbal gland carcinomas: 3/32, 5/51 Skin epitheliomas: 1/32, 0/51 Forestomach papillomas and achanthomas: 1/32, 1/51 Mammary gland tumours (malignant): 2/32, 1/51	-	99.97% purity No angiosarcomas (all sites) or hepatomas were observed in offspring. One Zymbal gland tumour at high dose (1/30) was the only tumour observed in dams. Despite the lack of controls, this study provides some evidence of the transplacental carcinogenicity of vinyl chloride. Data reported for both sexes combined.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (M, F) 124 wk Maltoni *et al.* (1981)	Perinatal Breeders and newborn offspring exposed together by inhalation to. 6000, 10000 ppm [15600, 26000 mg/m^3] 4 h/d, 5 d/wk, 5 wk 42–44/group (offspring)	In offspring Hepatic angiosarcomas: 17/42, 15/42 Liver angiomas: 1/42, 0/44 Extrahepatic angiosarcomas: 1/42, 0/44 Extrahepatic angiomas: 1/42, 3/44 Hepatomas: 20/42, 20/44 Zymbal gland carcinomas: 2/42, 1/44 Skin epitheliomas: 2/42, 1/44 Mammary gland tumours (malignant): 1/42, 0/44	-	99.97% No concurrent controls No tumours observed in breeders at sites where tumours occurred in offspring Data reported for both sexes combined
Rat, Sprague-Dawley (M, F) Lifetime Maltoni & Cotti (1988)	Perinatal Breeders exposed by inhalation to 0, 2500 ppm [0, 6500 mg/m^3], 4 h/d, 5 d/wk, 7 wk; dams became pregnant and delivered offspring. Dams exposed (7 h/d) for 69 additional wk with group I offspring; group II offspring exposed only for 8 additional wk 54–60 breeders (F)/group, 60–63 offspring/group/sex, 149–158 control offspring/group/sex	Hepatocarcinomas: Breeders (F): 0/60, 5/54 Offspring group I: (M) 27/64; (F) 38/63; group II: (M) 42/60; (F) 43/60; controls: (M) 1/158; (F) 0/149 Liver angiosarcomas: Breeders (F): 0/60, 27/54 Offspring group I: (M) 36/64; (F) 46/63; group II: (M) 24/60; (F) 28/60; controls: (M) 0/158; (F) 0/149 Neuroblastomas (see comments): Breeders (F): 0/60, 32/54 Offspring group I: (M) 31/64; (F) 27/63; group II: (M) 7/60; (F) 11/60; controls: (M) 0/158; (F) 0/149	NR [$P < 0.05$], all exposed group	99.97% purity The photomicrographs and the preferential location of the neuroblastomas in the anterior frontal lobes support the alternative diagnosis of an origin in the metabolically active olfactory neuroepithelium of the posterior nasal cavity (aesthesioneuroepithelioma)
Hamster, Golden (M) 109 wk Maltoni *et al.* (1981)	Inhalation 0, 50, 250, 500, 2500, 6000, 10 000 ppm [0, 130, 650, 1300, 6500, 15600, 26000 mg/m^3] 4 h/d, 5 d/wk, 30 wk 30/group, 60 controls	Liver angiosarcomas: 0/60, 0/30, 0/30, 2/30, 0/30, 1/30, 0/30 Skin epitheliomas: 3/60, 9/30*, 3/30, 7/30*, 3/30, 1/30, 7/30* Forestomach papillomas and acanthomas: 3/60, 3/30, 4/30, 9/30*, 17/30*, 10/30*, 10/30* Leukaemia: 8/60, 6/30, 6/30, 5/30, 9/30, 6/30, 5/30	NR *[$P < 0.05$]	99.97% purity Information on survival NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian golden (F) Lifetime Drew *et al.* (1983)	Inhalation 200 ppm [520 mg/m^3] 6 h/d, 5 d/wk 0 (untreated control); 6, 12, or 18 mo exposure of hamsters 8 wk old at onset; 6 mo exposure of hamsters 8, 14, or 20 mo old at onset; 12 mo exposure of hamsters 2, 8, 14 mo old at onset Initial group size NR	Haemangiosarcomas (all sites): 0/143, 13/88**, 4/52**, 2/103, 3/53*, 0/50, 0/52, 4/52**, 1/44, 0/43 Mammary gland carcinomas: 0/143, 28/87**, 31/52**, 47/102**, 2/52*, 0/50, 1/52, 31/52**, 6/44**, 0/42 Stomach adenomas: 5/138, 23/88**, 3/50*, 20/101**, 15/53**, 6/49*, 0/52, 3/50*, 10/44**, 3/41 Skin carcinomas: 0/133, 2/80, 9/48**, 3/90, 0/49, 0/46, 0/50, 2/80, 0/38, 0/30	*$P < 0.05$ **$P < 0.01$	Purity NR, commercial grade. Decrease in survival of exposed animals. Hamsters were necropsied when moribund or dead.
Chloroethylene oxide				
Mice, XVIInc./Z (M, F) ~80 wk Zajdela *et al.* (1980)	Subcutaneous 32 injections of 0 (control) or 0.1 mg chloroethylene oxide over 42 wk	Local tumours (mainly fibrosarcomas): 15/28 (M); 12/24 (F); 0/30 (M, controls)	[$P < 0.0001$]	Purity NR
Mice, XVIInc./Z (M) 52–54 wk Zajdela *et al.* (1980)	Skin painting with 1 mg chloroethylene oxide followed by TPA as a promoter 2 wk after initiation (3 × /wk, 42 wk)	Skin papillomas: 18/28 vs 4/28 (TPA controls). Skin carcinomas: 5/28 vs 0/28 (TPA controls)	[$P < 0.001$] $P < 0.02$	Purity NR Chloroethylene oxide tested as an initiator Chloroethylene oxide (1 mg) dissolved in 80 µL benzene

bw, body weight; d, day or days; h, hour or hours; F, female; M, male; h, hour or hours; mo, month or months; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; vs, versus; wk, week or weeks

a high incidence of tumours that were probably of olfactory neuroepithelial origin, but were formerly reported as cerebral neuroblastomas in some studies. Similar results were observed in co-exposed dams (Maltoni & Cotti, 1988). In a second study, rats were exposed to vinyl chloride for five weeks, beginning at birth. Angiosarcomas of the liver and hepatomas occurred at a high incidence in the offspring, but not in the dams that were co-exposed with the offspring (Maltoni *et al.*, 1981).

3.5 Carcinogenicity of metabolites

Chloroethylene oxide, a chemically reactive metabolite of vinyl chloride, was tested for carcinogenicity in a single study in mice by subcutaneous injection and in an initiation–promotion protocol by skin application. It caused a massive increase of fibrosarcomas at the site of injection and increased the incidence of squamous-cell papillomas and carcinomas of the skin at the site of application (Zajdela *et al.*, 1980).

4. Other Relevant Data

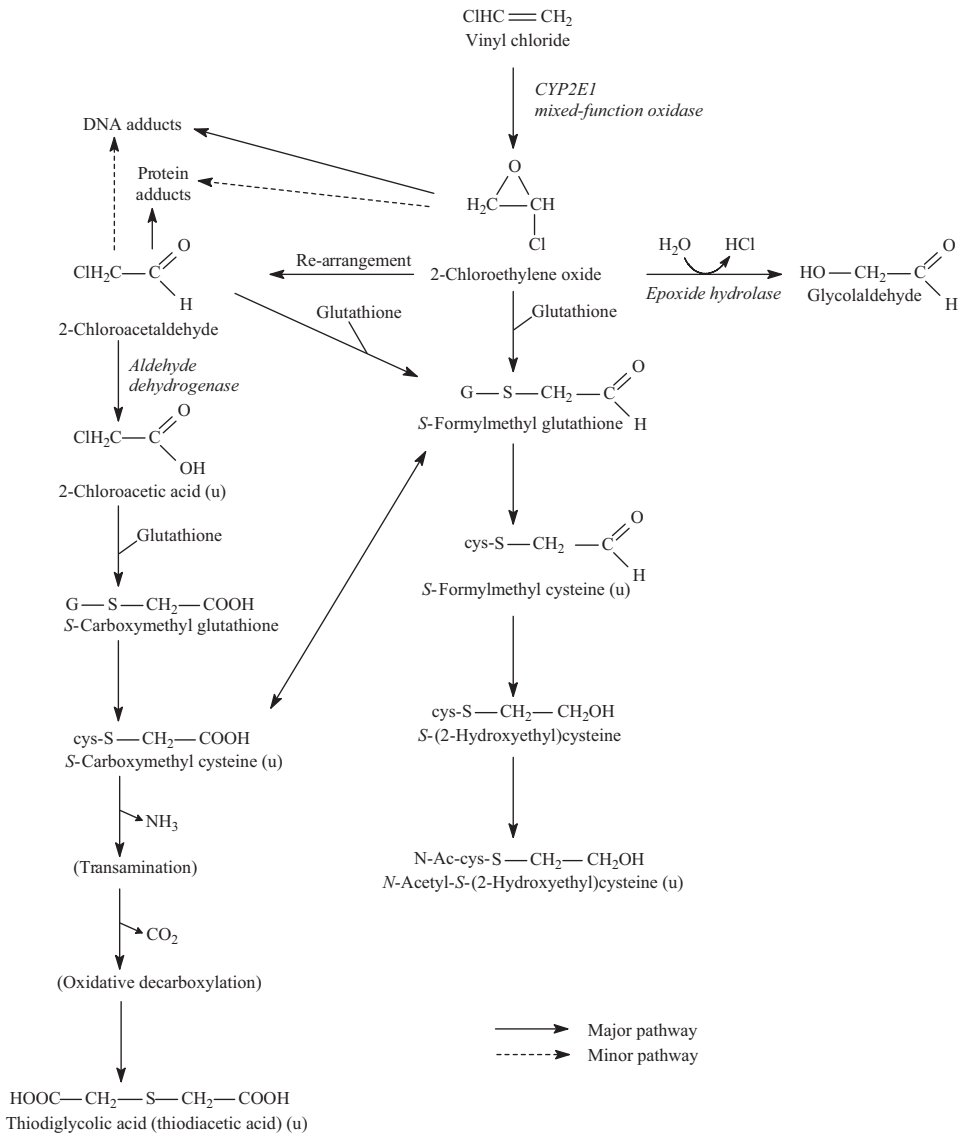
4.1 Kinetics and metabolism – studies in humans

Pulmonary absorption of vinyl chloride in humans appears to be rapid and the percentage absorbed is independent of the concentration inhaled. Adult male volunteers exposed for six hours to air containing 2.9–23.1 ppm [7.5–60 mg/m^3] vinyl chloride, retained on average approximately 42% of the inhaled amount (Krajewski *et al.*, 1980; cited in ATSDR, 2006). Pulmonary uptake is determined in part by the blood–air partition constant, which is 1.16 for vinyl chloride (Gargas *et al.*, 1989). Even if no data in humans were available, by assuming an identical solubility of vinyl chloride in rodent and human tissues, the tissue–blood partition constants would be twofold greater in humans (Clewell *et al.*, 2001), as a consequence of the twofold lower blood–air partition coefficient of vinyl chloride in humans compared with rats and mice.

In the postmitochondrial fractions of liver homogenates of humans and rats, large interindividual variations were noted in the metabolism of vinyl chloride, while the average activity was comparable between rat and human samples (Sabadie *et al.*, 1980). Vinyl chloride is primarily and rapidly metabolized in the liver (see Fig. 4.1), with a saturable mechanism (Reynolds *et al.*, 1975; Ivanetich *et al.*, 1977; Barbin & Bartsch, 1989; Lilly *et al.*, 1998; Bolt, 2005). The first step is oxidation in the liver, predominantly mediated by the human cytochrome P450 (CYP) isoenzyme 2E1 (WHO, 1999). Since CYP2E1 is present in several tissues at low levels – compared with concentrations in the liver – extrahepatic metabolism of systemically available vinyl chloride does occur. Inhibitors of CYP, such as 3-bromophenyl-4(5)-imidazole or 6-nitro-1,2,3-benzothiadiazole, reduce the metabolism of vinyl chloride *in vivo* (Bolt *et al.*, 1976). The primary metabolites of vinyl chloride are the highly reactive chloroethylene oxide, which is formed in a dose-dependent process and has a half-life of 1.6 minutes in aqueous solution at neutral pH (Barbin *et al.*, 1975; Dogliotti, 2006), and its rearrangement product chloroacetaldehyde (Bonse *et al.*, 1975). Both can bind to proteins, DNA and RNA and form ethenoadducts; chloroethylene oxide is the most reactive with nucleotides (Guengerich *et al.*, 1979).

Conjugation of chloroethylene oxide and chloroacetaldehyde with glutathione (GSH) eventually leads to the major urinary metabolites *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and thiodiglycolic acid (Plugge & Safe, 1977). The latter compound has been reported to be the major metabolite in the urine of exposed workers (Cheng *et al.*, 2001) with concentrations in

Fig. 4.1 Proposed metabolic pathways for vinyl chloride



From Barbin *et al.* (1975), Plugge & Safe (1977), Green & Hathway (1977), Guengerich & Watanabe (1979), Guengerich *et al.* (1979), Bolt *et al.* (1980), adapted from ATSDR (2006).
CYP, cytochrome P450; (u), excreted in urine

urine that correlated with environmental vinyl chloride concentrations of > 5 ppm (ATSDR, 2006). Chloroethylene oxide can also be detoxified to glycolaldehyde by microsomal epoxide hydrolase (mEH), while chloroacetaldehyde can be converted to chloroacetic acid by aldehyde dehydrogenase 2 (ALDH2) in the urine (Guengerich & Watanabe, 1979; ATSDR, 2006; IARC, 2008). Another route of elimination of vinyl chloride is exhalation of the unmetabolized compound, which occurs at low levels in humans (Müller *et al.*, 1978; Krajewski *et al.*, 1980; Pleil & Lindstrom, 1997). When volunteers were exposed for six hours to air containing 6.8–23.1 ppm [15–60 mg/m^3] vinyl chloride, the mean concentration in exhaled air ranged from 0.21 to 1.11 ppm [0.54–2.84 mg/m^3], representing 3.6 and 4.7%, respectively, of the inhaled amount of vinyl chloride (Krajewski *et al.*, 1980).

4.2 Kinetics and metabolism – studies in animals

Experimental studies on vinyl chloride have been evaluated in previous *IARC Monographs* (IARC, 1979, 1987, 2008). Comprehensive data on the mechanism of vinyl chloride-induced carcinogenicity are available, encompassing toxicokinetics, metabolism, biomarkers, and genotoxicity. Many key events in the pathway of vinyl chloride-induced hepatocarcinogenesis have been established (Bolt, 2005; Dogliotti, 2006; IARC, 2008).

The absorption, distribution, metabolism and elimination of vinyl chloride in rats and mice have been reviewed in *IARC Monograph* Volumes 19 and 97 (IARC, 1979, 2008) and elsewhere (WHO, 1999; ATSDR, 2006); the most relevant data are summarized below.

In animals, pulmonary and gastrointestinal absorption of vinyl chloride occurs readily and rapidly, while dermal absorption is probably not significant. In monkeys exposed (whole body, except the head) to atmospheres containing 7000 and 800 ppm vinyl chloride for, respectively, 2.0 and 2.5 hours, only 0.023–0.031% of the total available amount of vinyl chloride was absorbed via the dermal route (Hefner *et al.*, 1975a), whereas intestinal absorption and uptake in blood was virtually complete in 10 minutes in rats after single oral doses (44–92 mg/kg bw) in aqueous solution (Withey, 1976). Pulmonary absorption in rats amounted to about 40% of inhaled [^{14}C]-labelled vinyl chloride for initial exposure concentrations below 260 mg/m^3 [100 ppm] (Bolt *et al.*, 1976).

The tissue–blood partition constants determine the distribution volume of vinyl chloride, and range from 0.4 (muscle) to 10 (fat) in male rats (Barton *et al.*, 1995). The fat–air partition constant for vinyl chloride, reported by several authors, tends to be higher in females than in males (WHO, 1999). Following inhalation, vinyl chloride is distributed in several tissues such as brain, liver, spleen, kidney, adipose tissue and muscle, with the highest levels found in liver and kidney (Bolt *et al.*, 1976). It has also been detected in fetal blood and amniotic fluid of rats after a 2.5-hour exposure to ~2000–13000 ppm [5200–33800 mg/m^3]), which indicates its capability to cross the placental barrier (Ungváry *et al.*, 1978).

CYP2E1 appears to account for all metabolic activity in rat liver microsomes, with a maximum velocity (V_{max}) of 4674 pmol/mg protein/min and a Michaelis-Menten constant (Km) of 7.42 µmol/L (El Ghissassi *et al.*, 1998). Chloroacetic acid was metabolized in rats to two major urinary metabolites, *viz.* *S*-(carboxymethyl) cysteine and thiodiacetic acid (Yllner, 1971). *S*-(carboxymethyl)cysteine, *S*-(2-chloroethyl) cysteine and *N*-acetyl-*S*-vinylcysteine are metabolites of vinyl chloride in rats after oral administration (Watanabe *et al.*, 1976a; Green & Hathway, 1975 and 1977) and *N*-acetyl-*S*-(2-hydroxyethyl)cysteine is a metabolite after inhalation (Watanabe *et al.*, 1976b). Thiodiglycolic

acid was obtained as a common metabolite in rats dosed separately with either chloroacetaldehyde, chloroacetic acid or *S*-(carboxymethyl) cysteine. Therefore, the identification of the same *S*-containing metabolite from vinyl chloride-treated animals lends support to the hypothesis that chloroethylene oxide or chloroacetaldehyde are formed and react with GSH (Green & Hathway, 1977). Following oral administration of [^{14}C]-labelled vinyl chloride to rats, [^{14}C]-carbon dioxide (Green & Hathway, 1975; Watanabe *et al.*, 1976a), [^{14}C]-labelled urea and glutamic acid were identified as minor metabolites (Green & Hathway, 1975).

Saturation of the metabolism of vinyl chloride (Gehring *et al.*, 1978; Filser & Bolt, 1979) appears to occur at inhalation concentrations above 200 ppm [520 mg/m^3] in rhesus monkeys (Buchter *et al.*, 1980) and above 250 ppm [650 mg/m^3] in rats (Bolt *et al.*, 1977; Filser & Bolt, 1979). The plateau of incidence of ASL in carcinogenicity bioassays is also observed in rats at exposures above 250 ppm (reviewed in Bolt, 2005).

ATSDR (2006) summarized the kinetic constants obtained *in vivo* in male Sprague-Dawley rats (V_{max}, 58 µmol/h/kg; Km, 1 µM) and rhesus monkeys (V_{max}, 50 µmol/h/kg) (based on Buchter *et al.*, 1980; Barton *et al.*, 1995). The latter value (50 µmol/h/kg) was suggested to be a closer approximation to human metabolism than the value of 110 µmol/h/kg estimated for rats by Filser & Bolt (1979) (ATSDR, 2006).

Watanabe *et al.* (1978a) reported that the elimination rate of vinyl chloride was not altered during repeated exposures via inhalation (five days per week during seven weeks) compared with a single inhalation exposure (~13 000 mg/m^3 [5000 ppm]).

Urinary excretion of polar metabolites of vinyl chloride is the predominant route of elimination at low concentrations, and only very small amounts are expired in the air unchanged (Hefner *et al.*, 1975b). Once metabolic saturation is attained, vinyl chloride is eliminated via other routes, mainly exhalation of the parent chemical. Following exposure of male rats to 26 mg/m^3 [10 ppm] [^{14}C]-labelled vinyl chloride by inhalation during six hours, urinary [^{14}C] radioactivity and expired vinyl chloride (measured as [^{14}C]-labelled carbon dioxide) were recovered in amounts of 68% and 2%, respectively; after exposure to a 100-fold higher concentration, the proportion of radioactivity in the urine decreased to 56% and the amount expired increased to 12% (Watanabe *et al.*, 1976b). Moreover, the same authors showed that after single oral doses of 0.05, 1 or 100 mg/kg bw [^{14}C]-labelled vinyl chloride, urinary excretion of radioactivity was 68, 59 and 11%, respectively; expired [^{14}C]-labelled carbon dioxide accounted for 9, 13 and 3%, respectively; pulmonary elimination of [^{14}C]-labelled vinyl chloride represented only 1–3% of the lower dose and 67% of the higher dose (Watanabe *et al.*, 1976a). The route of elimination may depend upon the route of administration, since urinary excretion is favoured after oral or intra-peritoneal administration, which indicates a first-pass effect due to metabolism in the liver (Clewell *et al.*, 2001).

4.3 Reaction with cellular macromolecules

Vinyl chloride is a genotoxic carcinogen in animals and humans (Block, 1974; Creech & Johnson, 1974; Lee & Harry, 1974; Maltoni *et al.*, 1974, 1981). It is mutagenic, usually in the presence of metabolic activation, in various assays with bacteria, yeast or mammalian cells; it is also clastogenic *in vivo* and *in vitro*. Vinyl chloride induces unscheduled DNA synthesis, increases the frequency of sister chromatid exchange in rat and human cells, and increases the frequency of chromosomal aberrations and micronucleus formation in mice, rats, and hamsters *in vivo* (IARC, 2008).

Osterman-Golkar *et al.* (1976) reported the alkylation of haemoglobin – at cysteine and

histidine – and small amounts of alkylated histidine in proteins from the testis of mice exposed to [^{14}C]-labelled vinyl chloride. Binding of non-volatile metabolites of [^{14}C]-labelled vinyl chloride to liver macromolecules has been observed, both *in vitro* and in rats exposed by inhalation (Kappus *et al.*, 1976; Watanabe *et al.*, 1978a, b; Guengerich & Watanabe, 1979; Guengerich *et al.*, 1979; Bolt *et al.*, 1980; Guengerich *et al.*, 1981; Barton *et al.*, 1995). A decrease in non-protein sulfhydryl concentration was seen in rats after exposure to high concentrations of vinyl chloride (Jedrychowski *et al.* (1984). Kappus *et al.* (1975) and Laib & Bolt (1977) reported binding of vinyl chloride to RNA in an in-vitro incubation with rat-liver microsomes, and to liver RNA of rats exposed *in vivo*. Watanabe *et al.* (1978b) reported macromolecular binding proportional to the amount of vinyl chloride metabolized, but not proportional to the exposure concentration.

Chloroethylene oxide and chloroacetaldehyde can form etheno adducts with nucleic acid bases *in vitro* (Guengerich, 1992). Chloroethylene oxide yields the *N*7-(2-oxoethyl)guanine adduct (7-OEG), four etheno adducts – 1,N^6-ethenoadenine (εA), 3,N^4-ethenocytosine (εC), N^2,3-ethenoguanine (N^2,3-εG) and 1,N^2-ethenoguanine (1,N^2-εG) (Ciroussel *et al.*, 1990; Guengerich, 1992), and 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo[1,2-*a*]purine (HO-ethanoG) (Müller *et al.*, 1996). In rats, the DNA adducts εA and εC have been found in various organs after exposure to vinyl chloride by inhalation. 7-OEG was the major DNA adduct formed *in vivo* and was found in greater amounts in young animals (Swenberg *et al.*, 2000). However, 7-OEG has a short half-life of about 62 hours, while the etheno adducts are more persistent. For example, N^2,3-εG (which is 10–100-fold more abundant than other etheno adducts in exposed animals) has a half-life of about 30 days (Fedtke *et al.*, 1990). After exposure of rats to 500 ppm [1300 mg/m^3] vinyl chloride for eight weeks, the εA level was significantly increased above background in liver, lung, lymphocytes and testis, while the amount of εC was increased in liver, kidney, lymphocytes and spleen, but not in brain (Guichard *et al.*, 1996; Barbin, 1999). When adult rats were exposed to 1100 ppm [2860 mg/m^3] vinyl chloride for one or four weeks, there was a significant increase in the level of N^2,3-εG in hepatocytes and non-parenchymal cells, but not in the brain, with a linear increase at exposure concentrations from 0 to 100 ppm [260 mg/m^3] and a plateau at 100–1100 ppm [260–2860 mg/m^3]. In weanling animals there was a small, statistically significant increase in N^2,3-εG in the brain after five days of exposure and the amount of N^2,3-εG in hepatocytes was significantly greater than that measured in non-parenchymal cells after exposures to 10 and 100 ppm [26 and 260 mg/m^3] vinyl chloride (Morinello *et al.*, 2002a). These differential responses between weanlings and adults may contribute to the particular susceptibility of young rats to vinyl chloride-induced neuroblastomas and HCC (Maltoni & Cotti, 1988). There was no significant difference in N^2,3-εG-adduct levels, nor in the rate of repair between hepatocytes and non-parenchymal cells (Morinello *et al.*, 2002b), which confirms the earlier observation of Yang *et al.* (2000). Data on the occurrence and persistence of vinyl chloride-DNA adducts in humans are still lacking. Nair *et al.* (1995) used immunoaffinity purification of the etheno adducts and subsequent [^{32}P]-postlabelling, and reported values of 14.1 εA and 8.1 εC per 10^9 parent bases in non-neoplastic liver tissue of a vinyl chloride-exposed patient with HCC. These adducts may also result from lipid peroxidation (El Ghissassi *et al.*, 1995) and their level can be quite high in patients with unknown exposure (up to 0.5–40 εA and εC per 10^9 parent bases in liver-DNA samples) (Bartsch & Nair, 2000a, b).

Barbin *et al.* (1985) reported that the 7-OEG–DNA adduct lacks miscoding or promutagenic properties. In contrast, etheno adducts and related exocyclic DNA adducts (e.g. εA, εC,

N^2,3–εG, and HO-ethanoG) may be involved in base-pair substitution and other specific mutations in cancer-related genes (i.e. *RAS* oncogenes, *TP53* tumour-suppressor gene) (WHO, 1999). The DNA lesions εA, εC and *N*2,3-εG have demonstrated miscoding potential *in vitro* and *in vivo* (Singer *et al.*, 1987; Cheng *et al.*, 1991; Mroczkowska & Kuśmierek, 1991; Singer *et al.*, 1991; Basu *et al.*, 1993). The adduct εA causes A→G transitions and A→T transversions, εC causes C→A transversions and C→T transitions and εG causes G→A transitions (Bolt, 2005). The same mutation types are observed in *TP53* and *RAS* genes in vinyl chloride-induced tumours. Mutations in K_i-*RAS* are associated with vinyl chloride-induced angiosarcomas in humans but not in rats, and to a lesser extent with vinyl chloride-induced HCC (CAA61CTA Ha-*Ras* mutation) in rats (IARC, 2008). In half of the cases, these mutations led to the incorporation of aspartate instead of glycine. *TP53* mutations associated with exposure to vinyl chloride (frequently A→T transversions) are found in approximately half of the angiosarcomas in both humans and rats. The presence of mutated p21ras and p53 proteins in the blood of a high proportion of workers exposed to vinyl chloride and the positive correlation between the occurrence of the mutated proteins and cumulative exposure to vinyl chloride, suggest that the mutation is an early event (IARC, 2008).

Various assays have been designed to explore the mutagenic properties of DNA adducts introduced into oligonucleotides or into site-specific vectors. Vector plasmids have also been treated with 2-chloroethyleneoxide or 2-chloroacetaldehyde and propagated in *E. coli* or mammalian cells. The mechanism by which adducts cause mutations still remains unclear, as misincorporation events depend on the individual mechanisms of DNA polymerases (Choi *et al.*, 2006). HO-ethanoG and 1,N^2-εG block the replication process with many different polymerases, thereby causing base misincorporation (Langouët *et al.*, 1997, 1998; Guengerich *et al.*, 1999).

The induction of extrahepatic tumours (e.g. in the brain or lung) by vinyl chloride has been established experimentally, but the mechanism is not well elucidated (Bolt, 2005). Overall, data suggest that etheno adducts are probably involved in the initiation of hepatocarcinogenesis, but the effects of the observed tissue- and cell-specificity and the variability in various biomarkers such as mutant p53 and anti-p53 antibodies are not completely clear (Trivers *et al.*, 1995; Brandt-Rauf *et al.*, 1996). One source for this variability may be explained by differences in polymorphisms in genes (i.e. *CYP2E1*, *GSTT1*, *GSTM1*, *ALDH2*) that encode metabolising enzymes or DNA-repair proteins (i.e. the *XRCC1* gene) (Li *et al.*, 2003a).

4.4 Synthesis

Numerous studies on the toxicokinetics, metabolism, genotoxicity, and molecular biology of vinyl chloride provide strong evidence that the carcinogenicity of this chamical involves a genotoxic mechanism of action, mediated by reactive metabolites. The extensive information on the mechanism underlying vinyl chloride-induced carcinogenicity has established many key events in the pathway of vinyl chloride-induced liver carcinogenesis. These key events include metabolic activation to reactive metabolites, binding of the metabolites to DNA, promutagenic action of these adducts leading to G→A and A→T transitions, and the effects of such mutations on the functioning of proto-oncogenes and tumour-suppressor genes at the gene and protein levels, with tumourigenesis as the final outcome. Many of these key events identified in experimental animals have also been demonstrated in humans.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of vinyl chloride. Vinyl chloride causes angiosarcoma of the liver, and hepatocellular carcinoma.

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

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

There is strong evidence that the carcinogenicity of vinyl chloride operates by a genotoxic mechanism that involves metabolic activation to reactive metabolites, binding of the metabolites to DNA, promutagenic action of these adducts leading to mutations in proto-oncogenes and tumour-suppressor genes. Many of these key events identified in experimental animals have also been demonstrated in humans.

Vinyl chloride is *carcinogenic to humans (Group 1).*

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ISOPROPYL ALCOHOL MANUFACTURE BY THE STRONG-ACID PROCESS

Isopropyl alcohol and isopropyl alcohol manufacture (strong-acid process) were considered by previous IARC Working Groups in 1977 and 1987 (IARC, 1977, 1987). Since that time, new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation. A separate *Monograph* on 'Mists from Strong Inorganic Acids' – updating earlier evaluations on this agent (IARC, 1992) – appears elsewhere in this volume.

1. Exposure Data

1.1 Manufacturing processes

Isopropyl alcohol has been called the first petrochemical (IARC, 1992). It can be prepared via three different methods: indirect hydration of propylene, direct hydration of propylene, and catalytic hydrogenation of acetone. Indirect hydration, also called the sulfuric-acid process, was the only method used to produce isopropanol worldwide until the first commercial direct-hydration process was introduced in 1951. Each method has its advantages and disadvantages. For example, direct hydration is less corrosive than indirect hydration mediated by sulfuric acid. However, the direct method requires a pure propylene feed, in contrast to the indirect process, which can use a dilute, refinery stream (Lee *et al.*, 2003).

In the indirect-hydration process, propylene is reacted with sulfuric acid to produce mono- and diisopropyl sulfates, which are then hydrolysed to isopropanol. In the two-step strong-acid process, separate reactors are used for the propylene-absorption phase and the hydrolysis of the sulfate esters. The reaction occurs at high sulfuric acid concentration (> 80% wt) and low temperature (e.g. 20–30 °C). The weak-acid process is conducted in a single step at lower acid concentration (60–80% wt) and higher temperature (60–65 °C) (Logsdon & Loke, 2001).

1.2 Human exposure

1.2.1 Occupational exposure

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, based on data collected in the European Union (EU) from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). No results for isopropanol were reported. For data on EU workers exposed to strong inorganic acid mists containing sulfuric acid, see the *Monograph* on Mists from Strong Inorganic Acids in this volume.

From the National Occupational Exposure Survey (1981–83) it was estimated that approximately 4.7 million workers (including approximately 2.1 million women) in the United States of America (USA) were potentially exposed to isopropanol (NIOSH, 1990). No specific information on the numbers of workers exposed during isopropanol production was provided.

Although no data were available on exposure measurements at the workplace during isopropanol production, potential exposures from the indirect-hydration process include propylene, sulfuric acid, isopropanol, diisopropyl and isopropyl hydrogen sulfates, diisopropyl ether, propanal, acetone, sulfur oxides, polymeric oils and residues. In the past, benzene was used as an azeotroping agent to remove water from 'wet isopropanol', but nowadays diisopropyl ether or cyclohexane are preferentially used for this purpose (IARC, 1992; Papa, 2000; Logsdon & Loke, 2001).

2. Cancer in Humans

In *IARC Monographs* Supplement 7 it was concluded that there was *sufficient evidence* in humans for the carcinogenicity of work in the manufacture of isopropyl alcohol by the strong-acid process and *inadequate evidence* for the carcinogenicity of exposure to isopropyl alcohol and isopropyl oils (IARC, 1987). The carcinogenic hazards to humans of work in the manufacture of isopropyl alcohol by other methods, and of exposure to diisopropyl sulfate were not evaluated. The evaluation was based on an increased incidence of cancer of the paranasal sinuses observed in workers at factories where isopropyl alcohol was manufactured by the strong-acid process. The risk for laryngeal cancer may also have been elevated in these workers.

In *IARC Monograph* Volume 54 (IARC, 1992) an evaluation was made of exposure to mists from strong inorganic acids. The epidemiological data on isopropanol production in that *Monograph* are updated and reviewed elsewhere in the present volume, and partly overlap with the information given below.

2.1 Cohort studies

See also Section 2.1.3 in the *Monograph* on Mists from Strong Inorganic Acids in this volume.

Two cases of nasal sinus cancer and two cases of laryngeal cancer had occurred among an unspecified number of workers at a Baton Rouge, Louisiana, USA, isopropyl alcohol-production plant by about 1950 (Eckardt, 1974; Hueper, 1966). Subsequent analyses considered isopropyl alcohol workers in combination with employees in ethanol production and other production units (Lynch *et al.*, 1979; Hanis *et al.*, 1982; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-27-Table2.1.pdf). At this plant, a nested case–control study of workers in isopropyl alcohol manufacturing and others ascertained 50 cases of upper respiratory tract cancer among employees and former employees who had worked during at least 10–15 years. The study made use of an unidentified non-company tumour registry (see Table 2.1, on-line). Cases and controls were assessed for exposure to sulfuric acid and other agents; those with high exposure to sulfuric acid had a significantly elevated odds ratio (OR) of 5.2 (95%CI: 1.2–22.1) for pharyngeal, nasal sinus, or laryngeal cancer (Soskolne *et al.*, 1984).

Six cases of cancer occurred in the 1970s at an isopropyl alcohol-production unit in the USA, which began operation in 1943 and had employed 600 workers through 1976 (Fishbein, 1976). Mortality through 1978 was studied among 433 isopropyl alcohol-manufacturing workers in this facility (see Table 2.1, on-line): two buccal cavity/pharyngeal cancer deaths were reported (Enterline, 1982). Subsequent studies of workers

at this plant did not include data on the mortality experience of isopropyl alcohol-manufacturing workers (Enterline *et al.*, 1990; Marsh *et al.*, 1991).

Among 182 workers employed 1928–50 in another isopropyl alcohol-production unit in the USA, a statistically significant excess of sinus cancer occurred (four cases) (Hueper, 1966; Weil *et al.*, 1952). Subsequently, a case–control study that used reported lymphohaematopoetic cancer deaths as the cases was conducted. Workers ever having had exposure to alkyl sulfates, including diisopropyl sulfate, were at elevated risk for non-Hodgkin lymphoma (8 deaths; OR, 5.1; $P < 0.05$) (Ott *et al.*, 1989). A later mortality study analysed isopropyl alcohol-manufacturing workers together with ethyl alcohol-manufacturing workers at the same and another facility ($n = 1031$) (Teta *et al.*, 1992). Excesses of cancers of the larynx, buccal cavity and pharynx were observed, but based on very small numbers. There was one death due to sinus cancer (see Table 2.1, on-line).

Among 262 men employed in an isopropyl alcohol-manufacturing unit in the United Kingdom, nine cancer deaths had occurred by 1980, including one from nasal sinus cancer, corresponding to a 50-fold increased risk (see Table 2.1, on-line; Alderson & Rattan, 1980)

No further cancer mortality or incidence studies specifically updating any of these cohorts in isopropyl alcohol manufacture have been conducted, and no studies of other isopropyl alcohol-manufacturing plants have appeared in the scientific literature.

2.2 Case–Control Studies

Hu *et al.* (2002) conducted a study of carcinoma of the kidney (renal cell) in eight Canadian provinces. From cases and population-based cancer-free controls, data were collected on exposures during one year or more to 17 substances, including isopropyl oil. The OR – adjusted for age, province of residence, education, body-mass index, pack-years of cigarettes smoked, alcohol, and meat consumption – was 1.6 for men (95%CI: 1.0–2.6) and 1.2 for women (95%CI: 0.4–3.5) (see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-27-Table2.2.pdf).

Pan *et al.* (2005) conducted a similar study of cancer of the brain in eight Canadian provinces. Likewise, from cases and population-based cancer-free controls, data were collected on occupational exposures during one year or more to 18 substances, including isopropyl oil. Exposure to this type of oil was associated with an elevated but not significantly increased risk for brain cancer (see Table 2.2, on-line).

Overall, there is evidence from epidemiological studies that exposure of humans during the manufacture of isopropyl alcohol by the strong-acid process causes cancer of the nasal sinuses, based on three cohort studies. The evidence is inadequate to draw conclusions on other cancer sites.

3. Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

The kinetics of the toxic effects of inhaled acid mists on the respiratory tract depend on several interrelated factors, which include whether exposure occurs to a gas or an aerosol; the particle size, with small particles being more able to penetrate deeply into the lung (Martonen *et al.*, 1985; Jarabek *et al.*, 1989; US EPA, 1989); the solubility in water, with agents of higher solubility

being more likely to be deposited in the nose and mouth; the free hydrogen ion concentration; the breathing rate and pattern; the buffering capacity of the mucosal layer of the airways and the local deposition site (Utell *et al.*, 1989); and the presence of other chemicals carried along with the aerosol particle.

Acid mists that contain particles with a diameter of up to a few micrometers will be deposited in both the upper and lower airways. It is difficult to identify the principal site of deposition within the respiratory tract. For example, 90% of an aerosol of sulfuric acid (mass median aerodynamic diameter of particles, 5 μm) to which lead-acid battery workers are exposed, would be deposited in the extra-thoracic region of the respiratory tract, whereas only 50% of an aerosol with 2-μm particle size would be deposited in that same portion of the respiratory tract. This relationship between size and deposition renders estimation of the changes in pH of the mucus problematic, as diffuse deposition challenges the buffering capacity much less than does deposition of large particles at local sites (Gamble *et al.*, 1984; Jarabek *et al.*, 1989).

Assuming an average particle size of 1 μm and exposure concentrations of 0.4–1 mg/m^3, Amdur *et al.* (1952) showed that on average 77% of an inhaled aerosol of sulfuric acid was retained in the airways of exposed human subjects. Martonen *et al.* (1985) demonstrated that the hygroscopic growth of particles (≤ 1 μm) of several inorganic acids within the respiratory tract depended on temperature, humidity, particle size, respiratory characteristics and the hygroscopic nature of the acid.

The breathing pattern (i.e. mouth *vs* nose breathing, with normal augmentation through the mouth) also influences deposition. For all particle sizes, the dose deposited regionally below the nasopharynx is higher for mouth breathers. The effect of mouth breathing is most evident from the increasing deposition in the oropharynx, larynx and upper trachea (Jarabek *et al.*, 1989).

In the moist environment of the respiratory tract, sulfur trioxide – the anhydride of sulfuric acid – reacts instantaneously with water to form sulfuric acid (IARC, 1992); therefore, the toxicology of sulfur trioxide would be expected to be the same as that of sulfuric acid.

The medical condition called Barrett's oesophagus provides supporting evidence for an association between exposure to an acidic environment and cancer. Barrett's oesophagus refers to a metaplastic change of the lining of the lower end of the oesophagus, which is thought to be caused by chronic exposure to acid from gastric reflux. Genetic polymorphisms associated with an inflammatory response, DNA repair and chemical detoxication are all associated with the presence or progression of the condition. Changes in gene expression have been seen in metaplastic cells as a result of chronic inflammation, which could be due to genetic changes, epigenetic changes or modifications to signalling pathways (Shaheen & Richter, 2009).

4.1.2 Experimental systems

Generally, with respect to acid aerosol deposition similar effects were observed in animals. Regional deposition of sulfuric acid aerosols in experimental animals is also dependent on particle size (e.g. Dahl *et al.*, 1983). However, animal species differ from humans with regard to the dimensions and architecture of the respiratory tract, and deposition patterns of aerosols may vary accordingly (Jarabek *et al.*, 1989). A study in anaesthetized dogs showed that production of ammonia by the respiratory tract partially neutralizes acid aerosols, but larger-size aerosols are neutralized less efficiently than smaller-size aerosols (Larson *et al.*, 1982).

4.2 Genetic and related effects

4.2.1 Humans

Significant increases in the incidence of sister chromatid exchange (SCE), micronucleus formation and chromosomal aberrations in peripheral lymphocytes were detected in a study of 40 workers at a sulfuric acid plant in China, compared with 42 controls working and studying at a university in the same city as the factory. The controls were matched according to sex, age and smoking habits. The mean number of SCE/cell was 6.72 ± 0.22 for workers and 2.71 ± 0.31 for unexposed controls ($P < 0.01$); the mean frequency of micronuclei in cultivated lymphocytes was 0.168% in those from the workers and 0.071% from the control group ($P < 0.001$); and the mean frequency of several types of chromosomal aberration (including rings, translocations and di-centrics) per 100 metaphases was 0.963 for the workers and 0.227 for controls ($P < 0.01$). No positive correlation was observed between the frequency of sister chromatid exchange, micronuclei or chromosomal aberrations and length of employment of the workers. While there was no significant difference between smokers and non-smokers with regard to the frequencies of sister chromatid exchange and chromosomal aberrations, smokers among both the workers and the controls had significantly more micronuclei than non-smokers ($P < 0.001$) (Meng & Zhang 1990a, b).

4.2.2 Experimental systems

No data were available on genetic and related effects of exposures to acid mists in experimental systems; however, studies on genotoxic effects under extreme culture conditions with respect to pH have been reviewed (Scott *et al.*, 1991; Swenberg & Beauchamp, 1997).

The carcinogenic activity of sulfuric acid may be related to the genotoxicity of low pH conditions. Reduced pH environments enhance the depurination rate of DNA and the deamination rate of cytidine (Singer & Grunberger, 1983; IARC, 1992); it has been suggested that the fidelity of enzymes involved in DNA replication and repair may be reduced by low pH (Brusick, 1986). Also, low pH (5.4–6.5) is associated with induction of clastogenicity, sister chromatid exchange and chromosomal aberrations (Morita *et al.*, 1991, 1992; Morita, 1995).

Deamination of cytidine at CpG sites appears to be one of the mechanisms of mutation induction in the *p53* tumour-suppressor gene (Harris, 1993).

4.3 Toxicity relevant to carcinogenicity

Acid mists are irritating to mucous epithelia; they cause dental erosion and produce acute effects in the lungs (symptoms and changes in pulmonary function) (IARC, 1992).

Workers exposed to sulfuric acid mists had an increased incidence of symptoms and macroscopic and microscopic changes of the nasal mucosa, including squamous metaplasia and atypia, with an exposure-response relationship (Grasel *et al.*, 2003).

4.4 Synthesis

Little information on possible mechanisms of carcinogenicity of inorganic acid mists is available. The increased incidence of cancer of the paranasal sinuses in workers involved in the strong-acid process of isopropyl alcohol manufacture may be due to exposure to the strong acid mists and/or the presence of diisopropyl sulfate, an intermediate that shows sufficient evidence of carcinogenicity in experimental animals.

Available data suggest that localized low pH from inhalation of inorganic acid mists could damage DNA and lead to neoplasia. There is no evidence that would support the occurrence of DNA damage by any other mechanism of carcinogenesis.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of isopropyl alcohol manufacture by the strong-acid process. Isopropyl alcohol manufacture by the strong-acid process causes cancer of the nasal cavity.

No data on carcinogenicity of strong acid mists in experimental animals were available to the Working Group.

It is plausible that areas of localized low pH from inhalation of inorganic acid mists could damage DNA and increase cancer risks. There is no evidence to support DNA-damage induction by any other mechanism as the cause of the observed cancers due to exposure to inorganic acid mists.

Isopropyl alcohol manufacture by the strong-acid process is *carcinogenic to humans (Group 1).*

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MISTS FROM STRONG INORGANIC ACIDS

Occupational exposures to mists and vapours from sulfuric acid and other strong inorganic acids were considered by an IARC Working Group in 1991 (IARC, 1992). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation. A separate *Monograph* on 'Isopropyl Alcohol Manufacture by the Strong-acid Process' – updating earlier evaluations on this agent (IARC, 1977, 1987) – appears elsewhere in this volume.

1. Exposure Data

1.1 Manufacturing processes

Major industries with exposure to strong inorganic acid mists include those that manufacture phosphate fertilizer, isopropanol (isopropyl alcohol), synthetic ethanol (ethyl alcohol), sulfuric acid, nitric acid, and lead batteries. Exposure also occurs during copper smelting, and pickling and other acid treatment of metals (Suresh, 2009).

Minor uses of sulfuric acid include applications in petroleum refining, mining, metallurgy, and ore processing, in the synthesis of inorganic and organic chemicals, synthetic rubber and plastics, in the processing of pulp and paper, the manufacture of soap and detergents, cellulose fibres and films, inorganic pigments and paints, and in water treatment. The use of sulfuric acid is declining in some industries. For example, there is a trend in the steel industry to use hydrochloric acid instead of sulfuric acid in the metallurgic process of pickling, and hydrofluoric acid has replaced sulfuric acid for some applications in the petroleum industry (IARC, 1992; ATSDR, 1998; NTP, 2005).

1.2 Human exposure

1.2.1 Occupational exposure

Strong inorganic acid mists may be produced as a result of the use of inorganic acids, including sulfuric acid, in various industrial processes, as indicated in the previous section (IARC, 1992). The amount of vapour or mist that is produced varies with the process and the method. In pickling, for instance, mist may escape from tanks filled with acid, when hydrogen gas and steam rise from the surface of the solution (NTP, 2005).

CAREX (CARcinogen EXposure) is an international information system on occupational exposure to known and suspected carcinogens, based on data collected in the European Union from 1990 to 1993. The CAREX database provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry (Kauppinen *et al.*, 2000). Table 1.1 shows the results for strong inorganic acid mists containing sulfuric acid in the European Union by industry (CAREX, 1999).

From the National Occupational Exposure Survey (1981–83) it was estimated that

Table 1.1 Estimated numbers of workers exposed to strong inorganic acid mists containing sulfuric acid in the European Union

Industry, occupational activity	
Manufacture of industrial chemicals	78400
Manufacture of fabricated metal products, except machinery and equipment	67800
Construction	63600
Wholesale and retail trade and restaurants and hotels	63300
Medical, dental, other health and veterinary services	58000
Iron and steel basic industries	37100
Manufacture of other chemical products	33800
Manufacture of electrical machinery, apparatus, appliances and supplies	30800
Manufacture of machinery except electrical	29300
Food manufacturing	25300
TOTAL	700000

From CAREX (1999), see also Kauppinen *et al.* (2000)

approximately 776000 workers (including approximately 174000 women) in the United States of America (USA) were potentially exposed to sulfuric acid (NIOSH, 1990). [The Working Group noted that these numbers do not specifically reflect exposures to strong inorganic acid mists.]

Exposure to strong inorganic acid mists containing sulfuric acid may occur by inhalation, ingestion, and dermal contact. Exposure depends on many factors including particle size, proximity to the source, and control measures such as ventilation and containment. Data on particle-size distribution of acid mists are limited, and sampling methods have generally not differentiated between liquid and gaseous forms of the acids (IARC, 1992; NTP, 2005).

Sulfuric acid is used with other strong inorganic acids in many manufacturing processes, during which strong inorganic acid mists may be generated. Data from studies on exposure to inorganic acid mists published since the previous *IARC Monograph* (IARC, 1992) are briefly summarized below.

Sathiakumar *et al.* (1997) reviewed and categorized 25 epidemiological studies on the basis of high (> 1 mg/m^3; 8-hour time-weighted average), moderate (0.1–1 mg/m^3) and low (< 0.1 mg/m^3) exposure to sulfuric acid mists. Workers in the production of sulfuric acid and isopropanol, and in metal pickling were considered to have potentially high exposure, while workers in the production of soap and detergent, nitric acid, and ethanol were assumed to have moderate exposure. The lowest estimated exposures were for workers in copper and zinc refining and in the production of phosphate fertilizers and lead batteries. This grouping generally applies to exposures that occurred before 1970. In the early 1970s, the exposure started to decline due to the use of lower concentrations of the acids and improved industrial control measures.

Aerosol sampling was conducted at 24 locations in eight plants that manufactured phosphoric acid and concentrated fertilizer in Florida (USA) and in two locations as control sites elsewhere in Florida. In general, sulfate, fluoride, ammonium, and phosphate were the major chemical species found in the fertilizer plants. At the sulfuric acid tank, sulfate was the dominant substance, with a maximum PM_{10} sulfate concentration of 181 μg/m^3. The concentrations of sulfate-containing PM_{10}, including ammonium sulfate, calcium sulfate, and sulfuric acid were lower than 200 μg/m^3 at all locations (Hsu *et al.*, 2007a, b, 2008).

In a multidisciplinary cross-sectional study of 22 galvanic plants in Sao Paulo, Brazil from 1993 to 1996, ambient air was collected and personal exposure measurements were conducted on five consecutive working days. The geometric means for the concentration of sulphuric acid in ambient-air samples from five anodising plants varied from 34.7 to 2133.6 µg/m3 (total range, 7.2–2780.0 µg/m3); for personal samples, the geometric means ranged from 12.7 to 396.5 µg/m3 (total range, 5.3–865.6 µg/m3) (Grasel *et al.*, 2003).

Steenland (1997) described a follow-up study of a cohort of 1156 acid-exposed steelworkers employed in the USA between 1940 and 1965. The workers were followed until the end of October 1994. Historically, sulfuric acid was the most common acid used for pickling, but in the mid-1960s it was gradually replaced by hydrochloric, nitric, hydrofluoric, and hydrocyanic acid, with hydrochloric acid being the most common. For two of the three plants, personal exposure levels for sulfuric acid in air averaged 0.19 mg/m^3 in the period 1975–79 (time-weighted average, based on 15 samples) and the average concentration in the work area was 0.29 mg/m^3 (based on 34 samples). Exposures in the third plant were estimated to have been similar.

Exposure to mineral acid mists was assessed at two battery manufacturers (factories A and B, established in 1914 and 1929, respectively) and two steel works (factories C and D, established in 1948 and 1945, respectively) in the United Kingdom. In both factories exposure to sulfuric acid occurred principally during the formation and charging processes. Other exposures included those to asbestos – crocidolite and chrysotile –, lead, stibine, and pitch. Fixed-site monitoring of sulfuric acid was conducted from 1970 onwards and showed concentrations in air of 0.1–0.7 mg/m^3 in factory A, and 0.4–2.0 mg/m^3 in factory B. In the steel works C and D, sulfuric acid was used to clean steel before plating and galvanizing. Although its use was phased out in favour of hydrochloric acid in both factories, sulphuric acid continued to be used in the hot sheet finishing and plating areas in factory C, and in galvanizing processes in factory D. No occupational hygiene measurements of acid mists were available for either steel factory (Coggon *et al.*, 1996).

2. Cancer in Humans

Occupational exposures to mists from strong inorganic acids (e.g. sulfuric, hydrochloric, nitric and phosphoric acids) were last evaluated by a previous *IARC Monograph* (IARC, 1992), which concluded that there is *sufficient evidence* for the carcinogenicity to humans of occupational exposure to strong-inorganic-acid mists containing sulfuric acid.

2.1 Cohort studies

Industries with exposure to strong inorganic acids have been categorized with respect to potential average exposure levels to sulfuric acid mists: highest — metal pickling, sulfuric acid production, isopropanol production; moderate — soap and detergent production, nitric acid, and ethanol production; and low — copper and zinc refining, phosphate fertilizer production, and lead battery production (Sathiakumar *et al.*, 1997).

2.1.1 Metal pickling

Sulfuric acid and other inorganic acids have been used to pickle steel (i.e. remove scale and oxides from the metal surface) as part of the finishing process in the steel industry. Cohorts in Sweden, the USA and France have been studied (Mazumdar *et al.*, 1975; Ahlborg *et al.*, 1981; Beaumont *et al.*, 1987; Steenland *et al.*, 1988; Steenland & Beaumont, 1989; Steenland, 1997; Moulin *et al.*, 2000; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-28-Table2.1.pdf). Studies on cancer of the larynx showed statistically significant

excess risks in Sweden (Ahlborg *et al.*, 1981) and the USA (Steenland, 1997), while in a study from France non-significant excesses were found (Moulin *et al.*, 2000). In the US cohort, Steenland & Beaumont (1989) found a significant excess risk for cancer of the lung, considering a latency period of 20 years.

2.1.2 Sulfuric and nitric acid manufacture

Englander *et al.* (1988) reported a non-significant twofold excess of respiratory cancers and increases in bladder-cancer mortality in a Swedish sulfuric acid factory. Excesses of myeloid leukaemia were noted in an Italian sulfuric acid factory (Pesatori *et al.*, 2006), and increased risks for lung and pleural cancer were found in a Norwegian nitric acid plant (SIR = 4.5; 95%CI: 2.6–7.2) (Hilt *et al.*, 1985). An elevated lung-cancer mortality was observed among men working with acid mixtures in an Italian factory, but the increase was not statistically significant (Rapiti *et al.*, 1997). Likewise, at two US tanneries where sulfuric acid was used in the de-liming, bating and pickling subdepartments of the beamhouse, an elevated lung-cancer mortality was found, but the increase was not significant (Stern, 2003; see Table 2.1, on-line).

2.1.3 Manufacture of isopropanol and ethanol

See also the *Monograph* on Isopropyl Alcohol Manufacture by the Strong-acid Process in this volume.

In addition to acid mists, workers in isopropanol and ethanol production may be exposed to alloy/sulfates and isopropanol oils. Cohort studies in one factory in the United Kingdom and four in the USA where isopropyl alcohol and ethyl alcohol were manufactured by the strong-acid process showed a significant excess of cancers in the upper respiratory tract – nasal sinus, pharynx, larynx – and buccal cavity. Hueper (1966) found significant excesses of nasal sinus and larynx cancers combined (SIR 21.9, 95%CI: 7.9–56.1) based on two cases each. In the same facility, Lynch *et al.* (1979) reported significant excesses for cancer of the larynx. Subsequently, a nested case–control study among workers in the same plant assessed 50 upper respiratory tract cancer cases and controls with regards to exposure to sulfuric acid and other agents. Those with "high" sulfuric acid exposure had an increased risk (OR, 5.2; 95%CI: 1.2–22.1) for pharyngeal, sinus, or laryngeal cancer (Soskolne *et al.*, 1984; see Table 2.1, on-line).

Excess risks for buccal cavity and pharynx cancer were identified in an isopropyl alcohol production unit in the USA, which began operation in 1941 and had employed 433 workers through 1965 (Enterline, 1982).

Weil *et al.* (1952) reported a highly significant risk for nasal sinus cancer, based on four cases, in an isopropyl alcohol production facility in the USA. A case–control study of lympho-haematopoietic malignancies at the same plant showed an elevated risk for non-Hodgkin lymphoma among workers ever exposed to alkyl sulfates, including those in the isopropyl and ethyl alcohol production units, where sulfuric acid was used (Ott *et al.*, 1989). In a subsequent mortality study conducted in this facility, workers in isopropanol and ethanol production had a statistically significantly increased mortality risk for lympho- and reticulosarcoma (Teta *et al.*, 1992; see Table 2.1, on-line). Mortality from upper respiratory and lympho-haematopoietic cancers was also investigated in another isopropanol- and ethanol-manufacturing facility in the USA, but significant cancer excesses were not observed in this case (Teta *et al.*, 1992). In an isopropyl alcohol-manufacturing unit in the United Kingdom, nine cancer deaths had occurred by 1980, including one from nasal sinus cancer; this was statistically significant (Alderson & Rattan, 1980).

2.1.4 Soap manufacture

The only study of soap-manufacturing workers exposed to sulfuric acid vapours in the hydrolysis and saponification areas, found increased risks – not statistically significant – for laryngeal cancer incidence and mortality through 1983 (Forastiere *et al.*, 1987).

2.1.5 Manufacture of phosphate and nitrate fertilizer

Sulfuric acid is used to convert phosphate rock to phosphoric acid and superphosphate; nitric acid is the precursor of nitrate fertilizers. Many fertilizer plants manufacture these two acids on site (Al-Dabbagh *et al.*, 1986; Rafnsson & Gunnarsdóttir, 1990; Hagmar *et al.*, 1991; Fandrem *et al.*, 1993; Checkoway *et al.*, 1996). Several research groups studied phosphate-fertilizer manufacturing in the USA (Stayner *et al.*, 1985; Block *et al.*, 1988; Checkoway *et al.*, 1996). All found elevated lung cancer mortality, with higher rates for those who had been employed longer. Nitrate-fertilizer manufacture has been studied in Iceland, Norway, the Russian Federation, Sweden, and the United Kingdom (Al-Dabbagh *et al.*, 1986; Rafnsson & Gunnarsdóttir, 1990; Hagmar *et al.*, 1991; Fandrem *et al.*, 1993; Zandjani *et al.*, 1994; Bulbulyan *et al.*, 1996). None of these studies found statistically significantly elevated risks among workers potentially exposed to nitric acid mists except the study from Sweden (Zandjani *et al.*, 1994), which showed a standardized incidence ratio (SIR) for stomach cancer of 1.50 ($P < 0.05$) for 27 men hired before 1960, and an SIR for testicular cancer of 3.33 ($P < 0.05$) for 5 men hired during or after 1960. In a factory complex where both phosphate and nitrate fertilizers were manufactured, Bulbulyan *et al.* (1996) reported a statistically significant increase in stomach-cancer mortality for men in the sulfuric acid tower department (11 deaths, SMR 2.04, 95%CI: 1.02–3.66) (see Table 2.1, on-line).

2.1.6 Battery manufacture

Mortality for all cancers combined and for various separate respiratory cancers has been reported from four cohort studies of battery-manufacturing workers (one including cohorts from steel works as well) in the United Kingdom and the USA (Malcolm & Barnett, 1982; Cooper et. al., 1985; Coggon *et al.*, 1996; Sorahan & Esmen, 2004; see Table 2.1, on-line). In two studies, excesses of laryngeal cancer were found (Cooper *et al.*, 1985; Sorahan & Esmen, 2004). Within the United Kingdom cohort, a nested case–control analysis was carried out in two battery-manufacturing plants and two steel works, with respect to exposure to acid mists. The study included 15 cases with upper aerodigestive cancers (ICD-9 140–141, 143–149, 160–161) and 75 controls. The odds of cancer among those with high exposure to acids, or among those with five or more years of high exposure were increased compared with those with no exposure to acids, but this was not statistically significant (Coggon *et al.*, 1996).

2.1.7 Other industries

Two nested case–control studies, one in the Norwegian nickel-refining industry (Grimsrud *et al.*, 2005) and one in the chemical industry in the USA (Bond *et al.*, 1986; 1991) were reviewed. In the first study, a job-exposure matrix for sulfuric acid mist was developed, the second study developed a job-exposure matrix for hydrochloric acid; neither found an association between acid mist exposure and lung cancer.

2.2 Case–control studies

Case–control studies of cancer of the upper respiratory tract, larynx, lung, stomach, and other sites have evaluated whether exposure to acid mists affected the cancer risk (see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-28-Table2.2.pdf).

2.2.1 Cancer of the nasal cavity and sinuses

Incident cases of sinonasal (ICD-9 160) cancer in Piedmont, Italy, were matched with hospital controls from the ear/nose/throat and orthopaedics departments over a four-year period. For those whose histology was basocellular, mucoepidermoid, neuroendocrine, undifferentiated, or unspecified, ever having been exposed to acid mists at work was associated with a higher risk for these cancers (OR, 7.5; 95%CI: 2.0–28) (d'Errico *et al.*, 2009).

2.2.2 Cancer of the larynx

In Uruguay, 112 men diagnosed with laryngeal cancer between 1993 and 1995 were compared with 509 controls with cancer at other sites: there was an OR of 1.2 (95%CI: 0.6–2.5) for those who had been occupationally exposed to acid mists for up to 20 years, while the OR for 21 or more years of exposure was 1.8 (95%CI: 1.1–3.1) (De Stefani *et al.*, 1998). Soskolne *et al.* (1992) assessed the duration and intensity of exposure to sulfuric acid among laryngeal cancer cases in a case–control study in Canada and found a dose–response progression from ≤ 10 years of probable exposure (OR, 1.97; 95%CI: 0.6–6.1) to > 10 years of substantial exposure (OR, 5.6; 95%CI: 2.0–15.5). Zemła *et al.* (1987) asked 328 men with laryngeal cancer and 656 controls without cancer about "constant exposure to vapours of sulfuric, hydrochloric, or nitric acid" and reported a relative risk of 4.27 ($P < 0.001$). In five other studies (Olsen & Sabroe, 1984; Cookfair *et al.*, 1985; Brown *et al.*, 1988; Eisen *et al.*, 1994; Shangina *et al.*, 2006) no increased risks, or elevations that were not statistically significant, were found (see Table 2.2, on-line).

2.2.3 Cancer of the lung

Risks for cancer of the lung in association with exposure to acid mists, sulfuric or hydrochloric acid, or "any inorganic acid or base" have been reported from six case–control studies (see Table 2.2, on-line). No increased risks were found in three nested case–control studies (Bond *et al.*, 1986; Bond *et al.*, 1991; Moulin *et al.*, 2000; Grimsrud *et al.*, 2005) in the USA, France and Norway, or in an ecological study in Lithuania (Petrauskaite *et al.*, 2002).

2.2.4 Cancer at other sites

Three studies compared information on death certificates. The limitations of this type of study include possible misclassification, e.g. when the death certificate lists occupation and industry only for the most recent job rather than for the longest employment. Data on possible confounders are often lacking as well.

In two studies from the USA an increased risk for cancer of the stomach was found associated with exposure to sulfuric acid, which was derived from a job-exposure matrix applied to the occupation and industry indicated on the death certificates (Cocco *et al.*, 1998, 1999; see Table 2.2, on-line). Nearly 42000 certificates for deaths from stomach cancer were compared with certificates for decedents from non-malignant diseases. White men with low, medium, or high exposures to sulfuric acid, showed increasing ORs (p for trend < 0.01) for stomach cancer, compared with those not exposed to sulfuric acid. For black men the increase was more moderate, and for women – among whom only 17 of 16864 were rated as having high exposure to sulfuric acid – the ORs were lowest for the high-intensity group (Cocco *et al.*, 1999). A smaller study of cancer of the gastric cardia (ICD-9 151.0) compared 1056 men who died of this cancer with 5280 men who died of non-malignant disease. Men ever-exposed to sulfuric acid showed an OR of 1.2 (95%CI: 1.0–1.4), and increasing probability and increasing intensity of exposure were both associated with a significant *P* for trend (< 0.05 for probability, < 0.01 for intensity) (Cocco *et al.*, 1998).

Another US study based on death certificates evaluated breast-cancer risk and exposure to

acid mists, comparing 33509 cases and 117 794 controls. Homemakers had been excluded, as well as controls who had died of other cancers. Thus excluding women with a low probability of exposure, those with the highest level of exposure to acid mists had statistically significantly elevated ORs for breast cancer, i.e. 1.16 (for whites) and 1.44 (for blacks), after adjustment for age and imputed socioeconomic status (Cantor *et al.*, 1995).

3. Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Relevant Data

See the *Monograph* on 'Isopropyl Alcohol Manufacture by the Strong-acid Process' in this volume.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of mists from strong inorganic acids. Mists from strong inorganic acids cause cancer of the larynx.

Also, a positive association has been observed between exposure to mists from strong inorganic acids and cancer of the lung.

No data on the carcinogenicity of mists from strong inorganic acids in experimental animals were available to the Working Group.

While it is plausible that areas of localized low pH from inhalation of inorganic acid mists could damage DNA and increase cancer risks, the evidence supporting DNA-damage induction or any other mechanism as the cause of the observed cancers due to the inorganic acid mists is weak.

Mists from strong inorganic acids are *carcinogenic to humans (Group 1)*.

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OCCUPATIONAL EXPOSURES DURING IRON AND STEEL FOUNDING

Iron and steel founding was considered by previous IARC Working Groups in 1983 and 1987 (IARC, 1984, 1987). Since that time, new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Manufacturing process

Foundries produce shaped castings from re-melted metal ingots and scrap. Although foundry work is assumed to start with re-melting of ingots and scrap and to end with the fettling of castings, the industry is often so integrated that the distinction is not obvious. Machine shops are not normally part of the work environment where castings are produced; however, simple and accessory machining may be carried out, and these activities may be part of small foundry operations. The processes in iron and steel founding generally comprise pattern-making, moulding and core-making, melting, pouring and shake-out, and fettling. A detailed description of these production steps can be found in IARC (1984).

1.2 Human exposures

The iron and steel industry is very diverse in materials and processes, resulting in occupational exposures to a wide variety of substances. Substantial exposures to silica and carbon monoxide continue to occur in many foundries. Occupational exposures to airborne polycyclic aromatic hydrocarbons (PAHs) are also present, resulting mainly from the thermal decomposition of carbonaceous ingredients commonly added to foundry sand. In addition, some steel-foundry workers (e.g. fettlers) are exposed to airborne chromium and nickel compounds. The introduction of organic binder materials in the late 1950s has resulted in exposures of foundry workers to other chemicals, including phenol, formaldehyde, isocyanates and various amines.

Earlier exposure studies have been reviewed previously (IARC, 1984). More recent studies are presented here and summarized in Table 1.1.

1.2.1 Respirable dust and respirable quartz

Nearly all production workers in iron and steel foundries are exposed to silica dust and other mineral constituents of foundry sand (IARC, 1984). In a study from Sweden, the 'furnace and ladle repair workers' and fettlers were the highest exposed individuals. For the furnace workers (n = 33), geometric mean levels of respirable dust and respirable quartz were 1.2 mg/m^3 (range, 0.25–9.3) and 0.052 mg/m^3

Table 1.1 Exposures of workers in the iron and steel founding industry

Reference, Country	Year of study	Operation/Job/Task	No. of subjects	No. of samples	No. of smokers	Exposure	Air levels (μg/m³)		Urine levels (μmol/mol creatinine)	
							Geometric Mean	Range	Mean	Range or SD
Respirable dust and respirable quartz										
Andersson *et al.* (2009) Sweden	2005–2006	11 iron foundries		436		Respirable dust	580	76–31000		
				435		Respirable quartz	28	3–2100		
Chen *et al.* (2006) Taiwan, China	1990		1234	277	645	Respirable dust				
		Iron making								
		Sinter	112	27				90–5080		
		Coal preparation	40	9				20–430		
		Blast furnace	311	72				90–830		
		Steel making								
		Steel materials plant	192	30				130–7590		
		Basic oxygen furnace	174	47				170–310		
		Continuous steel casting	333	75				320–790		
		Steel slab rectifying	72	17				160–1140		
Binder compounds – methyl isocyanate, isocyanic acid and formaldehyde										
Westberg *et al.* (2005) Sweden	2001	Four foundries using the Hot Box core-binder system								
						Short-term samples:				
		Total		298		methyl isocyanate	4.9	< 4–68		
				297		isocyanic acid	24	< 4–280		
		Core maker		179		methyl isocyanate	4	< 4–60		
				178		isocyanic acid	22	< 4–130		
		Die caster		71		methyl isocyanate	10	< 4–68		
				71		isocyanic acid	48	< 4–280		
		Others		48		methyl isocyanate	3.4	< 4–12		
				48		isocyanic acid	12	< 4–66		

Table 1.1 (continued)

Reference, Country	Year of study	Operation/Job/Task	No. of subjects	No. of samples	No. of smokers	Exposure	Air levels (µg/m³)		Urine levels (µmol/mol creatinine)	
							Geometric Mean	Range	Mean	Range or SD
Westberg *et al.* (2005) (contd)						**8-hour TWA samples**				
		Total		64		methyl isocyanate	5.3	< 4–31		
				64		isocyanic acid	27	< 4–190		
				64		formaldehyde	120	14–1600		
		Core maker		39		methyl isocyanate	4.2	< 4–20		
				39		isocyanic acid	24	5.3–84		
				39		formaldehyde	200	50–1600		
		Die caster		15		methyl isocyanate	12	< 4–31		
				15		isocyanic acid	55	6–190		
				15		formaldehyde	63	21–220		
		Others		10		methyl isocyanate	3.5	< 4–8.2		
				10		isocyanic acid	14	< 4–32		
				10		formaldehyde	32	14–170		

Table 1.1 (continued)

Reference, Country	Year of study	Operation/Job/Task	No. of subjects	No. of samples	No. of smokers	Exposure	Air levels (µg/m³)		Urine levels (µmol/mol creatinine)	
							Geometric Mean	Range	Mean	Range or SD
Polycyclic aromatic hydrocarbons										
Hansen *et al.* (1994) Denmark	1988–89	**Iron foundry workers**		24		**Pyrene**				
		- melters		5			0.02			
		- transporters		2			0.0			
		- casters		9			0.22			
		- machine moulders		1			0.12			
		- hand moulders		2			0.22			
		- shake workers		3			0.01			
		- finishing workers		2			0.0			
						Benzo[a]pyrene				
		- melters					0.01			
		- transporters					0.0			
		- casters					0.02			
		- machine moulders					0.04			
		- hand moulders					0.03			
		- shake workers					0.0			
		- finishing workers					0.0			
						Sum of 15 PAHs				
		- melters					6.66			
		- transporters					5.78			
		- casters					10.91			
		- machine moulders					9.64			
		- hand moulders					5.63			
		- shake workers					11.17			
		- finishing workers					0.58			

Table 1.1 (continued)

Reference, Country	Year of study	Operation/Job/Task	No. of subjects	No. of samples	No. of smokers	Exposure	Air levels (µg/m³)		Urine levels (µmol/mol creatinine)	
							Geometric Mean	Range	Mean	Range or SD
Hansen *et al.* (1994) (contd)		**B[a]P exposed workers:**								
		- Foundry 1 – low		19						0.023
		- Foundry 2 – low		13						0.065
		- Foundry 1 – medium		14						0.030
		- Foundry 2 – medium		24						0.046
		- Foundry 1 – high		1						–
		- Foundry 2 – high		18						0.033
		Pyrene exposed workers:								
		- Foundry 1 – low		19						0.023
		- Foundry 2 – low		5						0.062
		- Foundry 1 – medium		5						0.013
		- Foundry 2 – medium		32						0.044
		- Foundry 1 – high		10						0.036
		- Foundry 2 – high		18						0.046
		PAH exposed workers[b]:								
		- Foundry 1 – low		0						–
		- Foundry 2 – low		0						–
		- Foundry 1 – medium		29						0.022
		- Foundry 2 – medium		29						0.041
		- Foundry 1 – high		5						0.043
		- Foundry 2 – high		26						0.053

[a] Calculated from short-term samples
[b] Sum of 15 PAHs

(range, 0.0098–0.83), respectively. For the fettlers (n = 115), geometric mean levels of respirable dust and respirable quartz were 0.69 mg/m^3 (range, 0.076–31) and 0.041 mg/m^3 (range, 0.004–2.1), respectively. Major improvements with respect to occupational hygiene in the foundries showed maximum average concentrations of respirable dust being almost equal to the minimum average concentrations for various jobs in the past (Andersson *et al.*, 2009). A study from Taiwan, China in a continuous steel-casting plant showed similar respirable dust levels (Chen *et al.*, 2006).

1.2.2 Carbon monoxide

Carbon monoxide (CO) is formed whenever there is incomplete combustion of carbonaceous material. In the foundry environment, CO is mainly produced by cupola melting and casting operations. Other sources of CO exposure are oil burners used for charge and ladle preheating, furnaces for annealing and carburizing, transport equipment powered by internal combustion engines and fettling operations such as welding and flame cutting. Workers in the furnace area may be exposed to gases leaking from the charging and bottom levels of the cupolas (IARC, 1984).

Results of recent measurements of exposure levels to CO in iron and steel foundries were not available to the Working Group.

1.2.3 Binder compounds

Organic binder materials for cores and moulds include furan, phenol-formaldehyde, urea-formaldehyde and urethane resins as well as oleo-resinous oils. These ingredients may volatilize into the workplace air during mixing, blowing, ramming, drying or baking operations. Curing reactions and thermal decomposition give rise to formation of additional compounds, which are released during pouring and shake-out. When organic binders are subjected to high temperatures, pyrolysis may produce gases and smoke aerosols. Only a few components of these emissions have been identified: aliphatic components include methane, ethane, ethylene, acetylene, and smaller amounts of high molecular-weight compounds; aromatic substances include benzene, toluene, xylenes, naphthalenes and a variety of PAHs in lower concentrations. Nitrogen compounds such as ammonia, cyanides and amines may be formed from the nitrogen-containing urea, ammonium salts and hexamethylenetetramine that are used as binder chemicals. Urethane resins may emit free isocyanates under moulding and pouring conditions. No-bake catalysts, based on arylsulphonic acids, may produce sulfur dioxide and hydrogen sulphide by thermal processes. If phosphoric acid is used as a catalyst, phosphine can be formed in the strongly reducing atmosphere of the hot emissions. In air, phosphine rapidly oxidizes to phosphorus oxide. Furan binders contain free furfuryl alcohol, which can volatilize during mixing, moulding or core-making. Similarly, furan and phenolic resins may emit formaldehyde, phenol and other derivatives by volatilization or thermal decomposition. Core oils and alkyd-isocyanate resins are partly composed of natural drying oils, and heating of these materials gives rise to acrolein, various aldehydes, ketones, acids and esters as well as aliphatic hydrocarbons. When organic solvents are used in sand binders, the vapours may add to the exposure of workers (Toeniskoetter & Schafer, 1977; IARC, 1984).

A study from Sweden by Westberg *et al.* (2005) showed that core-makers had higher average exposure to formaldehyde that casters who were more exposed to methyl-isocyanate and isocyanic acid. All four Swedish foundries using the Hot Box core-binder system were included in this study.

1.2.4 Polycyclic aromatic hydrocarbons

PAHs result from thermal decomposition of carbonaceous ingredients in foundry sand. During casting, PAHs are formed and partly vapourised under the extremely hot and reducing conditions at the mould-metal interface. They are then adsorbed onto soot, fume or sand particles and spread throughout the workplace during shake-out and other dusty operations. Although the mechanism of PAH formation is complex and variable, the reactions proceed via pathways that involve free radicals. Various radical species containing carbon atoms combine in rapid fashion at the temperature range of 500–800 °C. This pyro-synthesis is influenced by many variables, such as the composition of the gaseous atmosphere and the chemical structure of the carbonaceous material. Organic binders, coal powder and other carbonaceous additives are the predominant sources of PAHs in iron and steel foundries. In some cases, exhaust gases from engines, furnaces and ovens may increase the exposure of workers to these compounds (IARC, 1984).

Studies from the late 1980s in Denmark showed low levels of exposure to PAHs in the foundry workplace atmosphere (Hansen *et al.*, 1994; Table 1.1).

1.2.5 Metals

Metal fumes are formed by evaporation, condensation and oxidation of metals in air. Furnace tenders, melters, casters, ladle-men, pourers and crane drivers are exposed to fumes from molten metal; fettlers are exposed to metal fumes and dusts from grinding, welding and flame-cutting operations (IARC, 1984).

Apostoli *et al.* (1988) measured concentrations of thallium in urine of workers in two cast-iron foundries (with 9 and 12 subjects, respectively) employed in cupola furnace operating and iron casting. These workers were potentially exposed to coke dust. The mean urinary thallium concentrations were higher for the cast-iron foundry workers (n = 21; mean, 0.33 µg/l; range, 0.06–1.04) than for non-exposed individuals (n = 72; mean, 0.22 µg/l; range, 0.06–0.61), but lower than those of cement workers (n = 30; mean, 0.38 µg/l; range, 0.08–1.22).

In a study from the United Kingdom, concentrations of 14 elements in the blood of workers with low-alloy steel and stainless steel, and of workers exposed to lead- and cadmium-containing dusts were measured and compared with values of similar measurements in an unexposed control group. Concentrations of 20 elements in dust samples were also measured. The only elements that showed significantly elevated levels in whole blood were cadmium in workers with non-ferrous metals and lead in all workers. The values for lead were 97.2 ± 39.9 ppb in 19 workers with low-alloy steel, 74.9 ± 25.1 ppb in 20 workers with stainless steel, and 54.0 ± 18.2 ppb in 42 controls. The high concentrations cadmium and lead in the dusts confirmed the exposure of the workers (Triger *et al.*, 1989).

In a Danish study conducted over a 16-month period in 1996–1997, manganese concentrations were measured in air, and in blood from 24 furnace-men employed in three small-size foundries and from 21 scrap-recycling workers at another plant. Furnace-men who work in poorly ventilated smelting departments were found to have higher levels of manganese in their blood (approx. 2.5 ± 5 µg/L above reference values) despite low concentrations of manganese in fumes (0.002 ± 0.064 mg/m^3) (Lander *et al.*, 1999).

In a Taiwan, China study among 63 steel-production workers, Horng *et al.* (2003) measured urinary concentrations of cadmium, cobalt, nickel and lead by use of differential-pulse stripping voltammetry. Mean concentrations of the metals were elevated: cadmium: 9.52 µg/L, range, 3.19–22.07; cobalt: 8.18 µg/L, range, 3.06–23.30; nickel: 33.10 µg/L, range, 13.90–78.90; lead:

53.50 µg/L, range, 28.90–85.60. Age and duration of employment were correlated with the urinary concentrations of these metals. [The limited information provided on the actual job content of the workers did not give insight whether these workers were employed in primary steel production or in the steel foundry.]

1.2.6 Refractory ceramic fibres

In a study on refractory ceramic fibres (RCF) from Belgium, ferruginous bodies mimicking asbestos were detected in nine of 1800 bronchoalveolar lavage samples collected during 1992–1997 (Dumortier *et al.*, 2001). The nine individuals from whom these samples originated had been employed either as foundry worker, steel worker, or welder and the majority had worked on furnace insulation. Mean airborne fibre concentrations measured during installation or removal of RCF furnace insulation are close to 1 fibre/mL air (Maxim *et al.*, 1997), but concentrations as high as 23 fibres/mL have been detected (Strübel & Faul, 1994).

Two Finnish steel plants, three foundries and a repair shop were studied for the presence of airborne ceramic fibres under normal production and maintenance conditions (i.e. during the replacement of oven insulation). While ceramic fibres were found in most production phases (range < 0.01–0.29 fibres/cm^3), considerably higher fibre counts were obtained during maintenance work (range < 0.01–14.2 fibres/cm^3). Results from nasal sampling were found to correlate with the airborne fibre concentrations at the group level. The mean fibre concentrations varied from 34 to 930 fibres/cm^3 of lavage fluid (Linnainmaa *et al.*, 2007).

2. Cancer in Humans

2.1 Cohort studies

There are 13 cohort studies available on iron and steel founding workers in various parts of the world. Nearly all of these show a significantly increased risk for lung cancer, either in the entire cohort or in high-exposed subgroups (Koskela *et al.*, 1976; Gibson *et al.*, 1977; Tola *et al.*, 1979; Sitas *et al.*, 1989; Andjelkovich *et al.*, 1990, 1992, 1995; Moulin *et al.*, 1993; Sorahan *et al.*, 1994; Adzersen *et al.*, 2003; Hoshuyama *et al.*, 2006; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-29-Table2.1.pdf). There was a non-significantly increased lung-cancer risk in one study (Decoufle & Wood, 1979). In the cohort study from the United Kingdom (Sorahan *et al.*, 1994) an internal dose–response in terms of years of employment was found. A study from the USA showed a significantly increased lung-cancer risk after adjustment for smoking (Andjelkovich *et al.*, 1994). There are two additional cohorts, based on proportional mortality, that provide supporting evidence for an excess of lung cancer in foundry workers (Egan-Baum *et al.*, 1981; Silverstein *et al.*, 1986).

2.2 Case–control studies

Two population-based case–control studies, one from the USA (Blot *et al.*, 1983) and one from Poland (Becher *et al.*, 1989), showed a statistically significant excess of lung cancer in association with foundry work, with adjustment for smoking (see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-29-Table2.2.pdf).

2.3 Synthesis

Considering the consistent findings in the available cohort studies and the positive evidence from two case–control studies, the epidemiological data clearly support the notion that work in iron and steel foundries is associated with an increased risk for lung cancer. Chance, bias and confounding are not likely to explain the excess risk.

3. Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Relevant Data

4.1 Mechanistic evidence relevant to the carcinogenic hazards from occupational exposures during iron and steel founding

4.1.1 Experimental systems

In several studies extracts of particulates from samples collected at a steel foundry were mutagenic in *Salmonella typhimurium* strain TA98 in the presence or absence of an exogenous metabolic activation system. In another study filter extracts were mutagenic in *Salmonella typhimurium* strains TA98 and TA100 in the presence or absence of an exogenous metabolic activation system (IARC, 1984).

Exposures in the iron and steel founding industry are complex and include a wide variety of known genotoxic and carcinogenic substances including PAHs, metals (e.g. nickel, chromium) and formaldehyde. These agents have been previously reviewed by IARC (1983, 1990, 1995, 2010).

4.1.2 Humans

Workers in a Finnish iron foundry with occupational exposure to PAHs and control subjects without occupational exposure were examined for the presence in peripheral white blood cells of aromatic DNA adducts by use of ^{32}P-postlabelling. There was a significant correlation between the estimated exposures and DNA-adduct levels (Phillips *et al.*, 1988; Reddy *et al.*, 1991). The competitive ELISA assay was used to study another population of foundry workers with respect to the presence of aromatic DNA-adducts, which showed a positive trend with exposure (Santella *et al.*, 1993). Foundry workers studied over a period of five years were evaluated for aromatic DNA adducts in their leukocytes. During this time exposure to PAHs decreased and the level of DNA adducts decreased also. In the total group exposure was related to the level of DNA adducts (Perera *et al.*, 1994; Hemminki *et al.*, 1997; Perera *et al.*, 2000).

4.2 Synthesis

There is moderate evidence that extracts of particles collected from a steel foundry act via a genotoxic mechanism, based on bacterial mutation studies. There is weak evidence for a genotoxic mechanism of action for exposures during iron and steel founding, based on DNA-adduct studies.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during iron and steel founding. Occupational exposures during iron and steel founding cause cancer of the lung.

No data on the carcinogenicity to experimental animals of mixtures present in iron and steel founding were available to the Working Group.

Occupational exposures during iron and steel founding are *carcinogenic to humans (Group 1).*

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OCCUPATIONAL EXPOSURE AS A PAINTER

Occupational exposure as a painter was considered by previous Working Groups in 1988 and 2007 (IARC, 1989, 2010a). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Description of paint products

Paint is a suspension of finely divided pigment particles in a liquid composed of a binder (resin), a volatile solvent or water, and additives that impart special characteristics. The volatile components evaporate from the drying film after application, while the binder holds the pigment in the dry film, causing it to adhere to the substrate. Some high quality, hard gloss paints are referred to as enamels.

The basic components of paints vary widely in terms of chemical composition, depending on the colour, the durability, and other required properties of the paint. Table 1.1 lists the main substances and classes of chemicals present in paints, to which workers may be exposed in the painting trades.

Thousands of chemical compounds are used in paint products as pigments, extenders, binders, solvents, and additives. Azo pigments that contain 3,3′-dichlorobenzidine are common, although free aromatic amines are not present in significant quantities. Asbestos was used as a filler until the early 1990s. The main organic solvents used in paints are toluene, xylene, aliphatic compounds, ketones, alcohols, esters, and glycol ethers. Nowadays, solvent-based paints contain much less solvent – and less hazardous solvents – than a decade ago. In some cases the solvent content is reduced to such an extent that the amounts of volatile organic compounds (VOCs) released from the paint are similar to those from water-based paints.

Several hazardous chemicals (including benzene, phthalates (plasticizers), chromium, and lead oxides) have been reduced or replaced in paint in some countries, although they are still used elsewhere. The increasing use of water-based paints and powder coatings has promoted this trend. New formulations contain lower-toxicity solvents, biocides, and neutralizing agents, such as amines.

1.1.1 Pigments and fillers

Paints may contain pigments, dyes and fillers. Hazardous pigments and fillers, especially chromate- or lead-based substances, are increasingly replaced by other compounds, even though many of the new products have a lower performance in corrosion protection or mechanical properties of the paint layers. Many paints for industrial or individual use are lead- and chromate-free, especially in western Europe, but the situation is extremely diverse and complex across countries worldwide.

Table 1.1 Main substances (and classes of substances) to which workers may be exposed in the painting trades[a]

Material	Principal uses or sources of emissions	Agent evaluated	Evaluation
Acrylates (e.g. ethyl acrylate, methyl methacrylate)	Acrylic resins, ultraviolet curing paints	Ethyl acrylate Acrylic acid Methyl acrylate Methyl methacrylate	2B 3 3 3
Acrylic resins	Binders	As above	
Alcohols, aliphatic (e.g. methanol, isopropanol, *n*-butanol)	Solvents (lacquers), paint removers	Methanol Ethanol Isopropanol *n*-Butanol	– – 3 –
Alkalis (e.g. sodium hydroxide, potassium hydroxide)	Paint removers	–	–
Alkyd resins	Binders	–	–
Aluminium, powder	Pigment	–	–
Amides, aliphatic (e.g. dimethylformamide)	Solvents	Dimethylformamide	2A
Amines (mono), aliphatic (e.g. diethylamine) and alkanolamines (e.g. 2-amino-2-methyl-1-propanol)	Water-based paints	Triethanolamine	3
Amines (poly), aliphatic (e.g. diethylenetriamine)	Curing agents (epoxy resins)	–	–
Amines, aromatic (e.g. *meta*-phenylenediamine, 4,4-methylenedianiline)	Curing agents (epoxy resins)	*meta*-Phenylenediamine 4,4-Methylenedianiline	3 2B
Amino resins (e.g. urea-formaldehyde resins, melamine-formaldehyde resins)	Binders	See formaldehyde	
Ammonia	Water-based paints	–	–
Anhydrides, organic (e.g. maleic anhydride, phthalic anhydride, trimellitic anhydride)	Alkyd resin synthesis, curing agents (epoxy resins)	Succinic anhydride	3
Antimony compounds (e.g. antimony trioxide)	Pigments, fire retardant pigments	Antimony trioxide Antimony trisulfide	2B 3
Arsenic compounds (e.g. copper aceto-arsenate)	Antifouling agents	–	1
Asbestos	Filler, spackling and taping compounds, talc	Asbestos	1
Barium compounds (e.g. barium sulfate, barium carbonate)	Pigments	–	–
Benzoyl peroxide	Catalyst	Benzoyl peroxide	3
Bisphenol A	Epoxy resins		3
Cadmium compounds (e.g. cadmium sulfide, cadmium sulfoselenide)	Pigments	Cadmium and cadmium compounds	1

Table 1.1 (continued)

Material	Principal uses or sources of emissions	Agent evaluated	Evaluation
Calcium compounds (e.g. calcium sulfate, calcium carbonate)	Fillers	–	–
Camphor	Plasticizer	–	–
Carbon black	Pigment	Carbon black	2B
Cellulose ester resins (e.g. cellulose nitrate, cellulose acetate)	Binders	–	–
Chloracetamide	Fungicide (water-based paints)	–	–
Chlorofluorocarbons	Spray-can paint propellants	Chlorofluoromethane	3
Chromium and chromium compounds (e.g. chromic oxide, chromates)	Pigments	Chromium (III) compounds Chromium (VI) compounds Chromium, metallic	3 1 3
Clays (e.g. bentonite)	Fillers	–	–
Coal-tar and asphalt	Special waterproof coatings (ships, tanks, pipes)	Coal tar Coal-tar pitches Bitumen extracts Bitumen refined	1 1 2B 3
Cobalt compounds	Pigments, driers	Cobalt and cobalt compounds Cobalt, metallic	2B 2B
Copper and copper compounds (e.g. bronze powder, cuprous oxide)	Pigments, antifouling agents	–	–
Dyes and pigments, organic (e.g. aromatic azo dyes, phthalocyanines, rhodamine)	Pigments	CI Basic Red 9 } Magenta production } 2-naphthylamine } 4-aminobiphenyl } Auramine production } Benzidine } Benzidine-based dyes }	2B 1 1 1 1 1 1
Epichlorohydrin	Epoxy resins	Epichlorohydrin	2A
Epoxy resin	Binders	–	–
Esters, aliphatic (e.g. ethyl acetate, isopropyl acetate)	Solvents	–	–
Ethers, aliphatic (e.g. isopropyl ether, tetrahydrofuran) and glycol ethers (e.g. methyl cellosolve)	Solvents	2-Butoxyethanol 1-*tert*-Butoxypropan-2-ol	3 3

Table 1.1 (continued)

Material	Principal uses or sources of emissions	Agent evaluated	Evaluation
Formaldehyde	Amino resin varnishes, biocide (water-based paints)	Formaldehyde	1
Gasoline	Solvent	Gasoline	2B
Glycidyl ethers (e.g. *n*-butyl glycidyl ether and bisphenol A diglycidyl ether)	Epoxy resin diluents and constituents	Phenylglycidyl ether Triethylene glycol diglycidyl ether Bisphenol A diglycidyl ether	2B 3 3
Glycols (e.g. ethylene glycol)	Polyester resins, water-based paints	–	–
Hydrocarbons, aliphatic (e.g. hexanes, heptanes)	Solvents (naphthas, white spirits)	–	–
Hydrocarbons, aromatic (e.g. benzene, toluene, xylenes, trimethylbenzene)	Solvents (naphthas, white spirits), paint removers	Benzene Toluene Xylene Ethylbenzene	1 3 3 2B
Hydrocarbons, chlorinated (e.g. dichloromethane, 1,1,1-trichloroethane, carbon tetrachloride, trichloroethylene)	Solvents, paint removers, metal degreasers	Dichloromethane 1,1,1-Trichloroethane Carbon tetrachloride Trichloroethylene	2B 3 2B 2A
Hydrochloric acid (hydrogen chloride)	Catalyst (amino resins)	–	3
Iron compounds (e.g. iron oxides, ferric ferrocyanide)	Pigments	Ferric oxide	3
Isocyanates (e.g. 1,6-hexamethylene diisocyanate, toluene diisocyanate)	Two-component polyurethane resins	Toluene diisocyanate	2B
Isothiazolones (e.g. 1,2-benzisothiazolin-3-one)	Biocides in tinned foods	–	–
Kerosene	Solvent	Jet fuel	3
Ketones, aliphatic (e.g. acetone, methyl ethyl ketone, cyclohexanone, isophorone, diacetone alcohol)	Solvents, lacquers, paint removers	Cyclohexanone	3
Lead compounds (e.g. lead chromate, lead oxides, basic lead carbonate, lead naphthenate)	Primers, pigments, driers	Lead Lead compounds, inorganic	2B 2A
Magnesium compounds (e.g. magnesium carbonate)	Fillers	–	–
Manganese naphthenate	Drier	–	–
Mercury compounds (e.g. mercuric oxide, phenyl mercuric acetate)	Fungicides (water-based paints)	Mercury and inorganic mercury compounds	3
Methyl cellulose	Thickener (water-based paints)	–	–
Mica	Filler	–	–
Molybdenum compounds (e.g. lead molybdate)	Pigments	–	–
Nickel, metal powder	Pigment	Nickel compounds Nickel, metallic and alloys	1 2B 2B

Table 1.1 (continued)

Material	Principal uses or sources of emissions	Agent evaluated	Evaluation
Nitroparaffins (e.g. nitroethane, 2-nitropropane)	Solvents	2-Nitropropane	2B
Oils, vegetable (e.g. linseed oil, tung oil)	Binders	–	–
Oximes (e.g. methyl ethyl ketoxime)	Anti-oxidants, anti-skinning agents	–	–
Petroleum solvents (e.g. Stoddard solvent, VM & P naphtha)	Solvents, paint removers	Petroleum solvents	3
Phenol	Phenol-formaldehyde resins, paint remover (formerly)	Phenol	3
Phenol-formaldehyde resins	Binders	See phenol, and formaldehyde	
Phenols, chlorinated (e.g. pentachlorophenol)	Fungicides (water-based paints)	Polychlorophenols and their sodium salts Pentachlorophenol	2B 2B
Phosphates, organic (e.g. tricresyl-*ortho*-phosphate, tributyl phosphate)	Plasticizers	–	–
Phthalate esters (e.g. dibutyl phthalate, dioctyl phthalate	Plasticizers	Di(2-ethylhexyl)phthalate Butyl benzyl phthalate	3 3
Polychlorinated biphenyls	Plasticizers	Polychlorinated biphenyls	2A
Polycyclic aromatic hydrocarbons	Special waterproof coatings (ships, tanks, pipes)	Selected polycyclic aromatic hydrocarbons	–[b]
Polyester resins	Binders	–	–
Polyurethane resins	Binders	Polyurethane foams	3
Polyvinylacetate resins	Binders	Polyvinyl acetate	3
Pyrolysis fumes	Removal of paint by burning; heat-curing operations	–	–
Rosin	Binder	–	–
Rubber, synthetic (e.g. butyl rubber, styrene-butadiene rubber)	Binders (special paints, water-based paints)	Rubber industry	1
Shellac resin	Binder	–	–
Silica, amorphous (e.g. diatomaceous earth)	Filler	Silica, amorphous	3
Silica, crystalline (e.g. quartz)	Filler, sand-blasting operation	Silica, crystalline	1
Silicates (e.g. sodium silicate, aluminium silicate)	Fillers	–	–
Stearates (e.g. aluminium stearates, zinc stearates)	Soaps, flattening agents	–	–
Strontium compounds (e.g. strontium chromate, strontium sulfide)	Pigments	Strontium chromate see chromium and chromium compounds	
Styrene	Polyester resins	Styrene	2B

Table 1.1 (continued)

Material	Principal uses or sources of emissions	Agent evaluated	Evaluation
Styrene oxide	Diluent (epoxy resins)	Styrene-7,8-oxide	2A
Sulfuric acid	Metal cleaner	–	–
Talc	Filler	Talc containing asbestiform fibres Talc, not containing asbestiform fibres	1 3
Tin, metal powder	Lacquers (tinplate containers)	–	–
Tin, organic compounds (e.g. tri-*n*-butyltin oxide, dibutyltin laurate)	Antifouling agents, catalysts	–	–
Titanium dioxide	Pigment	Titanium dioxide	2B
para-Toluenesulfonic acid	Catalyst (amino resins)	–	–
Turpentine	Solvent	–	–
Vinyl acetate	Polyvinylacetate resins	Vinyl acetate Vinyl chloride – vinyl acetate copolymers	2B 3
Zinc and compounds (e.g. zinc metal powder, zinc oxide, zinc chromate)	Pigments, catalysts, bodying agents	Zinc chromate see chromium and chromium compounds	

[a] Updated from IARC (1989)

[b] Groups 1–3; see (IARC, 2010d) for details

–, not evaluated by IARC

(a) Pigments

Pigments can be classified as inorganic and organic (Bentley & Turner, 1998; Stoye & Freitag, 1998; Brock *et al.*, 2000; Smith, 2002) and they are generally added in considerable proportion (3–60% by weight) to paint formulations to provide colour, opacity, and sheen. Pigments also affect the viscosity, flow, toughness, durability, and other physical or chemical characteristics of the coating (e.g. corrosion-protective properties). The diameter of pigment particles is generally less than 3 μm, but for special performance the particle size can be up to 15 or 20 μm (Oyarzún, 2000).

Today the most common pigment employed in paint is the white pigment titanium dioxide, TiO_2 (IARC, 2010b). It occurs in two different crystal forms – rutile and anatase – with distinct colour properties. The rutile crystal structure has an almost 25% greater opacity than the anatase form. Because of its chemical inertness, extreme whiteness, excellent covering power and lack of toxicity compared with white lead, titanium dioxide is the predominant component in the manufacture of white paint, representing 90% of all pigments on the market worldwide. The most important black pigment in paints is carbon black (micro-crystalline carbon, 10–40 nm, graphite-similar), which belongs to the inorganic pigments (Buxbaum & Pfaff, 2005; IARC, 2010b).

In the 1960s, there were probably more than 200 different organic pigments used in paints. At the time, azo pigments such as Benzidine Yellow were considered to have relatively low toxicity, and were widely used. These pigments have relatively low solubility, and although they are based on the aromatic amine 3,3′-dichlorobenzidine, the free amine is not readily bioavailable. Three 3,3′-dichlorobenzidine-based paint pigments were commonly used in architectural finishes in the mid-to-late 1960s. Benzidine was used as the basis for the paint-pigment pyrazolone maroon (IARC, 2010c). The free aromatic amines used in the synthesis of azo pigments can be found in trace amounts as impurities. The aromatic amines 4-aminobiphenyl, benzidine, 2-naphthylamine and 2-methyl-4-chloroaniline [4-chloro-*ortho*-toluidine] have been found in azo pigments (IARC, 2010c).

(b) Dyes

Dyes, unlike pigments, are soluble in paint medium. Dyes are used only in a few instances or products, because they provide much less long-term stability against light and other influences. Examples of use of dyes are in transparent wood stains (Zollinger & Iqbal, 2003), and as transparent colourants in automobile coatings (Streitberger & Dössel, 2008).

1.1.2 Binders (resins)

The ‘vehicle’ part of paints contains components collectively termed ‘binders’ or film formers. Almost all binders in modern paint films are composed of polymer materials such as resins and drying oils, whose main functions are to provide film hardness, gloss, and surface adhesion, as well as resistance of the film to weather influences, air pollutants that stimulate corrosion through the atmosphere, acids, alkalis, and other agents (Stoye & Freitag, 1998; Brock *et al.*, 2000; Müller & Poth, 2006). A large variety of natural and synthetic binders or resins, mostly synthetic, have been used in paints.

(a) Natural resins and oils

Shellac and insect exudations are natural oleoresins that have been used in paints for centuries. Another useful natural resin is rosin (colophony), which is obtained as a residue after distilling pine oleoresin for the production of turpentine. Vegetable and fish oils have long been used as binders in traditional paints and varnishes. White linseed oil has been the most important oil in standard exterior paints, despite its relatively slow drying rate. Other important

oils include castor oil, tall oil, soya-bean oil, coconut oil, cottonseed oil, tung oil and various fish oils (Brock *et al.*, 2000).

(b) Synthetic resins

A wide variety of synthetic resins have been commercially available since the early 1900s. Those that have been most frequently used in paints, varnishes and lacquers include cellulose-based resins, phenolic, alkyd, vinyl, acrylic and methacrylic resins, polyester and polyurethane resins, chlorinated rubber derivatives, styrene-butadiene, and silicone oils. Mixtures of synthetic resins provide characteristic properties that cannot be obtained from a single resin. While the amount of resin in paint varies, concentrations of 20–60% by weight are not uncommon.

1.1.3 Solvents

Since the early 1900s, the number of solvents in paints has increased considerably to encompass a broad range of petroleum and coal-tar distillates, alcohols, esters, ketones, glycols, synthetic glycol ethers and esters (mainly ethylene-derived), and propylene glycol derivatives, as well as a large variety of mixtures of these chemical classes. The choice of solvent depends on properties such as adequate polarity, possibility of hydrogen-bonding, volatility and vapour pressure, cooling effects during atomization, surface tension, viscosity, flash point, flammability and – more and more importantly – lack of adverse physiological effects. In western Europe, derivatives of ethylene-glycol monoethyl-ether (ethyl glycol) have been removed from many formulations since the 1980s. Since 1990, the use of styrene has been restricted by legislation in the European Union. Water-based coatings generally require water-soluble solvents such as glycol ethers (butyl glycol), *n*-butanol or, less commonly, *N*-methyl-pyrrolidone.

1.1.4 Additives

Additives are defined as chemicals that have a specific function or impart a special property to paints or coatings. They are present at low concentrations (generally 0.1–5% by weight) and include surfactants and dispersing agents, driers, rheological agents, plasticizers, biocides, anti-skinning agents, antifoam agents (de-foamers), corrosion inhibitors, light (UV) stabilizers and catalysts (Stoye & Freitag, 1998; Brock *et al.*, 2000). Many additives are adapted to the new paint systems by modification of existing products rather than by the development of new ones.

(a) Surfactants and dispersing additives

Anionic, cationic, amphoteric or non-ionic surfactants are used in paints as pigment dispersants (in both non-aqueous and aqueous systems), emulsifying agents, protective colloids, wetting agents and antifoaming agents. Dispersants employed in non-aqueous paints include lecithin, zinc or calcium naphthenate or octoate, oleates, oleic acid, polyurethanes, polyamides and other chemicals. Dispersants in aqueous paints include polyphosphates, pyrophosphates, salts of arylalkyl-sulfonic acids and salts of polycarboxylic acids, e.g. polyacrylic acid (Oyarzún, 2000; Müller & Poth, 2006). Surfactants used in water-based paints include aluminium stearate, cellulose ethers, polydimethyl siloxanes, polyethylene, alkali metal phosphates and sodium dioctyl sulfosuccinate.

A variety of other surface-active agents are added to paints to control flow, levelling, sagging, settling and viscosity. These include hydrogenated castor oils, lecithin, metallic soaps (e.g. linoleates, palmitates and stearates), treated montmorillonite clays, peptized oil gels, polyol esters, siloxan-polyester resins, silicas, and soap solutions (Brock *et al.*, 2000; Müller & Poth, 2006). Mineral oils and specially modified siloxanes are used as antifoaming agents.

(b) Driers

Driers (siccatives) used in solvent-based and water-based paints containing unsaturated polymers are principally metal salts – lead, calcium, cobalt, manganese, zirconium, vanadium, barium, zinc, cerium and lanthanum – of naphthenic acid, tall-oil acid, 2-ethylhexanolic acid and neodecanoic acid, generally at concentrations ranging from 0.3 to 0.8% (Brock *et al.*, 2000). Cobalt-based driers are the most commonly used commercially as active catalysts in both air-drying and heat-cure systems. Other metal-containing siccatives serve as auxiliary driers and are generally used in combination with cobalt- and manganese-based driers. Lead-containing products were at one time the major auxiliary driers, but legislation that limits the amount of lead used in coatings has practically eliminated its use during the period 1990–2000 (IARC, 2006). The most suitable replacements for lead are reported to be zirconium, calcium and cobalt-zirconium compounds (Müller & Poth, 2006).

(c) Rheological additives

The rheological properties of a coating material influence its optimal performance during application ('good flow without dripping') as well as its storage life. Water-soluble hydrophilic colloids that are used as rheological additives include agents such as gum arabic, gum tragacanth, starch, sodium alginate, methyl cellulose, hydroxyethyl cellulose, polyvinyl alcohol, ammonium caseinate, polyurethane derivates, and polyacrylates. Acrylate salts, casein and cellulose-derived compounds are widely used in acrylic paints, while the major thickeners for styrene–butadiene paints are alkali-soluble proteins (e.g. soy-bean proteins). Methyl cellulose and hydroxyethyl cellulose are common thickeners for polyvinyl acetate paints (Brock *et al.*, 2000).

Agents used in water-based and solvent-based paints as rheological additives not derived from cellulose include maleic anhydride copolymers, mineral fillers such as colloidal attapulgite (IARC, 1997), treated magnesium montmorillonite clays, pyrogenic silicic acid (SiO_2), natural products (e.g. alginic acid, casein and soya-bean protein), polyacrylamides, polyacrylic acid salts and acid-containing cross-linked acrylic emulsion copolymers (Brock *et al.*, 2000).

(d) Plasticizers

Plasticizers are generally added in quantities of up to about 2% by weight and include dibutyl-, diethyl-, diethylhexyl- and dioctylphthalates. To a lesser extent, plasticizers also contain the low molecular-weight esters of adipic and sebacic acid, tributyl phosphate, and castor oil. Polyester resins, including maleic residues, sulfonamides, tri-*ortho*-cresyl phosphate and chlorinated diphenyls, are used occasionally (Stoye & Freitag, 1998).

(e) Biocides (fungicides, preservatives, and mildew killers)

Water-based paints contain organic substances and represent an ideal growth medium for fungi, algae and bacteria. With the reduced content of residual monomers and organic solvents (which often have anti-microbial action), there is a greater risk for microbial contamination in the new formulations. The growth of microorganisms in the coating or subsequently in the film can be reduced or even prevented by adding chemical biocides to the paint in concentrations below 1% by weight (Brock *et al.*, 2000; Schwartz & Baumstark, 2001).

In-can preservatives protect the paint against microbial growth during production, transportation and storage. Substances commonly used for this purpose are formaldehyde – now less and less common – and its reaction products with alcohols, amines and amides, as well as *N*,*S*-heterocyclic compounds such as isothiazolinones and

chloroacetamide (Brock *et al.*, 2000). *In-film preservatives*, also encompassing *antifouling additives* in marine paints, protect the applied paint against attack by bacteria, moulds, algae or mosses. Substances currently in use for this purpose include several *S*- and *N*-containing chemicals, cyclic compounds such as dithiocarbamates, thiophthalimide derivates, benzimidazole derivates and trialkyl compounds, as well as ecologically harmful substances such as organic mercury compounds (Brock *et al.*, 2000).

(f) Anti-skinning agents

Anti-skinning agents are added to paints to retard the formation of skin on the surface of the liquid coating, in either closed or open cans, without delaying the drying of the product. The principal anti-skinning agents are oximes (e.g. methyl ethyl ketoxime, butyraldoxime, cyclohexanone oxime) and phenol derivatives (methoxyphenol, *ortho*-aminophenol, poly-hydroxyphenol). Small quantities of cresols, guaiacol, hydroquinone (IARC, 1999), isobutoxysafrol and lignocol have also been used as anti-skinning agents.

(g) Corrosion inhibitors

Corrosion inhibitors can be divided into inorganic pigments and organic inhibitors (Brock *et al.*, 2000). Red lead and chromate-containing pigments are both chemically and electrochemically active. Pigments containing red lead are still used in heavy-duty anti- corrosion systems, because they possess excellent protection properties. Some zinc chromates are still essential for the protection of aluminium on aircraft. Lead- and chromate-containing anti-corrosion pigments are increasingly being substituted by phosphates (zinc, chromium(III), aluminium, calcium and magnesium phosphates). Zinc-dust primers are widely used in the protection of steel structures. The synthetic micaceous iron-oxide pigment haematite (Fe_2O_3) acts via a physical mechanism, mainly by the barrier effect of its crystal-lattice structure (platelets). The most important compound in the group of organic inhibitors is the zinc salt of 5-nitrophthalic acid.

(h) Asbestos

In the early twentieth century, asbestos was used as a filler to improve the technical properties of paints, particularly those used in shipyards and on bridges. The paints may have contained up to about 20% asbestos by weight. Usage decreased after about 1950, although special textured paints or coatings continued to be widely used in home decoration until the early 1990s. These paints contained approximately 5% chrysotile asbestos by weight (Williams *et al.*, 2007).

(i) Nanoparticles

The use of 0.5–5% (w/w) nanoparticles (10–100 nm) remarkably improves the properties of paint layers in terms of scratch resistance, hardness, gloss, weather stability, and cross-linking and hardening properties. Nanoparticles are present as single particles only at the time of manufacturing. They increase in effective size by agglomeration and by absorption of polymers and surface-active agents onto their surface. During drying of the paint, the particles continue to agglomerate and are incorporated irreversibly into the polymer matrix.

1.2 Human exposure

Workers in the painting industry are potentially exposed to the chemicals found in paint products during their application and removal. Exposure to dichloromethane occurs during paint stripping from wood and metal surfaces. Diisocyanate is present in some binders and is released during painting. Silica is used in the preparation of surfaces before painting. As bystanders during construction or demolition activities, painters may also be exposed to asbestos or crystalline silica. During the

application of paint, workers are exposed primarily to solvents, whereas the mechanical removal of paint mainly leads to exposure to pigments and fillers. In the past, exposure to hazardous substances frequently exceeded current occupational exposure limits, but exposure levels have generally decreased over time.

Exposure, both by inhalation and via skin contact, occurs specifically in operations that involve manual handling during preparation of the paint, such as weighing ingredients (pigments, extenders, resins, additives), loading them into mixing equipment, adding solvents to mills, and cleaning equipment (mixers, mills, reactors, kettles, tanks, filters). Additional exposure to solvents occurs during thinning, tinting and shading, during filling operations, and during the filtering of varnishes. The cooking of varnishes may produce emissions of various aldehydes such as acrolein, of phenol, ketones, glycerine and fatty acids as well as dusts or vapours of maleic, phthalic and fumaric anhydrides during the loading of kettles. The production of powder coatings can be associated with significant exposure to dust from resin powders, pigments, curing agents and other additives. In the manufacture of radiation-curable coatings, exposures may occur to monomers such as ethyl acrylate, other acrylates, and photo-initiators. While inhalation and cutaneous contact are the major routes of exposure, ingestion related to personal work habits constitutes another potential route of entry.

Bio-monitoring of workers exposed to paints has shown elevated levels of paint compounds or their metabolites in blood and urine. Appropriate selection and use of personal protective equipment can substantially reduce uptake, although painters do not generally wear respirators or gloves.

The main substances to which workers may be exposed are listed in Table 1.1. Quantitative studies of occupational exposure in the major painting trades are summarized in IARC (2010a).

As indicated above, the use of 0.5–5% (w/w) nanoparticles (10–100 nm) remarkably improves several properties of paint. Because these particles agglomerate and become incorporated irreversibly into the polymer matrix, painters are not exposed to single nanoparticles as such. Since nanoparticles are made by special manufacturers and sold as aqueous or solvent-based slurry because of their strong potential for agglomeration, workers in paint manufacture do not come into contact with nanoparticles (Aitken *et al.*, 2006).

2. Cancer in Humans

Occupational exposure as a painter was classified as a Group-1 carcinogen in *IARC Monograph* Volume 47 (IARC, 1989), based on an increased risk for lung cancer, and reaffirmed in *Monograph* Volume 98 (IARC, 2010a), based also on increased risks for mesothelioma and bladder cancer. The recent Working Group noted that there was *limited evidence*, based primarily on studies of maternal exposure, that painting is associated with childhood leukaemia. The epidemiological evidence on occupational exposure as a painter did not allow identification of the specific carcinogenic agent in paint.

2.1 Cancer of the lung

2.1.1 Cohort studies

Eighteen independent cohort studies of painters – excluding reports with substantial population overlap – have investigated the association between occupation as a painter and lung cancer (OPCS, 1958, 1972, 1979, 1986, 1996; Guralnick, 1963; Dunn & Weir, 1965; Menck & Henderson, 1976; Whorton *et al.*, 1983; Dubrow & Wegman, 1984; Gubéran *et al.*, 1989; Hrubec *et al.*, 1995; Alexander *et al.*, 1996; van Loon *et al.*, 1997; Boice *et al.*, 1999; Steenland & Palu,

1999; Pronk *et al.*, 2009; Pukkala *et al.*, 2009; see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-30-Table2.1.pdf). Overall, these studies have shown a significantly increased incidence or mortality from lung cancer, with a magnitude of effect that was relatively consistent between studies. Several of these studies were adjusted for tobacco smoking (Dunn & Weir, 1965; Hrubec *et al.*, 1995; van Loon *et al.*, 1997; Pronk *et al.*, 2009).

2.1.2 Case–control studies

Thirty independent case–control studies of lung cancer that reported on the association with occupation as a painter demonstrated relatively consistent increased risks for lung cancer (generally ranging between 1.10 and 2.70) (Wynder & Graham, 1951; Breslow *et al.*, 1954; Viadana *et al.*, 1976; Williams *et al.*, 1977; Milne *et al.*, 1983; Kjuus *et al.*, 1986; Lerchen *et al.*, 1987; Levin *et al.*, 1988; Ronco *et al.*, 1988; Vineis *et al.*, 1988; Zahm *et al.*, 1989; Bethwaite *et al.*, 1990; Burns & Swanson, 1991b; Siemiatycki, 1991; Morabia *et al.*, 1992; Notani *et al.*, 1993; Wu-Williams *et al.*, 1993; Finkelstein, 1995; De Stefani *et al.*, 1996, 2005; Muscat *et al.*, 1998; Wünsch-Filho *et al.*, 1998; Jahn *et al.*, 1999; Pezzotto & Poletto, 1999; Brüske-Hohlfeld *et al.*, 2000; Matos *et al.*, 2000; Pohlabeln *et al.*, 2000; Bouchardy *et al.*, 2002; Richiardi *et al.*, 2004; Baccarelli *et al.*, 2005; Zeka *et al.*, 2006; see Table 2.2, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-30-Table2.2.pdf). Twenty-seven of these studies showed positive associations (with statistically significant or borderline significant results in 15 studies) and three studies had odds ratios below 1, but not statistically significant (Morabia *et al.*, 1992; Wünsch-Filho *et al.*, 1998; Baccarelli *et al.*, 2005). All except six studies adjusted for tobacco smoking (Wynder & Graham, 1951; Breslow *et al.*, 1954; Milne *et al.*, 1983; Bethwaite *et al.*, 1990; Finkelstein, 1995; Bouchardy *et al.*, 2002).

2.1.3 Meta-analyses

Two comprehensive meta-analyses of the epidemiological literature on painters and lung cancer have been published since the previous *Monograph* (IARC, 2010a).

One meta-analysis included 39 studies (23 case–control and 16 cohort studies) (Bachand *et al.*, 2010). Summary risk estimates were derived and sensitivity analysis performed to evaluate smoking, socioeconomic status and exposure variables. Overall summary risk estimates for lung cancer were 1.29 (95%CI: 1.10–1.51) for case–control studies, and 1.22 (95%CI: 1.16–1.29) and 1.36 (95%CI: 1.34–1.41) for cohort studies, respectively. The 20 case–control studies that adjusted for smoking gave a summary relative risk (RR) of 1.32 (95%CI: 1.10–1.59). Only one of the cohort studies included in the meta-analysis adjusted for smoking and an external adjustment for smoking demonstrated an increased mortality from lung cancer in painters. [The Working Group noted that the methods for external adjustment for smoking were not clearly described.]

Guha *et al.* (2010a) performed a meta-analysis to assess the association between occupation as a painter and lung cancer. Forty-seven independent studies (18 cohort and 29 case–control studies) were included. Overall, a statistically significant increased risk for lung cancer was observed (meta-relative risk, 1.35; 95%CI: 1.29–1.41). When the analysis was restricted to smoking-adjusted estimates, the summary relative risk was 1.35 (95%CI: 1.21–1.51). On the basis of data from three studies that investigated risk in never smokers, the meta-relative risk was 2.00 (95%CI: 1.09–3.67). The increased risk persisted when the analysis was restricted to studies that adjusted for other occupational exposures (RR 1.57; 95%CI: 1.21–2.04). A duration–response relationship was also identified: the meta-relative risk for < 10 years of exposure was 1.13 (95%CI: 0.77–1.65) and 1.95 (95%CI: 1.26–3.02) for > 10

years of exposure; similarly, the meta-RR was 1.37 (95%CI: 0.89–2.13) for < 20 years of exposure and 2.00 (95%CI: 1.01–3.92) for > 20 years (the reference category was no exposure).

2.2 Mesothelioma

The association between occupation as a painter and mesothelioma was investigated in four cohort studies (Malker *et al.*, 1990; Peto *et al.*, 1995; Brown *et al.*, 2002; Pukkala *et al.*, 2009) and two case–control studies (Teschke *et al.*, 1997a; Pan *et al.*, 2005). An increase in mortality from mesothelioma was observed in each of the four cohort studies, with borderline significant relative risks ranging from 1.31 to 1.70. The two case–control studies on mesothelioma also showed an increased risk (OR, 4.5; 95%CI: 1.0–23.7; 6 exposed cases; and OR, 2.6; 95%CI: 1.3–5.3; 31 exposed cases, respectively) for persons ever employed as painters.

[The Working Group noted that it is improbable that the presence of asbestos would completely explain the excess of lung cancer; if this had been the case, a more pronounced excess of mesothelioma would have been observed.]

2.3 Cancer of the urinary bladder

2.3.1 Cohort studies

The association between occupational exposure as a painter and urinary bladder cancer was investigated in 11 cohort studies, excluding reports with substantial population overlap (OPCS, 1958, 1972, 1979, 1986; Guralnick, 1963; Whorton *et al.*, 1983; Gubéran *et al.*, 1989; Hrubec *et al.*, 1995; Steenland & Palu, 1999; Zeegers *et al.*, 2001; Pukkala *et al.*, 2009) see Table 2.3, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-30-Table2.3.pdf). Two of the cohort and record-linkage studies controlled for tobacco smoking (Hrubec *et al.*, 1995; Zeegers *et al.*, 2001). Overall these studies showed consistent increases in incidence of or mortality from urinary bladder cancer.

2.3.2 Case–control studies

Several case–control studies have investigated the association between urinary bladder cancer and occupation as a painter. Thirty-one independent case–control studies were identified (Wynder *et al.*, 1963; Cole *et al.*, 1972; Decouflé *et al.*, 1977; Williams *et al.*, 1977; Howe *et al.*, 1980; Schoenberg *et al.*, 1984; Morrison *et al.*, 1985; Coggon *et al.*, 1986; Iscovich *et al.*, 1987; Risch *et al.*, 1988; Silverman *et al.*, 1989a, b; Bethwaite *et al.*, 1990; La Vecchia *et al.*, 1990; Burns & Swanson, 1991a; Barbone *et al.*, 1994; Teschke *et al.*, 1997b; Golka *et al.*, 1999, 2008; Bouchardy *et al.*, 2002; Pelucchi *et al.*, 2002; Zheng *et al.*, 2002; Kogevinas *et al.*, 2003; Colt *et al.*, 2004; Gaertner *et al.*, 2004; Band *et al.*, 2005; Reulen *et al.*, 2007; Dryson *et al.*, 2008; Ramanakumar *et al.*, 2008; Kobrosly *et al.*, 2009 ; see Table 2.4, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-30-Table2.4.pdf). Three studies showed no association (Howe *et al.*, 1980; Colt *et al.*, 2004; Ramanakurmar *et al.*, 2007) and six reported odds ratios less than 1.0, although none were statistically significant (Morrison *et al.*, 1985 – the United Kingdom and Japanese populations; Williams *et al.*, 1977; Coggon *et al.*, 1986; Iscovich *et al.*, 1987; Gaertner *et al.*, 2004). Twenty-two case–control studies demonstrated an increased risk for bladder cancer associated with occupation as a painter. Although the results of three studies were statistically significant (Silverman *et al.*, 1989a, b; Golka *et al.*, 1999; Band *et al.*, 2005) and those of six studies were of borderline statistical significance (Wynder *et al.*, 1963; Decouflé *et al.*, 1977; Risch *et al.*, 1988; Bethwaite *et al.*, 1990; Zheng *et al.*, 2002; Kogevinas *et al.*, 2003), all studies that showed an increased risk for bladder cancer among painters were relatively consistent in the magnitude of the effect reported.

2.3.3 Meta-analyses

Five meta-analyses have also demonstrated a significant or borderline significant increased incidence of or mortality from bladder cancer among persons occupationally exposed as a painter (Yamaguchi *et al.*, 1991; Chen & Seaton, 1998; Bosetti *et al.*, 2005; Bachand *et al.*, 2010; Guha *et al.*, 2010b). The two most recent meta-analyses are highlighted below.

The meta-analysis by Bachand *et al.* (2010) included 40 case–control and 11 cohort studies. Overall bladder-cancer summary risk estimates were 1.28 (95%CI: 1.17–1.41) for case–control and 1.14 (95%CI: 1.06–1.22) and 1.27 (95%CI: 1.16–1.38) for cohort morbidity and mortality studies, respectively. The 33 case–control studies that adjusted for smoking gave a summary RR of 1.30 (95%CI: 1.17–1.44). None of the cohort studies adjusted for smoking. When an external adjustment for smoking was applied to the meta-analysis of the cohort studies, an increased incidence of and mortality from bladder cancer persisted. [The Working Group noted that the methods for the external adjustment for smoking were not clearly described.]

A separate meta-analysis of 41 independent studies (11 cohort and record-linkage studies and 30 case–control studies) conducted by Guha *et al.* (2010b) showed a meta-relative risk of 1.25 (95%CI: 1.16–1.34). This association did not change significantly when the analysis was restricted to population-based studies or studies that adjusted for smoking and other potentially confounding occupational exposures. Furthermore, exposure-response analyses suggested that the risk increased with duration of employment: those exposed < 10 years had a lower risk (meta-RR, 1.41; 95%CI: 1.00–2.01) than those exposed > 10 years (meta-RR, 1.81; 95%CI: 1.20–2.75) (reference category was no exposure).

2.4 Childhood leukaemia

2.4.1 Maternal exposure

The association between maternal exposure during painting and childhood leukaemia was evaluated in nine population-based case–control studies (van Steensel-Moll *et al.*, 1985; Lowengart *et al.*, 1987; Buckley *et al.*, 1989; Shu *et al.*, 1999, 2004; Schuz *et al.*, 2000; Freedman *et al.*, 2001; Alderton *et al.*, 2006; Scélo *et al.*, 2009; see Table 2.5, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-30-Table2.5.pdf). Most of the studies presented results for the combined exposure to paints, stains, and lacquers. Shu *et al.* (2004) presented a case-only analysis that examined whether maternal exposure to paint was associated with development of mutation in the *RAS* gene in acute lymphoblastic leukaemia (ALL) cases compared with ALL cases without *RAS* mutation and, therefore, is not directly relevant to the discussion of whether maternal exposure during painting increases the risk for childhood leukaemia compared with healthy controls.

In five studies significant positive associations were found between maternal exposure during painting, either before or during pregnancy, and acute leukaemia (van Steensel-Moll *et al.*, 1985; Lowengart *et al.*, 1987), acute non-lymphoblastic leukaemia (ANLL) (Buckley *et al.*, 1989); and ALL (Shu *et al.*, 1999; Schuz *et al.*, 2000). All these studies controlled for age and/or sex, race, social class (measured through income, socioeconomic status, or degree of urbanization) or other variables. Additionally, borderline significant positive associations were found with ALL (Freedman *et al.*, 2001; Scélo *et al.*, 2009) and non-significantly elevated ORs for ALL and acute myeloid leukaemia (AML) (Alderton *et al.*, 2006). Furthermore, significant exposure-response relationships, according to duration of maternal exposure to paint, were observed in two studies (Buckley *et al.*, 1989; Shu *et al.*, 1999).

2.4.2 Paternal exposure

The association between paternal exposure during painting and childhood leukaemia was considered in 12 population-based case–control studies (Fabia & Thuy, 1974; Hakulinen *et al.*, 1976; Kwa & Fine, 1980; Hemminki *et al.*, 1981; Sanders *et al.*, 1981; Gold *et al.*, 1982; van Steensel-Moll *et al.*, 1985; Lowengart *et al.*, 1987; Buckley *et al.*, 1989; Shu *et al.*, 1999; Schuz *et al.*, 2000; McKinney *et al.*, 2003; (see Table 2.6, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-30-Table2.6.pdf). In three of the four case–control studies on ALL, non-significant positive associations were found (van Steensel-Moll *et al.*, 1985; Schuz *et al.*, 2000; McKinney *et al.*, 2003) and in the only case–control study of ANLL (Buckley *et al.*,1989) a significant, positive association was observed. Of the eight studies that considered combined leukaemia subtypes, positive associations were found in five (either significant or borderline significant) (Fabia & Thuy, 1974; Hemminki *et al.*, 1981; Gold *et al.*, 1982; Lowengart *et al.*, 1987; McKinney *et al.*, 2003), while there was no association in two studies (Kwa & Fine, 1980; Sanders *et al.*, 1981).

2.5 Lympho-haematopoietic cancers

The risk for lymphatic and haematopoietic cancers among painters was evaluated in 21 case–control studies (Persson *et al.*, 1989, 1993; Lindquist *et al.*, 1987; La Vecchia *et al.*, 1989; Bethwaite *et al.*, 1990; Heineman *et al.*, 1992; Scherr *et al.*, 1992; Blair *et al.*, 1993; Demers *et al.*, 1993; Mele *et al.*, 1994; Nordström *et al.*, 1997; Clavel *et al.*, 1998; Persson & Fredrikson, 1999; Blair *et al.*, 2001; Costantini *et al.*, 2001; Adegoke *et al.*, 2003; Dryver *et al.*, 2004; Kato *et al.*, 2005; Colt *et al.*, 2007; Ramanakumar *et al.*, 2008; Purdue *et al.*, 2009). Although increased risks were observed, the results were inconsistent and the data are inadequate to draw a conclusion about the association between occupation as a painter and the risk for lymphatic and haematopoietic cancers.

2.6 Other cancers

A few case–control studies of cancers of the upper aerodigestive tract (oral cavity, nose, pharynx, nasopharynx, larynx, and oesophagus), stomach, pancreas, liver, colon, rectum, kidney, brain, prostate, testis, ovary and breast, and of melanoma and soft–tissue sarcoma have been conducted among painters (Tarvainen *et al.*, 2008; IARC, 2010a). Results were inconclusive for all sites.

2.7 Synthesis

The Working Group reviewed a large body of epidemiological evidence evaluating the association between occupational exposure as a painter and the risk for lung cancer (30 case–control studies, 18 cohort studies), urinary bladder cancer (30 case–control studies, 11 cohort studies) and mesothelioma (2 case–control studies, 4 cohort studies). This evidence demonstrates that occupational exposure as a painter is causally associated with the risk for cancer of the lung and urinary bladder, and for mesothelioma. The Working Group noted that the magnitude of the effect was consistent across studies and the elevated risks persisted after controlling for other occupational exposures and for tobacco smoking. Increased risks for these cancers were also observed in never smokers and current non-smokers. Several studies that demonstrated significant positive duration-response relationships support this evaluation.

The Working Group noted that no particular agent could be identified from epidemiological studies as the specific cause of the excess of lung and urinary bladder cancers. It is improbable that the presence of asbestos in paint would completely explain the excess of lung cancer; if

this had been the case, a more pronounced excess of mesothelioma would have been observed. There is little information from epidemiological studies on the risk associated with the use of paint pigments that are known lung carcinogens, such as chromium or cadmium.

There is evidence suggesting a causal association between maternal exposure during painting and childhood leukaemia in the offspring. Although there were few studies and exposure assessments were relatively poor, all of the studies showed significant positive associations after adjusting for potential confounders such as age and/or sex, race and social class, although confounding or recall bias could not be ruled out.

The evidence is inconclusive for cancer at other sites.

3. Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Relevant Data

The chemicals discussed below are common components of paints: benzene, toluene, chlorinated solvents (dichloromethane, trichloroethylene), xylenes, metals (cadmium, chromium, inorganic lead), styrene, PAHs, and aromatic azo dyes.

4.1 Toxicokinetics and metabolism

4.1.1 Solvents

(a) Aromatic hydrocarbons

The aromatic hydrocarbons present in paints (benzene, toluene, xylenes and styrene) are absorbed mainly through inhalation, although oral or dermal exposure could be important as well. They are metabolized primarily in the liver by oxidation, in a process catalysed mainly by the CYP2E1 enzyme:

(i) Benzene

The toxicokinetics of benzene is reviewed in the *Monograph* on Benzene in this volume.

(ii) Toluene

Toluene is metabolised to benzyl alcohol followed by oxidation to benzoic acids, which are excreted as conjugates with glycine or with UDP-glucuronate (ATSDR, 2000a).

(iii) Xylene

All three isomers of xylene are metabolised to methylbenzyl alcohol and conjugated with glycine to form methylhippuric acid. Aromatic hydroxylation of xylene to xylenol occurs to only a limited extent in humans. About 90% of the absorbed xylene is excreted in the urine as methylhippuric acid, and less than 2% as xylenol. Approximately 5% is eliminated unchanged in exhaled air (ATSDR, 2007b).

(b) Chlorinated solvents

(i) Dichloromethane

Dichloromethane (DCM) is absorbed mainly into the bloodstream after inhalation and is found in highest concentration in adipose tissue and liver. DCM can be metabolised by the cytochrome P450(CYP)-associated enzyme CYP2E1 to form formyl chloride, CO and CO_2, and by GSTT1-1 to carbon dioxide via a postulated glutathione-conjugate (*S*-chloromethyl glutathione), and to formaldehyde. Both pathways can give rise to toxic metabolites. After inhalation exposure, humans eliminate dichloromethane mainly in expired air, but also in the urine (ATSDR, 2000b).

(ii) Trichloroethylene

Trichloroethylene (TCE) is also absorbed mainly into the bloodstream after inhalation and is widely distributed in the liver, kidneys, and the cardiovascular and nervous systems.

Trichloroethylene is mainly metabolized in the liver through an oxidative pathway by CYP isoenzymes and through conjugation with

glutathione (Davidson & Beliles, 1991; Lash *et al.*, 2000a), leading to the formation of major metabolites such as chloral hydrate, trichloroethanol and trichloroacetic acid. Four CYP isoforms play a role in TCE metabolism: CYP2A1/2, CYP2B1/2, CYP2C11/6 and CYP2E1 (Koop *et al.*, 1985; Nakajima *et al.*, 1988; Guengerich & Shimada, 1991; Lash *et al.*, 2000a). The resulting metabolites are thought to be associated with liver toxicity and liver carcinogenesis in animals. The glutathione conjugation pathway leads to the formation of dichlorovinyl glutathione and dichlorovinyl cysteine. The latter can be further metabolized by β-lyase to reactive chemical species that are thought to play a role in the toxicity of TCE in the proximal renal tubules and in the renal carcinogenicity of TCE in animals. The CYP pathway is thought to predominate and formation of reactive species via the glutathione *S*-transferase (GST) pathway is less important (Lash *et al.*, 2000b).

4.1.2 Metals

Metal compounds used as paint pigments such as cadmium, chromium, and inorganic lead, are predominantly absorbed in the lung. Dermal absorption is generally low and depends on the chemical properties of the compound, the vehicle, and the integrity of the skin. Absorbed metals are distributed to the organs and, in the case of lead, are concentrated in the bone. Elimination of metals varies from several days to several years.

(a) Cadmium

Cadmium (Cd) enters the body mainly by inhalation in the working environment, whereas the general population is exposed via food and drinking-water. Fractional intestinal absorption is influenced by dietary factors and increases with dietary Cd concentration, while pulmonary fractional absorption depends partly on the solubility of cadmium. Cd and other agents induce synthesis of metallothionein, a protein that binds to cadmium and transfers it via the blood, primarily to the liver and the kidney. In the kidney, the complex is filtered through the renal glomeruli and then reabsorbed from the filtrate in the proximal tubules where the protein portion is rapidly degraded to release Cd. As a result, most of the body burden of Cd is retained in the liver and kidneys (where the half-life is estimated to be 7–16 years (IARC, 2012). Excretion occurs mainly via the urine and, in individuals without renal dysfunction, primarily reflects the amount of cadmium retained in the kidneys (IARC, 1993).

(b) Chromium

The toxicokinetics of chromium compounds depend on the solubility and particle size, on the valence state of the chromium atom and the nature of its ligands. Absorption of chromium(VI) compounds is higher than that of chromium(III) compounds, the latter occurring via passive diffusion and phagocytosis. Absorption of inhaled chromium compounds takes place in the lung via transfer across cell membranes and in the gastrointestinal tract from particles cleared from the lungs. Absorption after dermal exposure depends on the physical and chemical properties of the compound, the vehicle, and the integrity of the skin. Once taken up in the blood, chromium compounds are distributed to all organs, with highest concentrations in kidney, liver and bone (NTP, 2010).

Particles containing chromium can be retained in the lung for years after occupational exposure (ATSDR, 2000c). After exposure by inhalation, excretion occurs predominantly via the urine, and after oral exposure via the faeces, due to low absorption of chromium compounds from the gastrointestinal tract (ATSDR, 2008).

(c) Lead compounds, inorganic lead

Lead compounds have been used in paints as primers, pigments and driers. Lead can be absorbed after inhalation, oral or dermal exposure. Patterns and rates of particle deposition are highly dependent on size and ventilation rate. However, all lead settled deep in the lung is eventually absorbed. Dermal absorption of inorganic lead is negligible, although slightly increased by high perspiration in humans. Absorption from the gastrointestinal tract in both humans and experimental animals is strongly influenced by age, fasting/fed status, nutrition, solubility, and particles size. Absorbed lead is rapidly distributed from plasma into erythrocytes, soft tissues and – mainly – bone. Bone can be a significant source of endogenous lead, in particular when the resorption rate is increased, such as during pregnancy, lactation, and just after the menopause. After oral ingestion, absorbed lead is primarily excreted in the urine and, via the bile, in the faeces. Inorganic lead that has not been absorbed in the gastrointestinal tract is excreted in the faeces (IARC, 2006).

4.1.3 Other compounds

(a) Styrene

Styrene is used as polyester resin in paints. After inhalation, oral intake, or dermal absorption, styrene is rapidly distributed throughout the body, with the highest concentrations found in adipose tissue (IARC, 1994, 2002). In humans, styrene is metabolised to the predominant first metabolite, styrene-7,8-oxide, principally by CYP2E1, CYP2F, but also by CYP2B6. Isolated erythrocytes are also capable of non-enzymatic conversion of styrene totyrene-7,8-oxide. After its oxidation, a large percentage of styrene is excreted as urinary mandelic and phenylglyoxylic acids; glutathione conjugates represent a minor fraction of the metabolites of styrene-7,8-oxide (IARC, 1994, 2002).

(b) PAHs

PAH exposure during painting occurs through the use of special waterproof coatings or by pyrolysis of paint residues during removal. There are more than 100 different PAHs, which generally occur as complex mixtures rather than single compounds, but their identity is unknown in paints. Therefore, the toxicokinetics is discussed in broad general terms. Little is known about the toxicokinetics of PAH mixtures or individual PAHs in humans and most of the available data come from benzo[*a*]pyrene in experimental studies (described in IARC, 2010d; see also the *Monograph* on Benzene elsewhere in this volume).

(c) Aromatic amines and azo dyes

The toxicokinetics of aromatic amines and azo dyes, such as 4-aminobiphenyl, benzidine, benzidine-based dyes and 2-naphthylamine, are described in *IARC Monograph* Volume 99 (IARC, 2010c) and in the *Monographs* on these agents elsewhere in this volume.

4.2 Genetics and related effects

4.2.1 Genotoxicity

(a) Chromosomal aberrations, micronuclei, and sister chromatid exchange

Six of eight studies on chromosomal aberrations among painters reviewed in *IARC Monograph* Volume 98 (IARC, 2010a) showed consistent and significant elevated frequencies, and three of these studies reported an association with years of employment (Silva & Santos-Mello, 1996; Pinto *et al.*, 2000; Gajalakshmi *et al.*, 2002) while the others did not report analyses on duration of employment (Capomazza & Botta, 1990; Piña-Calva *et al.*, 1991; Testa *et al.*, 2005).

Several chromosomal abnormalities could be detected in the bone marrow of patients with acute myeloid leukaemia (AML). In a study by

Crane *et al.* (1996), routine cytogenetic data from 213 patients (129 enrolled in the period 1976–1983 and 84 enrolled in the period 1986–1990) with AML were correlated with environmental exposures to organic chemicals (e.g. benzene), paints, pesticides, and other substances such as dyes, glues, or varnishes. A suggestive association was found between exposure to paints and the –7/7q chromosomal abnormality but this was non-significant and only observed in the set of patients enrolled between 1986 and 1990.

In a study in Columbia, chromosomal aberrations were evaluated in 200 unexposed control subjects and in 200 car-painters recruited from several workshops. Painters were exposed for at least five years to the same commercial thinners, a complex mixture which contains toluene, isobutane, xylene, hexane, ethyl-benzene, and octane. After exclusion of current smokers, ex-smokers and those under medical treatment, the chromosomal aberration frequency was significantly higher in exposed workers compared with controls. The chromatid-type aberration was the most common aberration found in both groups, and was significantly higher in painters compared with controls, whereas no statistical difference was detected between frequencies of chromosome-type aberration in exposed workers and controls (Hoyos-Giraldo *et al.*, 2009).

Five of six studies reported significant increases in the frequency of micronuclei among painters (Diaz *et al.*, 1990; Di Giorgio *et al.*, 1994; Lemasters *et al.*, 1997, 1999; Pinto *et al.*, 2000; Martino-Roth *et al.*, 2003; Testa *et al.*, 2005). Chromosomal aberrations and micronuclei were found both in cultured lymphocytes and in buccal cells. Significantly increased frequencies of sister chromatid exchange were found in four of seven studies among painters (Haglund *et al.*, 1980; Kelsey *et al.*, 1988, 1989; Cullen *et al.*, 1992; Sardas *et al.*, 1994; Lemasters *et al.*, 1997, 1999; Pinto *et al.*, 2000; Testa *et al.*, 2005). Exposure–response relationships with duration of employment were reported in three of these studies (Sardas *et al.*, 1994; Lemasters *et al.*, 1997, 1999; Pinto *et al.*, 2000).

(b) DNA strand-breaks

Increased levels of DNA single-strand breaks among painters were reported in three of four studies (Oesch *et al.*, 1994; Fuchs *et al.*, 1996a, b; Zhu *et al.*, 2001; Martino-Roth *et al.*, 2003); a dose-gradient with years or weeks worked and the cytogenetic end-point which remained after adjusting for smoking was found in two (Oesch *et al.*, 1994; Zhu *et al.*, 2001). These data strongly suggest that occupational exposures in painting lead to increased levels of DNA damage.

(c) Aromatic DNA adducts

In a study among 208 Korean workers, aromatic DNA adducts assessed by ^{32}P-postlabelling tended to be higher in paint users (particularly coal-tar paint users) compared with on-site controls. When the data from general painters and coal-tar painters were combined, they showed higher adduct levels than on-site controls (Lee *et al.*, 2003).

4.2.2 Genetic effects for some individual constituents of paints

(a) Benzene

See Section 4 of the *Monograph* on Benzene in this volume.

(b) Toluene

Toluene is mainly converted to benzyl alcohol and excreted as hippurate. Human data are inconclusive with regards to the genotoxicity of toluene. Studies in exposed workers are limited by concurrent exposure to other chemicals, small cohort size, and a lack of historical exposure monitoring, and it is likely that the methods are not sufficiently sensitive to detect small, but significant, manifestations of genetic toxicity (ATSDR, 2000a). In some cytogenetic studies in

occupationally exposed populations, increases in chromosomal aberrations (two studies), micronuclei (one study) and DNA strand-breaks (one study) have been reported. Genotoxicity testing in experimental systems (rats, mice and cultured mammalian cells including studies on DNA strand-breaks) has been limited and has produced mostly negative results (Chen *et al.*, 2008). DNA-adduct formation has not been detected (IARC, 1999).

Higher frequencies of micronucleated polychromatic erythrocytes (PCE) have been observed in mice following co-exposure to benzene and toluene via inhalation at lower and intermittent co-exposures, compared with mice exposed to benzene or toluene alone (Wetmore *et al.*, 2008). The authors suggested that, at the doses used in this study (50 ppm for benzene and 100 ppm for toluene), toluene could enhance benzene-induced clastogenic or aneugenic bone-marrow injury.

(c) Xylene

Genotoxicity studies on mixtures of xylenes and on the individual isomers of xylene have provided consistently negative results in a variety of *in vitro* and *in vivo* assays and test systems (bacteria, yeast, cultured mammalian cells, mice, rats, and humans). Xylenes may cause DNA fragmentation indirectly, i.e. at cytotoxic concentrations because of nucleases released from lysosomes in dying cells. There is some evidence from bacterial test systems suggesting that xylene metabolites, specifically *meta*-xylenol, *para*-xylenol, 2,4-dimethylphenol, and *ortho*-methylbenzyl alcohol, are non-mutagenic (ATSDR, 2007b).

(d) Dichloromethane

Dichloromethane is consistently mutagenic in microorganisms. Weaker and less consistent responses are seen in mammalian systems, predominantly in mice, both *in vitro* and *in vivo*. The compound induced SCE, chromosome breakage, chromosome loss and DNA single-strand breaks in human cells, while results in rodent cells were inconclusive or negative (IARC, 1999). Mechanistic studies have established a link between GST-mediated metabolism of dichloromethane and its genotoxicity and carcinogenicity in mice. The GST enzyme responsible for the metabolism of dichloromethane is expressed to a significantly greater extent in mouse tissues than in rat, hamster or human tissues. The available data suggest a plausible mechanism for the development of liver and lung tumours in mice, but not in rats exposed to dichloromethane (IARC, 1999).

(e) Trichloroethylene

Studies of structural chromosomal aberrations, aneuploidy and SCE in peripheral lymphocytes of workers exposed to TCE were inconclusive but suggested clastogenic effects (IARC, 1995; ATSDR, 1997). TCE did not induce chromosomal aberrations, dominant lethal mutations, SCE or unscheduled DNA synthesis in rodents, whereas an increased induction of micronuclei and DNA single-strand breaks or alkali-labile sites was observed. Although TCE may not be genotoxic, several of its metabolites are reactive and potentially genotoxic substances, suggesting that genotoxic effects may be a concern for workers exposed to trichloroethylene (ATSDR, 1997; Lash *et al.*, 2000b; Tabrez & Ahmad, 2009). Several isomers of 1,2-dichlorovinyl-cysteine, a product of TCE metabolism in the kidney, are mutagenic in the *in vitro* Ames assay. These products have been identified in the urine of workers exposed to TCE.

(f) Cadmium and chromium

The genotoxic effects of cadmium and chromium are described in Section 4 of the *Monograph* on these two metals in *IARC Monograph* Volume 100C (IARC, 2012).

(g) Inorganic lead

Equivocal results have been published with respect to the mutagenicity of water-soluble lead compounds in mammalian cells in culture; in most classical test systems, effects were weak or restricted to toxic doses. In cultures of various mammalian cells and in lead-exposed animals, lead acetate, lead chromate and lead nitrate induced DNA strand-breaks. Chromosomal aberrations and micronuclei have been shown consistently in mammalian cells in culture, in experimental animals (in bone-marrow cells of lead-exposed animals) and in several cases also in humans occupationally exposed to lead. In some studies, these effects were correlated with blood-lead concentrations. However, with respect to epidemiological studies, confounding exposures cannot be ruled out (IARC, 2006).

Two mechanisms may underlie lead-induced genotoxicity, namely a disruption of pro-oxidant/antioxidant balance, at least in part through interaction with the sulfhydryl groups of key enzymes, and as a result of interference with DNA-repair systems. There is little evidence that lead interacts directly with DNA at blood-lead concentrations normally encountered. The involvement of reactive oxygen species (ROS) in lead-induced genotoxicity has been shown at different experimental levels. Molecular mechanisms may be enhanced lipid peroxidation, inhibition of antioxidant defence systems, catalysis of Fenton-type reactions and the inhibition of aminolevulinic acid dehydratase. The latter reaction leads to the accumulation of the haeme precursor aminolevulinic acid, with the subsequent generation of ROS and induction of oxidative DNA damage (IARC, 2006).

Lead interacts with proteins, including those involved in DNA repair. Lead has been shown to inhibit the apurinic/apyrimidinic endonuclease (APE1) at low concentrations (in the micromolar range) in cultured AA8 cells, leading to accumulation of a-purinic sites and to an increase in methylmethane sulfonate-induced mutagenicity (McNeill *et al.*, 2007). This latter mechanism may be responsible for enhancing the genotoxicity of other agents. Furthermore, lead interferes with the repair of DNA double-strand breaks via interaction with the stress-response pathway induced by the ATM (ataxia-telangiectasia mutated) protein (Gastaldo *et al.*, 2007). Low concentrations of lead stimulate cell growth via mobilization of free intracellular Ca^{2+} and activation of protein kinase C (PKC), which triggers a signal-transduction cascade leading to stimulation of DNA synthesis (IARC, 2006).

(h) Styrene

Data from studies in experimental systems (*in vitro* and *in vivo*) and from studies in humans indicate that exposure to styrene can result in the formation of DNA adducts. However, mice, but not rats, develop lung tumours after exposure to styrene, even though both species form DNA adducts, also in organs other than the lung. Circulating styrene 7,8-oxide – the active metabolite of styrene – may also play a role. Since the concentration of styrene in blood is two orders of magnitude higher in the rat than in the mouse, the lung tumours in mice probably develop as a result of in situ formation of styrene 7,8-oxide, which causes cytotoxicity, or increased cell proliferation, or DNA-adduct formation. It is likely that the proposed mechanism involving conversion of styrene to styrene 7,8-oxide in mouse Clara cells is not operative in human lungs to a biologically significant extent. However, based on the observations in human workers regarding styrene 7,8-oxide in blood, and DNA adducts and chromosomal damage in lymphocytes, it cannot be excluded that this and other mechanisms are important for organs other than the lung (IARC, 2002).

(i) PAHs

Genotoxic effects of PAHs are described in *IARC Monograph* Volume 92 (IARC, 2010d) and the *Monograph* on benzene in this volume.

After metabolic activation, PAH mixtures are genotoxic in humans and individual PAHs are genotoxic in experimental systems. In the complex mixtures to which humans are exposed, some of the genotoxic effects of PAHs can be ascribed to benzo[*a*]pyrene and are described in Volume 96 (IARC, 2010d) and in the *Monograph* on Benzene in this volume.

(j) Aromatic amines and azo dyes

The genotoxic effects of aromatic amines and azo dyes such as 4-aminobiphenyl, benzidine, benzidine-based dyes and 2-naphthylamine, are described in *IARC Monograph* Volume 99 (IARC, 2010c), and in the *Monographs* on these specific substances elsewhere in this Volume.

4.2.3 Indirect effects potentially related to genotoxicity

(a) Haematological and immunological effects

Haematological changes were observed in several studies of painters. These included reduced levels of total white blood cells, T-cells and natural killer cells (Moszczyński *et al.*, 1996; Rothman *et al.*, 1997; Kim *et al.*, 1999). Furthermore, an increased prevalence of leukopenia, anaemia and granulocytopenia was observed among painters. Immunological changes were also reported in several studies. These effects included specific immunoglobulin (G and E) responses to hexamethylene diisocyanate – an aliphatic diisocyanate used in the manufacture of paints and surface coating, which can induce asthma (Grammer *et al.*, 1988; Cartier *et al.*, 1989; Baur *et al.*, 1996; Tee *et al.*, 1998; Redlich *et al.*, 2001; Pronk *et al.*, 2007) and increased proliferation of lymphocytes after *in vitro* stimulation with this substance (Redlich *et al.*, 2001; IARC, 2010a).

4.3 Susceptible populations

Several studies have addressed the interplay between genetic factors, biological and clinical endpoints and were reviewed in *IARC Monograph* Volume 98 (IARC, 2010a). In one study considering bladder cancer as an endpoint, the slow acetylation status (*N*-acetyltransferase 2 phenotype) was over-represented in painters (n = 16) with bladder cancer (88%) compared with their healthy colleagues (65%) (n = 26) (Golka *et al.*, 2001). The effects of the *NAT2* polymorphism are also described for arylamines in Volume 99 (IARC, 2010c).

Few studies have addressed the effect of genetic polymorphism on biological endpoints. In one study no significant associations were detected between any of the biomarker responses (chromosomal aberrations, SCE, micronuclei) and either the *GSTM1* or *GSTT1* genotype. However, the small size of the study (25 car painters and 37 unexposed controls) does not allow definite conclusions to be made on the relationship between genetic polymorphisms and biomarkers (Testa *et al.*, 2005).

In another study, among 181 painters using coal-tar paints (n = 111) or general paints (n = 70) and 27 on-site controls, no gene–environment interactions between *GSTM1* (all workers, 51% *GSTM1*-null) or *GSTT1* (all workers, 54% *GSTT1*-null) and aromatic DNA-adduct formation was found among any of the groups exposed (Lee *et al.*, 2003).

Only one recent study has addressed the interplay between genetic polymorphisms and biological endpoints. In this study the effect of polymorphisms in genes involved in metabolism of xenobiotics (*CYP2E1*, *GSTM1*) and in DNA repair ($XRCC1^{194}$ *Arg/Trp*, *Trp/Trp*, $XRCC1^{280}$ *Arg/His*, $XRCC3^{241}$ *Thr/Met*) on chromosomal aberration (CA) frequency was investigated (Hoyos-Giraldo *et al.*, 2009). A significant effect was observed of the *CYP2E1 C1/C1* genotype, which increased the CA frequency in exposed

workers. Exposed workers with the *GSTM1-null* genotype had a statistically significantly elevated CA frequency compared with controls and exposed workers with a *GSTM1*-positive genotype. Exposed workers with *XRCC1*^{194}*Arg/Trp* and *Trp/Trp* genotypes had statistically higher CA frequencies compared with those with the *XRCC1*^{194}*Arg/Arg* genotype. Also, there was an association between the *XRCC1*^{280}*Arg/Arg* and *XRCC3*^{241}*Thr/Thr* genotypes and a significant increase of CA frequency in exposed workers. The authors suggested that these wild-type genotypes may decrease the capacity to repair DNA single- and double-strand breaks and influence the formation of chromosomal aberrations (Hoyos-Giraldo *et al.*, 2009).

In most studies that measured a variety of cytogenetic end-points and markers of genotoxicity, elevated levels of genetic damage were reported in painters. Mechanistic data reviewed by ATSDR (1997, 2000a, b, 2007a, b) and by previous *IARC Monograph* evaluations on selected specific chemicals that had been or still are prevalent in exposures during painting, strongly support a role of these substances in the induction of haematopoietic malignancies (benzene, trichloroethylene), liver cancer (trichloroethylene), lung cancer (cadmium, chromium, PAHs) and bladder cancer (aromatic azo dyes).

4.4 Synthesis

The multiple genetic and cytogenetic effects observed among workers employed as painters or in the paint industry provide strong evidence in support of genotoxicity as one mechanism underlying the observed increase in cancer risk. However, due to the complexity and changing nature of the exposure mixtures and the potential interactions between exposures as a painter, other mechanisms are also likely. While it is clear that exposures to some agents in the paint industry have decreased over time, recent cytotoxicity studies and the ongoing exposures to multiple mutagens and carcinogens continue to raise concerns about cancer risks.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposure as a painter. Occupational exposure as a painter causes mesothelioma, and cancers of the urinary bladder and lung.

Also, a positive association has been observed between maternal exposure to painting (including pre-conception and during pregnancy) and childhood leukaemia in the offspring.

No data in experimental animals relevant to exposure as a painter were available to the Working Group.

The multiple genetic and cytogenetic effects observed among workers employed as painters and the information on individual chemicals to which painters are exposed provide strong evidence to support genotoxicity as a mechanism underlying the observed increase in cancer risk. However, due to the complexity and changing nature of the exposure mixtures and the potential interactions between exposures as painters, other mechanisms are also likely. While it is clear that exposures as a painter to some agents have been reduced over time, recent genotoxicity studies and the exposure to multiple mutagens and carcinogens continue to raise concerns about cancer risks.

Occupational exposure as a painter is *carcinogenic to humans (Group 1)*.

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OCCUPATIONAL EXPOSURES IN THE RUBBER-MANUFACTURING INDUSTRY

Occupational exposures in the rubber-manufacturing industry were considered by previous IARC Working Groups in 1981 and 1987 (IARC, 1982, 1987). Since that time new data have become available, which have been incorporated in this *Monograph,* and taken into consideration in the present evaluation.

1. Exposure Data

In the context of this *Monograph*, the rubber industry is restricted to the rubber-manufacturing industry, including the production of tyres and general rubber goods and the process of re-treading. The production of synthetic polymers in chemical plants is not discussed.

1.1 Manufacturing process

Rubber manufacturing generally comprises the following operations: raw materials handling, weighing and mixing; milling; extruding and calendering; component assembly and building; 'curing' or vulcanizing; inspection and finishing; storage and dispatch. A detailed description of these steps in the production process can be found in IARC (1982).

Although the stages described below are applicable to the majority of rubber goods manufactured from solid polymer, a substantial proportion of rubber production involves the use of liquid latex. This applies to the manufacture of dipped rubber goods (such as rubber gloves and some footwear), foam-latex products (such as mattresses, cushions, etc.), and extruded thread products (such as elasticated fabrics and surgical hose).

1.1.1 Raw materials handling, weighing and mixing

All the materials required for the manufacture of the finished product are assembled. The raw polymer, either natural or synthetic is brought together at this stage with a variety of compounding chemical additives before being introduced into a mixer. The extensive range of chemicals required and the volume of raw material handled can give rise to substantial quantities of airborne dust.

1.1.2 Milling

From the mixer, the uncured rubber compound usually passes to one or more milling machines, where it is thoroughly blended to ensure an even dispersion of its chemical constituents. At this stage, considerable heat is generated, and, although many technical improvements have been introduced in recent years, the job of mill operator still involves a considerable degree of physical exertion and exposure to fumes arising from the heated compound.

1.1.3 Extruding and calendering

The extruders force the rubber compound through a die into various forms, which are then cut to appropriate lengths. Strips of softened rubber compound are fed into multiple-roll milling machines (calenders) to form rubber sheeting, or to apply the rubber directly onto woven textile fabric, which can then be wound off onto a roll. During such manufacturing operations, fumes are often generated.

1.1.4 Component assembly and building

At this stage, solvents are frequently used, with the possibility of inhalation of solvent vapours or of direct effects of the solvent on the skin of the operator.

1.1.5 Curing or vulcanizing

Heat is applied to the product, usually by use of steam, in a curing mould, press, or autoclave. Operators working in the area are exposed both to heat from the presses and to fumes from the heated rubber products. Chemical reactions take place throughout the manufacturing process, and may give rise to new, more volatile chemicals.

1.1.6 Inspection and finishing

This involves the handling of cured rubber products, often while still hot. It usually involves direct and extensive skin-contact with the surface of the finished article (during inspection) and may also involve exposure to vulcanizing fumes. Grinding, trimming, repair, painting and cleaning may also entail exposure to rubber dust, fumes and solvents.

1.1.7 Storage and dispatch

Large quantities of stored rubber goods may release considerable amounts of toxic substances, either as vapours or as constituents of the 'bloom' on the surface of finished goods.

1.2 Chemicals used in the rubber-production process

A wide variety of natural or synthetic elastomers, fillers (e.g. carbon black, precipitated silica or silicates) and additives are used in compounding to create the necessary properties of the final rubber product. The actual chemicals used in this process have changed over time and vary extensively depending on the manufacturing sector (e.g. tyres, general rubber goods, re-treading), and on the specific plant.

Compounding ingredients are classified as vulcanising agents (e.g. elemental sulfur, sulfur donors such as organic disulphides and higher sulphides, peroxides, urethane cross-linking agents); vulcanization accelerators (e.g. sulphenamides, thiazoles, guanidines, thiurams, dithiocarbamates, dithiophosphates, and miscellaneous accelerators such as zinc isopropyl xanthate and ethylene thiourea); vulcanization activators (e.g. zinc oxide, magnesium oxide, lead oxide); retarders and inhibitors of vulcanization (e.g. benzoic acid, salicylic acid, phthalic anhydride, *N*-nitrosodiphenylamine (NDPA), *N*-(cyclohexylthio)phthalimide); antidegradants; antioxidants (e.g. phenolics, phosphites, thioesters, amines, bound antioxidants such as quinone-diimines, miscellaneous antioxidants such as zinc and nickel salts of dithiocarbamates); antiozonants (e.g. *para*-phenylenediamines, triazine derivatives, waxes); anti-reversion agents (e.g. zinc carboxylates, thiophosphoryl derivatives, silane coupling agents, sulphenimide accelerator, hexamethylene-1,6-bis thiosulphate disodium dehydrate, and 1,3-bis(citranimidomethyl) benzene); plasticisers and softeners (e.g. petroleum products such as petroleum waxes and mineral oils, coal-tar products such as coumarone resin, pine products, synthetic softeners, and other products such as vegetable oils and fats); and miscellaneous ingredients (such as peptising agents, blowing agents, bonding agents, and pigments) (Datta & Ingham, 2001).

1.3 Human exposure

Workers in the rubber-manufacturing industry are exposed to dusts and fumes from the rubber-making and vulcanization processes. Potential exposures include *N*-nitrosamines, polycyclic aromatic hydrocarbons, solvents, and phthalates. Inhalation is the main route of exposure, although workers may have dermal exposure as well (e.g. to cyclohexane-soluble compounds). Details on historical occupational exposures in the rubber-manufacturing industry can be found in the previous *IARC Monograph* (IARC, 1982).

Data from studies published since the previous evaluation (IARC, 1982) are summarized below. These are mainly from Europe and North America. Hardly any current exposure data from Asia, where production of rubber goods has increased considerably during the last two decades, was available to the Working Group.

Several industry-wide surveys have been carried out in the United Kingdom (Dost *et al.*, 2000) and in the Netherlands (Kromhout *et al.*, 1994; Vermeulen *et al.*, 2000). In these studies, inhalable dust concentrations, curing-fume concentrations and solvents were measured. A recent European Concerted Action created a large exposure database for the rubber-manufacturing industry in five countries (the United Kingdom, Germany, the Netherlands, Poland, and Sweden). The Improved Exposure Assessment for Prospective Cohort Studies and Exposure Control in the Rubber-Manufacturing Industry (EXASRUB) database contains results of 59609 measurements collected from 523 surveys in 333 factories between 1956 and 2003. The database consists primarily of measurements of *N*-nitrosamines ($n = 21202$), rubber dust ($n = 13655$), solvents ($n = 8615$) and rubber fumes ($n = 5932$) (de Vocht *et al.*, 2005). The long timespan and the presence of longitudinal data from several countries provide insight into long-term temporal trends in exposure concentrations in the rubber-manufacturing industry.

1.3.1 Dust from rubber processing

An industry-wide survey in the Netherlands in 1998 showed geometric mean concentrations of inhalable dust that varied from 0.8 to 1.9 mg/m^3 and from 0.2 to 2.0 mg/m^3 when analysed by plant and by department, respectively. Actual inhalable dust concentrations depended to a large extent on specific conditions within the departments of the 10 plants involved in the study (Kromhout *et al.*, 1994). Comparison of the exposure levels nine years later revealed a reduction rate of 5.7% per annum for exposure to inhalable particulate matter. On average, median inhalable dust concentrations went down from 1.00 mg/m^3 to 0.59 mg/m^3 between 1988 and 1997. The steepest decline was observed in companies and departments with the highest exposure levels in 1988 and in workers with long employment. However, the highest concentrations were still seen in the compounding and mixing departments (Vermeulen *et al.*, 2000).

Dost *et al.* (2000) reported on exposure data collected in an industry-wide inventory in the United Kingdom during 1995–97 from 29 re-treading plants, 52 producers of general rubber goods, and seven producers of new tyres. The results show similar patterns at somewhat elevated levels.

These findings were confirmed in an analysis of dust-exposure data (13380 inhalable and 816 respirable dust measurements collected between 1969 and 2003) in the EXASRUB database. Geometric mean inhalable dust concentrations changed by −4% (range −5.8 to +2.9%) per year. Significant reductions were found in all five participating countries for 'handling of crude materials and mixing and milling' (–7% to –4% per year) and for 'miscellaneous workers' (–11% to –5% per year). Average geometric mean personal exposure levels ranged from 0.72 mg/m^3 in the Netherlands to 1.97 mg/m^3 in Germany. Up to 4–5-fold differences were observed between the countries in the early eighties, but these

differences diminished considerably in the two decades afterwards. In most countries, personal measurements appeared to be on average 2–4 times higher than stationary measurements (de Vocht *et al.*, 2008).

1.3.2 Fumes from rubber curing

Heating and curing of rubber compounds generates a visible fume. This fume has a complex chemical composition, which makes detailed analysis rather difficult. The cyclohexane-soluble fraction (CSF) of total particulate matter has been used as an indicator of fume contamination in the areas in which the samples were taken. Such monitoring studies are reviewed below.

In the 1988 Dutch industry-wide survey, Kromhout *et al.* (1994) reported a geometric mean CSF concentration of 0.39 mg/m^3 (n = 163) in the curing departments of 10 factories. Considerable variation was seen between the companies, with a range of geometric mean concentrations of 0.21–1.16 mg/m^3.

Median exposures reported for the United Kingdom industry-wide study were highest in the general rubber goods companies at 0.40 mg/m^3, intermediate for re-treading plants at 0.32 mg/m^3 and lowest for manufacturers of new tyres at 0.22 mg/m^3. Process-specific CSF concentrations in rubber goods production were as follows: 0.40 mg/m^3 in moulding, 0.33 mg/m^3 in extrusion, 0.18 mg/m^3 in milling. For re-treading, levels were 0.32 mg/m^3 for pressing, 0.19 mg/m^3 for extruding and 0.10 mg/m^3 for autoclaving (Dost *et al.*, 2000).

Analysis of 5657 CSF measurements in the EXASRUB database collected between 1977 and 2003 showed an annual decrease in concentration of 3% (range –8.6% to 0%). Steepest declines were seen in curing (–8.6% per year) and maintenance and engineering departments (–5.4% per year) (de Vocht *et al.*, 2008).

1.3.3 N-nitrosamines

Nitrosamines in the rubber-manufacturing industry are formed in the vulcanising process, with its extensive use of chemicals such as tetramethyl thiuram disulfide, zinc-diethyldithiocarbamate and morpholinomercaptobenzothiazole.

Exposures to volatile nitrosamines were measured at 24 French rubber-manufacturing plants from 1992 to 1995. A total of 709 exposure measurements (109 in the personal breathing zone, and 600 area samples) were collected. The following five different nitrosamines were identified: *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodibutylamine, *N*-nitrosopiperidine, and *N*-nitrosomorpholine (NMor). Eighty samples, in which the concentrations were either zero or not quantifiable were excluded. NDMA was the most frequently encountered nitrosamine (detected in 98% of the remaining 629 samples) and represented the most important fraction of the total nitrosamine concentration. For all nitrosamines present, 141 of the concentrations measured exceeded 2.5 µg/m^3. The salt-bath curing process generated particularly high nitrosamine levels, with 90% of the 96 measurements showing concentrations higher than 2.5 µg/m^3, many values even exceeding 20 µg/m^3 (Oury *et al.*, 1997).

Time trends of personal exposure to NDMA and to NMor over two decades (1980–2000) in the German rubber-manufacturing industry were analysed and compared with exposures observed in the Netherlands, Poland, Sweden, and the United Kingdom over the same time period. A total of 2319 NDMA and 2316 NMor measurements contained in the EXASRUB database were analysed. Results from Germany accounted for 88% and 85% of the data for these two amines, respectively. For both NDMA and NMor, the average geometric mean concentration in Germany was 0.13 µg/m^3. Geometric mean concentrations of NDMA ranged from

0.05 μg/m^3 in the Netherlands to 0.34 μg/m^3 in Sweden, while those of NMor ranged from 0.03 μg/m^3 in the United Kingdom to 0.17 μg/m^3 in Poland and Sweden. Exposure to NDMA and NMor decreased on average 2–5-fold in the German rubber-manufacturing industry over this time period, mainly due to the introduction of modern curing systems. Comparable levels were observed in the other European countries (de Vocht *et al.*, 2007).

In a study from Italy, personal exposures to nine airborne *N*-nitrosamines (NDMA, NDEA, *N*-nitrosodi-*n*-propylamine, *N*-nitrosodiisopropylamine, *N*-nitrosodi-n-butylamine, N-nitrosopiperidine, N-nitrosopyrrolidine, and NMor) were measured in 34 workers from four Italian factories that manufactured rubber drive belts for automobile engines. Airborne levels were very low and, in most cases, below the limit of detection of 0.06 μg/m3 (Iavicoli & Carelli, 2006)

Personal exposures to six nitrosamines (NDMA, NDEA, *N*-nitrosodi-*n*-butylamine, *N*-nitrosomorpholine, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine) were measured in the rubber-manufacturing industry in Sweden (Jönsson *et al.*, 2009). The exposures ranged from less than the limit of detection to 36 μg/m^3, and differed with the vulcanization method used. Workers involved in salt-bath vulcanization had the highest level of exposure (median, 4.2 μg/m^3).

Although average levels of *N*-nitrosamines are nowadays well below the current exposure limits, exposure to these chemicals has not been eliminated and incidental high exposures do still occur.

1.3.4 PAHs

In a 1997 cross-sectional study of 116 Dutch male workers in the rubber-manufacturing industry, Peters *et al.* (2008) collected urine samples on weekdays and on Sundays, and determined the concentration of 1-hydroxypyrene. The concentrations were significantly higher in workweek samples compared with those collected on Sunday. However, this increase was not uniform across tasks and only reached statistical significance for the curing department ($P = 0.003$).

1.3.5 Solvents

Kromhout *et al.* (1994) measured exposures to solvents in 10 rubber-manufacturing plants in the Netherlands in the late 1980s. The extent of use of individual solvents varied widely and total solvent concentrations were reported. The quantitative assessment of exposure to solvents was restricted to paraffins (hexane, heptane and octane); aromatic compounds (toluene, xylene, trimethylbenzene, naphthalene and isopropylbenzene); chlorinated hydrocarbons (trichloroethylene and 1,1,1-trichloroethane); ketones, alcohols and esters (methylisobutylketone, 2-ethoxyethanol and isobutylacetate). These were chosen on the basis of information on solvents, cements, and release and bonding agents used in the 10 plants. The geometric mean concentration by plant varied from 0.5–46.9 mg/m^3 and ranged from 0.4–34.6 mg/m^3 by department, with the highest exposures reported in the pretreating departments.

1.3.6 Phthalates

Two studies reported on exposure to phthalates, which are used as plasticizers in the rubber-manufacturing industry. A total of 386 spot-urine samples were collected from 101 Dutch workers employed in nine different factories, and analysed for the presence of phthalic acid and 2-thiothiazolidine-4-carboxylic acid. Samples were collected on Sunday and during the workweek on Tuesday, Wednesday, and Thursday. Geometric mean concentrations of phthalic acid showed a significant 2-fold increase (paired *t*-test; $P < 0.05$) during the workweek compared with the concentrations measured on Sunday

(GM, 83 µg/l), with absolute increases of approximately 70 µg/l. The concentrations did not differ markedly between Tuesday, Wednesday and Thursday (GM, 148 µg/l, 152 µg/l and 164 µg/l, respectively). Increases seemed to be restricted to specific factories and/or departments (e.g. moulding and curing) (Vermeulen *et al.*, 2005).

In a pilot biomonitoring study in several industries, Hines *et al.* (2009) reported that workers from a rubber boot manufacturing plant had 3-fold higher geometric mean concentrations of diethylhexyl-phthalate metabolites in post-shift urine than the concentrations measured in the general population.

1.3.7 Dermal exposure

Kromhout *et al.* (1994) and Vermeulen *et al.* (2000) reported on dermal exposures to cyclohexane soluble compounds in the rubber-manufacturing industry in the Netherlands. Dermal CSF levels decreased in a similar pattern as inhalation exposures over a 9-year period (1988–1997).

2. Cancer in Humans

The literature reviewed in previous *IARC Monographs* (IARC, 1982, 1987) provided *sufficient evidence* of a causal association between exposures in the rubber-manufacturing industry and cancer. The recent Working Group decided to review evidence from individual studies that appeared after the earlier evaluation (IARC, 1982) making use of a systematic review by Kogevinas *et al.* (1998). Evidence from meta-analyses published by Stewart *et al.* (1999), Borak *et al.* (2005) and Alder *et al.* (2006) was not considered since these studies combined a variety of exposure circumstances that would tend to dilute any observed effect. The Working Group realized that the complexity of occupational exposure in the rubber-manufacturing industry had so far precluded a clear conclusion about an association between increased cancer mortality and incidence and exposure to particular chemicals (except historically well known associations between 2-naphthylamine and bladder cancer, and benzene and leukaemia). Future studies in the rubber-manufacturing industry may overcome this problem by making use more systematically of the wealth of exposure data available in the industry (de Vocht *et al.*, 2005, 2009).

2.1 Cancer of the bladder

In the previous *IARC Monograph* (IARC, 1982) it was concluded that there was *sufficient evidence* of an excess occurrence of urinary bladder cancer in workers in the rubber-manufacturing industry. The first evidence appeared when a substantial excess of bladder cancer was noted among workers in this industry in the United Kingdom (Case *et al.*, 1954).

2.1.1 Cohort studies

Among workers in the British rubber-manufacturing industry, the death rate from bladder cancer during 1936–1951 was almost twice that of the general population (Case & Hosker, 1954). Studies in other countries also showed an excess of bladder cancer in workers in this industry, but these studies were based on small numbers.

Kogevinas *et al.* (1998) conducted a systematic review of epidemiological studies on cancer in the rubber-manufacturing industry. This review included cohort and case–control studies published after the previous evaluation (IARC, 1982), which were conducted in facilities that manufactured and repaired tyres, manufactured cables and other rubber goods. The authors found that moderately increased risks for bladder cancer were reported in 6 of 8 cohort studies of workers employed in the rubber-manufacturing industry in different regions of the world. In four studies that reported results by calendar period, the risk was highest among workers employed before 1950 (Delzell & Monson, 1984a,

b, 1985a, b; Gustavsson *et al.*, 1986; Negri *et al.*, 1989; Szeszenia-Dabrowska *et al.*, 1991). One of these studies reported potential co-exposure to 2-naphthylamine (Szeszenia-Dabrowska *et al.*, 1991).

Two cohort studies of Polish workers published before 1998 were updated and an excess mortality risk for bladder cancer was reported (see Table 2.1, available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-31-Table2.1.pdf). In the most recent follow-up of a cohort of Polish workers involved in the manufacture of rubber footwear, non-statistically significant increased risks for bladder cancer were found among men and women (Szymczak *et al.*, 2003). In a cohort study among workers in rubber-tyre manufacture in Poland (Wilczyńska *et al.*, 2001), no increased risk for bladder cancer was observed among all workers, but analysis of a subcohort of men involved in mixing and weighing of raw materials, milling, extruding and calendaring, showed a non-significantly increased risk. A job-exposure matrix (JEM) for occupational exposure to aromatic amines was used in the analysis of this cohort (de Vocht *et al.*, 2009). This JEM was set-up with data from EXASRUB, which provided estimates of geometric mean concentrations for airborne chemicals in each department in the factory during the study period. Internal analyses showed an increased risk in the highest two quartiles of exposure to aromatic amines.

2.1.2 Synthesis

Studies in the rubber-manufacturing industry with documented exposure to 2-naphthylamine clearly show an increased risk of cancer of the urinary bladder. More recent studies that included workers with no recorded exposure to 2-naphthylamine identified moderately increased risks for bladder cancer. [The Working Group could not rule out that the increased risks in recent studies were attributable to exposure to 2-naphthylamine, or whether other exposures in this industry contributed to this risk.]

2.2 Leukaemia

It was concluded in the previous *IARC Monograph* (IARC, 1982) that there was *sufficient evidence* of an excess occurrence of leukaemia in workers in the rubber-manufacturing industry.

2.2.1 Cohort studies

Kogevinas *et al.* (1998) noted four cohort studies that found moderately increased risks for leukaemia among workers in the rubber-manufacturing industry in the USA (Norseth *et al.*, 1983; Delzell & Monson, 1984a, b, in two departments of a rubber plant in Akron, Ohio), in Italy (Bernardinelli *et al.*, 1987), and in Germany (Weiland *et al.*, 1996), while four studies did not report an excess risk (Gustavsson *et al.*, 1986; Negri *et al.*, 1989; Sorahan *et al.*, 1989; Carlo *et al.*, 1993). The magnitude of the risk varied between studies, with the highest risks found in studies conducted in North America. The results supported the conclusion that the excess risk for leukaemia was attributable to exposure to solvents, particularly benzene. The authors indicated that a variety of solvent mixtures, with or without benzene, had been used in rubber cements, glues, binding agents, and release agents.

Cohort studies on leukaemia that were published since the review paper mentioned above (Kogevinas *et al.* (1998) are summarized in Table 2.2 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-31-Table2.2.pdf). Straif *et al.* (1998) reported an excess risk for leukaemia in a cohort of male workers employed in one of five large plants in Germany that produced tyres or general rubber goods (SMR, 1.5; 95%CI: 1.0–2.1). An increased risk was observed in work area I (Preparation of Materials) where solutions were made up,

and in work area II (Technical Rubber Goods). Non-significant excesses were also seen in other areas. Longer duration of employment was associated with increased incidence of leukaemia in work area I, particularly among those workers with 10 or more years of employment (SMR, 3.0; 95%CI: 1.5–5.6).

Li & Yu (2002a) conducted a nested case–control study (7 cases of leukaemia, 28 controls) in a rubber-manufacturing facility, and reported an excess risk for leukaemia in workers of the inner-tube department, but not in other departments of the plant. The odds ratio for leukaemia was 7.81 (95%CI: 0.8–78.8) for one or more years of work in the inner-tube department.

2.2.2 Case–control study

McLean *et al.* (2009) conducted a population-based case–control study in New Zealand, with 225 cases of leukaemia and 471 controls. Full occupational histories were obtained by interview. Among those reporting working as rubber/plastics machine-operators (9 cases, 4 controls), the age-, sex- and smoking-adjusted risk estimate was 3.8 (95%CI: 1.1–13.1). The strongest findings, nonetheless, were for plastics rather than for the rubber-manufacturing industry.

2.2.3 Synthesis

The Working Group concluded that there was an increased risk for leukaemia among workers in the rubber-manufacturing industry. The excess risks may be associated with exposure to solvents, in particular benzene.

2.3 Malignant lymphoma including multiple myeloma and other lymphopoietic cancers

It was concluded in the previous review (IARC, 1982) that there was *limited evidence* of an excess occurrence of lymphoma among rubber-manufacturing workers. Excess occurrence of lymphoma had been noted in workers exposed to solvents in departments like footwear production and tyre manufacture (Veys, 1982).

2.3.1 Cohort studies

Kogevinas *et al.* (1998) reported excess risks for malignant lymphoma, including multiple myeloma, ranging from 1.7 to 3.6 in three cohort studies in the USA (Norseth *et al.*, 1983; Delzell & Monson, 1984a, b, in a rubber plant in Akron, Ohio) and Italy (Bernardinelli *et al.*, 1987), while there was no excess risk in two other cohort studies, in Italy and the United Kingdom (Negri *et al.*, 1989; Sorahan *et al.*, 1989). Delzell & Monson (1984b, 1985b) reported excess risks for multiple myeloma in certain departments of a rubber plant in Akron, Ohio, as did Gustavsson *et al.* (1986) in Sweden.

Cohort studies published since 1998 are included in Table 2.2, on-line. In Germany, Mundt *et al.* (1999) observed an increased risk for lymphatic system cancers among women employed in one of five large plants that produced tyres or general rubber goods. All cases were seen among women hired after 1950. In the United Kingdom, an increased mortality risk for multiple myeloma was found among men and women in 41 British rubber factories that manufactured tyres and general rubber goods (Dost *et al.*, 2007). Women also had increased multiple-myeloma incidence (SRR, 8.1; 95%CI: 1.7–23.7). Excess mortality was observed among workers in the general rubber sector (seven deaths observed, one expected).

Wilczyńska *et al.* (2001). did not find an overall increase in mortality risk for cancers of lymphatic and haematopoietic tissues in a rubber-tyre plant in Poland.

2.3.2 Synthesis

The Working Group concluded that there is *sufficient evidence* of excess malignant lymphoma among workers in the rubber-manufacturing industry.

2.4 Cancer of the lung

In the previous *IARC Monograph* (IARC, 1982) it was concluded that there was suggestive evidence of an excess incidence of lung cancer among rubber-manufacturing workers, but that the evidence for a causal association with occupational exposures was *limited*.

2.4.1 Cohort studies

Kogevinas *et al.* (1998) noted that the more recently reviewed studies tended to confirm a moderate excess risk for lung cancer. Positive findings were reported in five cohort studies (Delzell & Monson, 1985a, in the curing department; Gustavsson *et al.*, 1986; Zhang *et al.*, 1989; Szeszenia-Dabrowska *et al.*, 1991; Solionova & Smulevich, 1993). These risks were found among workers in tyre-curing departments, mixing and milling, in vulcanization workers, and in a study on jobs with high exposure to fumes or solvents. In three studies excess risks up to 1.5 were reported (Delzell & Monson, 1984b, in the aerospace-product department; Sorahan *et al.*, 1989; Weiland *et al.*, 1996), while in five cohort studies excess risks were not found (Norseth *et al.*, 1983; Delzell & Monson, 1984a, 1985b, in industrial-products and reclaim departments; Bernardinelli *et al.*, 1987; Negri *et al.*, 1989; Carlo *et al.*, 1993).

Cohort studies on lung cancer published after the above-mentioned review (Kogevinas *et al.*, 1998) are listed in Table 2.3 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-31-Table2.3.pdf). In most studies moderate but consistent increases in risk for lung cancer were found; two studies reported no increase in risk (Dost *et al.*, 2007; deVocht *et al.*, 2009).

From a study of a cohort of German women employed in rubber-manufacturing plants, Mundt *et al.* (1999) reported an increased risk for lung cancer mortality. Stronger associations were observed for certain periods of employment. Among a cohort of German men, a significantly increased risk for lung cancer mortality was observed (Straif *et al.*, 2000a). Using internal comparisons, the authors showed increased risks among those employed during one year or more in work areas that involved preparation of materials, technical rubber goods and tyre production (Straif *et al.*, 1999). Through retrospective, semiquantitative estimates of exposures to nitrosamines, asbestos and talc, an increased risk for lung cancer in association with high exposure levels for asbestos was observed. An exposure characterization in which categories of medium and high exposure levels of talc were combined with medium exposure to asbestos revealed an exposure-response relationship with lung-cancer incidence (Straif *et al.*, 2000a).

Szymczak *et al.* (2003) reported excess lung-cancer mortality among men and women employed in a rubber-footwear plant in Poland. There were increased risks by duration of employment, but no trend was observed. A population-based cohort study of non-smoking women in China also showed an increased risk for lung cancer, after controlling for exposure to second-hand smoke, education level and family history of lung cancer (Pronk *et al.*, 2009).

2.4.2 Case–control studies

The findings of population-based case–control studies are listed in Table 2.4 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-31-Table2.4.pdf). Most notably, in two large multicentre studies of non-smokers, increased risks for lung cancer were found among

women who reported having been employed in the rubber-manufacturing industry (Pohlabeln *et al.*, 2000; Zeka *et al.*, 2006).

2.4.3 Synthesis

Overall, the cohort studies suggest an increased lung-cancer risk among workers in the rubber-maufacturing industry. This conclusion is supported by the findings of population-based case–control studies. The Working Group concluded that there is evidence of excess lung cancer among workers in the rubber-manufacturing industry.

2.5 Cancer of the larynx

In a previous *IARC Monograph* (IARC, 1987) it was indicated that cancer of the larynx had been reported as occurring in excess in workers in the rubber-manufacturing industry, but this excess was not consistent.

2.5.1 Cohort studies

In his review, Kogevinas *et al.* (1998) reported a small but consistent excess risk for laryngeal cancer in seven cohorts, but indicated that the available evidence did not permit an evaluation to be made of the specific agents that may be associated with the increased risk for this cancer.

Straif *et al.* (2000a) found increased mortality from laryngeal cancer among workers in the German rubber-manufacturing industry (see Table 2.3, on-line). The authors indicated that the excess risk may be associated with employment in weighing and mixing and with exposure to asbestos, talc or carbon black. Dost *et al.* (2007) did not find an increased risk for laryngeal cancer mortality and incidence among workers in the British rubber-manufacturing industry. De Vocht *et al.* (2009) did not find increased mortality from laryngeal cancer in a plant that manufactured rubber tyres. They also did not find an association with exposure to aromatic amines or inhalable aerosol.

2.5.2 Synthesis

The Working Group concluded that there was inconsistent evidence of excess laryngeal cancer among workers in the rubber-manufacturing industry. [Tobacco smoking is a risk factor for laryngeal cancer, yet in many studies no adjustment for smoking status was made.]

2.6 Cancer of the stomach

In the previous *IARC Monograph* (IARC, 1982) it was concluded that there was *sufficient evidence* of an excess of stomach cancer among workers in the rubber-manufacturing industry, and limited evidence of a causal association with occupational exposures.

2.6.1 Cohort studies

The conclusions of the previous Working Group (IARC, 1982) were supported by cohort studies of male workers in specific rubber factories. A study in a rubber plant in Akron, Ohio (USA) showed an excess of stomach cancer primarily among workers involved in jobs early in the production line, where exposures are mainly to particulate matter, but also to some fume from uncured rubber (Delzell & Monson, 1982). A case–control analysis of stomach cancer among male workers in the same plant showed a positive association with work early in the production line and with jobs in curing and maintenance (McMichael *et al.*, 1976). Further analysis, according to estimated exposure to specific agents, showed a positive association with exposure to talc (Blum *et al.*, 1979). In one study in the United Kingdom, mortality from stomach cancer was increased among all workers, but particularly among men in jobs early in the production process (Parkes *et al.*, 1982). In a second study in the United Kingdom,

excess mortality from stomach cancer was also observed among all workers, but not among particular occupations (Baxter & Werner, 1980).

Kogevinas *et al.* (1998) reported low excess risks for stomach cancer in seven cohort studies. The risk was elevated mainly in mixing and milling departments in two studies (Wang *et al.*, 1984; Gustavsson *et al.*, 1986) and in jobs with high exposure to dust in a third study (Sorahan *et al.*, 1989). Kogevinas *et al.* (1998) indicated that cohort studies published after 1982 either did not confirm the presence of an excess risk or suggested the presence of only a slightly elevated risk.

Cohort studies on stomach cancer published since the above-mentioned review are listed in Table 2.5 (available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-31-Table2.5.pdf). In case–cohort study in China of workers in a rubber-manufacturing plant, Li & Yu (2002b) reported an increased risk for stomach cancer. Increased risks were also reported by duration of work in inner tyre-tube manufacturing and milling departments. Mundt *et al.* (1999) found excess risks for stomach cancer among German women employed in the rubber-manufacturing industry, which was stronger among workers hired after 1960.

Straif *et al.* (2000a) reported a moderately increased risk for stomach cancer among male workers in the German rubber-manufacturing industry. An exposure-effect association with talc was observed, but no association with nitrosamines. Data appeared to indicate an association with carbon black, but after adjustment for talc- and asbestos-containing dusts, the risk estimate was lower and no longer significant.

On the basis of internal comparisons, Neves *et al.* (2006) found an increasing risk for stomach cancer among workers in the rubber-manufacturing industry employed in small companies in comparison with workers at large companies, with 10-year lagging and control for confounding (RR, 3.47; 95%CI: 2.57–4.67). Company size was used as a surrogate of probability of exposure to carcinogenic substances. De Vocht *et al.* (2009) found a moderate excess risk for stomach cancer, particularly among workers in the maintenance department of a tyre-manufacturing plant, while Dost *et al.* (2007) showed a modest excess of stomach-cancer incidence among male workers in a study of British rubber plants.

2.6.2 Synthesis

The Working Group concluded that there was evidence of an excess of stomach cancer among rubber-manufacturing workers.

2.7 Cancer of the oesophagus

The previous *IARC Monograph* (IARC, 1982) determined that there was *inadequate evidence* for excess occurrence of cancer of the oesophagus among workers in the rubber-manufacturing industry.

2.7.1 Cohort studies

Kogevinas *et al.* (1998) reported an increased risk for oesophageal cancer in four cohorts (Delzell & Monson, 1985b, in reclaim-department workers; Sorahan *et al.*, 1989; Szeszenia-Dabrowska *et al.*, 1991, Weiland *et al.*, 1996). Other cohorts studies showed no effect.

Straif *et al.* (2000b) reported a significantly increasing trend for oesophageal cancer with increasing exposure to nitrosamines (see Table 2.5, on-line). Tests for trend and associations were also significant for cancers of the lip and oral cavity. In Poland, Szymczak *et al.* (2003) reported a significant excess risk among rubber-footwear workers.

2.7.2 Synthesis

The Working Group concluded that there was some evidence for an excess risk for cancer of the oesophagus among workers in the

rubber-manufacturing industry. [The Working Group noted that in none of the studies adjustments were made for tobacco or alcohol use.]

2.8 Cancer of the prostate

The previous *IARC Monograph* (IARC, 1982) concluded that the evidence of excess risk for prostate cancer was *limited* and that the evidence for a causal association with occupational exposures was inadequate.

2.8.1 Cohort studies

Kogevinas *et al.* (1998) reported excess risks for prostate cancer in five studies (Norseth *et al.*, 1983; Delzell & Monson 1984a, in the industrial-products department; Bernardinelli *et al.*, 1987; Solionova & Smulevich, 1993; Weiland *et al.*, 1996). Other studies did not report any excess (Delzell & Monson, 1984b, 1985b; in the aerospace-products and re-claim departments; Gustavsson *et al.*, 1986; Sorahan *et al.*, 1989; Szeszenia-Dabrowska *et al.*, 1991).

Since then, only one case–cohort study that investigated the association between prostate cancer and work in the rubber-manufacturing industry has been published (Zeegers *et al.*, 2004). In this study a non-statistically significant increased risk for prostate cancer was found.

2.8.2 Synthesis

The Working Group concluded that there is weak evidence of excess risk for prostate cancer among workers in the rubber-manufacturing industry.

2.9 Other cancers

The previous *IARC Monograph* (IARC, 1982) determined that for cancers of the brain, thyroid and pancreas, the evidence was *inadequate* for an excess in occurrence of these cancers and for a causal association with occupational exposures.

2.9.1 Cohort studies

Kogevinas *et al.* (1998) reported that findings for other cancer sites were not consistent between studies, or were derived from too few studies. Since this review, studies on workers in the rubber-manufacturing industry with excess cancers of the brain, pancreas, gallbladder, cervix and liver have been reported (see Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-31-Table2.6.pdf).

2.9.2 Synthesis

The Working Group concluded that there is little evidence of excess risks for cancers at sites other than those mentioned above, being associated with work in the rubber-manufacturing industry. [Excess risks found in single studies may be related to specific exposure circumstances occurring in particular rubber-manufacturing plants. One problem in evaluating findings for other cancer sites is that reporting may have been incomplete in cohort and case–control studies, with possibly preferential reporting of positive findings.]

3. Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Relevant Data

The rubber-manufacturing industry has used and still uses a wide variety of substances that belong to many different chemical categories, e.g. carbon black, aromatic amines, PAH, *N*-nitrosamines, mineral oils, other volatile organic compounds from curing fumes, trace amounts of monomers from synthetic rubber like 1,3-butadiene, acetonitrile, styrene, vinyl chloride, ethylene oxide, etc. (See Section 1). For this reason, it has been difficult to relate the observed

Table 4.1 Biomonitoring studies and cytogenetic assays among workers in the rubber-manufacturing industry

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Degrassi *et al.* (1984) Italy	Exposed: Vulcanizers (n = 34) in a rubber plant Controls: Workers (n = 16) in the same plant and living in the same geographic area	Airborne particulate matter ranged from 0.5 to 3.4, with an average (± SD) of 1.1 (± 1.1) mg/m^3. Exposure duration (± SE) was 8.2 ± 0.8 yr	Chromosomal aberrations (per 100 cells) SCE (per cell)	1.9 ± 1.4 (excl. gaps) 5.2 ± 1.3	2.1 ± 1.5 (excl gaps) 5.2 ± 0.7	NS Cigarette smoking was associated with increased SCE in exposed and controls. Chromosomal aberrations were not correlated
Hema Prasad *et al.* (1986) India	Exposed: Workers (n = 35) employed for 3–12 yr in a rubber factory. There were 20 unexposed controls (not specified)	NR	Chromosomal aberrations (per 100 cells)	Ranged from 1.57 to 2.75, increased with longer time at work	0.6 per 100 cells	$P < 0.05$ No information is given about smoking.
Sasiadek (1992) Poland	Exposed: Vulcanizers (14 women, 7 men; 14 were smokers) in a rubber plant. Controls: Non-exposed women (n = 7) and men (n = 7), of whom 5 were smokers	Exposure duration was 14.2 ± 9.7 yr (range 2–35 yr)	Chromosomal aberrations (per 100 cells) SCE (per cell)	2.2 ± 1.06 (incl. gaps) 16.1 ± 3.5	0.9 ± 1.0 (incl. gaps) 10.0 ± 1.5	$P < 0.01$ $P < 0.001$
Sasiadek (1993) Poland	Exposed: Vulcanizers (19 women, 7 men; 10 were smokers) in a rubber plant. Controls: Non-exposed women (n = 15) and men (n = 10), of whom 10 were smokers	Exposure duration was 0.5–30 yr (mean 15.6 ± 9.5 yr).	SCE (per cell)	13.2 ± 2.9 (range 9–20)	9.8 ± 1.8 (range 7–14)	$P < 0.001$

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Ward *et al.* (1996) USA	Workers in a butadiene-production plant (10 high-exposed, 10 low-exposed) in Texas, USA. Non-exposed controls from elsewhere (n = 9). All 29 were non-smokers. Second study: follow-up after 8 mo. Ongoing study among workers in a styrene-butadiene rubber plant in the same area. Data are presented on 16 high-exposed (5 smokers) and 9 low-exposed (3 smokers) subjects	Exposure survey by the company: mean level 3.5 ± 7.25 ppm From 8-h personal breathing zone air samples: 0.30 ± 0.59, 0.21 ± 0.21, and 0.12 ± 0.27 ppm for high-, intermediate- and low-exposure areas Passive dosimeters worn during the 8-h shift: of 40 samples, 20 were > 0.25 ppm, 11 were > 1 ppm	a) *HPRT* mutants, lymphocytes b) butadiene metabolite in urine (see comments)	3.99 ± 2.81 (high) and 1.20 ± 0.51 (low) *HPRT* mutants/10^{-6} cells 5.33 ± 3.76* (high) 2.27 ± 0.99 (medium), 2.14 ± 0.97 (low) mutants/10^{-6} cells *Non-smokers:* 7.47 ± 5.69 (high)** 1.68 ± 0.85 (low) *Smokers:* 6.24 ± 4.37 (high)** 3.42 ± 1.57 (low)	1.03 ± 0.12 *HPRT* mutants per 10^{-6} cells	$P < 0.02$ *$P < 0.02$ ** $P < 0.01$ *Comment:* dihydroxybutane mercapturate, 1,2-dihydroxy-4(*N*-acetyl-cysteinyl) butane, was measured in urine by GC/MS. The high-exposure groups (butadiene-monomer plant only) had significantly higher levels.
Moretti *et al.* (1996) Italy	Workers at 4 rubber plants (n = 19; 9 smokers) and 20 age-matched (± 5 yr) blood donors as controls (8 of whom were smokers)	NR	a) mutagenicity in urine b) urinary excretion of thioethers	a), b): no differences between exposed and controls		
			c) DNA damage in lymphocytes	median migration distance in Comet assay: 37.99 µm	median migration distance: 33.81 µm	$P > 0.05$
			d) SCE	5.51 ± 0.82 / metaphase	6.06 ± 1.15	$P > 0.05$
			e) MN formation	22.84 ± 15.82 MN per 1000 binucleated cells	13.74 ± 4.42	$P < 0.05$

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Major *et al.* (1999) Hungary	Subjects (n = 29, among whom 24 were smokers; 23 men, 6 women) with mixed industrial exposure during 3–20 yr in the rubber-manufacturing industry. Controls were living and/or working in the vicinity of chemical plants, but had no occupational exposure to chemicals (industrial controls)	Exposures included aromatic solvents, dust, tar, lubricating oil. No quantitative data given		*Exposed*	*Industrial controls*	
			a) chromosomal aberrations per 2900 metaphases scored	3.38 ± 0.26	1.60 ± 0.62	$P < 0.01$
			b) PCD: - mitoses with ≤ 3 chromosomes	11.45 ± 1.43	1.57 ± 0.44	$P < 0.01$
			- mitoses with > 3 chromosomes	6.00 ± 1.18	0.32 ± 0.10	$P < 0.01$
			c) aneuploidy	5.64 ± 0.44	6.20 ± 0.43	NS
Somorovská *et al.* (1999) Slovak Republic	Workers (27 men, 2 women; 18 smokers, 11 non-smokers) in a rubber tyre factory. The industrial controls comprised 22 clerks (8 men, 14 women; 14 smokers, 8 non-smokers) from the same factory. A second control group comprised 17 men and 5 women (7 smokers, 15 non-smokers) who worked in a laboratory in Bratislava	Air sampling was followed by analysis of styrene, toluene, butadiene, PAHs, alkanes, and alkenes	a) DNA breakage (Comet assay) b) chromosomal aberrations c) MN assay	*Sample 1* (1996): 33% DNA in tail *Sample 2* (1997): 45% DNA in tail	*Factory controls:* 13% DNA in tail *Laboratory controls:* 22% DNA in tail	$P < 0.00001$
				1 aberration/100 cells	*Factory controls:* 0.4 aberr./100 cells *Laboratory controls:* 0.2 aberr./100 cells	$P < 0.00001$
				6.5 MN/2000 cells	*Factory controls:* 2.1 MN/2000 cells *Laboratory controls:* 1.5 MN/2000 cells	$P < 0.00001$

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Zhu *et al.* (2000) Guangzhou, China	Workers [197 men (130 smokers) and 174 women (6 smokers)] at a factory that produced tyres, pads and other products. Among these, 281 were in rubber-processing jobs, and 90 controls were in management. There were 318 drinkers and 53 non-drinkers	Environmental monitoring of dust, toluene, xylene, gasoline, H_2S, SO_2	DNA breakage (Comet assay; results given as tail moment)	*All rubber workers:* 1.77 (1.64–1.90)* μm *Finishing:* 1.81 (1.48–2.21) μm *Calendering:* 1.77 (1.54–2.03) μm *Vulcanizing:* 1.64 (1.46–1.83) μm *Mixing:* 2.54 (1.95–3.31)** μm	*Managerial workers:* 1.52 (1.36–1.71) μm	* $P = 0.04$ *Comment*: Non-drinking, non-smoking mixers also had higher tail moment than comparable managers: 2.25 (1.66–3.03) vs 1.39 (1.18–1.63) μm ($P = 0.049$) ** $P = 0.002$
Ma *et al.* (2000) Texas, USA	Male non-smoking workers at a styrene-butadiene polymer plant. Controls were employees at the University of Texas Medical Branch	Breathing-zone air sampling with personal monitors	Analysis of *HPRT* variants and mutants, and of exon deletions in the *HPRT* gene in lymphocytes, with a multiplex PCR assay	*HPRT* variants per 10^{-6} cells: 6.86 ± 3.25 ($n = 12$) *HPRT* mutants per 10^{-6} cells: 17.63 ± 5.05 ($n = 10$)	2.36 ± 1.04 ($n = 8$) 8.47 ± 2.88 ($n = 11$)	$P < 0.05$ $P < 0.05$
Ward *et al.* (2001) Texas, USA (study conducted in 1998)	Workers in a BD rubber plant: 22 in a high-exposure and 15 in a low-exposure group, with levels of 1.71 ± 0.54 (SE) and 0.07 ± 0.03 (SE) ppm butadiene, respectively	Exposure to 1,3-BD was monitored with organic vapour monitors and varied from 4.04 ± 3.45 ppm (tank farm) to 0.29 ± 0.33 (laboratory). Low areas* had 0.05 ± 0.06 ppm *packaging, baling, warehouse, shipping	*HPRT* mutant analysis in lymphocytes	*High-exposure group:* All ($n = 22$)–10.67 ± 1.51 (SE) Non-smokers ($n = 12$)–8.64 ± 1.60 Smokers ($n = 10$)–13.10 ± 2.57	*Low-exposure group:* All ($n = 15$)–3.54 ± 0.61 Non-smokers ($n = 14$)–3.46 ± 0.65 Smokers–4.61	$P = 0.001$ $P = 0.011$ *Comment*: increases in *HPRT* variant frequency of about threefold are seen at average BD exposure levels of 1–3 ppm

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Ammenheuser *et al.* (2001) Texas, USA	Workers (n = 24) in the reactor, recovery, tank farm and laboratory area of a BD rubber plant represented a high-exposure group. Workers (n = 25) in blending, coagulation, baling, shipping, the control room and utility areas were a low-exposure group	Workers were asked to wear an organic vapour monitor during one 8-h work-shift, to measure exposure to butadiene/styrene. Lower detection limit: 0.25 ppm BD	*HPRT* mutant analysis in lymphocytes	*High-exposure group:* Non-smokers (n = 19)– 6.8 ± 1.2* (SE) Smokers (n = 5)– 6.1 ± 2.0	*Low-exposure group:* Non-smokers (n = 20)– 1.8 ± 0.2 Smokers (n = 5)–3.3 ± 0.5	* $P < 0.0005$
Vermeulen *et al.* (2002) the Netherlands	Workers in the rubber-manufacturing industry (n = 52; all non-smokers)	Mutagenicity on likely skin-contact surfaces (high, ≥ 25 revertants/cm^2; low, < 25 rev/cm^2) and in ambient air (high, ≥ 210 rev/m^3 low, < 210 rev/m^3) tested in YG1041 of *S. typhimurium*	DNA-adduct analysis in exfoliated bladder cells collected from 24-h urine, by ^{32}P-postlabelling. Samples from 32 slow and 20 fast acetylators (based on *NAT2* analysis)	Of 52 urine samples, 46 gave reliable data for the presence of three main adducts: 1 in 41 samples, 2 in 13 samples, 3 in 29 samples	NR	The 'slow *NAT2*' subjects had lower levels of adducts 1–3 than the fast acetylators. ($P < 0.04$; $P = 0.32$; $P = 0.15$, resp) No information is given on the identity of the adducts
Laffon *et al.* (2006) Portugal	Exposed male workers (n = 32) and non-exposed male controls (n = 32) in a rubber tyre factory in Oporto, of whom 39% were smokers	NR	a) thio-ethers in post-shift urine	0.41 ± 0.05 mM	0.24 ± 0.02 mM	$P < 0.01$
			b) micronucleus test (MN per 1000 cells)	2.34 ± 0.33	1.84 ± 0.29	NS
			c) SCE/cell	4.35 ± 0.20	4.38 ± 0.17	NS
			d) DNA-breakage (Comet assay) (tail length, µm)	44.72 ± 0.66	48.25 ± 0.71	$P < 0.01$

Table 4.1 (continued)

Reference	Description of exposed and controls	Exposure levels	Cytogenetic/genotoxic end-point	Response in exposed	Response in controls	Comments
Peters *et al.* (2008) the Netherlands	Workers (n = 116; 45 smokers, 71 non-smokers) in the Dutch rubber-manufacturing industry, selected on the basis of their function in the production process. Urine and blood were collected	NR		*Weekday samples:*	*Sunday samples:*	
			a) Hydroxypyrene in urine (result for non-smokers)	0.15–0.19 µmol/mol creatinine	0.12 µmol/mol creatinine	$P < 0.0001$
			b) Mutagenic activity in urine (revertants/g creatinine) of workers in compounding and mixing	10 511	6522	$P < 0.05$
			c) DNA adducts in urothelial cells and in peripheral blood monocytes	Increased compared with control		
Musak *et al.* (2008) Czech Republic	Workers in a tyre plant (n = 177; 69 smokers) and 172 controls (49 smokers)	Personal samplers worn in breathing zone. Average BD level in the mixing department was 2.6 ± 0.2 mg/m^3	Chromosomal aberrations (per 100 metaphases)	2.5 ± 1.8	1.7 ± 1.2	$P = 0.055$
Wickliffe *et al.* (2009) Texas, USA	Workers in a BD rubber plant (see Ward *et al.*, 2001 above)	Current exposures: mean 93.5 ppb, median 2.5 ppb	*HPRT* mutant analysis in lymphocytes	Current, low, exposures to butadiene in this plant do not seem to increase the *HPRT* mutant frequency. However, older workers showed increased *HPRT* mutant frequencies, likely due to previous chronic exposure to higher levels of butadiene.		

BD, styrene-butadiene; h, hour or hours; *HPRT,* hypoxanthine-guanine phosphoribosyltransferase; mo, month or months; MN, micronucleus; NR, not reported; NS, not significant; PCD, premature chromosome condensation; SCE: Sister-chromatid exchange; SD standard deviation; SE standard error; vs, versus; yr, year or years

cancer hazards in the rubber-manufacturing industry to exposure to specific chemicals.

Table 4.1 presents a list of bio-monitoring studies and cytogenetic assays among workers in the rubber-manufacturing industry in various countries and at different times. These studies have focused on analysis of chromosomal aberrations, sister-chromatid exchange, micronucleus formation, premature chromosome condensation, DNA breakage, DNA-adduct formation, mutagenicity in urine, and mutation in the *HPRT* gene. For each of these endpoints, in most studies a positive response has been observed in exposed workers compared with non-exposed controls. It is noted that the studies listed in Table 4.1 span a period of approximately 25 years.

The multiple genetic and cytogenetic effects observed among workers employed in the rubber-manufacturing industry provide strong evidence to support genotoxicity as one mechanism for the observed increase in cancer risk. However, due to the complexity and changing nature of the exposure mixture and the potential interactions between exposures in this industry, other mechanisms are also likely to play a role.

While it is clear that exposures to some agents in the rubber-manufacturing industry have been reduced over time, the outcome of recent cytogenetic studies continues to raise concerns about cancer risks.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures in the rubber-manufacturing industry. Occupational exposures in the rubber-manufacturing industry cause leukaemia, lymphoma, and cancers of the urinary bladder, lung, and stomach.

lso, a positive association has been observed between occupational exposures in the rubber-manufacturing industry and cancers of the prostate, oesophagus, and larynx.

No data in experimental animals with relevance to the rubber-manufacturing industry were available to the Working Group.

The multiple genetic and cytogenetic effects observed among workers employed in the rubber-manufacturing industry provide strong evidence to support genotoxicity as one mechanism for the observed increase in cancer risks. However, due to the complexity and changing nature of the exposure mixture and the potential interactions between exposures in the rubber-manufacturing industry, other mechanisms are also likely to play a role. While it is clear that exposure to some agents in the rubber-manufacturing industry has been reduced over time, the results of recent cytogenetic studies continue to raise concerns about cancer risks.

Occupational exposures in the rubber-manufacturing industry are *carcinogenic to humans (Group 1).*

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LIST OF ABBREVIATIONS

4-ABP	4-aminobiphenyl
2-NA	2-naphthylamine
AIDS	acquired immune-deficiency syndrome
ALL	acute lymphocytic leukemia
ANLL	acute nonlymphocytic leukemia
AhR	aryl hydrocarbon receptor
ASCR	Association of Swiss Cancer Registries
ASL	angiosarcoma of the liver
ATSDR	Agency for Toxic Substances and Disease Registry
B[*a*]P	benzo[*a*]pyrene
BCME	bis(chloromethyl)ether
b.p.	boiling point
BSF	benzene-soluble fraction
BSM	benzene-soluble materials
bw	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service (of the American Chemical Society)
CI	confidence interval
CMME	chloromethyl methyl ether
CNS	central nervous system
CPTV	coal-tar pitch volatiles
CSF	cyclohexane-soluble fraction
CYP	cytochrome P450
DCM	dichloromethane
DEHP	diethylhexylphthalate
DHBMA	1,2-dihydroxybutyl mercapturic acid
DLC	dioxin-like compound
DMSO	dimethyl sulfoxide
EH	epoxide hydrolase
EPA	Environmental Protection Agency (USA)
EU	European Union
EXASRUB	Improved Exposure Assessment for Prospective Cohort Studies and Exposure Control in the Rubber-Manufacturing Industry
FISH	fluorescence *in situ* hybridisation
GST	glutathione *S*-transferase
HCC	hepatocellular carcinoma

HPRT	hypoxanthine-guanine phosphoribosyltransferase
HRR	hazard rate ratio
IH	industrial hygiene
IP	Institute for Petroleum
IRR	incidence rate ratio
ISCO	International Standard Classification of Occupations
ISIC	International Standard Industrial Classification
MBT	2-mercaptobenzothiazole
MBS	morpholinomercaptobenzothiazole
MDS	myelodysplastic syndromes
mEH	microsomal epoxide hydrolase
MGP	manufactured gas plant residues
MHBMA	monohydroxy-3-butenyl mercapturic acid
MHBVal	*N*-(2-hydroxy-3-butenyl)valine
MN	micro nucleus
MOCA	4,4′-methylene-bis-(2-chloroaniline)
MPD	myeloproliferative disorder
MT	metallothionein
NAT	*N*-acetyltransferase
NACE	Nomenclature Générale des Activités Économiques dans les Communautés
NDBA	*N*-nitrosodibutylamine
NDEA	*N*-nitrosodiethylamine
NDMA	*N*-nitrosodimethylamine
NDPA	*N*-nitrosodiphenylamine
NG	not given
NIOSH	National Institute for Occupational Safety and Health (USA)
NMor	*N*-nitrosomorpholine
NPIP	*N*-nitrosopiperidine
NTP	National Toxicology Program (USA)
OR	odds ratio
OSHA	Occupational Safety and Health Administration (USA)
PAH	polycyclic aromatic hydrocarbons
PARP	poly(ADP-ribose) polymerase
PBNA	phenyl-β-naphthylamine
PCB	polychlorobiphenyl
PCDD	polychlorinated dibenzo-*para*-dioxins
PCP	pentachlorophenol
PCR	polymerase chain reaction
PeCDF	2,3,4,7,8-Pentachlorodibenzofuran
PKC	protein kinase C
POG	pediatric oncology group
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PVC	polyvinyl chloride
PyrVal	*N,N*-(2,3-dihydroxy-1,4-butadiyl) valine
RDD	random digit dialling
ROS	reactive oxygen species

RR	relative risk
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SES	socioeconomic status
SIC	Standard Industrial Classification
SOC	Standard Occupational Classification
SIR	standardised incidence ratio
SMR	standardised mortality ratio
SRR	standardized rate ratio
TCDD	tetrachlorodibenzo-*p*-dioxin
TCE	trichloroethylene
TEF	toxicity equivalence factor
TEQ	toxic equivalence quotient: unit of measurement for TCDD-like compounds that is defined as the amount of TCDD that would produce the same toxicity as a mixture of TCDD-like compounds
THBVal	*N*-(2,3,4-trihydroxybutyl)valine
TWA	time-weighted average
USA	United States of America
VA	Veterans Administration
VC	vinyl chloride
VCM	vinyl chloride monomer
VOC	volatile organic compound
wk	week
yr	year
ZDEC	zinc-diethyldithiocarbamate

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