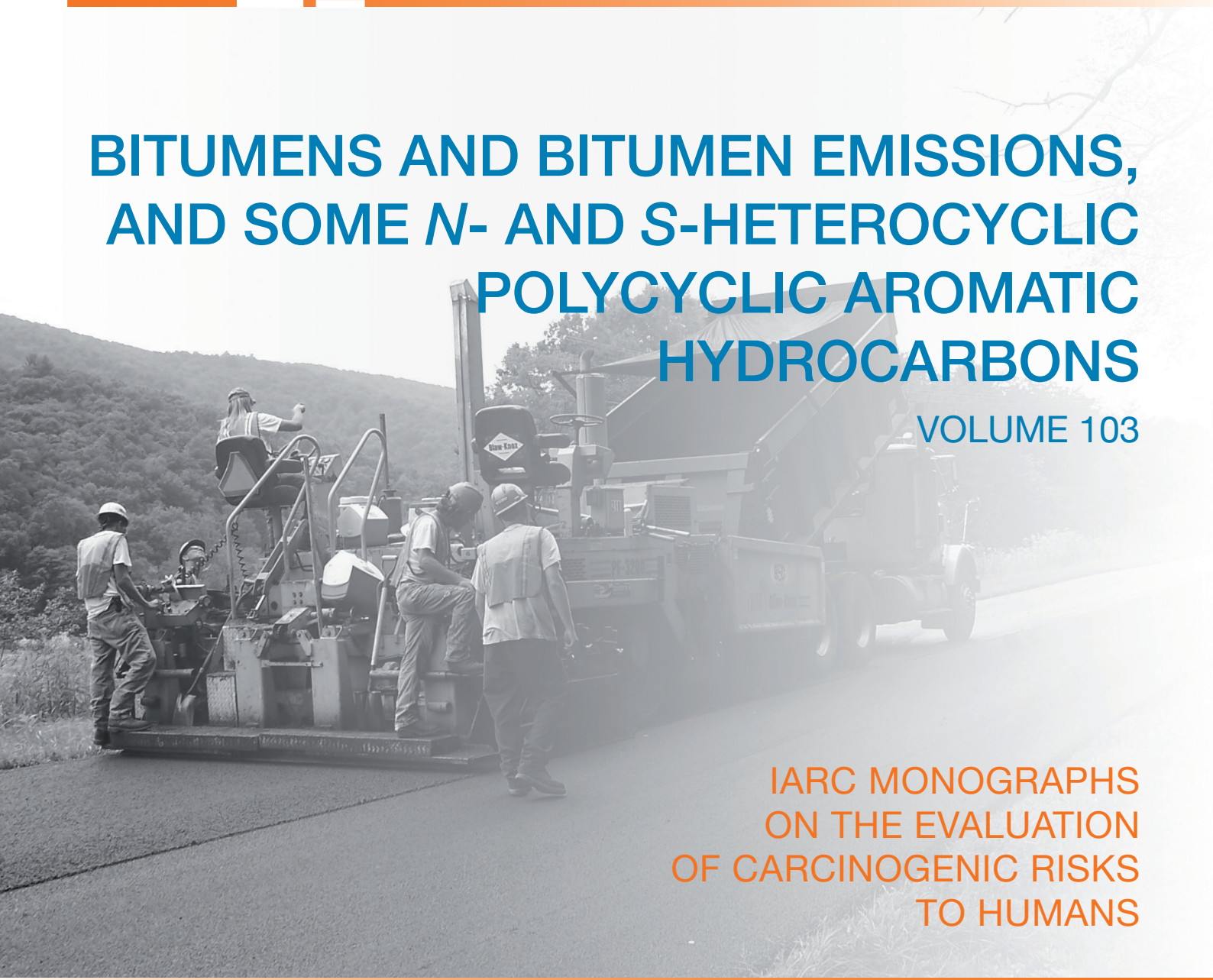
IARC MONOGRAPHS

BITUMENS AND BITUMEN EMISSIONS, AND SOME *N*- AND *S*-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS

VOLUME 103



IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

BITUMENS AND BITUMEN EMISSIONS, AND SOME *N*- AND *S*-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS

VOLUME 103

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, 11-18 October 2011

LYON, FRANCE - 2013

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IARC MONOGRAPHS

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

This volume was made possible, in part, through Cooperative Agreement CR 834012 with the United States Environmental Protection Agency, Office of Research and Development. The contents of this volume do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

Distributed by WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland
(tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int).

IARC Library Cataloguing in Publication Data

Bitumens and bitumen emissions, and some *N*- and *S*-heterocyclic aromatic hydrocarbons / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2011: Lyon, France)

(IARC monographs on the evaluation of carcinogenic risks to humans ; v. 103)

1. Carcinogens, Environmental – adverse effects 2. Heterocyclic Compounds – adverse effects 3. Neoplasms – chemically induced 4. Polycyclic Hydrocarbons, Aromatic – adverse effects 5. Vehicle Emissions – toxicity

I. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans II. Series

ISBN 978 92 832 1326 0 (NLM Classification: W1)
ISSN 1017-1606

PRINTED IN FRANCE

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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 4 years or anticipated in the future are identified here. Minor pertinent interests are not listed and include stock valued at no more than US$1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are listed as significant pertinent interests.

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PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

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

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

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

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

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

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

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

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

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

3. Selection of agents for review

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

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

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

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate

or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

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

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

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

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

5. Meeting participants

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

(a) The Working Group

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

(b) Invited Specialists

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

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

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

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

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

6. Working procedures

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

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

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

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

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

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

B. SCIENTIFIC REVIEW AND EVALUATION

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

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

Exposure data
Studies of cancer in humans
Studies of cancer in experimental animals
Mechanistic and other relevant data
Summary
Evaluation and rationale

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

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

1. Exposure data

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

(a) General information on the agent

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

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

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

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

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

(b) Analysis and detection

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

(c) Production and use

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

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are

obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d) Occurrence and exposure

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

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

(e) Regulations and guidelines

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

2. Studies of cancer in humans

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

(a) Types of study considered

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

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

In correlation studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population

to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

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

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

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

(b) Quality of studies considered

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

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

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

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

confounding related to the difference in risk factors between an external reference group and the study population.

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

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

(c) Meta-analyses and pooled analyses

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

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

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

(d) Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they

allow only indirect inferences about mechanisms of carcinogenesis.

(e) Use of biomarkers in epidemiological studies

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

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

(f) Criteria for causality

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

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

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall database are considered. Data on biomarkers may be employed in

an assessment of the biological plausibility of epidemiological observations.

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

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

3. Studies of cancer in experimental animals

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

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

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

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

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

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

(a) Qualitative aspects

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

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

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

transition to malignancy, the agent should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

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

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

(c) Statistical analyses

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

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

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

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

4. Mechanistic and other relevant data

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

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

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

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

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

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

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

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

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

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

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

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

(c) Other data relevant to mechanisms

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

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

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

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

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and

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

6. Evaluation and rationale

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

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

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

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

(a) Carcinogenicity in humans

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

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

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

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

***Evidence suggesting lack of carcinogenicity*:** There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In

addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

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

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

(b) Carcinogenicity in experimental animals

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

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

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

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

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

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

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

(c) Mechanistic and other relevant data

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

physicochemical parameters and analogous biological agents.

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

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

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

(d) Overall evaluation

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

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

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

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental

animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

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

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

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

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

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

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

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

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

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

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

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

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity*

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

(e) Rationale

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

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GENERAL REMARKS

Background

This one-hundred-and-third volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of bitumens and bitumen emissions, and of some *N*- and *S*-heterocyclic polycyclic aromatic hydrocarbons (referred to as azaarenes and thiaarenes, respectively). This volume is the fourth in a series of *IARC Monograph* volumes evaluating exposures related to air pollution. Indeed, the *IARC Monographs* Advisory Group that met in 2004 recommended that IARC develop such series, in recognition of the large contribution of air pollution to the global burden of cancer. Agents and related exposures evaluated thus far according to this recommendation include non-heterocyclic polycyclic aromatic hydrocarbons in Volume 92 (IARC, 2010a); particles and fibres in Volume 93 (IARC, 2010b) and indoor air pollution in Volumes 95 and 100E (IARC, 2010c, 2012).

This *Monograph* concerns only bitumens produced by petroleum refining and not naturally occurring bitumens. Thus the term "bitumens", as used in this volume, refers to the products derived from residues resulting from vacuum distillation of selected petroleum crude oils. These materials are called "asphalt", "petroleum asphalt" or "asphalt cement" in North America; in this volume, the term "asphalt" is used to describe mixtures of bitumen and mineral matter. Bitumens must be distinguished from coal tars, which are products of the destructive distillation of coals, and also from coal-tar pitches, which are residues from the distillation of coal tars.

A summary of the findings of this volume has appeared in *The Lancet Oncology* (Lauby-Secretan *et al.*, 2011).

Previous evaluations of the agents covered

An overview of the previous IARC evaluations for the agents covered in this volume is given in Table 1.

Categorization of bitumens into classes

The Working Group that met in February 1984 for Volume 35 categorized bitumens into eight classes representing the major types used in industry (IARC, 1985). The Working Group for the present *Monograph* reconsidered these categories and defined six classes according to current uses

Table 1 Previous IARC evaluations of the agents under review

Agent	*Monograph* volumes [a]	IARC Group	Level of evidence [b]	
			Humans	Animals
Bitumens and bitumen emissions				
Bitumens	35, Sup7	3	I	-
Steam-refined bitumen extracts [class 1]	35, Sup7	2B	-	S
Air-refined bitumen extracts [class 2]	35, Sup7	2B	-	S
Steam- and air-refined bitumen mixtures [classes 1 and 2]	35, Sup7	2B	-	S
Cracking-residue bitumens [class 8]	35, Sup7	-	-	L
Steam-refined bitumens, undiluted [class 1]	35, Sup7	-	-	L
Air-refined bitumens, undiluted [class 2]	35, Sup7	-	-	I
N-heterocyclic polycyclic aromatic hydrocarbons				
Benz[*a*]acridine	32, Sup7	3	-	I
Benz[*c*]acridine	3, 32, Sup7	3	-	L
Dibenz[*a*,*h*]acridine	3, 32, Sup7	2B	-	S
Dibenz[*a*,*j*]acridine	3, 32, Sup7	2B	-	S
Dibenz[*c*,*h*]acridine	-	-	-	-
Carbazole	32, Sup7, 71	3	-	L
7*H*-Dibenzo[*c*,*g*]carbazole	3, 32, Sup7	2B	-	S
S-heterocyclic polycyclic aromatic hydrocarbons				
Dibenzothiophene	-	-	-	-
Benzo[*b*]naphthol[2,1-*d*]thiophene	-	-	-	-

[a] Sup7, Supplement 7 of the *IARC Monographs*

[b] Level of evidence in humans and experimental animals: I, inadequate evidence; L, limited evidence; S, sufficient evidence

Table 2 Comparison of the classes of bitumen as defined by the Working Group for Volume 35 and by the Working Group for Volume 103

Volume 35		Volume 103	
Class	**Definition**	**Class**	**Definition**
Class 1	Penetration bitumens	Class 1	Straight-run bitumens
Class 4	Hard bitumens		
Class 2 [a]	Oxidized bitumens	Class 2	Oxidized bitumens
Class 3	Cutback bitumens	Class 3	Cutback bitumens
Class 5	Bitumen emulsions	Class 4	Bitumen emulsions
Class 6	Blended or fluxed bitumens	Class 5	Modified bitumens
Class 7	Modified bitumens		
Class 8	Thermal bitumens	Class 6	Thermally-cracked bitumens

[a] It is noteworthy that class 2 "oxidized bitumens" (CAS No. 64742-93-4) comprises two grades of oxidized bitumens, namely fully-oxidized (penetration index > 2) and air-rectified (semi-blown) (penetration index ≤ 2). These grades differ by their degree of oxidation during production, which leads to very different characteristics and uses. Air-rectified bitumens have applications similar to those of class 1 bitumens.

(see Table 2). Class 1 "straight-run bitumens" now encompasses the former class 1 "penetration bitumens" and class 4 "hard bitumens," while classes 6 and 7 have been merged into a single class 5 "modified bitumens," as shown in Table 2.

The influence of solvents

Bitumens are produced as a solid or highly viscous material that can be softened or solubilized in solvents for use in industrial applications and in experimental settings. The individual constituents of bitumens have variable solubility and the choice and amount of solvent used will influence the physical form of the resulting material and the composition of the liquid and solid phases. Certain solvents may selectively extract specific constituents from bitumen, and the presence of solvents is likely to alter dermal-penetration characteristics and may influence the carcinogenic outcome.

In earlier studies of carcinogenicity in experimental animals, various solvents, including benzene, toluene or cyclohexane/acetone, were used to prepare either bitumen or bitumen condensates for dermal application. Interpretation of these studies is challenging due to the use of these different solvents. Indeed, this raised some concern in relation to the possibility that the dissolved and/or suspended study material may be different from the original neat material to the extent that it should be defined as a different class of bitumen.

The influence of temperature

Bitumens are produced as a solid or highly viscous material and are heated to form a molten liquid that can be used for industrial applications such as roofing and paving. Softer grades of paving bitumen are typically heated to 140 °C, while harder paving grades and oxidized bitumens are heated to higher temperatures. The variable physicochemical properties of the individual constituents of

bitumen mean that the composition and physical form of the emissions from heated bitumens are dependent on the temperature to which the bitumen is heated. This variability presents a significant challenge when assessing airborne exposure for epidemiological studies and when designing studies in experimental animals.

While in earlier studies in animals bitumen was typically applied neat or diluted in a solvent, on the skin or by subcutaneous injection (see above), studies of carcinogenicity in experimental animals conducted since the late 1980s have investigated the carcinogenic activity of bitumen-fume condensates generated at temperatures between 120 and 316 °C, in both skin and inhalation models. The condensates are liquids of lower viscosity in which the lighter constituents of lower relative molecular mass have been concentrated. Results of studies with condensates generated at > 199 °C strongly suggest that temperature plays an important role in determining the degree of exposure and also the carcinogenic potential of bitumen emissions.

Use of coal tar for road paving and roofing

Human exposure to bitumens and their emissions comes almost exclusively from occupational exposure during manufacture and use of the products. The potential for confounding by other occupational exposures is a concern in the study of the carcinogenicity of bitumens and their emissions because many workers with occupations that involve exposure to bitumens may also experience, today or in the past, exposure to coal tars, which are established human carcinogens.

In road paving, coal tars were used as such or mixed with bitumens until the early 1960s in many European countries. From the early 1960s to the mid-1970s, coal-tar use declined dramatically, but continued in some countries such as Germany and France until 1996 in specialized surface-dressing operations (Burstyn *et al.*, 2003). Coal tar was frequently used in the USA in road paving until the Second World War, and decreased drastically thereafter. Since then, coal tars have been used in some non-road applications, such as airfields, and as a pavement sealer for parking lots, driveways and bridges. Some coal-tar mixes were used in South Africa and Australia in the 1960s and 1970s in container terminals, car parks and bus terminals, which are subject to fuel spills. No information on use of coal tar for road paving in other countries was available to the Working Group.

Roofers may also be exposed to coal tar during the process of tearing off old roofing materials made with coal tar.

Studies of carcinogenicity in experimental animals

The current review of available studies on the carcinogenicity of bitumens in experimental animals indicated that of the 26 reported studies in mice, fewer than half were adequately conducted or reported to allow evaluation of carcinogenicity. In rats, of the three reported studies (one on injection, two on inhalation) there was only one adequate study (inhalation). Both reported dermal studies in rabbits were also inadequate. All the inadequate studies were published before 1980. A similar proportion of inadequate studies was also observed in the studies reviewed for the *N*- and *S*-heterocyclic polycyclic aromatic hydrocarbons. Studies were judged to be inadequate on the basis

of poor study design or poor reporting, no inclusion of information about controls, limited or no histopathology or information on survival.

Although naturally occurring bitumens were not considered for this *Monograph*, it is interesting to report here a study on their carcinogenicity. Mice received lifetime dermal exposure to tar sands (containing approximately 80% sand, 10% water, and 10% hydrocarbons) or to an oily emulsion of it (created by first treating the tar sands with hot water, steam, and sodium hydroxide and then removing the solids and water). The mice treated with tar sand did not develop skin tumours (0/40), while two skin tumours developed in those treated with the oily emulsion (2/40; one papilloma and one carcinoma) (McKee *et al.*, 1986; McKee & Lewis, 1987).

Combining data on experimental carcinogenicity and epidemiological findings

In evaluating the carcinogenicity of bitumens in experimental animals, the Working Group was faced with the challenge of determining which class of bitumen was used in a study, based on the description of the study materials. The current categorization into six classes of bitumens, compared with the eight classes defined by the previous Working Group (see Table 2), and the poor description of the material used in some early studies sometimes made it difficult to attribute the study material to the proper class of bitumen.

Unlike the data for animals, the epidemiological studies were reported for four major types of occupational exposure, namely road paving, roofing, mastic-asphalt work, and several other occupations involving exposure to bitumens and bitumen emissions, including manufacturing of bitumens and asphalt products. Each of these occupational situations could involve worker exposure to several different classes of bitumens with attendant challenges for comparing or combining the data for humans and animals.

New development of products and processes

Recent research reported significant reductions in exposure levels among paving workers in Europe since 1960 (Burstyn *et al.*, 2003). The discontinuance of coal-tar use in Europe and technological advances in bitumen manufacture have contributed to reducing worker exposures. Application temperature is widely recognized as an important parameter in the generation of bitumen fume. More recently, warm-mix asphalt has been developed as a method that allows asphalt to be produced and placed on the road at significantly lower temperatures than conventional asphalt mixes. Lowering the mixing and application temperature by 10–38 °C (50–100 °F) has the potential to reduce emissions surrounding paving workers. However, these technologies may take time to introduce, particularly in low- and medium-resource countries.

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BITUMENS AND BITUMEN EMISSIONS

Bitumens and bitumen emissions were considered by previous IARC Working Groups in 1984 and 1987 (IARC, 1985, 1987). Since then, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent: definitions and classifications

1.1.1 Introduction

Bitumens are engineering materials produced by the distillation of crude oil during petroleum refining and exist in numerous forms and types. Bitumens are dark viscous liquids or semi-solids that are non-volatile at ambient temperatures and soften gradually when heated. In North America, bitumen is commonly known as "asphalt cement" or "asphalt binder". "Asphalt" is the term used for a mixture of small stones, sand, filler and bitumen (~5%), which is used as a road-paving material. Bitumen emissions are defined as the complex mixture of aerosols, vapours, and gases from heated bitumen and products containing bitumen. Although the term "bitumen fume" is often used in reference to total emissions, bitumen fume refers only to the aerosolized fraction of total emissions (i.e. solid particulate matter, condensed vapour, and liquid bitumen droplets). Accordingly, the term "bitumen emissions" is more appropriate for referring to total content of bitumen in air.

Different grade specifications of bitumen, based on physical properties, can be achieved for specific applications either directly via refining or by blending. For example, the basic product is often referred to as "straight-run" bitumen and is commonly used in road-paving applications. This basic product can be further processed by blowing air through it at elevated temperatures to produce "oxidized" bitumen, which is commonly used in roofing applications. While these are the two products most commonly used in industry, there are four additional classes that are produced to achieve specific physical characteristics by modification of the production process (see Section 1.1.3).

Bitumens should not be confused with coal-derived products such as coal tars or coal-tar pitches, which are distinctly different substances. While bitumens are derived from petroleum, coal-tar products are derived from the high-temperature carbonization of bituminous coals (> 1000 °C) and are by-products of gas and coke production. Coal-tar products contain much higher concentrations of polycyclic aromatic hydrocarbons (PAHs) than bitumens, particularly in the three- to seven-ring size range. In contrast, bitumens contain higher concentrations of paraffinic and napthenic hydrocarbons

Table 1.1 Ranges of PAH concentrations in bitumens and coal-tar pitches, and in fume (BSM) from bitumen and from coal-tar pitch

PAH	Bitumens (μg/g)	Coal-tar pitches (μg/g)	Bitumen fume (ppm) 160–250 °C	Coal-tar pitch fume (ppm) 160–210 °C
Phenanthrene	0.32–7.3	19850–25700	107–842	$2.0–2.5 \times 10^5$
Anthracene	0.01–0.32	4600–7310	3.6–22	$0.56–0.76 \times 10^5$
Fluoranthene	0.1–0.72	29000–36000	13–32	$0.76–0.92 \times 10^5$
Pyrene	0.17–1.5	21300–27200	15–134	$0.44–0.55 \times 10^5$
Chrysene	0.8–3.9	11200–22670	33–157	$0.056–0.11 \times 10^5$
Perylene	0.04–3.9	2770–3500	1.7–15	119–456
Benzo[*a*]anthracene	0.14–1.1	20400–24510	12–40	$0.059–0.12 \times 10^5$
Benzo[*k*]fluoranthene	ND–2.2	5250–6010	ND–2.6	377–1216
Benzo[*a*]pyrene	0.22–1.8	11360–15170	2.9–8.5	553–2022
Benzo[*g,h,i*]perylene	1.2–5.7	3430–3530	6.0–15	34–200
Anthanthrene	ND–0.11	1231–1728	ND	9–69
Dibenzo[*a,i*]pyrene	ND–0.6	127–164	ND–0.5	ND–0.6
Coronene	ND–0.4	ND–120	ND–11	ND

BSM, benzene-soluble matter; ND, not detected, below the limit of detection; PAH, polycyclic aromatic hydrocarbon
From Brandt & de Groot (1985)

and their derivatives, whose large size and viscosity result in limited solubility (Table 1.1). Puzinauskas & Corbett (1978) provide a concise review of the differences between bitumens and coal-tar products (IARC, 2010).

Similarly, bitumen should not be confused with petroleum pitch, which is the highly aromatic residue produced by thermal cracking (i.e. extreme heat treatment) of selected petroleum fractions. The properties and chemical composition of petroleum pitch are therefore quite different from those of refined bitumen. While the term "petroleum pitch" is not used consistently (this term is used to describe different materials in different areas), petroleum pitches are principally used as binders in the manufacture of metallurgical electrodes (IARC, 2010).

1.1.2 Chemical properties and physical characteristics of bitumens

Bitumens contain a complex mixture of aliphatic compounds, cyclic alkanes, aromatic hydrocarbons, PAHs and heterocyclic compounds containing nitrogen, oxygen and sulfur atoms, and metals (e.g. iron, nickel, and vanadium). However, most of the available analytical data are focused on the characterization of PAHs. Table 1.2 lists the PAHs and volatile organic compounds present in bitumens or in bitumen emissions that have been evaluated by IARC. Elemental analyses indicate that most bitumens contain primarily hydrocarbons, i.e. carbon, 79–88%; hydrogen, 7–13%; sulfur, traces to 8%; oxygen, 2–8%; nitrogen, 3%; and the metals vanadium and nickel in parts per million (Speight, 2000). The exact chemical composition of a bitumen varies depending on the chemical complexity of the original crude petroleum and the manufacturing processes. In addition, the products of other refining processes, e.g. flux or solvent from petroleum distillate, may be blended with bitumen to achieve the desired performance specifications. Consequently, no two bitumen products are chemically identical, and chemical analysis cannot be used to define the exact chemical structure or chemical composition of bitumens.

PAHs are present in crude oils (Bingham *et al.*, 1979) and generally in lower amounts in bitumens

Table 1.2 IARC evaluation of compounds identified in bitumens or their emissions

Agent	Levelof evidence		IARC Group	Volume	Year of publication
	Humans	Animals			
Acenaphthene	I	I	3	92	2010
Anthanthrene	I	L	3	92	2010
Anthracene	I	I	3	92	2010
Benzene	S	S	1	29, Sup 7, 100F	2011
Benz[*a*]acridine	I	I	3	103	2011
Benz[*c*]acridine	I	L	3	103	2011
Benzo[*a*]anthracene	I	S	2B	92	2010
Benzo[*a*]fluorene	I	I	3	92	2010
Benzo[*a*]pyrene	I	S	1[a]	92, 100F	2011
Benzo[*b*]fluoranthene	I	S	2B	92	2010
Benzo[*b*]fluorene	I	I	3	92	2010
Benzo[*b*]naphtha[*2,1-d*]thiophene	I	L	3	103	2011
Benzo[*e*]pyrene	I	I	3	92	2010
Benzo[*k*]fluoranthene	I	S	2B	92	2010
Carbazole	I	S	2B	103	2011
Chrysene	I	S	2B	92	2010
Coronene	I	I	3	32, Sup 7	1987
Dibenz[*a,h*]acridine	I	S	2B	103	2011
Dibenz[*a,j*]acridine	I	S	2A[a]	103	2011
Dibenz[*c,h*]acridine	I	L	2B[a]	103	2011
7H-Dibenzo[*c,g*]carbazole	I	S	2B	103	2011
Dibenzo[*a,i*]pyrene	I	S	2B	92	2010
Dibenzo[*a,l*]pyrene	I	S	2A[a]	92	2010
Dibenzothiophene	I	I	3	103	2011
Ethylbenzene	I	S	2B	77	2000
Fluoranthene	I	L	3	92	2010
Fluorene	I	I	3	92	2010
Indeno[*1,2,3-cd*]pyrene	I	S	2B	92	2010
1-Methylphenanthrene	I	I	3	92	2010
3-Methylchrysene	I	L	3	92	2010
4-Methylchrysene	I	L	3	92	2010

Table 1.2 (continued)

Agent	Levelof evidence		IARC Group	Volume	Year of publication
	Humans	Animals			
5-Methylchrysene	I	S	2B	92	2010
Naphthalene	I	S	2B	82	2002
Perylene	I	I	3	92	2010
Phenanthrene	I	I	3	92	2010
Phenol	I	I	3	47, 71	1999
Picene	I	L	3	92	2010
Pyrene	I	I	3	92	2010
Styrene	L	S	2B	60, 82	2002
Tetrachloroethylene	L	S	2A	63	1995
Toluene	I	ESLC	3	47, 71	1999
Triphenylene	I	I	3	92	2010
Xylene [*m*+*p*-]	I	I	3	47, 71	1999
Xylene [*o*-]	I	I	3	47, 71	1999

[a] Upgraded based on strong mechanistic evidence

ESLC, evidence suggesting lack of carcinogenicity; I, inadequate evidence; L, limited evidence; S, sufficient evidence

(Brandt & Molyneux, 1985; Brandt *et al.*, 1985a, b). This is because the principal refinery process used for the manufacture of bitumens, namely vacuum distillation, removes the majority of compounds of lower relative molecular mass with lower boiling-points, including PAHs with three to seven fused rings, and because the maximum temperatures involved in the production of vacuum residue range from 350 °C to 450 °C and are not high enough to initiate significant PAH formation. Although most of the PAHs are removed during the manufacturing process, residues of two- to seven-ring PAHs are found both in solid bitumens and bitumen emissions. Bitumen emissions tend to contain proportionally more two-ring PAHs, such as naphthalene, and less five-ring PAHs, such as benzo[*a*]pyrene, than solid bitumens.

Bitumen products are tailored to needs on the basis of required physical properties rather than on chemical composition. Bitumens are soluble in carbon disulfide, chloroform, ether and acetone, partially soluble in aromatic organic solvents, and insoluble in water at 20 °C (IPCS, 2004). Until the 1990s, bitumen specifications in both Europe and the USA relied primarily on mechanical tests of hardness and viscosity. At that time, the Strategic Highway Research Program (SHRP) introduced the performance grade (PG) system, which replaced the penetration and viscosity grading systems for both conventional, unmodified bitumens and polymer-modified bitumens in the USA. The PG system is used to assess and designate engineering properties at temperatures that are representative of the climatic conditions in which the bitumens will be used (Asphalt Institute & Eurobitume, 2011). Conventional notation for PG binders is a two-number system where the first number represents the maximum pavement design temperature (°C), while the second number represents the minimum likely pavement design temperature (°C) that can be used without failure (e.g. PG 64–28). Table 1.3 summarizes the ASTM requirements by performance grade.

Older specification systems are still recognized alongside the newer generation of performance-based systems. The important characteristics of bitumen production are summarized below.

(a) Penetration

The penetration test (or "pen" test) is used to measure the hardness of bitumens, lower penetration indicating greater hardness. In testing, a container of bitumen is kept at the standard test temperature, 25 °C, in a temperature-controlled water bath. A steel needle of specified dimensions is allowed to bear on the surface of the bitumen for 5 seconds under a load of 100 g (British Standards Institution, 1974). The distance that the needle penetrates, in tenths of a millimetre (dmm), is the penetration measurement. Specifications for penetration-graded bitumens typically state the penetration range for a grade (e.g. 50/70). On the basis of this test, bitumens have been classified into five standard grades of penetration (from hardest to softest): 40–50, 60–70, 85–100, 120–150, and 200–300 dmm (NIOSH, 2001a).

(b) Softening-point

In the softening-point test, the temperature of a sample of bitumen in the form of a disc is raised at 5 °C per minute while being subjected to loading by a small steel ball. As the temperature rises, the bitumen softens and the particular temperature at which the disc of bitumen is deformed by a distance of 1 inch [2.54 cm] is recorded as the softening-point in °C (British Standards Institution, 1983a).

(c) Viscosity

The viscosity of bitumen can be measured in several ways. For example, vacuum capillary viscometers are used for definition by grade; for products of relatively low viscosity, simple

Table 1.3 Bitumen specifications by performance grade

	PG 46	PG52	PG 58	PG 64	PG 70	PG 76	PG 82
Grade range	**–34 to –46**	**–10 to –46**	**–16 to –40**	**–10 to –40**	**–10 to –40**	**–10 to –34**	**–10 to –34**
Average 7-day maximum pavement design temperature (°C)	< 46	< 52	< 58	< 64	< 70	< 76	< 82
Minimum pavement design temperature (°C)	>–34 to >–46	>–10 to >–46	>–16 to >–40	>–10 to >–40	>–10 to >–40	>–10 to >–34	>–10 to >–34
Original binder							
Flash-point temperature, D92; min. (°C)	230	230	230	230	230	230	230
Viscosity, D 4402: max. 3 Pa × s, test temperature (°C)	135						
Dynamic shear, D7175: G*/sinδ, min. 1.00 kPa; 25 mm plate, 1 mm gap; test temperature at 10 rad/s (°C)	46	52	58	64	70	76	82
Rolling thin film oven residue (T 240)							
Mass loss, max. %	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dynamic shear, D7175: G*/sinδ, min. 2.20 kPa; 25 mm plate, 1 mm gap; test temperature at 10 rad/s (°C)	46	52	58	64	70	76	82
Pressure ageing vessel residue (PP 1)							
PAV ageing temperature (°C)	90	90	100	100	100 (110)	100 (110)	100 (110)
Dynamic shear, D7175: G* × sinδ, max. 5000 kPa; 8mm plate, 2 mm gap; test temperature at 10 rad/s (°C)	10 to 4	25 to 7	25 to 13	31 to 16	34 to 19	37 to 25	40 to 28
Creep stiffness, D 6648: S, max. 300 MPa; m-value, min. 0.300; test temperature at 60 s (°C)	–24 to –36	0 to –36	–6 to –30	0 to –30	0 to –30	0 to –24	0 to –24
Direct tension, D6723: failure strain, min. 1.0%; test temperature at 1.0 mm/min. (°C)	–24 to –36	0 to –36	–6 to –30	0 to –30	0 to –30	0 to –24	0 to –24

PAV, pressure ageing vessel; PG, performance grade
Adapted from ASTM (2008)

orifice-type viscometers are generally used (British Standards Institution, 1983b). Viscosity is typically calculated from the time required for the bitumen binder to flow between two successive marks. The viscosity grade of bitumen (AC-2.5, AC-5, AC-10, AC-20, AC-30, and AC-40) or bitumen residue (AR-4000, AR-8000, and AR-16 000) indicates viscosity in hundreds of poises (gram per centimetre per second) at 60 °C (NIOSH, 2001a).

(d) Temperature susceptibility

Tests of ductility are used to determine the ability of bitumen to stretch at intermediate temperatures (4–25 °C). "Dog-bone" shaped specimens are pulled at a constant rate until the sample breaks. Penetration index is used as an indication of temperature susceptibility.

(e) Solubility

The solubility test ASTM D2042 (ASTM, 2010) is used to measure the purity of the bitumen. Active cementitious constituents will be soluble in trichloroethylene while non-cementing matter is not. As a prerequisite of eligibility to be graded in the Superior Performing Asphalt Pavements (Superpave) system, insoluble matter cannot exceed 1%.

(f) Flash-point

The flash-point is the temperature at which bitumen fume may flash, or spark. As an example, this temperature is usually 230 °C or higher for common paving bitumens. The flash-point provides an indication of fire hazard and the test is frequently used to indicate whether a given product has been contaminated with materials of lower flash-point.

1.1.3 Classification of refined petroleum bitumens

For the purposes of this *Monograph*, bitumens have been categorized into six classes. Examples of typical specifications for straight-run bitumens (class 1), oxidized bitumens (class 2) and cutback bitumens (class 3) are provided in the tables in this Section. Emulsion bitumens (class 4), modified bitumens (class 5) and thermal ly cracked bitumens (class 6) are used in a range of applications with specifications that vary depending on the intended use, but are not as widely used as classes 1 and 2.

(a) Straight-run or paving bitumens (class 1)

Straight-run or paving bitumens (CAS No. 8052-42-4; EINECS No. 232-490-9) are usually produced from the residue from atmospheric distillation of petroleum crude oil by applying further distillation under vacuum, solvent precipitation, or a combination of these processes (see Section 1.2.1). In Australia, class 1 bitumens are called "viscosity-graded asphalt cements"; in the USA, they are called "asphalt binders". An additional straight-run bitumen product includes residues obtained via further separation by a de-asphalting process (Asphalt Institute & Eurobitume, 2011). The types and levels of PAHs found in bitumens of class 1 are shown in Table 1.4 and Table 1.5.

In Europe, straight-run bitumens are defined by the upper and lower limits of penetration values. For example, a nominal 200-PEN grade has a range of 170–230 in the British Standards Specification (British Standards Institution, 1982a, b). Penetration grades commonly used vary from 15-PEN to 450-PEN, though terminology varies by country. In France, the 180–220 grade has limits of 180–220, while in Germany the B200 grade may vary from 160 to 210 in penetration value. Specific ranges of softening-point are required for particular straight-run bitumens to ensure that the penetration index (PI, a measure

Table 1.4 Content of PAHs (mg/kg) in eight samples of straight-run bitumens (class 1)

PAH	n[a]	Bitumen[b]							
		A	B	C	D	E	F	G	H
Anthracene	3	ND	ND	ND	ND	ND	ND	ND	ND
Phenanthrene	3	2.3	0.4	3.5	1.3	0.6	35*	1.1	2.3*
Pyrene	4	0.6	1.8	4.0	8.3	0.9	38	0.3	0.08
Fluoranthene	4	+	+	2.0	+	+	5	ND	ND
Benzofluorenes	4	+	+	+	+	+	+	+	ND
Benz[*a*]anthracene	4	0.15	2.1	1.1	0.7	0.9	35	0.2	ND
Triphenylene	4	0.25	6.1	3.1	3.4	3.8	7.6	1.0	0.3
Chrysene	4	0.2	8.9	2.3	3.9	3.2	34	0.7	0.04
Benzo[*a*]pyrene	5	0.5	1.7	1.3	2.5	1.6	27	0.1	ND
Benzo[*e*]pyrene	5	3.8	13	2.9	3.2	6.5	52	1.6	0.03
Benzo[*k*]fluoranthene	5	+	ND	+	+	+	ND	ND	ND
Perylene	5	ND	39	2.2	6.1	2.9	3.0	0.1	ND
Anthanthrene	6	ND	Tr	Tr	Tr	+	1.8	ND	ND
Benzo[*g,h,i*]perylene	6	2.1	4.6	1.0	1.7	2.7	15	0.6	Tr
Indeno[*1,2,3-cd*]pyrene	6	Tr	ND	Tr	Tr	Tr	1.0	ND	ND
Picene	6	+	+	+	+	+	1.0	+	ND
Coronene	7	1.9	0.8	0.5	0.2	0.9	2.8	0.9	ND

[a] Number of aromatic rings
[b] Estimate includes alkyl derivatives
+, not estimated but present in small amount; ND, not detected; Tr, trace
Adapted from Wallcave *et al.* (1971)

Table 1.5 Content of 14 PAHs (mg/kg) in some straight-run (class 1) and oxidized (class 2) bitumens[a]

PAH	n[b]	Class 1			Class 2			
		80/100	80/100	50/60	80/100	85/40	110/30	95/25
Phenanthrene	3	7.3	5.0	1.7	5.0	0.32	1.7	2.4
Anthracene	3	0.32	0.27	0.015	0.17	0.01	0.03	0.07
Fluoranthene	4	0.72	0.46	0.41	0.39	0.15	0.4	0.46
Pyrene	4	1.5	1.0	0.26	1.1	0.17	0.3	0.29
Chrysene	4	1.5	3.3	0.47	3.9	0.90	1.0	0.80
Benz[*a*]anthracene	4	1.1	0.89	0.14	0.63	0.33	0.3	0.23
Perylene	5	3.3	0.69	0.044	0.25	0.14	0.08	0.20
Benzo[*k*]fluoranthene	5	0.19	ND	0.024	ND	0.051	0.10	0.04
Benzo[*a*]pyrene	5	1.8	0.92	0.22	1.1	0.49	0.35	0.48
Benzo[*g,h,i*]perylene	6	4.2	2.3	1.67	2.7	1.3	1.2	2.0
Anthanthrene	6	0.11	0.04	0.006	0.02	0.01	ND	0.03
Dibenzo[*a,l*]pyrene	6	ND	ND	ND	ND	ND	ND	ND
Dibenzo[*a,i*]pyrene	6	0.50	ND	0.05	0.60	ND	0.3	0.10
Coronene	7	ND	ND	0.40	ND	ND	ND	ND

[a] Bitumens obtained from a range of crude oils originating from the Middle East, Venezuela and Mexico.
[b] Number of aromatic rings
ND, not detected; PAH, polycyclic aromatic hydrocarbon
From Brandt & de Groot (1985)

Table 1.6 Specifications for straight-run bitumens (class 1) by penetration grade

Property		Test method	Penetration grade									
			15 PEN	25 PEN	35 PEN	40 PEN HD	50 PEN	70 PEN	100 PEN	200 PEN	300 PEN	450 PEN
Penetration at 25 °C		BS 4 691	15 ± 5	25 ± 5	35 ± 7	40 ± 10	50 ± 10	70 ± 10	100 ± 20	200 ± 30	300 ± 45	450 ± 65
Softening-point (°C)	Min.	BS 4 692	63	57	52	58	47	44	41	33	30	25
	Max.		76	69	64	68	58	54	51	42	39	34
Loss on heating for 5 h at 163 °C		BS 2000:Part 45										
Loss by mass (%)	Max.		0.1	0.2	0.2	0.2	0.2	0.2	0.5	0.5	1.0	1.0
Drop in penetration (%)	Max.		20	20	20	20	20	20	20	20	25	25
Solubility in trichloroethylene by mass (%)	Min.	BS 4 690	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5	99.5

Max., maximum; Min., minimum; PEN, penetration grade
From IARC (1985)

Table 1.7 Specifications for hard bitumens (class 1)

Property		Test method	Grade	
			H 80/90	H 100/120
Softening-point (°C)	Min.	BS 4692	80	100
	Max.		90	120
Penetration at 25 °C	Min.	BS 4691	6	2
	Max.		12	10
Loss on heating for 5 h at 163 °C by mass (%)	Max.	BS 2000: Part 45	0.05	0.05
Solubility in trichloroethylene by mass (%)	Min.	BS 4690	99.5	99.5

Max., maximum; Min., minimum
From IARC (1985)

of change in penetration with temperature) does not vary by more than the allowable amount. Table 1.6 summarizes the specifications for class 1 bitumens by penetration grade.

Under the American PG system, PG 64–22 bitumens, for example, provide enough stiffness to prevent permanent deformation or rutting at pavement temperatures as high as 64 °C and low-temperature cracking at temperatures as low as –22 °C. The standard PG specifications are described in AASHTO M320–04 and ASTM D6373 (Asphalt Institute & Eurobitume, 2011).

Hard bitumens are a subset of straight-run bitumens that have low penetration values (i.e. < 15) and are generally designated by the prefix H (HVB in Germany) combined with the softening-point range, e.g. H 80/90. Hard bitumens are brittle in nature and are commonly used in mastic applications. While the nomenclature is derived from the softening-point range, each grade also has a defined penetration range, giving these materials a PI of 0 to +2.0. Table 1.7 summarizes the specifications of hard bitumens by grade.

In the past decade, warm-mix asphalt technologies have been developed for use in road-paving applications, allowing application temperatures to be lowered to 100–140 °C rather than the higher temperatures associated with the application of conventional paving bitumen (140–160 °C). Warm-mix asphalts are produced by adding one of three additives to straight-run bitumens: water (application temperature of 129–135 °C), organics/waxes or chemicals/surfactants (application temperature of 116–121 °C). The resulting road-paving materials allow for lower mixing temperatures, improved coating of the mineral aggregate, better compaction, and lower application temperatures (Prowell *et al.*, 2011).

(b) Oxidized bitumens (class 2)

Oxidized bitumens or blown bitumens (CAS No. 64742-93-4; EINECS No. 265-196-4) are produced by passing air through hot, soft bitumens under controlled temperature conditions, a process that reduces temperature susceptibility and increases resistance to stress. Intense oxidation produces a fully-oxidized product, which has a PI of +2.0 to +8.0 and is used in roofing applications (Asphalt Institute & Eurobitume, 2011). Mild oxidation (i.e. a mild degree of air blowing) produces air-rectified (semi-blown) bitumen, a different product that has a PI ≤ +2.0 and applications similar to those for class 1 bitumens (Asphalt Institute & Eurobitume, 2011). In the USA, oxidized bitumens are also known as "air-blown asphalts" or "roofing asphalts". The types and levels of PAHs found in class 2 bitumens are shown in Table 1.5.

Oxidized bitumens are classified by the ranges of allowable values for penetration and softening-point. For instance, a common grade

Table 1.8 Specifications for oxidized bitumens (class 2)

Property		Test method	Grade					
			75/30	85/25	85/40	95/25	105/35	115/15
Softening-point (°C)	Min.	BS 4692	70	80	80	90	100	110
	Max.		80	90	90	100	110	120
Penetration at 25 °C		BS 4691	30 ± 5	25 ± 5	40 ± 5	25 ± 5	35 ± 5	15 ± 5
Loss on heating for 5 h at 163 °C by mass (%)	Max.	BS 2000: Part 45	0.2	0.2	0.5	0.2	0.5	0.2
Solubility in trichloroethylene by mass (%)	Min.	BS 4 690	99.5	99.5	99.5	99.5	99.5	99.5

Max., maximum; Min., minimum
From IARC (1985)

such as 85/25 has a mean value of 85 for the permissible softening-point range of 80–90 °C and a mean value of 25 for the penetration range of 20–30. In the USA, the specifications for oxidized bitumens are based on softening-point and penetration tests at three temperatures (0 °C, 25 °C and 46 °C). Common grades in this class of bitumens are 85/25, 85/40, 100/40, 105/35, 105/13 and 115/15. Oxidized bitumens are somewhat rubbery in nature and exhibit low-temperature dependence. Table 1.8 outlines the specifications for class 2 bitumens by grade.

(c) Cutback bitumens (class 3)

Cutback bitumens or fluxed bitumens are produced by adding an agent to straight-run bitumens or oxidized bitumens for the purpose of reducing (i.e. "cutting back") viscosity and rendering the products more fluid for ease of handling. Since cutback bitumens include different combinations of multiple products (blends), there is no CAS No. available for cutback bitumens. Examples of agents that are suitable for blending include solvent extracts (aromatic by-products from the refining of base oils), thermally-cracked residues, or certain heavy petroleum distillates with final boiling points > 350 °C. Coal-tar products are also sometimes used as fluxes (see IARC, 2010). When volatile diluents from petroleum crudes (i.e. white spirit, naphtha, kerosene or gas oil) are used, the initial properties of bitumens are recovered when the diluent evaporates. However, when non-volatile agents such as coal tar are used, there is limited evaporation (Asphalt Institute & Eurobitume, 2011). In the USA, cutback bitumens are sometimes referred to as "road oils".

Grades of cutback bitumens are designated by a value in seconds required for a given quantity of the product to flow through a standard orifice at a fixed temperature. Typical products used in road applications are made by cutting back 100-PEN bitumens with 8–14% kerosene. They are designated by the midpoints of the viscosity limits adopted. European specifications for typical products are given by the British Standards Institution (1982a), while specifications in the USA are given by the ASTM. Cutback bitumens are viscous to highly fluid materials at ambient temperatures. Table 1.9 outlines the specifications for class 3 bitumens by grade.

(d) Bitumen emulsions (class 4)

Bitumen emulsions are fine dispersions of bitumen droplets in water, primarily of straight-run bitumens (class 1), although cutback bitumens (class 3) and modified bitumens (class 5) can also be used. Accordingly, there is no CAS No. available for bitumen emulsions. Bitumen emulsions are manufactured using high-speed

Table 1.9 Specifications for cutback bitumens (class 3)

Property		Test method	Grade		
			50 s	100 s	200 s
Viscosity (STV) at 40 °C, 100-mm cup		BS 2000: Part 72	50 ± 10	100 ± 20	200 ± 40
Distillation to 225 °C (% by volume)	Max.	BS 2000: Part 72	1	1	1
Distillation to 360 °C (% by volume)	Max.		8 to 14	6 to 12	4 to 10
Penetration at 25 °C of residue from distillation to 360 °C		BS 4691	100 to 350	100 to 350	100 to 350
Solubility in trichloroethylene by mass (%)	Min.	BS 4690	99.5	99.5	99.5

Max., maximum; Min., minimum; STV, standard tar viscometer
From IARC (1985)

shearing devices, such as colloid mills. The bitumen content can range from 30% to 70% by weight. They can be anionic, cationic or non-ionic depending on the surfactant used (Asphalt Institute & Eurobitume, 2011). In the USA, they are referred to as "asphalt emulsions".

(e) Modified bitumens (class 5)

Modified bitumens contain appreciable quantities (typically 3–15% by weight) of special additives, such as polymers, crumb rubber, elastomers, sulfur, polyphosphoric acid and other products used to modify their properties. This is a variable class of bitumens that are modified for use in specialized applications (Asphalt Institute & Eurobitume, 2011). Accordingly, there is no CAS No. available for modified bitumens.

(f) Thermally-cracked bitumens (class 6)

Thermally-cracked bitumens (CAS No. 92062-05-0; EINECS No. 295-518-9) are produced by extended high-temperature distillation of a petroleum residue (440–500 °C). The thermally-cracked residue produced by this process is vacuum-distilled and further treated to create a hard material used in blending bitumens (Asphalt Institute & Eurobitume, 2011). Thermally-cracked bitumens may contain levels of PAHs of up to 272 µg/kg (Yanysheva *et al.*, 1963). Thermally-cracked bitumens are not produced in the USA.

1.1.4 PAH composition of class 1 and class 2 products and their emissions

Trumbore *et al.* (2011) evaluated the effect of oxidation on the concentrations of PAHs in bitumens. Five samples of straight-run bitumen were laboratory-oxidized to a range of softening-points used for common roofing products. This resulted in a reduction of four- to six-ring PAHs in the oxidized products. [Since workers are exposed to bitumen emissions generated at temperatures that vary by product, it is important to consider the concentration and composition of bitumen emissions generated from different products when heated to the temperatures at which they are typically applied.]

Cavallari *et al.* (2012a) characterized temperature-dependent emissions from 20 samples of straight-run bitumens (typically used in paving) and five samples of oxidized bitumens (typically used in roofing), obtained directly from contractors. Emissions were generated in a laboratory at eight different temperatures ranging from 120 °C to 315 °C. Two of the evaluated temperatures (120 °C, 150 °C) were consistent with those used in paving applications and showed that emissions from straight-run bitumens included two to three-ring PAHs but rarely four- to six-ring PAHs. In comparison, three of the temperatures evaluated (180 °C, 205 °C, 230 °C) were consistent with those used in hot-appplied roofing applications and showed that emissions from oxidized

bitumens included two- to three-ring PAHs and four- to six-ring PAHs at much greater frequency and significantly higher concentrations. In multivariate models, PAHs were found to significantly increase with increasing temperature, with a stronger effect for oxidized bitumens than for straight-run bitumens. Table 1.10 summarizes the PAH results in laboratory-generated emissions by bitumen type and temperature.

While the above experiment was conducted to characterize the chemical composition under occupational conditions, bitumen emissions are also generated for experimental purposes. Accordingly, emission-generation systems are developed to produce emissions in a laboratory setting that are similar to those in the field. For example, Binet *et al.* (2002) analysed PAHs and sulfur-containing PAHs in laboratory-generated emissions from straight-run bitumen samples at 170 °C, selected to represent the upper range of temperatures used in paving applications (Table 1.11).

1.1.5 Naturally occurring bitumens

Natural bitumens form from petroleum as a result of the evaporation of light fractions and of oxidation under the influence of hypergenesis. The petroleum first changes into thick and highly viscous maltha, then into hard and easily fusible bitumens. Further change in natural bitumens usually leads to the formation of asphaltite. Natural bitumens can be recovered for specialized industrial purposes.

1.2 Methods of analysis

1.2.1 Bitumens

The chemical composition of bitumens depends on the chemical complexity of the original crude petroleum and the manufacturing processes, and can be determined by global methods based on their spectrometric properties, or by class separation following chromatography coupled with mass spectrometry (MS) detection for identification of individual chemical compounds. Using solvent precipitation and adsorption chromatography, the chemical characterization of bitumens is based on their separation into four broad classes of compounds: asphaltenes, resins, cyclic compounds, and saturates (IARC, 1985).

(a) Fourier transform infrared

This technique is used to detect and analyse the oxygenated species (ketones, acids, bases) contained in bitumen. With modified bitumens, it is used to identify and quantify added polymers such as styrene–butadiene type copolymers (Masson *et al.*, 2001).

(b) Simulated distillation

Simulated distillation is a type of gas chromatography in which the results are expressed as the boiling-point of the products. It can be used to detect the presence of compounds that are volatile at 100–300 °C. This method (ASTM D2887) is used particularly for class 3 and 4 bitumens and is also useful as a mean of monitoring changes in volatile products in the pavement (ASTM, 2009).

(c) Gel-permeation chromatography

Gel-permeation chromatography is useful for separating compounds with very different molecular sizes (Jennings *et al.*, 1993), and can also be used to identify polymers that have been added to the bitumen (class 5).

(d) Class separation by adsorption chromatography

Adsorption chromatography is a technique to separate bitumens into fractions – asphaltenes, resins, cyclic compounds and saturates – using an alumina column or silica-gel chromatography. The method ASTM D2007-11 may be used (ASTM, 2011).

Table 1.10 Concentrations of PAHs (μg/m³) in laboratory-generated emissions, by temperature, for straight-run bitumens (*n* = 20 samples, *n* = 1600 measurements) and oxidized bitumens (*n* = 5 samples, *n* = 400 measurements)

	Temperature regime 1[a]				Temperature regime 2[a]					
	120 °C		150 °C		180 °C		205 °C		230 °C	
	% BDL	GM (GSD)	% BDL	GM (GSD)	% BDL	GM (GSD)	% BDL	GM (GSD)	% BDL	GM (GSD)
Two-ring PAHs										
Acenaphthene	85	[b]	0	1.80 (1.66)	0	57.9 (1.46)	0	89.9 (1.37)	0	108.2 (1.42)
Fluorene	100	[b]	48	[b]	0	7.62 (5.54)	0	18.3 (2.93)	0	27.6 (2.44)
2-Methylnaphthalene	5	1.20 (1.59)	0	4.51 (2.07)	0	25.3 (1.64)	0	77.5 (1.49)	0	110.5 (1.41)
Naphthalene	0	1.32 (1.43)	0	3.77 (1.63)	0	47.4 (1.51)	0	89.8 (1.56)	0	81.0 (1.87)
Three-ring PAHs										
Anthracene	97	[b]	0	0.81 (5.36)	48	[b]	38	6.66 (25.7)	0	17.2 (10.1)
Fluoranthene	88	[b]	55	[b]	0	8.29 (1.66)	0	14.7 (1.37)	0	20.6 (1.44)
Phenanthrene	100	[b]	0	2.26 (1.53)	0	54.4 (1.46)	0	78.4 (1.45)	0	100.5 (1.42)
Four-ring PAHs										
Benz[*a*]anthracene	97	[b]	17	0.23 (2.23)	0	5.84 (1.84)	0	12.1 (1.44)	0	20.0 (1.39)
Benzo[*b*]luoranthene	97	[b]	85	[b]	33	0.24 (3.07)	0	2.39 (2.81)	0	4.71 (3.11)
Benzo[*k*]fluoranthene	96	[b]	94	[b]	70	[b]	24	0.67 (7.42)	0	1.53 (8.03)
Chrysene	99	[b]	100	[b]	17	0.35 (1.68)	10	0.66 (1.78)	0	1.46 (1.36)
Pyrene	99	[b]	81	[b]	0	5.21 (3.36)	0	6.71 (3.32)	0	8.86 (3.21)
Triphenylene	98	[b]	97	[b]	58	[b]	10	0.31 (1.84)	10	0.55 (2.11)
Five-to six-ring PAHs										
Benzo[*a*]pyrene	96	[b]	97	[b]	80	[b]	38	0.13 (1.86)	0	0.49 (1.54)
Benzo[*e*]pyrene	97	[b]	94	[b]	93	[b]	39	0.12 (1.92)	15	0.19 (1.97)
Dibenz[*a,h*]anthracene	97	[b]	97	[b]	96	[b]	50	[b]	49	[b]
Dibenzo[*g,h,i*]perylene	98	[b]	97	[b]	94	[b]	50	[b]	50	[b]
Indeno[*1,2,3-cd*]pyrene	97	[b]	97	[b]	98	[b]	93	[b]	19	0.14 (1.63)

[a] Samples were evaluated over two temperature regimes: standard application temperatures for warm-mix asphalt (100–140 °C) during paving (regime 1), and standard application temperatures for type II, III, and IV build-up roofing applications (175–240 °C) (regime 2).

[b] GM (GSD) were not calculated for samples with >40% below detection limit (BDL)

% BDL, percent below the detection limit; GM, geometric mean; GSD, geometric standard deviation; PAH, polycyclic aromatic hydrocarbon

From Cavallari *et al.* (2012a)

Table 1.11 Concentrations of PAHs and S-heterocyclic PAHs in laboratory-produced bitumen emissions (class not reported); TPM concentration, 5 mg/m³; temperature, 170 °C

PAH	N[a]	Fume (n = 3)		Vapours (n = 3)	
		µg/m³	% of TPM	µg/m³	% of TPM
Naphthalene	2	0.10	0.002	53	1.06
Methylnaphthalenes	2	0.05	0.001	134	2.68
1-Ethylnaphthalene	2	ND	ND	20	0.4
Dimethylnaphthalenes	2	0.41	0.008	104	2.08
2,3,5-Trimethylnaphthalene	2	0.27	0.005	27	0.54
Biphenyl	2	ND	ND	4.3	0.086
2-Methylbiphenyl	2	ND	ND	0.8	0.016
Acenaphthylene	3	ND	ND	1.7	0.034
Acenaphthene	3	ND	ND	4.1	0.082
Anthracene	3	0.01	< 0.001	0.5	0.01
Fluorene	3	0.14	0.003	12.1	0.24
Phenanthrene	3	1.85	0.037	7.7	0.15
1-Methylphenanthrene	3	1.62	0.032	1.4	0.028
2-Methylphenanthrene	3	1.58	0.032	1.3	0.026
3,6-Dimethylphenanthrene	3	0.16	0.003	ND	ND
Benzo[*a*]anthracene	4	0.06	0.001	ND	ND
Benzo[*a*]fluorene	4	0.17	0.003	ND	ND
Benzo[*b*]fluorene	4	0.01	< 0.001	ND	ND
Chrysene	4	0.12	0.002	ND	ND
Fluoranthene	4	ND	ND	ND	ND
Pyrene	4	0.14	0.003	ND	ND
Methylchrysenes	4	0.23	0.005	ND	ND
1-Methylpyrene	4	0.19	0.004	ND	ND
Benzo[*a*]pyrene	5	0.04	< 0.001	ND	ND
Benzo[e]pyrene	5	0.06	0.001	ND	ND
Benzo[*k*]fluoranthene	5	0.06	0.001	ND	ND
Benzo[*b*]naphtho[1,2-*d*]thiopene	4	0.10	0.002	ND	ND
Benzo[*b*]naphtho[2,1-*d*]thiopene	4	0.33	0.007	ND	ND
Benzo[*b*]naphtho[2,3-*d*]thiopene	4	0.04	0.001	ND	ND
Dibenzothiopene	3	2.64	0.053	ND	ND
Sum of two-ring PAHs		ND	ND	343	6.86
Sum of three-ring PAHs		13	0.26	29	0.58
Sum of four–five ring PAHs		1.08	0.022	ND	ND
Sum of *S*-PAHs		3.12	0.062	ND	ND

[a] Number of aromatic rings
ND, not detected; TPM, total particulate matter
From Binet *et al.* (2002)

(e) Analysis of PAHs

Due to the existence of numerous structural isomers of the PAHs, chromatographic separation either by gas chromatography (GC) coupled with universal flame ionization detection (FID), MS or high-performance liquid chromatography (HPLC) coupled with ultraviolet or fluorescence detection (FD) is generally employed for isomer-specific identification and quantification. Different official methods for PAH analysis have been proposed: National Institute for Occupational Safety and Health (NIOSH) Method 5506 (NIOSH, 1998) for PAHs by HPLC-FD; NIOSH Method 5515 (NIOSH, 1998) for PAHs by GC-FID; and Method 5800 (NIOSH, 1998) for PAHs by HPLC-FD with supplementary clean-up. However, these methods present a poor clean-up scheme, limited theoretical plate-separation power and weak selectivity of FID to achieve the reliable determination of PAHs in such complex matrices. HPLC provides a useful fractionation technique for isolating PAHs from complex sample mixtures and allows quantification with selective detectors after further separation, for example, by GC-MS (Vu-Duc *et al.*, 1995, 2007). Individual PAHs in bitumen emissions may be analysed using intensive clean-up procedures followed by GC-ion trap MS (Huynh *et al.*, 2007).

The development of standard reference materials with certified values for PAHs in complex environmental matrices allows evaluation of new analytical techniques (Wise *et al.*, 1993; Vu-Duc *et al.*, 1995; Schubert *et al.*, 2003). Intensive clean-up procedures followed by GC-MS analytical methods were proposed to overcome the difficulties of quantification of PAHs in bitumens (Vu-Duc *et al.*, 1995, 2007; Sauvain *et al.*, 2001; Huynh *et al.*, 2007). Sulfur-containing PAHs were also identified and quantified by such methods (Binet *et al.*, 2002; Vu-Duc *et al.*, 2007) (see *Monograph on Some* N- *and* S-*heterocyclic polycyclic aromatic hydrocarbons*, in this volume).

1.2.2 Bitumen emissions

Bitumen emissions are defined as complex mixtures of aerosols, vapours and gases from heated bitumens and products that contain bitumen. Although the term "bitumen fume" is often used in reference to total emissions, bitumen fume refers here only to the aerosolized fraction of total emissions (i.e. solid particulate matter, condensed vapour and liquid bitumen droplets). Accordingly, the term "bitumen emissions" is more appropriate for referring to total content of bitumens in air.

A variety of methods for sample collection and analysis are available for evaluating bitumen emissions. Originally, methods focused on inhalable particulates (aerosols fraction) and its solvent extractable fraction. More recent methods address both the aerosol fraction and the vapour fraction. The chemical composition (e.g. PAHs) of the collected fractions can then be determined.

All the following methods use an active sampling technique with personal air pumps to draw air through a sampling medium. The standardized NIOSH Method 5042 has been developed for determination of total particulate matter (TPM) and benzene-soluble fraction (BSF) or cyclohexane-soluble fraction collected on filters only (NIOSH, 1998). Each fraction is analysed separately by gravimetric analysis. A sampling method based on infrared spectrophotometric absorption of aerosols and vapours of bitumen emissions (filter plus XAD-2 cartridge) was developed in Germany (IFA method 6305). This method uses bitumen condensate as a reference standard (DFG, 2011). In the USA, NIOSH method 5506 uses a filter followed by an XAD-2 tube to capture the vapour fraction. This method uses HPLC fluorescence to analyse the PAHs (NIOSH, 1998).

A field study was performed to compare the IFA method 6305 and a modified NIOSH 5506 method using GC-time of flight-MS instead of

HPLC (Kriech *et al.*, 2010). The resulting concentrations from the aerosol, vapours, and aerosol plus vapour fractions showed strong correlation, but the absolute values were higher with the NIOSH method.

Individual PAHs in bitumen emissions may be analysed using an intensive clean-up procedure followed by GC-ion trap MS (Huynh *et al.*, 2007). Luminescence spectroscopy was used as an alternative method to quantify, without identification, a subset of PACs in condensates of bitumen fume (Osborn *et al.*, 2001).

1.2.3 Dermal exposure to PAHs

Unlike for air sampling, there are no standard methods for the assessment of dermal exposure. Dermal exposure to PAHs from bitumens can be measured based on sampling by hand washing or pads on the skin followed by PAH analysis by HPLC or GC-MS.

In the hand-washing method, hands are washed before and after the working shift with 3 mL sunflower oil which is rubbed on the hands for 1 minute. The oil is then wiped with a cleaning tissue, which is extracted with dichloromethane. PAHs are analysed with HPLC-FD (Jongeneelen *et al.*, 1988).

In the exposure-pad method, polypropylene filters are attached to both wrists of the worker for the whole working shift. After sampling the filters are extracted with a mixture of cyclohexane and dichloromethane. PAHs are analysed by GC-MS (Jongeneelen *et al.*, 1988).

1.2.4 Biomonitoring of PAHs

Uptake of PAHs by inhalation and dermal contact can be monitored by measuring the concentration of metabolites of PAHs in the urine of exposed workers.

Urinary 1-hydroxypyrene (1-OHP) is a metabolite of pyrene, a compound commonly detected in bitumen emissions. Urine samples are hydrolysed enzymatically, purified in solid-phase and 1-OHP is analysed by HPLC-FD (Jongeneelen *et al.*, 1988; Lintelmann *et al.*, 1994).

The same method can be used to determine the main metabolites (i.e. 1-, 2-, 3-, 4- and 9-hydroxyphenanthrenes) of phenanthrene, another major PAH in bitumen emissions.

Another major PAH in bitumen emissions is naphthalene, which is metabolized to 1- and 2-naphthol. Urine samples are hydrolysed with an acid, purified in the solid phase, and naphtols are analysed by GC-MS (Keimig & Morgan, 1986). Alternatively, enzymatic hydrolysis and HPLC-FD can be used (Hansen *et al.*, 1992).

Unmetabolized PAHs (naphthalene, phenanthrene and anthracene) can be found in urine and may be analysed by head-space solid-phase microextraction coupled with GC-MS (Sobus *et al.*, 2009a).

1.3 Production and use

1.3.1 Production volumes

The widespread availability of bitumens resulting from oil refining is a comparatively modern development. Bitumens have been produced in the USA by vacuum distillation of crude petroleum since 1902, when 18 000 tonnes were produced. By 1907, the quantity made from this source equalled the amount recovered from sources of natural bitumen (IARC, 1985). By 1938, annual consumption had grown to 5 million tonnes (Chipperfield, 1984). Production in the USA reached 29 million tonnes in 1978, but dropped steadily to 20 million tonnes in 1982 (IARC, 1985). By 2000, bitumen production had risen again, reaching 30 million tonnes for paving and non-paving applications (IPCS, 2004). Several European countries were producing substantial quantities of bitumens by the 1920s and experienced similar increases thereafter. Table 1.12 summarizes the estimated quantity of bitumens used between 1960 and

Table 1.12 Annual use of bitumens by country (million tonnes)

Country	Year				
	1960	1976	1980	1982	2004
Austria	0.1	0.6	0.6	0.5	NA
Belgium, the Netherlands, Luxembourg	0.3	1.3	1.1	0.8	NA
Canada	1.5	2.8	3.4	NA	3.0
Finland	NA	NA	NA	0.4	0.3
Denmark, Norway, Sweden	0.4	1.3	1.0	1.3	0.9
France	1.2	3.1	2.8	2.4	4.2
Germany	1.4	3.9	3.4	3.0	2.3
Italy	0.6	NA	1.9	1.9	NA
Japan	NA	NA	4.7	4.4	3.0
New Zealand	0.1	0.1	0.1	0.1	0.2
South Africa	0.1	0.3	0.3	0.3	0.3
United Kingdom of Great Britain and Northern Ireland	1.1	1.9	1.8	2.0	2.3
United States of America	18.9	25.5	27.3	23.2	27.4

NA, not available
From IARC (1985) and IBEF (2006)

2004 in selected countries. Table 1.13 provides a more complete overview of global consumption by country in 2004 and 2005 (estimated). As of 2007, approximately 85% of bitumens were being used in paving applications, 10% in roofing applications, and 5% in other specialized applications such as waterproofing, insulation, and pipe coatings (Asphalt Institute & Eurobitume, 2011). Table 1.14 provides an estimate of the pattern of bitumen use by class. It is estimated that the current annual world use of bitumens is more than 102 million tonnes (Asphalt Institute & Eurobitume, 2011).

1.3.2 Production processes

Bitumens are derived from the distillation of crude petroleum oils that give substantial amounts of heavy residue, typically from 10–50%, although crude oils giving a greater yield of residue are sometimes used. While the manufacturing process can alter the physical properties of bitumens, the chemical properties do not change unless thermal cracking [breakage of bitumen molecules at high temperatures] occurs as in the production of class 6 bitumens (NIOSH, 2001a).

The processes used in bitumen production are summarized below and illustrated in Fig. 1.1 (for a more detailed summary of these processes, see Chipperfield, 1984; NIOSH, 2001a).

(a) Distillation

The first stage in oil refining is atmospheric distillation. Crude petroleum is heated to 340–400 °C (644–752 °F) and introduced at atmospheric pressures into a distillation tower in which the most volatile components vapourize. More volatile components rise higher in the tower than less volatile components. When the temperature drops below the boiling-point of a specific component, that component condenses and is collected in a tray. The remaining residuum is called "straight-reduced bitumen" (Roberts *et al.*, 1996; Speight, 2000). Raising the temperature to 400–560 °C increases the likelihood of cracking and causes the more volatile components (and even the components with higher boiling-points) to be released from the residuum.

Table 1.13 Annual consumption of straight-run bitumens (class 1) and bitumen emulsions (class 4)

Country	Annual consumption (tonnes)			
	2004		Estimation 2009[a]	
	Paving bitumens	Bitumen emulsions	Paving bitumens	Bitumen emulsions
Afghanistan	NR	NR	500	NR
Algeria	NR	NR	950 000	35 000
Angola	NR	NR	45 000	3 000
Argentina	328 000	20 000	500 000	74 000
Australia	NR	NR	[b]849 000	[b]80 000
Austria	NR	20 500	[b]500 000	[b]20 000
Bangladesh	NR	NR	50 000	NR
Belgium	209 000	9 300	200 000	6 700
Benin	34 400	650	[b]10 000	[b]1 500
Botswana	30 000	12 000	20 000	4 000
Brazil	1 200 000	400 000	1 600 000	[b]440 000
Bulgaria	132 000	5 014	[b]140 000	[b]5 000
Cambodia	11 000	5 200	24 000	9 000
Cameroon	NR	NR	16 000	NR
Canada	2 675 000	350 000	3 450 000	[b]250 000
Chile	180 000	18 000	325 000	[b]30 000
China	NR	NR	15 200 000	418 000
Colombia	200 000	15 000	342 000	[b]55 000
Congo	NR	NR	15 000	1 500
Croatia	NR	NR	[b]200 000	5 000
Czech Republic	347 650	36 950	430 000	35 000
Democratic Republic of the Congo	NR	NR	18 000	NR
Denmark	180 000	22 000	140 000	14 000
Ecuador	180 000	10 000	236 000	23 000
Egypt	NR	NR	820 000	NR
El Salvador	36 000	3 200	31 000	1 800
Estonia	60 000	15 000	69 300	31 500
Finland	306 000	8 100	[b]300 000	[b]10 000
France	3 186 707	990 520	3 040 000	950 000
Gabon	NR	NR	5 000	2 500
Germany	2 170 000	120 000	3 800 000	170 000
Greece	NR	25 000	300 000	[b]10 000

Table 1.13 (continued)

Country	Annual consumption (tonnes)			
	2004		Estimation 2009[a]	
	Paving bitumens	Bitumen emulsions	Paving bitumens	Bitumen emulsions
Guatemala	60 000	3 500	63 000	3 500
Honduras	18 000	1 500	20 000	2 000
Hungary	160 000	5 310	160 000	15 000
Iceland	30 000	1 442	22 000	1 800
India	3 500 000	90 000	4 959 000	224 000
Indonesia	600 000	15 000	805 000	20 100
Iran, Islamic Republic of	NR	NR	2 400 000	45 000
Ireland	600 000	130 000	600 000	120 000
Israel	NR	NR	230 000	15 000
Italy	NR	NR	2 016 000	115 000
Japan	2 730 000	223 985	1 768 000	192 000
Kenya	6 500	2 000	13 000	4 000
Korea, Republic of	1 807 310	57 073	1 941 400	77 600
Lao People's Democratic Republic	7 500	444	12 000	3 000
Latvia	55 100	13 500	[b]30 000	5 000
Lithuania	NR	NR	3 700	2 000
Madagascar	NR	4 500	[b]3 000	[b]1 000
Malaysia	857 000	36 000	650 000	45 000
Mauritius	10 000	1 500	11 200	1 000
Mexico	1 400 000	620 000	1 851 000	650 000
Morocco	NR	62 000	363 000	78 000
Mozambique	25 000	1 500	10 000	2 500
Myanmar	NR	NR	10 000	1 000
Namibia	12 000	12 000	15 000	1 000
Nepal	NR	NR	30 000	12 000
Netherlands	NR	NR	300 000	30 000
New Zealand	160 000	20 000	171 000	14 000
Nicaragua	27 000	1 500	[b]30 000	2 000
Nigeria	NR	NR	186 000	NR
Norway	264 000	6 800	360 000	8 000
Oman	NR	NR	200 000	NR
Pakistan	NR	NR	324 000	4 200

Table 1.13 (continued)

Country	Annual consumption (tonnes)			
	2004		Estimation 2009[a]	
	Paving bitumens	Bitumen emulsions	Paving bitumens	Bitumen emulsions
Peru	NR	NR	7 300	1 700
Philippines	NR	NR	70 000	4 000
Poland	800 000	85 000	1 300 000	130 000
Portugal	611 608	66 171	450 000	40 000
Romania	290 000	37 000	270 000	48 000
Russian Federation	NR	NR	3 441 000	300 000
Saudi Arabia	NR	NR	500 000	75 000
Singapore	5 000	1 000	45 000	3 000
Slovakia	8 580	153	117 000	6 800
South Africa	250 000	80 000	415 000	75 000
Spain	1 200 000	347 623	1 950 000	265 000
Sri Lanka	NR	15 000	58 000	2 000
Sweden	375 000	64 000	507 000	64 000
Switzerland	NR	NR	160 000	17 000
Thailand	861 150	147 070	585 000	119 000
Tunisia	20 000	17 000	[b]160 000	[b]15 000
Turkey	133 000	80 000	2 000 000	56 020
United Arab Emirates	NR	NR	400 000	3 000
United Kingdom	2 196 000	149 842	1 370 000	135 000
United Republic of Tanzania	NR	NR	30 000	3 000
United States of America	25 000 000	2 400 000	[b]20 352 000	2 250 000
Uruguay	60 000	3 000	[b]60 000	[b]3 000
Venezuela	120 000	12 000	300 000	[b]10 000
Viet Nam	30 000	18 000	495 000	14 000
Zambia	8 000	2 400	10 000	3 300
Zimbabwe	2 500		8 000	100
Total	**57 463 005**	**7 031 247**	**88 238 400**	**2 263 000**

[a] Data not consolidated, presented at World Congress on Emulsion, October 2010.
[b] Estimation based on incomplete information from that country.
NR, not reported
From IBEF (2006)

Table 1.14 Bitumen use pattern (%) by class

Class	Western Europe	Japan	USA
Straight-run bitumens [class 1]	74	86	70
Oxidized bitumens [class 2]	12	6	13
Cutback bitumens [class 3]	8	7[a]	10
Bitumen emulsions [class 4]	4		7
Modified bitumens [class 5]	< 2	< 1	0

[a] Classes 3 and 4 combined
Adapted from IARC (1985)

The atmospheric residue of very heavy crude oils is sometimes used for bitumen production and is generally distilled further to yield various products. Atmospheric distillation followed by vacuum distillation of the residuum helps separate remaining volatile components with higher boiling points. The use of vacuum distillation prevents thermal degradation of distillates and residue by reducing pressure. The resulting products are called "vacuum-processed bitumens" (NIOSH, 2001a).

Vacuum residues from particular crude oils meet specification requirements for straight-run bitumens (class 1). Steam is sometimes injected into the residue to aid distillation in a process known as steam stripping, and bitumens produced in this way are referred to as "vacuum-processed, steam-refined" (class 1).

(b) Air blowing

Oxidized bitumens (class 2) are produced by extended air blowing of vacuum residues, propane-precipitated bitumens, or mixtures of vacuum residues and atmospheric residues or waxy distillates. Catalysts such as ferric chloride (0–2%) and phosphorus pentoxide (0–4%) are used in a few refineries to speed the reaction or to modify the properties of the resultant bitumens, referred to as "catalytic air-blown bitumens" (class 2) (Speight, 2000). The blowing process dehydrogenates the residue, resulting in oxidation and condensation polymerization. The content of asphaltenes is considerably increased, while the content of cyclics is decreased (IARC, 1985).

Limited air blowing, known as "air rectification", may be used to produce bitumens for paving or industrial uses with properties similar to those of class 1 (Asphalt Institute & Eurobitume, 2011).

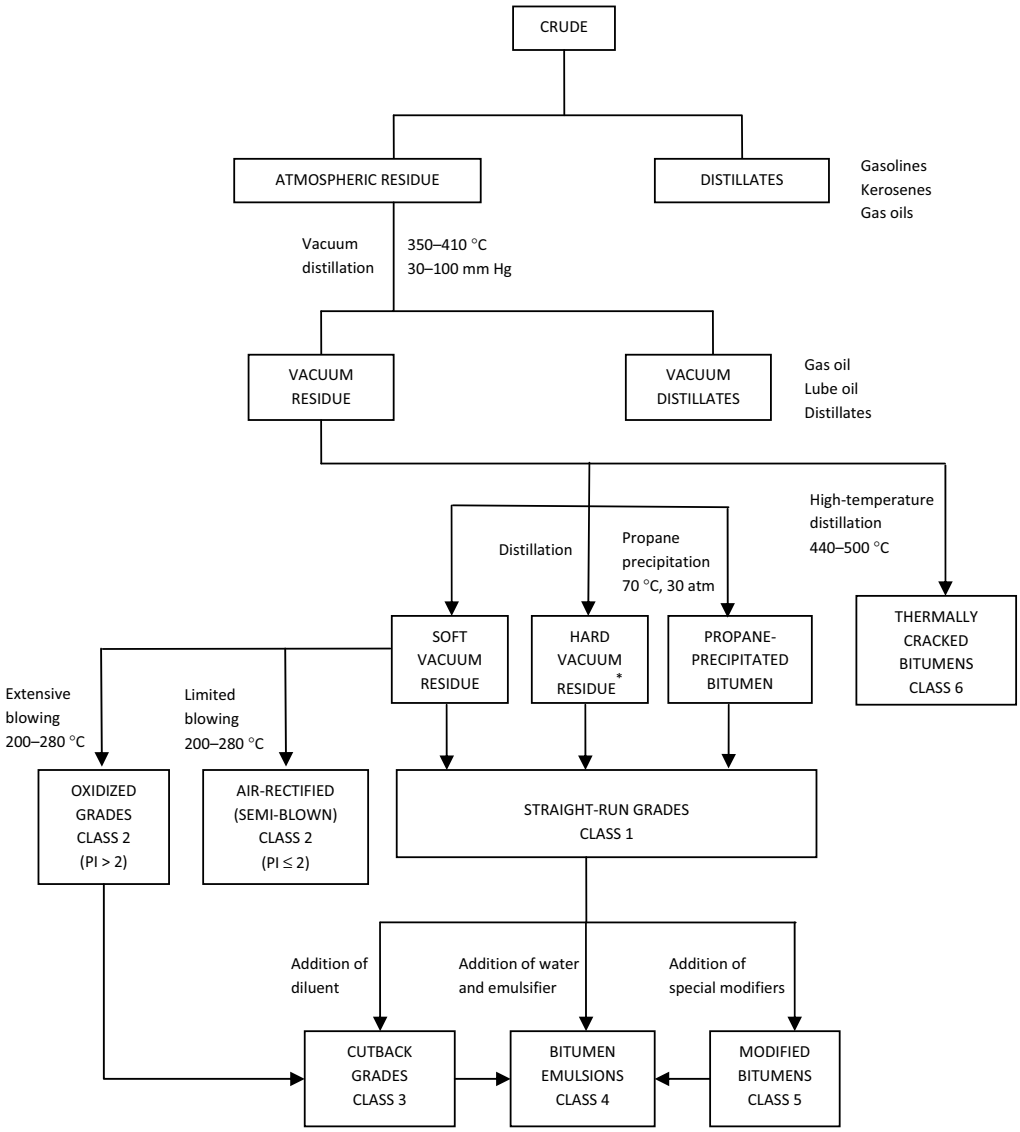
(c) Solvent precipitation

Some crude oils contain components of high boiling-point that are difficult to recover even when high vacuum is used. Such materials are therefore separated from the vacuum residue using solvent precipitation, usually with propane or butane. The resulting product precipitated is called "propane-precipitated bitumen", although in a strict sense this is a class 1 bitumen as defined in this *Monograph*. In the USA, propane-precipitated bitumens are also referred to as "solvent-refined asphalt" or "propane-derived asphalt". Solvent-precipitated bitumens, which are harder and less resistant to temperature changes than other bitumens, have a higher content of asphaltenes than the vacuum residues from which they are produced, but a lower content of saturates than would be obtained by distillation of the vacuum residue (IARC, 1985).

(d) Transportation and storage

Class 1 and class 2 bitumens are normally delivered in bulk by pipeline, tanker truck, or railcar, in liquid form at temperatures of 90–230 °C, depending on the type of bitumen and local practice. Cutback bitumens are usually

Fig. 1.1 Main processing methods in the production of bitumens



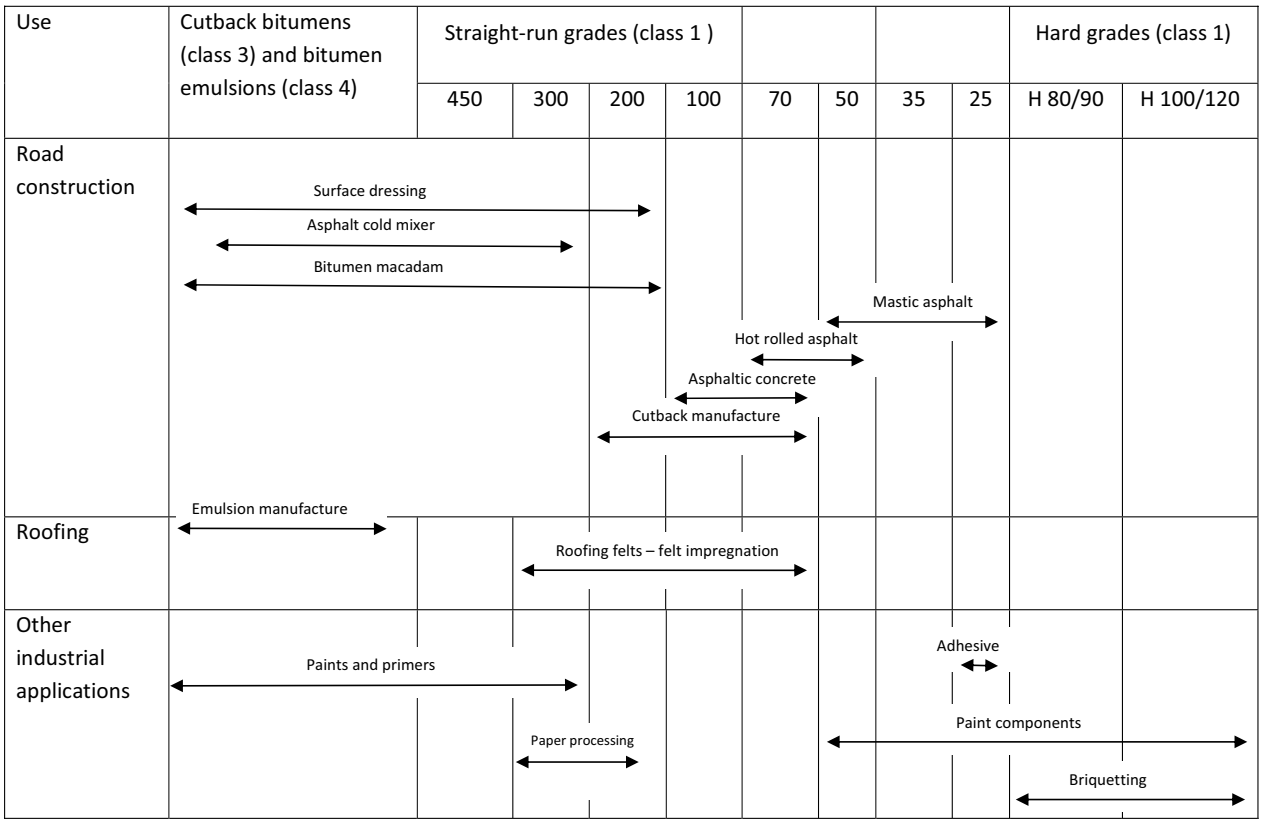
* Used for mastic asphalt
Compiled by the Working Group

stored at 50–80 °C, although storage temperatures of up to 230 °C have been noted. Lower temperatures are usually maintained with steam coils in the tanks. Emulsions are stored and transported between 20 °C and 90 °C. Saturated and coating bitumens are normally stored at 200–260 °C (NIOSH, 2001b; NAPA & EAPA, 2011).

1.3.3 Uses

The major applications of bitumen are in paving for roads and airfields, hydraulic uses (such as dams, water reservoirs and sea-defence works), roofing, flooring and protection of metals against corrosion. More than 80% of bitumens are used in the many different forms of road construction and maintenance. Fig 1.2a describes the principal uses of cutback and

Fig. 1.2a Principal uses of cutback, straight-run and hard bitumens, and bitumen emulsions



Compiled by the Working Group

emulsions, straight-run and hard bitumens, and Fig 1.2b describes the principal uses of oxidized bitumens.

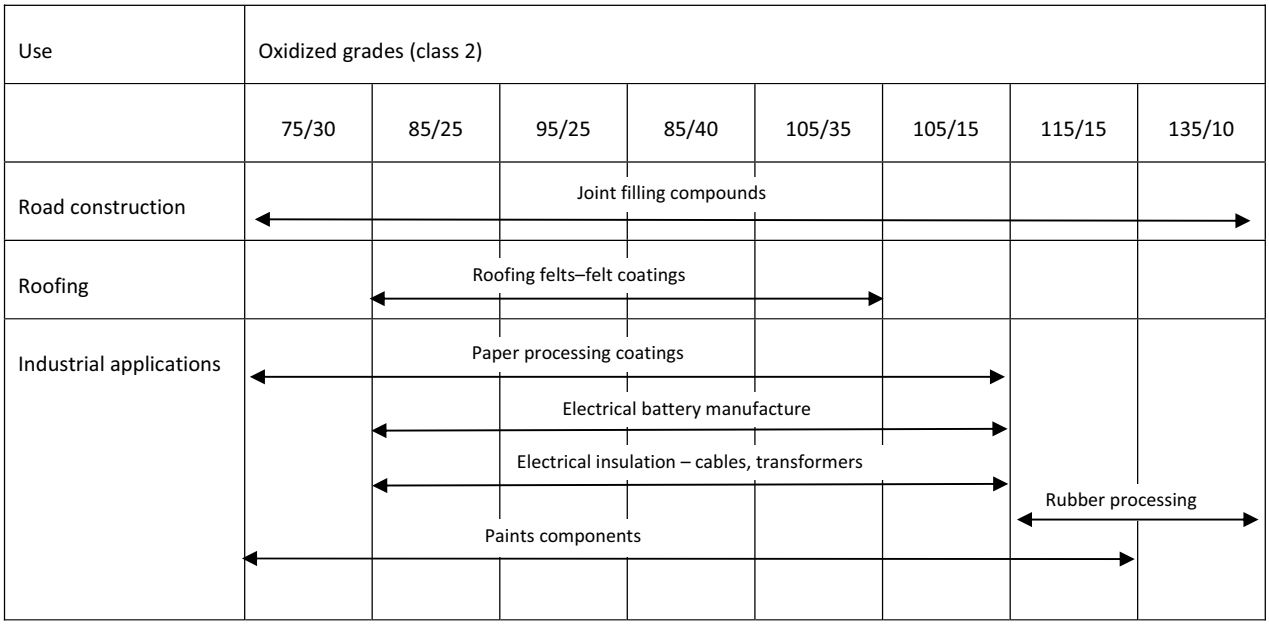
(a) Manufacture of products containing bitumen

The manufacture of roofing felt is based on the use of hot straight-run bitumens (class 1, typically 200-PEN) to impregnate, during immersion, a dry felt made from waste paper or rags. A surface coating is then applied to both sides of the saturated felt using oxidized bitumens (class 2, e.g. 85/40 or 105/35), which sometimes contains added filler. Impregnated felts are also used for damp-proof courses in masonry. Oxidized bitumens are also used in roofing applications such as shingles.

(b) Paving

Asphalt mixes are manufactured by heating and drying mixtures of graded crushed stone, sand and filler (the mineral aggregate) and mixing with straight-run or air-rectified bitumens (typically 4–10% by weight), which serve mainly as a binder to hold the aggregate together. At the construction site, the asphalt mix is fed through a mechanical laying machine, which spreads and compacts the mix. The application temperature of the hot mix asphalt is usually between 112 °C and 162 °C (NIOSH, 2001a). Asphalt mixes include asphaltic concrete, bitumen macadams, and hot rolled asphalts. Special techniques can be adopted to mix aggregate or sands with cutback bitumens (class 3) or emulsions (class 4). These may be carried out with only minor heating or at

Fig. 1.2b Principal uses of oxidized bitumens



Compiled by the Working Group

ambient temperature and are therefore referred to as "asphalt cold mixes".

Although straight-run and air-rectified bitumens are the main types used in paving asphalt mixes, as described above, cutback (class 3) and emulsified (class 4) bitumens are commonly used to provide a waterproof layer under new pavement surfaces and sometimes to improve bonding between various layers of asphalt pavement. They are also used in some surface sealing applications and to produce a cold-mix patching material (NAPA & EAPA, 2011).

Bitumens are laid onto roads by a placement and compaction crew of about five to nine people. These jobs, as pictured in Fig. 1.3, include paver operators, screed operators, labourers/rakers and roller operators. Paver operators (pavers, paving machine operators) drive the paver machine, which receives asphalt from delivery trucks and distributes it on the road in preparation for the roller machine. Screed operators work behind the paver, controlling the even spread of the asphalt mat with a spreading augur before compaction. Mobile rakers work behind the paver, shovelling and raking excess asphalt material to fill in voids and prepare joints for rolling. Labourers often work as rakers, but also handle other tasks that may be more removed from the asphalt fume. Roller operators (rollers) drive the machinery that compacts the asphalt mat and have the mobility to work at varying distances from the paving machine. A foreman supervises the crew, often coming into close proximity to the screed (NAPA & EAPA, 2011).

In place of new mineral aggregates and bitumens, reclaimed asphalt pavement is commonly added to asphalt mix for use in highway pavements and other applications. The proportion of reclaimed asphalt pavement used depends on several factors, but can contribute to as much as 30% of highway mixtures and 60% in other applications (NAPA & EAPA, 2011). Specifications vary in the amount of reclaimed asphalt pavement allowed for particular pavements. Regulations prohibit the recycling of reclaimed asphalt pavement with a given coal-tar

Fig. 1.3 Typical job composition of a road paving crew

Courtesy of the National Asphalt Pavement Association

content in most European countries and in the USA (see Section 1.5.2).

Surface dressing and surface treatments are used to seal minor roads (i.e. low traffic volume) or to maintain road surfaces that have suffered abrasion and loss of skid resistance. Straight-run bitumens (class 1), cutbacks (class 3) or emulsions (class 4) are sprayed onto the surface being treated to give a uniform film to which chippings are applied, followed by light rolling.

Coal tar, which is similar in appearance to bitumen, was used in global paving industries until the 1990s. Coal tar is a by-product of processing coal by thermal degradation in a coking plant and of making oil from coal in the Fischer–Tropsch process. As a result of these two processes, coal tar has a much higher PAH content than bitumen, which is produced by petroleum refining. The differential use of coal tar in different countries worldwide was based mostly on economics and on the availability of bitumen. In Europe, for example, coal tar was blended with bitumen and used in all layers of paving until oil production increased and coal fell out of economic favour in the 1970s and 1980s. Coal tar was eventually phased out in Europe in the 1990s and controls were put in place to prevent coal tar from re-entering pavement as a result of recycling. Coal tar has not been widely used in the USA since the Second World War

Fig. 1.4a Example of cold-applied bitumen

Courtesy of the National Roofing Contractors Association

and is limited to a few non-road applications, such as a sealer in airfield pavement (NAPA & EAPA, 2011). More information on use of coal tar is available in *IARC Monograph* Volume 92 (IARC, 2010).

(c) Roofing

The roofing industry primarily uses oxidized bitumens (class 2) in applications that vary widely according to the type of roofing product and application temperature. Bitumen roofing products can be cold-applied (e.g. bitumen shingles on steep-sloped roofs of residential buildings), soft-applied (e.g. bitumen membranes on low-sloped roofs), or hot-applied (i.e. hot liquid bitumens as the bonding agent on gently sloping roofs) (Asphalt Roofing Manufacturers Association, 2011). Over the past 20 years, cold-applied roofing systems have largely replaced hot-applied roofing. In Europe and North America, cold-applied bitumen accounts for 81% of the production of bitumen roofing, while soft-applied (13%) and hot-applied (6%) bitumen are much less common (Asphalt Roofing Manufacturers Association, 2011).

In cold-applied roofing applications, workers install bitumen shingles using fasteners, typically roofing nails or staples. In soft-applied roofing applications, workers use either propane torches or hot-air welders to heat the polymer-modified bitumen membranes as the material is unrolled to ensure adequate adhesion to the other elements of the system (Asphalt Roofing Manufacturers Association, 2011). Fig 1.4a and

Fig. 1.4b Example of soft-applied bitumen

Courtesy of the National Roofing Contractors Association

Fig. 1.4b show roofing workers applying these types of roofing system.

In hot-applied roofing, bitumens are typically heated on-site in a kettle (180–230 °C) and pumped to the roof via a supply line or brought to the roof in buckets. A worker remains on the ground and tends the kettle (i.e. filling, fluid and temperature checks, and skimming-off debris), while other workers tear off the old roof and put down the new roof. The rooftop workers first tear off the old roof, which is typically an old bitumen roof that may or may not contain coal tar. Once the bitumen has been delivered to the rooftop, it may be drawn directly into mop carts or buckets for manual installation jobs in which it is applied much like mopping a floor. Fig 1.4c and Fig. 1.4d show workers applying a hot bitumen roof (Asphalt Roofing Manufacturers Association, 2011).

(d) Mastic-asphalt applications

Mastic asphalt is a mixture of relatively hard-grade, straight-run bitumens (class 1), coarse aggregate, and/or sand, and/or limestone fine aggregate, and/or filler. Mastic asphalt may also contain additives (polymers, natural bitumens, wax or pigments) (class 5). Its application temperature is high, usually 200–250 °C. It is pourable, spreads well when hot, and forms a waterproof and durable surface (Fig. 1.5; European Mastic Asphalt Association, 2009).

Mastic asphalts are used in Europe, but are practically nonexistent in the USA and Canada. Their use in Asia has begun to grow recently. They are used in bridge decks, as flooring in houses and industrial buildings, in heavy traffic motorways, rooftop car parks, hydraulic constructions (canal slopes, riverbanks) and in flat-roof waterproofing (European Mastic Asphalt Association, 2009).

Fig. 1.4c Example of hot-applied bitumen (kettleman)

Courtesy of the National Roofing Contractors Association

Fig. 1.4d Example of hot-applied bitumen (mopper)

Courtesy of the National Roofing Contractors Association

Fig. 1.5 Example of mastic-asphalt application

Courtesy of European Mastic Asphalt Association

Mastic asphalt was often manufactured in the past in mobile mixing plants at the work-site. Nowadays it is manufactured in specially designed stationary plants. From there it is transported to the processing location in mixers with a heating system mounted on a truck or chassis of a trailer. From the transportation mixer it is transferred into dumpers or carts. If necessary, it may also be poured into metal or wooden buckets or wheelbarrows to reach the actual processing site. Recently special pumps have been developed for the transfer of mastic asphalt to the application site. Both interior and exterior applications are often done manually. Mastic asphalt is hand-spread to the desired thickness and levelled with a wooden float or screed. Mechanical pavers are used for large surfaces, e.g. in the paving of highways. As a rule, the surface of mastic asphalt is coated with sand or aggregates. In road construction, aggregate pre-coated with bitumen is usually spread evenly and pressed into the still warm mastic asphalt (European Mastic Asphalt Association, 2009).

The term "stone-mastic asphalt" describes a paving mixture with a high stone content, used

in some countries. It should not be confused with the "mastic-asphalt application" described here.

(e) Other specialized applications

(i) Waterproofing

For waterproofing operations, polymer-modified bitumen membranes and bitumen paints that often contain a specialized cutback-bitumen product integrated with relatively small amounts of other materials are used. Emulsified bitumens that can be applied at lower or ambient temperatures have largely replaced cutback bitumens for this application (NAPA & EAPA, 2011).

(ii) Electrical and sound insulation

The electrical properties of bitumens (primarily class 2) enable them to be used in wrappings and jointing compounds for heavy-duty cables. Bitumens (classes 2 and 5) find wide use for sound insulation, e.g. in car bodies and floor mats, and in floor mountings for factory machinery (NAPA & EAPA, 2011).

(iii) Pipe coatings

To protect pipelines for oil, water, etc. coatings of bitumen enamel are applied to the cleaned, primed, metal surface. The enamel is made of oxidized bitumens (class 2, e.g. 115/15) with the addition of up to 30% of an inert filler, such as slate dust. The primer is a cutback (class 3) of the oxidized bitumens with a volatile solvent (white spirit/mineral or white spirit) (NAPA & EAPA, 2011).

(iv) Briquettes

Briquetting was previously a process by which fine materials (e.g. coal dusts, metal tailings) were mixed with a bitumen binder to form conveniently handled blocks or pellets for use as fuel in the metal industry and in power plants. The most suitable bitumens for this purpose were hard and of a low PI, e.g. 85/2 or 90/1 (softening-point/penetration at 25 °C). Other grades, such as 15-PEN or H 80/90, may also have been used (NAPA & EAPA, 2011).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

(a) Natural occurrence

Natural bitumens are widespread in regions where oil-bearing rocks occur on or not far below the Earth's surface, and seep spontaneously to the surface. Bitumen products occur naturally as rock asphalt deposits such as uintahite (from Utah, USA) and as lake asphalt (e.g. in Trinidad). Deposits of natural bitumen occur around the world, including Pitch Lake, Trinidad; the Dead Sea; Venezuela; and Switzerland (IPCS, 2004) (see Section 1.1.5).

(b) Air

Releases from bitumens into the air occur in the vicinity of hot-mix asphalt plants and road-laying operations, and near factories. Bitumen-producing refineries are also a source of releases into the air. In the production of roofing felts, emissions of particulates including bitumen fume were found to be 1.35 mg/g bitumens for controlled and 3.15 mg/g bitumens for uncontrolled conditions. In a bitumen-blowing operation, releases of particulates into the air ranged from 0.29 to 3.65 mg/g bitumens for well controlled and uncontrolled operations, respectively (EPA, 1978).

Kebin *et al.* (1996) reported on the percentage of polar, aromatic and saturated fractions measured in air samples collected 2–84 m from a highway in Denmark. [The Working Group noted that diesel and gasoline exhaust from traffic, and residues from tyre abrasion, were likely to have contributed to the composition of these fractions.]

(c) Soil and sediment

PAHs and trace elements were found in creek-bed sediment near a seal-coated parking lot in Austin, Texas.

In an experimental setting, parking lots and test plots sealed with coal tar, with "asphalt", or left unsealed, and unsealed concrete [asphalt] pavements were sprayed with distilled deionized water to simulate rainfall and the wash-off was collected for analyses. The highest PAH levels were reported for effluent from pavement sealed with coal-tar emulsion, followed by bitumen-sealed and unsealed pavements (US Department of the Interior, 2004).

(d) Water

Bitumen emissions can end up in water through surface runoff from land, and fallout and rainout from the atmosphere. Concentrations of PAHs and selected heavy metals were determined in water samples collected from water draining from road surfaces and from water upstream and downstream from the point of discharge from road surfaces into stream sites in California, USA. The concentrations of PAHs in all stream and road runoff samples were below the detection limit of 0.5 µg/L (Cooper & Kratz, 1997).

Leaching tests of bitumen-based materials have been conducted in laboratories. Six samples of paving bitumen and four samples of roofing bitumen were leached according to the United States Environmental Protection Agency (EPA) method SW846–1311 (Kriech *et al.*, 2002a). None of the roofing samples tested leached any of the 29 PAHs analysed. Four of the paving samples did not leach any of the 29 PAHs, and the leachates of two paving samples contained detectable amounts of naphthalene and phenanthrene. The levels were below the detection limit of 0.1 µg/L, except for naphthalene with a value of 0.18 µg/L.

Leaching tests on samples of reclaimed asphalt pavement from Florida, USA, detected none of 16 EPA-priority pollutant PAHs in the leachates of these samples (Brantley & Townsend, 1999). The concentrations of PAHs with more than two rings in leachate water from ten samples of bitumen and asphalt were 4–50 ng/L (Brandt & de Groot, 2001).

(e) Food vegetation

Kebin *et al.* (1996) reported on the percentage of polar, aromatic and saturated fractions measured in plant samples collected at 5–10 m from a main road in Denmark. [The Working Group noted that diesel and gasoline exhaust from traffic, and residues from tyre abrasion, were likely to have contributed to the composition of these fractions.]

1.4.2 Occupational exposures

(a) Number of workers exposed

No reliable estimates were available to the Working Group concerning the number of workers exposed to bitumen. It is most likely that the largest number of workers is exposed in road-paving and roofing operations. Furthermore, occupational exposures occur in the production of bitumen, production of roofing material and in asphalt-mixing plants. Conservative estimates stemming from the early 2000s for western Europe mention 4000 asphalt-mixing plants and 100 000 paving crewmen (Boffetta & Burstyn, 2003). In 2007 the estimates of number of workers employed in the hot-mix asphalt industry in the USA, as presented by the National Asphalt Pavement Association, totalled 300 000 individuals. The number of mixing plants was estimated at 4000 (Acott, 2007). In Delhi, India, alone some 25 hot-mix plants are currently in operation (Chauhan *et al.*, 2010). Paving is a worldwide activity that is reflected in the presence of 400 mixing plants in Mexico, 60 in South Africa and 45 in New Zealand. In China, more than 6500 small plants exist that produce about one third of the volume produced in Europe (NAPA & EAPA, 2011).

In Europe, the statistical office Eurostat provides figures for the number of workers employed in construction of roads and railways (NACE 42.1), construction of utility projects (42.3) and roofing activities (NACE 43.91) (Eurostat, 2010). For the first quarter of 2011, these numbers totalled more than 10 million workers. Of these workers, approximately 10% were women.

(b) Occupational exposure to bitumen and its emissions

Different sampling methods (e.g. different inhalable samplers used), analytical methods and measurement strategies (short-duration, shift-long, personal *versus* static sampling) have been used across countries and over time, which complicates interpretation of time trends and differences between regions and countries. In the following sections, recent exposures to bitumen emissions and its specific ingredients are described for workers involved in bitumen-production plants, in the production of bitumen-containing materials, and for workers applying bitumen and bitumen products in road paving, roofing, and more specialized applications such as mastic flooring and waterproofing. Data on historical exposure up to 1984 are reported in a previous *IARC Monograph* (IARC, 1985).

A review by NIOSH (2001a) presents a detailed picture of exposure to bitumens in the USA. Analysis of data from a large number of studies of exposure suggested that personal exposures to bitumen fume (measured as TPM) were highest during asphalt flooring and waterproofing activities, followed by manufacture of roofing products, bitumen refining, roofing application and at asphalt-mixing plants, with lowest exposures to bitumen fume encountered during road-paving activities. The BSF of the collected fume showed a similar pattern.

In Europe, Rühl *et al.* (2006) reported on 1272 samples (mainly 2-hour task-based measurements) collected in Germany between 1991 and 2005. Higher inhalation exposures to bitumen emissions were observed for workers engaged in (indoor) laying of mastic asphalt. Roofing work was next, followed by hot-mix paving and work in the mixing plant. Production of bitumen and industrial production of bitumen-containing products (e.g. sheets and shingles) showed median concentrations of bitumen fume that were mainly < 1 mg/m^3.

Results from personal measurements of inhalation and dermal exposure of workers exposed to bitumen and its emissions during production and use of bitumen are presented in Tables 1.15 and Table 1.16, respectively, by industry and application. Table 1.17 summarizes the results of biomonitoring of urinary 1-OHP.

(i) Production and transport of bitumens

Boogaard (2007) reported the exposure of process operators at a refinery producing bitumen monitored in three separate surveys. The arithmetic mean urinary concentrations of 1-OHP of the operators were relatively low and varied between 0.12 and 0.17 µmol/mol creatinine. A recent study from France focusing on workers transporting bitumen showed (model based on task-based measurements of exposure during loadings) average concentrations in fumes as follows: total PAHs, 3.51 ng/m^3; benzo[*a*]pyrene, 2.3 ng/m^3; and pyrene, 5.7 ng/m^3 (Deygout *et al.*, 2011).

(ii) Production of bitumen-containing materials

In the USA, a study among workers in 19 plants manufacturing bitumen-roofing products involved the use of bitumens of class 1 and class 2 (Calzavara *et al.*, 2003). The reported average concentration of TPM was relatively high at 2.47 mg/m^3, but the average BSF concentration was relatively low (0.11 mg/m^3).

(iii) Road paving

For a large European multicentre epidemiological study, Burstyn *et al.* (2000a, b) built a large exposure-measurement database

Table 1.15 Inhalation exposure of workers exposed to bitumens and bitumen fume by inhalation, by type of production and application

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Bitumen production										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	64			0.5	10	mg/m^3
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	6			0.2	2.9	mg/m^3
Hicks (1995)		USA	TPM	7–9 h	44		0.18 (3.7)			mg/m^3
			RPM	7–9 h	8		0.26 (3.6)			mg/m^3
			BSF	7–9 h	44		0.16 (3.7)		13	mg/m^3
			PYR	7–9 h	9		All < LOD			µg/m^3
			BaP	7–9 h	44		0.15 (1.3)			µg/m^3
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	4	0.4		< 0.1	1.0	mg/m^3
			11-PAHs	109–437 min	4	33		3.8	95	ng/m^3
Deygout *et al.* (2011)		France	16-PAHs	466 min	6	0.31	0.23			µg/m^3
			NAP	466 min	6	2 755	1 894			ng/m^3
			PYR	466 min	6	5.7	5.4			ng/m^3
Posniak (2005)		Poland	PYR	Full shift	3	0.003		ND	0.01	µg/m^3
			BaP	Full shift	3	0.007		ND	0.013	µg/m^3
Deygout *et al.* (2011)		France	BaP	466 min	6	2.3	2.2			ng/m^3
Roofing material manufacturing										
Hicks (1995)		USA	TPM	405 min	34		1.4 (3)		13	mg/m^3
			BSF	405 min	34		0.27 (4.4)		3.7	mg/m^3
Gamble *et al.* (1999)		USA	TPM	405 min	77		0.6		6.2	mg/m^3
			BSF	405 min	77		0.08		1.3	mg/m^3
Calzavara *et al.* (2003)		USA	TPM	405 min	58		2.47 (2.51)	< 0.03	13.3	mg/m^3
			BSF	405 min	58		0.11 (0.08)	< 0.01	0.42	mg/m^3
Asphalt mixing and transport										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	80			0.25	45	g/m^3

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Hicks (1995)		USA	TPM	7–9 h	33		0.78 (2.8)			mg/m³
			RPM	7–9 h	6		0.24 (3.1)			mg/m³
			BSF	7–9 h	33		0.15 (2.8)			mg/m³
			PYR	7–9 h	8		All < LOD			µg/m³
			BaP	7–9 h	33		All < LOD			µg/m³
Conventional paving										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	298			0.12	15.5	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	12			0.2	15.1	mg/m³
Burstyn *et al.* (2000a)	1960–90	Europe	TPM	6–8 h	1 193	1.91	0.28	< LOD	260	mg/m³
			Vapoursz	6–8 h	510	7.59	1.86	< LOD	290	mg/m³
			BaP	6–8 h	487	95.8	8.58	< LOD	8 000	ng/m³
Heikkilä *et al.* (2002)	1992–96	Finland	Bitumen fume	6–8 h	70			0.01	3.9	mg/m³
			TPM	6–8 h	70			0.2	4.2	mg/m³
Deygout & Le Coutaller (2010)	2000–06	France	TPM	177–506 min	46	[0.43]	0.26 (2.73)	0.01	2.61	mg/m³
Hugener *et al.* (2009)		Switzerland	TPM					ND	3.8	µg/m³
Hicks (1995)		USA	TPM	7–9 h	37		0.37 (1.7)			mg/m³
Watts *et al.* (1998)	1994	USA	TPM	16 h				111	345	ng/m³
Burr *et al.* (2002)	1994–97	USA	TPM	8 h	78			0.01	0.89	mg/m³
Kriech *et al.* (2002b)		USA	TPM		44		0.23	0.09	0.64	mg/m³
Mickelsen *et al.* (2006)		USA	TPM	8 h	132	0.35		0.26	0.75	mg/m³
Hicks (1995)		USA	RPM	7–9 h	7		0.18 (1.5)			mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	11			0.1	0.5	mg/m³
Deygout & Le Coutaller (2010)	2000–06	France	BSF	177–506	45	[0.21]	0.13 (2.66)	< LoQ	3.35	mg/m³
Jongeneelen *et al.* (1988a)	1986	The Netherlands	CSM		27		0.2–0.6			mg/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Hicks (1995)		USA	BSF	7–9 h	37		0.24 (3)		3.7	mg/m^3
Burr *et al.* (2002)	1994–97	USA	BSF	8 h	32			0.01	0.82	mg/m^3
Kriech *et al.* (2002b)		USA	BSF		44		0.06	0.06	0.31	mg/m^3
Mickelsen *et al.* (2006)		USA	BSM	8 h	132	0.13		0.04	0.56	mg/m^3
Deygout & Le Coutaller (2010)	2000–06	France	Vapour fraction	177–506 min	37	[1.52]	0.75 (3.29)	0.05	11.13	mg/m^3
Kriech *et al.* (2002b)		USA	TOM		45		1.23	0.33	8.32	mg/m^3
Heikkilä *et al.* (2002)	1992–96	Finland	Total PAHs	6–8 h	65			0.15	52.6	µg/m^3
Väänänen *et al.* (2003)	1999–2000	Finland	Total PAHs	8 h	35		5.7	0.87	46	µg/m^3
Heikkilä *et al.* (2002)	1992–96	Finland	4–6 ring PAHs	6–8 h	65			< 0.05	0.93	µg/m^3
Campo *et al.* (2006)	2003	Italy	15 PAHs	4 h	147			127	2 973	ng/m^3
Hugener *et al.* (2009)		Switzerland	16 PAHs					36	510	µg/m^3
Burr *et al.* (2002)	1994–97	USA	2–3 ring PAHs	8 h	48			0.01	191	µg/m^3
			4–7 ring PAHs	8 h	48			0.01	25	µg/m^3
McClean *et al.* (2004a)	1999–2000	USA	Total PAHs	Full shift	109			0.3	40	µg/m^3
Campo *et al.* (2006)	2003	Italy	NAP	4 h	147			2	2 319	ng/m^3
Heikkilä *et al.* (2002)	1992–96	Finland	PYR	6–8 h	66	< 0.01–0.12				µg/m^3
Väänänen *et al.* (2003)	1999–2000	Finland	PYR	8 h	35			0.01	1.2	µg/m^3
Campo *et al.* (2006)	2003	Italy	PYR	4 h	147			< 0.6	282.2	ng/m^3
Posniak (2005)		Poland	PYR	Full shift	13	0.043		nd	0.24	µg/m^3

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Hicks (1995)		USA	PYR	7–9 h	9		0.17 (1.3)			µg/m³
Watts *et al.* (1998)	1994	USA	PYR	16 h				1.6	4.2	ng/m³
McClean *et al.* (2004a)	1999–2000	USA	PYR	Full shift	109			0.01	1.7	µg/m³
Heikkilä *et al.* (2002)	1992–96	Finland	BaP	6–8 h	66	< 0.01			< 0.01	µg/m³
Väänänen *et al.* (2003)	1999–2000	Finland	BaP	8 h	14	0.01–0.07	0.03	< 0.01	0.32	µg/m³
Campo *et al.* (2006)	2003	Italy	BaP	4 h	147			< 0.003	40.25	ng/m³
Posniak (2005)		Poland	BaP	Full shift	13	0.006		ND	0.034	µg/m³
Hugener *et al.* (2009)		Switzerland	BaP					0.003	0.2	µg/m³
Hicks (1995)		USA	BaP	7–9 h	37	all < LOD	NA			µg/m³
Watts *et al.* (1998)	1994	USA	BaP	16 h				0.9	4.4	ng/m³
McClean *et al.* (2004a)	1999–2000	USA	BaP	Full shift	109			0.01	0.03	µg/m³
Heikkilä *et al.* (2002)	1992–96	Finland	VOC	6–8 h	70			0.2	65.5	mg/m³
Modified asphalt paving										
Väänänen *et al.* (2006)	2003	Finland	Bitumen fume	4 h	20	0.13	0.11	0.05	0.29	mg/m³
			TPM	4 h	20	0.4	0.4	< LOQ	1.1	mg/m³
Watts *et al.* (1998)	1994	USA	TPM	16 h				164	389	ng/m³
Burr *et al.* (2002)	1994–97	USA	TPM	8 h	87			0.01	0.91	mg/m³
	1994–97	USA	BSF	8 h	37			0	0.75	mg/m³
Väänänen *et al.* (2006)	2003	Finland	Vapours	4 h	20	0.9	0.9	0.4	1.9	mg/m³
			Total PAHs	4 h	18	1.6	1.42	0.54	3.45	µg/m³
Burr *et al.* (2002)	1994–97	USA	2–3 ring PAHs	8 h	60			0.6	540	µg/m³
			4–7 ring PAHs	8 h	60			0.1	27	µg/m³
Väänänen *et al.* (2006)	2003	Finland	PYR	4 h	18		< 0.015	< 0.015	0.038	µg/m³
Watts *et al.* (1998)	1994	USA	PYR	16 h				4.5	57	ng/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Väänänen *et al.* (2006)	2003	Finland	BaP	4 h	18				< 0.01	µg/m³
Watts *et al.* (1998)	1994	USA	BaP	16 h				0.9	3.5	ng/m³
Roofing										
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	182			0.25	18.2	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	9			0.5	6.4	mg/m³
Hicks (1995)		USA	TPM	7–9 h	38		0.55 (2.5)			mg/m³
Kriech *et al.* (2004)		USA	TPM	5.1 h	35		0.94		10	mg/m³
Hicks (1995)		USA	RPM	7–9 h	6		0.15 (2.2)			mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	9			0.2	5.4	mg/m³
Hicks (1995)		USA	BSF	7–9 h	38		0.25 (3.4)			mg/m³
Kriech *et al.* (2004)		USA	BSF	5.1 h	35		0.33		9.6	mg/m³
Wolff *et al.* (1989)	1987	USA	Total PAHs	Full shift	13	5.8–23.0				µg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	11 PAHs	109–437 min	3			24	364	ng/m³
Wolff *et al.* (1989)	1987	USA	PYR	Full shift	18	2.6–5.4				µg/m³
Hicks (1995)		USA	PYR	7–9 h	18		0.21–0.34			µg/m³
			BaP	7–9 h	72		0.16			µg/m³
Wolff *et al.* (1989)	1987	USA	BaP	Full shift	18	0.9–1.5				µg/m³
Mastic										
Spickenheuer *et al.* (2011)	2001–08	Germany	Aerosols and vapours, tunnel	315 min		6	7.84			mg/m³
Rühl *et al.* (2006)	1991–2005	Germany	Aerosols and vapours	2 h	608			0.13	77	mg/m³
Breuer *et al.* (2011)	2001–08	Germany	Aerosols and vapours	315 min	320		3.46	LOQ	41.68	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	TPM	109–437 min	12			2.9	18.2	mg/m³

Table 1.15 (continued)

Reference	Time period	Country	Agent	Duration of sampling	*n*	AM	GM/Median (SD)	Min.	Max.	Unit
Breuer *et al.* (2011)	2001–08	Germany	TPM	315 min	320			LOQ	33.02	mg/m³
Brandt *et al.* (1985a, b)	1979–82	Europe	BSF	109–437 min	12			1.8	13.6	mg/m³
			11 PAHs	109–437 min	7			285	2 971	ng/m³
Heikkilä *et al.* (2002)	1992–96	Finland	BaP	6–8 h	2	0.01				µg/m³
			PYR	6–8 h	2	0.19				µg/m³
Joint filling										
Rühl *et al.* (2006)	1991–05	Germany	Aerosols and vapours	2 h			20	0.32	5.5	mg/m³

AM, arithmetic mean; BaP, benzo[*a*]pyrene; BSF, benzene-soluble fraction; CSM, cyclohexane-soluble matter; h, hour; LOD, limit of detection; LOQ, limit of quantification; Max., maximum; Min., minimum; min, minute; NA, not applicable; NAP, naphthalene; ND, not detected; PAHs, polycyclic aromatic hydrocarbons; PYR, pyrene; RPM, respirable particulate matter; TOM, total organic matter; TPM, total particulate matter; VOC, volatile organic compounds

Table 1.16 Dermal exposure of workers exposed to bitumens and bitumen fume, by type of production and application

Reference	Time period	Country	Agent	*n*	AM	GM/ Median	Min.	Max.	Unit
Mixing and conventional paving									
Väänänen *et al.* (2005)	1999–2000	Finland	Total native PAHs	22		4.6–10	1.8	78	ng/cm^2
			Total PAHs	30		7.8	0.71	63	ng/cm^2
Väänänen *et al.* (2006)	2003	Finland	Total PAHs	18	1.6	1.4	0.71	3.5	ng/cm^2
McClean *et al.* (2004a)	1999–2000	USA	Total PAHs, paving	59		89	< 38	751	ng/cm^2
			Total PAHs, milling	39		< LOD	< 38	757	ng/cm^2
			Total PAHs, road construction	11		45	< 38	246	ng/cm^2
			PYR, paving	59		3.5	< 2.6	25	ng/cm^2
			PYR, milling	39		< LOD	< 2.6	7.1	ng/cm^2
			PYR, road construction	11		< LOD		2.6	ng/cm^2
Väänänen *et al.* (2005)	1999–2000	Finland	PYR	22		0.45–2.9	0.07	24	ng/cm^2
			PYR	30		0.88	< 0.05	24	ng/cm^2
Väänänen *et al.* (2006)	2003	Finland	PYR	18	0.19	0.12	< 0.09	0.48	ng/cm^2
Jongeneelen *et al.* (1988a)	1986	the Netherlands	PYR, wrist pad	39		< 10–24			ng
			PYR, on hands	35		37.4–216			µg
McClean *et al.* (2004a)	1999–2000	USA	BaP	59		< LOD	< 0.6	2.5	ng/cm^2
			BaP	39		< LOD	< 0.6	1.2	ng/cm^2
			BaP	11		< LOD	< 0.6	1.2	ng/cm^2
Väänänen *et al.* (2005)	1999–2000	Finland	BaP	22		< LOD–0.04	< 0.02	0.11	ng/cm^2
Roofing									
Wolff *et al.* (1989)	1987	USA	Total PAHs	7	600 (544)				ng

Table 1.16 (continued)

Reference	Time period	Country	Agent	*n*	AM	GM/ Median	Min.	Max.	Unit
McClean *et al.* (2007a)	1998	USA	Total PAHs, roof work	71		898 (4.5)	48	34 014	ng/cm^2
			Total PAHs, tear-off	41		886 (4.6)	49	33 538	ng/cm^2
			Total PAHs, put-down	56		344 (3.6)	48	21 437	ng/cm^2
			Total PAHs, kettleman	19		299 (3.7)	40	4 558	ng/cm^2
			PYR, roof work	71		11 (5.5)	< 2.4	221	ng/cm^2
			PYR, tear-off	41		11.5 (4.8)	< 2.4	168	ng/cm^2
			PYR, put down	55		3.8 (5.4)	< 2.4	150	ng/cm^2
			PYR, kettleman	19		4.5 (5.5)	< 2.4	34	ng/cm^2
Wolff *et al.* (1989)	1987	USA	PYR	10	171 (197)				ng
McClean *et al.* (2007a)	1998	USA	BaP, roof worker	71		3.3 (12)	< 0.5	59	ng/cm^2
			BaP, tear-off	41		4.6 (5.8)	< 0.5	84	ng/cm^2
			BaP, put-down	54		1 (11)	< 0.5	59	ng/cm^2
			BaP, kettlemen	18		0.9 (18)	< 0.5	20	ng/cm^2
Wolff *et al.* (1989)	1987	USA	BaP	10	83 (88)				ng

AM, arithmetic mean; BaP, benzo[*a*]pyrene; LOD, limit of detection; Max., maximum; Min., minimum; PAH, polycyclic aromatic hydrocarbon; PYR, pyrene

Table 1.17 Urinary concentrations of 1-hydroxypyrene (1-OHP) in workers exposed to bitumens and bitumen fume, by type of production and application

Reference	Time period	Country	Time of sampling	*n*	AM	GM/ Median	Min.	Max.	Unit
Bitumen production									
Boogaard (2007)		Europe	Post-shift	> 121	0.12–0.17		< LOD	2.18	µmol/mol creatinine
Preuss *et al.* (2003)		Germany	Post-shift	70 NS		0.08	0.02	0.35	µg/g creatinine
Mixing and conventional paving									
Väänänen *et al.* (2006)	2003	Finland	Pre-shift	8 NS	0.17	0.15	0.06	0.26	µmol/mol creatinine
			Post-shift	8 NS	0.27 (0.15)	0.22	0.06	0.55	µmol/mol creatinine
Szaniszló & Ungváry (2001)		Hungary	Post-shift	< 10 NS	0.2 (0.23)				µmol/mol creatinine
Cavallo *et al.* (2003)		Italy	Pre-shift	18	609	381	222	2 460	ng/g creatinine
			Post-shift	18	680	516	59	1 774	ng/g creatinine
Campo *et al.* (2006)	2003	Italy	Pre-shift	56 NS		252	< 50	2 114	ng/L
			Post-shift	56 NS		506	< 50	3 799	ng/L
Cavallo *et al.* (2006)		Italy	Pre-shift	19 (9 S, 10 NS)	0.27				µg/g creatinine
			Post-shift	19 (9 S, 10 NS)	0.81				µg/g creatinine
Loreto *et al.* (2007)		Italy	Post-shift	16	0.89 (0.43)				ng/mL urine
Jongeneelen *et al.* (1988b)		The Netherlands	Pre-shift	3	0.5				µmol/mol creatinine
			Post-shift	3	0.6				µmol/mol creatinine
Järvholm *et al.* (1999)	1990	Sweden	Pre-shift	28 NS		0.96	0.04	3.8	µmol/L
			Post-shift	28 NS		0.96	0.23	4	µmol/L
Burgaz *et al.* (1992)		Turkey	Post-shift	39 (18 NS)	0.61 (0.38)				µmol/mol creatinine
Burgaz *et al.* (1998)	1997	Turkey	Post-shift	28 (12 NS)	0.78 (0.46)		0.32	2.20	µmol/mol creatinine
Karakaya *et al.* (1999)		Turkey	Post-shift	16	0.92 (0.14)				µmol/mol creatinine
Karakaya *et al.* (2004)		Turkey	Post-shift	6 S 4 NS	0.65 0.38				µmol/mol creatinine

Table 1.17 (continued)

Reference	Time period	Country	Time of sampling	*n*	AM	GM/ Median	Min.	Max.	Unit
Karaman & Pirim (2009)	2008	Turkey	Pre-shift	26	0.18 (0.07)				µmol/mol creatinine
			Post-shift	26	0.39 (0.21)				µmol/mol creatinine
Heikkilä *et al.* (2002)	1992–96	Finland	Pre-shift	15 NS	4		< 0.1	26.4	nmol/L
			Post-shift	15 NS	3.6		< 0.1	33	nmol/L
Väänänen *et al.* (2003)	1999–2000	Finland	Pre-shift	25 NS		0.15	< 0.06	3.9	µmol/mol creatinine
			Post-shift	26 NS		0.24	< 0.06	2.2	µmol/mol creatinine
McClean *et al.* (2004b)		USA	Pre-shift Post-shift	64 51		0.4–1.4 1.6–2			µg/g creatinine
Modified asphalt paving									
Väänänen *et al.* (2006)	2003	Finland	Pre-shift	7 NS	0.45	0.39	0.1	0.64	µmol/mol creatinine
			Post-shift	7 NS	0.46	0.45	0.3	0.64	µmol/mol creatinine
Paving with bitumen and coal tar									
Jongeneelen *et al.* (1988b)		The Netherlands	Pre-shift	[58]	0.8–2.3				µmol/mol creatinine
			Post-shift	[55]	0.9–3.2				µmol/mol creatinine
Sellappa *et al.* (2011)	2010	India	Post-shift	20 S 16 NS	2.09 (1.23) 1.13 (0.95)				µmol/mol creatinine
Roofing									
McClean *et al.* (2007a)	1998	USA	Pre-shift	54 (62% S)		0.5–1			µmol/mol creatinine
			Post-shift	63 (62% S)		0.6–1.3			µmol/mol creatinine
Mastic									
Pesch *et al.* (2011)	2001–08	Germany	Pre-shift	120 NS		193	94	385	ng/L
			Post-shift	120 NS		419	216	678	ng/L
Milling									
McClean *et al.* (2004b)		USA	Post-shift	10	0.4–0.5	0.31–0.62			µg/g creatinine

AM, arithmetic mean; GM, geometric mean; Max., maximum; Min., minimum; NS, non-smokers; S, smokers

(Asphalt Worker Exposure database). This database contained almost 1200 measurements of bitumen fume (measured as TPM or inhalable dust) and 500 measurements of organic vapour. PAH concentrations were also available for almost 500 measurements. The database covered 30 years from the late 1960s to 1999.

The arithmetic mean exposure was 1.9 mg/m^3 (range, < limit of detection [LOD] – 260 mg/m^3), 7.6 mg/m^3 (range, < LOD – 290 mg/m^3) and 95.8 ng/m^3 (< LOD – 8000 ng/m^3) for bitumen fume, bitumen vapour and benzo[*a*]pyrene respectively. Concentrations in the solvent-extracted fraction were strongly correlated with inhalable dust levels; 93% of inhalable dust emitted in road paving consisted of organic particulate matter. The correlation between vapour and fume concentrations appeared to be low.

Time trends from multivariate empirical (statistical) models in which differences in strategy, sampling and analytical methods, and use of coal tar (benzo[*a*]pyrene model only) were accounted for, showed that exposures decreased by a factor of two to three each decade (Burstyn *et al.*, 2003a). For road pavers from eight European countries, the model predicted a decrease in exposure to bitumen fume from 1.2–2.0 mg/m^3 in 1960 to 0.2–0.5 mg/m^3 in the mid-1990s (see Fig. 1.6) A temporary increase was estimated when recycling of old asphalt (which often contains coal-tar layers) started in the mid-1960s. Since the mid-1970s, however, exposures have again come down steadily, due to banning of recycling of asphalt layers with coal tar. For organic vapour and benzo[*a*]pyrene, the decreasing trends were slightly steeper (see Fig. 1.7 and Fig. 1.8).

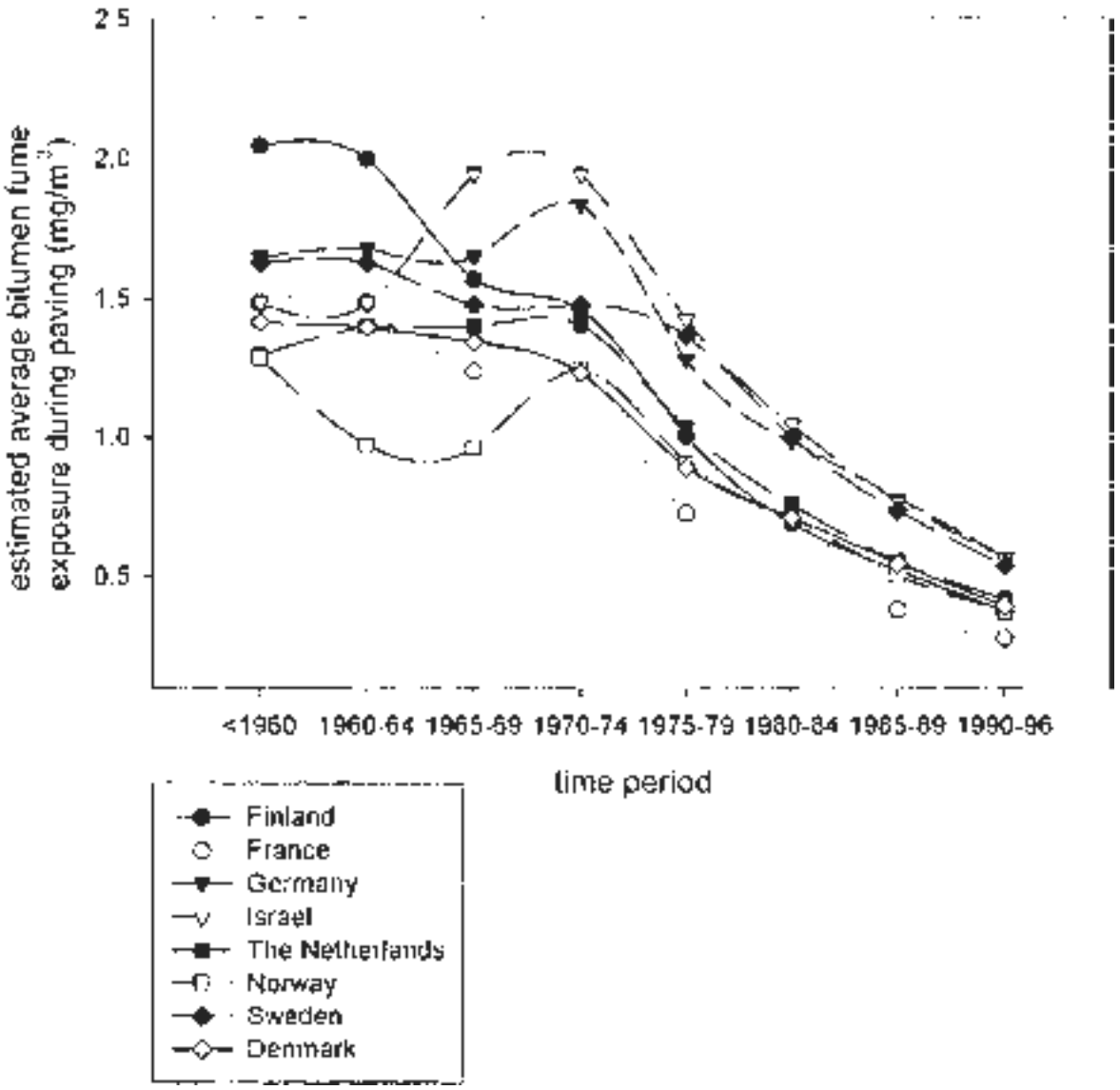
Studies carried out in 1999–2000 among road pavers working in resurfacing (n = 20), as well as millers (n = 12) and road-construction workers (n = 6) not exposed to hot-mix asphalt (McClean *et al.*, 2004a) showed that inhalation exposures varied considerably by task, crew, use of recycled asphalt product and work rate. Geometric mean breathing-zone concentrations of total PAHs reached 4.1 µg/m^3 among pavers, 1.4 µg/m^3 for millers, and was hardly detectable (among 36% of workers) for road-construction workers. Benzo[*a*]pyrene concentrations did not exceed 0.03 µg/m^3. Geometric mean dermal exposure levels to total PAHs measured with patches on the wrists reached 89 ng/cm^2 among pavers, 45 ng/cm^2 among road-construction workers and was only detected in 26% of the samples from millers. Applying lag models to urinary 1-OHP concentrations measured in the same workers, the impact of exposure to total PAHs on uptake of pyrene was estimated to be eight times greater with dermal exposure than with inhalation (McClean *et al.*, 2004b). Re-analyses of these data addressing other urinary biomarkers of exposure, such as naphthalene and phenanthrene, showed that toxicokinetic processes probably have less influence on variance in urinary biomarkers than dermal exposure and effects of covariates such as smoking (Sobus *et al.*, 2009b).

Kriech *et al.* (2002b) carried out a study among 45 pavers at 11 hot-mix paving sites across the USA. Geometric mean exposures were 0.23 mg/m^3 for bitumen fume, 0.06 mg/m^3 for BSF, and 1.23 mg/m^3 for total organic matter (TOM). Mixture temperatures varied between 120 and 165 °C.

Another recent study on 12 paving workers during four working weeks (144 worker-days) assessed inhalation and dermal exposure to PAHs under different scenarios. This intervention study found that lower application temperatures and dermal protection reduced inhalation and dermal exposure to bitumen-derived PAHs (Cavallari *et al.*, 2012a, b).

A recent study by Kriech *et al.* (2011) looked at the effect of substitution of hot-mix by warm-mix asphalt. Of the six warm-mixes, five resulted in 30–60% reductions in concentrations of TOM in the pavers' breathing zones.

Fig. 1.6 Assessed time trend in average exposure to bitumen fume (pavers only)

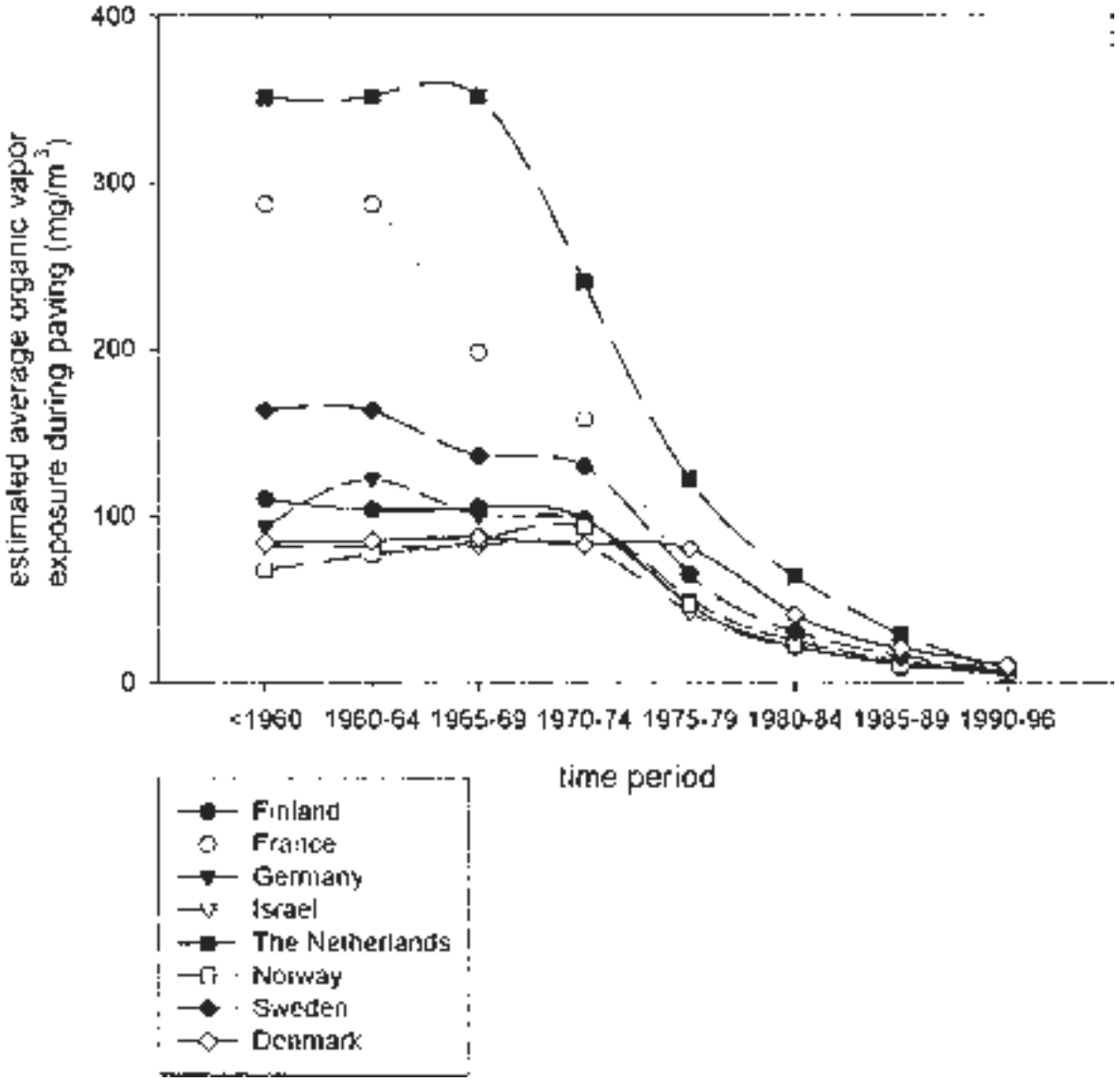


From Burstyn *et al.* (2003a)

Heikkilä *et al.* (2002) measured pre- and post-shift concentrations of urinary 1-OHP in 32 road pavers at 13 paving sites in Finland. The workers had been exposed to 11 different asphalt mixtures. Post-shift concentrations of 1-OHP were significantly higher ($P < 0.05$) among pavers (AM, 6.6 nmol/L; standard deviation [SD], 9.8) than among controls (AM, 1.6 nmol/L; SD, 2.6), and twice as high among pavers who were smokers (AM, 7.4 nmol/L; SD, 9.0) compared with non-smokers (AM, 3.6 nmol/L; SD, 8.3) ($P < 0.05$).

Also in Finland, Väänänen *et al.* (2006) investigated the exposure of road pavers to asphalt containing waste plastic and tall-oil pitch. Exposure was monitored over one working day at four paving sites among 16 road pavers who used mixtures of conventional asphalt or mixtures containing waste material. The concentrations of 11 aldehydes in air were 515 µg/m³ and

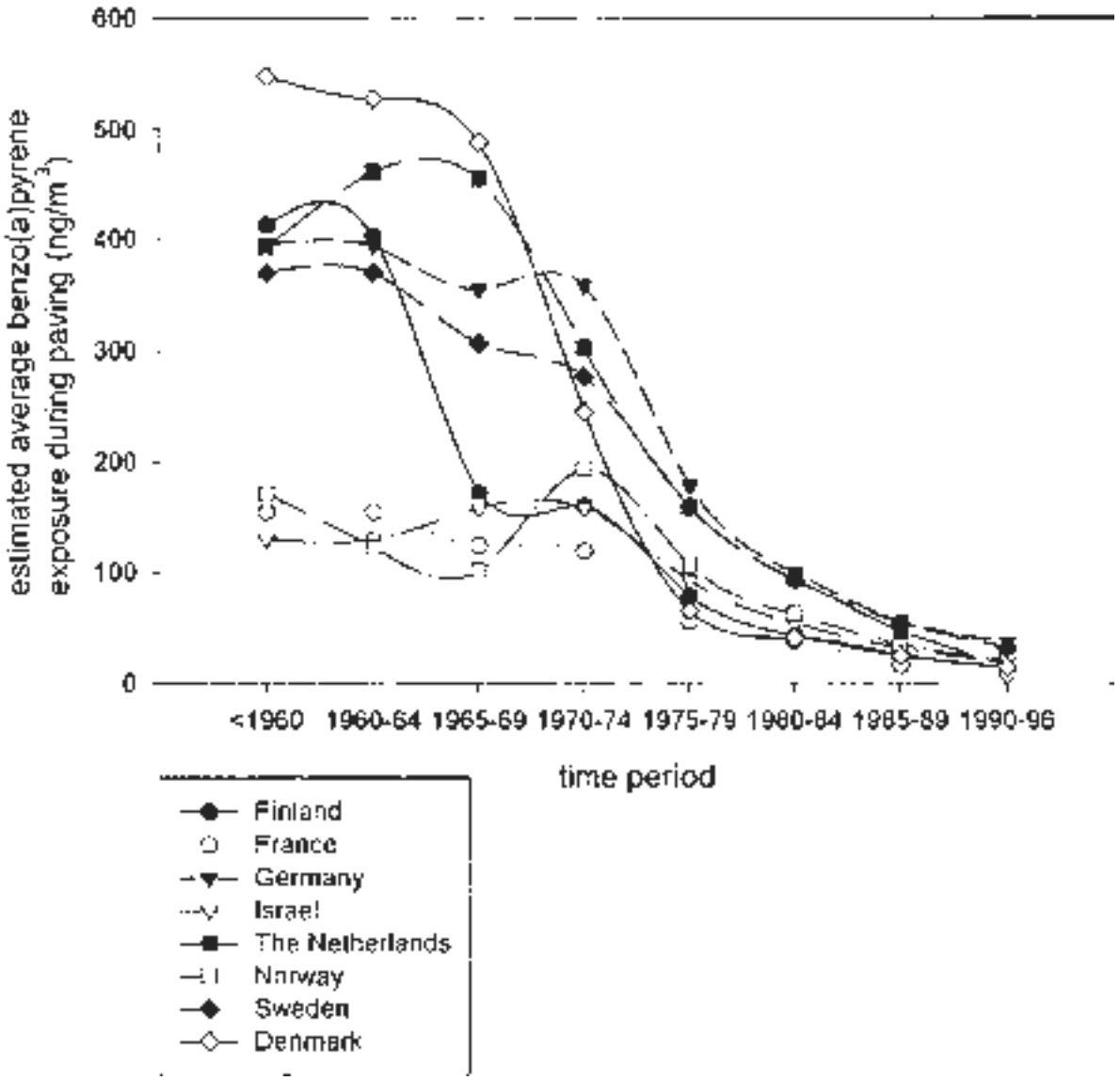
Fig. 1.7 Assessed time trend in average exposure to organic vapour (pavers only)



From Burstyn *et al.* (2003a)

902 µg/m^3 for asphalt and stone-mastic asphalt, respectively, at the worksites where tall-oil and waste plastic were used, being around 4 and 14 times greater than at the corresponding work-sites where conventional asphalt was used. Eight hydroxy-PAHs were measured, and the parent PAHs naphthalene, phenanthrene and pyrene were quantified in urine samples collected before and after the working shift. The post-shift concentrations (mean ± SD, µmol/mol creatinine) in workers using conventional asphalt of 1-naphthol/2-naphthol, combined 1-, 2-, 3-, 4- and 9-phenanthrol, and 1-OHP were 18.1 ± 8.0, 2.41 ± 0.71 and 0.66 ± 0.58 for smokers; and 6.0 ± 2.3, 1.70 ± 0.72 and 0.27 ± 0.15 for non-smokers, respectively. For asphalt workers using mixtures that contained waste material, the concentrations were 22.0 ± 9.2, 2.82 ± 1.11 and 0.76 ± 0.18 (smokers); and 6.8 ± 2.6, 2.35 ± 0.69 and 0.46 ± 0.13 (non-smokers). Similarly, PAH

Fig. 1.8 Assessed time trend in average exposure to benzo[*a*]pyrene (pavers only)



From Burstyn *et al.* (2003a)

concentrations were significantly higher in smokers than in non-smokers.

In Italy, Campo *et al.* (2006) compared the exposure from asphalt workers exposed to bitumen fume and diesel exhausts (n = 100) with road-construction workers (n = 47) exposed to diesel exhausts only. Total PAHs and 15 individual PAHs were monitored; median concentrations of individual PAHs ranged from 0.33 to 426 ng/m^3. 1-OHP concentrations increased during the day and over the working week. Work activities contributed in the same order of magnitude as cigarette smoking to the observed concentrations of 1-OHP. Campo *et al.* (2007) reported urinary unmetabolized PAHs. Median concentrations of urinary naphthalene, phenanthrene, fluoranthene and pyrene in end-shift samples were 117, 50, 8 and 6 ng/L among the asphalt workers and 104, 19, 5 and 4 ng/L among the road-construction workers. From the same

study, Buratti *et al.* (2007) reported on hydroxy-PAH concentrations quantified in urine samples collected at three different time-points during the week. The urinary concentrations of hydroxy-PAH increased with time: median concentrations of 2-hydroxyfluorene, 3-hydroxyphenanthrene and 1-hydroxypyrene in non-smokers were 0.29, 0.08 and 0.18 ng/L at baseline; 0.50, 0.18 and 0.29 ng/L pre-shift; and 1.11, 0.44 and 0.44 ng/L post-shift, respectively. Each hydroxy-PAH showed a characteristic profile, reflecting differences in half-lives. In non-smokers, positive correlations were found between vapour-phase PAHs and hydroxy-PAHs, both in pre- and post-shift samples. Concentrations of hydroxy-PAHs in smokers were two to five times higher than those in non-smokers. A recent study of dermal exposure among 24 road pavers in the same country showed dermal exposure in the range of a few nanograms per cm^2 (Fustinoni *et al.*, 2010).

In a recent study among road pavers in France, exposure data were collected for 2000–06. Geometric mean concentrations of 0.26, 0.13 and 0.75 mg/m^3 [arithmetic means, 0.43, 0.21 and 1.52 mg/m^3] were reported for TPM, BSF and bitumen vapours, respectively (Deygout & Le Coutaller, 2010).

Sellappa *et al.* (2011) reported on a study among pavers laying "tar bitumens", which, according to the authors, are increasingly being used in India and are applied hot. This binder consists of 70% bitumen and 25–30% tar. Mean urinary concentrations of 1-OHP among the road pavers was high (1.68 ± 0.93 µmol/mol creatinine) and significantly higher than among controls (0.55 ± 0.42 µmol/mol creatinine).

(iv) Roofing

In a study in the USA, 26 bitumen roofers employed in removing old roofs, putting down new roofs and operating the bitumen kettle were monitored to evaluate the effect of dermal exposure to PAHs on urinary concentrations of 1-OHP (McClean *et al.*, 2007a). Dermal concentrations of PAHs were about four times higher for workers tearing off old roofs than for those attending the kettle (812 ng/cm^2 versus 181 ng/cm^2). These concentrations were 2–20 times higher than those reported for road pavers. Exposure to coal tar was associated with an 35-times increase in dermal exposure to benzo[*a*]pyrene. As with the study in pavers (McClean *et al.*, 2004b), a distributed lag model showed that dermal exposure had a significant effect on urinary concentrations of 1-OHP. The presence of coal-tar pitch appeared to be the primary determinant of dermal exposure to PAHs and particularly for benzo[*a*]pyrene; when controlling for exposure to coal-tar pitch, dermal exposure to bitumen also had an effect.

Full-shift breathing-zone measurements for TPM, BSF and PAHs were significantly higher for roofers exposed to coal tar than for roofers not exposed to coal tar (Toraason *et al.*, 2001, 2002). Similarly, urinary concentrations of 1-OHP were higher in roofers exposed to coal tar than in roofers not exposed to coal tar. TPM or BSFs were not associated with urinary 1-OHP, but PAH levels were highly correlated with urinary 1-OHP.

A study by Kriech *et al.* (2004) of 26 roofing workers using built-up roofing asphalt (BURA) type III (class 2) at six sites in the USA found exposures for TPM that ranged from 0.29 to 10.3 mg/m^3. The BSF ranged from 0.02 to 9.63 mg/m^3, and TOM from 0.49 to 11.8 mg/m^3. At the six sites, the higher values were observed for the men operating the bitumen kettle.

(v) Specialized applications as mastic flooring and waterproofing

Using the Asphalt Worker Exposure database described above (Burstyn & Kromhout, 2000), the concentrations of bitumen fume and benzo[*a*]pyrene during mastic-laying operations were estimated to be higher, on average, than those in road paving by a factor of six and of eight, respectively. A study on dermal exposure with an observational assessment method indicated

again higher exposures for mastic workers versus road pavers, by a factor of two to five (Agostini *et al.*, 2011).

The German Human Bitumen study monitored a non-random sample of 320 bitumen-exposed workers and 69 non-exposed construction workers from 2001 to 2008 at 57 construction sites in Germany. With the main focus being whether respiratory effects would be noticeable among workers exposed at concentrations above the former German exposure limit of 10 mg/m^3, the selection of workers and type of application studies was biased towards worst-case exposure situations (e.g. > 90% were indoor mastic applications) (Breuer *et al.*, 2011; Raulf-Heimsoth *et al.*, 2011a; Spickenheuer *et al.*, 2011). Shift-long median exposures to bitumen fume (measured as inhalable dust) were relatively high at 3.1 mg/m^3 (based on bitumen condensate reference). Median concentrations of bitumen aerosols and vapours combined reached 5.1 mg/m^3. The concentrations of urinary 1-OHP and of the sum of 1-, 2+9-, 3- and 4-hydroxyphenanthrene (OHPhe) in 317 exposed non-smoking workers increased during a shift, from 193 to 419 ng/L and from 618 to 1414 ng/L, respectively (Pesch *et al.*, 2011).

(c) Coexposures

In addition to bitumen emissions, road-paving workers and roofers may also be exposed to other chemical agents. Coal tars have been used on their own or mixed with bitumens in road paving in many countries worldwide, and may still be in use in some places.

Chemical components of coal tar, namely benzo[*a*]pyrene, may occur in emissions when old asphalt containing coal tar is recycled. Also, removal of old roofing materials containing coal tar can result in considerable dermal and inhalation exposure to coal tar (McClean *et al.*, 2007a).

Road-paving workers are exposed to diesel exhaust because paving machines are usually powered by diesel fuel. They may also be exposed to diesel and gasoline engine exhaust from background traffic. Diesel fuel, gas oil, kerosene and organic solvents have also been used to clean equipment. Organic solvents and aliphatic amines are also used as components of some application mixes (Burstyn *et al.*, 2000c). Substitution of biodiesel for diesel oil as a cleaning agent was shown to reduce inhalation and dermal exposure to PAHs (Cavallari *et al.*, 2012a, b).

Mineral dusts including crystalline silica from gravel or sand may occur in air in mixing plants, paving sites and during milling of existing asphalt roads. Mineral dusts may also be generated due to (mechanical) sweeping of roads and roadsides. There is also evidence that asbestos and lime have been added infrequently to paving mixtures (Burstyn *et al.*, 2000c).

The modification of bitumen with recycled or waste materials such as crumb rubber, sulfur, ground roofing shingles, foundry sand, fly ash, contaminated soil, plastics, and waste fibres, gives rise to some specific exposures. The thermal degradation products of polyethylene and polypropylene include aldehydes, ketones, hydrocarbons, formic acid and acetic acid. Styrene and alcohols may be emitted from polystyrene, and hydrogen chloride and phthalates from polyvinyl chloride (Väänänen *et al.*, 2006).

(d) Synopsis

The characterization of occupational exposure to bitumens and their emissions is very complex. Exposure to particulate matter, volatile organic compounds and various polycyclic aromatic compounds is common among bitumen workers; however, the concentration and composition of exposure is highly variable and depends on where and under which circumstances the bitumen and bitumen-containing products are being used. The highest exposures to fume and vapours have been described for mastic-asphalt workers and roofers applying hot bitumen, while mixing-plant workers and pavers are exposed at lower concentrations.

In field studies, it is difficult to determine the extent to which such differences in exposure are due to differences in bitumen or differences in application practices, although there is strong evidence that higher application temperatures are associated with higher exposures. For example, the typical temperature ranges used in mastic applications (200–250 °C) and in hot-bitumen roofing applications (180–230 °C) are higher than those in paving applications (110–170 °C). Studies have shown consistently that inhalation and dermal exposures among mastic workers are higher than among conventional paving workers.

Coexposure to coal-tar pitch can significantly confound measurements of exposure to bitumens. Among roofing workers, exposure to coal tar was associated with increases of thirty-five times in dermal exposure to benzo[*a*]pyrene and six times in dermal exposure to PAHs. Similarly, exposure to benzo[*a*]pyrene by inhalation among road pavers was estimated to be a factor of five higher when coal tar was present.

Data on time trends are primarily available for road paving in Europe, where exposures have decreased by a factor of two to three each decade since 1970 for bitumen fume, bitumen vapour and benzo[*a*]pyrene.

1.5 Regulations and guidelines

1.5.1 Limits for occupational exposure

Limits for occupational exposure to bitumen emissions have been set in more than 50 countries or regions (Table 1.18), although many countries do not have limits. The legal status of such limits varies between binding regulatory limits and voluntary guidelines. They are based on different analytical metrics and measurement methods. More than half of the regulated countries use a limit of 5 mg/m^3 for the 8-hour time-weighted average (TWA) concentration, typically as TPM. Slightly fewer than half use a TWA limit concentration of 0.5 mg/m^3, measured typically as BSF of the inhalable fraction of bitumen fume. Ten countries have set a limit for short-term exposure in addition to the TWA-based limit.

Limits for occupational exposure have also been set in many countries for some agents that may occur in fume or vapours emitted from hot bitumens. Such agents include PAHs, naphthalene, benzo[*a*]pyrene and aldehydes.

No regulations or guidelines specifically concerning bitumens or bitumen emissions in ambient air, drinking-water or food were available to the Working Group.

1.5.2 Regulation and policies for reclaimed asphalt pavement

(a) France

A threshold for PAHs in reclaimed asphalt material containing coal tar has been set at 50 mg/kg (French Government, 2010).

(b) Sweden

The Swedish Road Administration has developed guidelines on how to handle recycling of asphalt containing tar (SRA, 2004). The asphalt is chemically identified as "containing coal tar" when the concentration of the 16 EPA-priority PAHs is > 70 mg/kg of solid matter.

(c) Switzerland

Coal tar was used for paving in Switzerland until 1991. Regulations have limited the use of materials containing coal tar (BAFU, 2006). Reclaimed asphalt material containing PAHs at concentrations < 5000 mg/kg can be recycled into both hot (asphalt plant) or cold (*in situ*) applications, as well as in unbound form, storable in landfill for inert waste. It is also possible to recycle asphalt crust containing PAHs at concentrations of up to 20 000 mg/kg, but only for cold applications. At concentrations above 20 000 mg/kg, the waste is directed to a landfill (Hugener *et al.*, 1999, 2010).

Table 1.18 National occupational exposure limits (OELs) for bitumen emissions

Country	OEL (mg/m³)	Basis	Analytical metric
Australia	5		Bitumen fume
Belgium	5	TWA	TPM
Canada-Quebec	5	TWA	TPM
Denmark	1	TWA	Cyclohexane-soluble fraction
	2	STEL	Cyclohexane-soluble fraction
European Union	No limit		
Finland	5	TWA	Organic dust (used also for bitumen fume)
France	No limit		
Germany	No limit		
Hungary	No limit		
Ireland	0.5	TWA	Benzene-soluble fraction of aerosol
	10	STEL (15 min)	Benzene-soluble fraction of aerosol
Italy	No limit		
Japan	No limit		
New Zealand	5	TWA	
Norway	5	TWA	TPM
Poland	5	TWA	TPM
	10	STEL	TPM
Portugal	0.5	TWA	Benzene-soluble inhalable particulates
Republic of Korea	0.5	TWA	
Singapore	5	TWA	TPM
Spain	0.5	Daily limit	Benzene-soluble inhalable particulates
Switzerland	10	TWA	Total hydrocarbons
United Kingdom of Great Britain and Northern Ireland	5	TWA	TPM
	10	STEL (10 min)	TPM
USA			
NIOSH	5	STEL (15 min)	TPM
ACGIH	0.5	TWA	Benzene-soluble inhalable particulates

ACGIH, American Conference of Governmental Industrial Hygienists; min, minute; NIOSH, National Institute of Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; STEL, short-term exposure limit; TPM, total particulate matter; TWA, 8-hour time-weighted average

From NAPA & EAPA (2011), GESTIS (2011)

(d) The Netherlands

If intended for recycling, reclaimed asphalt material containing coal tar should not contain 10 specified PAHs (anthracene, benzo[*a*] anthracene, benzo[*a*]fluoranthene, benzo[*g,h,i*] perylene, benzo[*k*]fluoranthene, chrysene, fluoranthene, indeno[1,2,3-*c,d*]pyrene, naphthalene and phenanthrene) in excess of a concentration of 75 mg/kg (The Netherlands, 2000).

(e) USA

In the USA there are no regulations for reclaimed asphalt pavement (RAP) at the federal level, and specifications vary by state (Mundt *et al.*, 2009). Currently, most state departments of transportation limit the percentage of RAP allowed in the mix; maxima range between 10% (e.g. Iowa, Washington) and 30% (e.g. Florida, Pennsylvania), with 20% most commonly reported. In some states, the amount of RAP used in the mix has decreased over time (e.g. Kansas, Ohio, and Washington),

while it has increased in others (e.g. Indiana and Pennsylvania). Restrictions for RAP use include: no RAP in rubber asphalt (Florida); no recycling of pavements containing coal tar (Minnesota); no poor quality or "dirty" RAP (Texas and Virginia); and RAP to be used only from known sources (Washington). Six states (Kansas, New York, Ohio, Pennsylvania, California, and Utah) specifically reported no current restrictions on use of RAP.

2. Cancer in Humans

2.1 Introduction

2.1.1 Introduction to studies of cancer in humans

Studies designed to evaluate cancer incidence or mortality attributable to exposure to bitumens and bitumen emissions have employed cohort and case–control designs. There have also been several occupational surveys that used death certificates or other routinely collected administrative data to provide information on this issue. These studies, which serve primarily as hypothesis-generating exercises in the early stages of evaluation of a scientific issue, are typically analysed using proportionate mortality methods. Such studies are less valuable when hypothesis testing is required because of the limitations of routine data and of the proportionate mortality ratio as an indicator of association. Every attempt was made, however, to obtain and review articles that specifically indicated a focus on cancer and possible exposure to asphalt or bitumen.

2.1.2 Strengths and limitations of epidemiological studies

The potential for confounding from other occupational exposures can bias estimates of relative risk and this is a particular problem in the study of bitumen because many occupations linked to bitumen exposure may also involve exposure to coal tars, which are established human carcinogens. Studies that obtained information on both exposures for direct adjustment can be used to evaluate the potential for such confounding, not only in that study, but in other studies of similar occupational groups, by providing an indication of how likely confounding from occupational exposure to coal tars, or other occupational exposures, may be.

Studies that characterize exposure to bitumens only on the basis of broad occupational titles (such as roofer or road-construction worker) may experience exposure misclassification because workers classified under a given job title may have different exposures if they perform different job tasks. Moreover, some studies are based on exposure data obtained from a census or death certificates at a single point in time, which does not reflect job mobility. Other studies combine several occupations to form the exposure group, which may lead to even more serious misclassification. All these methods can lead to non-differential misclassification of exposure, which may in turn detract from the ability to detect associations between occupational exposure and cancer.

Exposure assessment in some studies is only qualitative, and sometimes as crude as "ever *versus* never". This approach can be useful, but can also encompass considerable misclassification of exposure. In hypothesis-testing studies, quantitative assessment of exposure is necessary to identify exposure–effect patterns.

Publication bias can occur when positive findings are published preferentially. Such publication bias could create a literature that appears to indicate an abundance of positive associations. This can be a particular problem for case–control studies where many occupations/exposures can be evaluated, but where details regarding exposure are weak. Publication bias may be a more serious problem during the early years of

concern about a health issue. However, as the issue grows in importance and visibility, results from all studies, regardless of the findings, are more likely to make their way into the scientific literature. There was no information available regarding publication bias on the risk of cancer associated with exposure to bitumen; however, it has been an important concern since bitumens and their emissions were first evaluated by IARC in 1984 (IARC, 1985).

Studies of the risk of cancer associated with bitumens and bitumen emissions have mainly focused on workers employed in roofing, paving and mastic-asphalt operations. These operations occur in very different locations and have different histories regarding the materials used and potential coexposures. In all, coal tars have been used at some time (see Section 1).

Cohort and case–control studies can have different strengths and limitations. The quality of each study depends upon how reliably and accurately the disease of interest is characterized, the quality of exposure assessment, and controls for confounding. These factors must be evaluated for each study and cannot be arbitrarily determined by study type. In general, it is easier to assess occupational exposures in cohort studies because they are grounded in industry, which provides considerable information on work practices and exposure. Case–control studies can usually deal with confounding more easily because information is typically solicited directly from study subjects, which is not the case with occupational cohort studies. Nested case–control studies within an occupational cohort are also used to obtain information directly from participants, or surrogate respondents.

2.2 Cohort studies

2.1.2 IARC multicentre cohort study

See Table 2.1

The findings from the IARC multicentre cohort study are reviewed in the following three sections. The first reviews the findings of the cohort study by occupational group (Boffetta *et al.*, 2001, 2003a) and the findings obtained by applying a job–exposure matrix to investigate disease risks related to exposure to specific substances (Boffetta *et al.*, 2003b; Burstyn *et al.*, 2000a, 2003a). In the second section, analyses of several subcohorts published separately are reviewed (Bergdahl & Järvholm, 2003; Burstyn *et al.*, 2003b, 2007; Hooiveld *et al.*, 2003; Randem *et al.*, 2004; Behrens *et al.*, 2009). In the third section, findings from the nested case–control study are reviewed (Olsson *et al.*, 2010). The nested case–control study focused on cancer of the lung, included cases of cancer of the lung diagnosed from 1980 onwards, and additional incident cases of cancer of the lung.

(a) IARC multicentre study

The IARC multicentre cohort study of European workers exposed to bitumen comprised 79 822 workers from the asphalt industry, roofing industry, and related trades in Denmark, Finland, France, Germany, Israel, the Netherlands, Norway and Sweden. [The studies by Engholm & Englund (1995) and Pukkala (1995) overlapped with the present multicentre study. It is unclear to what extent the studies by Hansen (1989a, b) also overlapped with the present study. The study by Boffetta *et al.* (2003a, b) provided additional assessment of exposure that was superior to that in the individual studies.]

For all countries except Sweden, study participants were identified from records of road-paving and asphalt-mixing companies. For Sweden, participants were identified from the records of the Swedish construction industry's Organisation for Working Environment Safety and Health (*Bygghälsan*); all road pavers and asphalt mixers, as well as a sample of other construction workers, were included. In all countries, all male workers active for at least one

Table 2.1 IARC multicentre cohort study and risk of cancer

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Boffetta *et al.* (2001, 2003a) IARC Multicentre cohort study in Denmark, Finland, France, Germany, Israel, the Netherlands, Norway, and Sweden	29 820 bitumen workers, employed for at least one season in the bitumen industry.	Beginning 1953–79 and ending 1995–2000	Job tasks obtained from company records. A job-exposure matrix was constructed to assess exposure to bitumen fume and other potential occupational carcinogens	Lung cancer	Bitumen workers (SMR)	330	1.17 (1.04–1.30)	Age, calendar period, country
					Bitumen workers (RR using construction workers as ref.)		1.09 (0.89–1.15)	No smoking data available. No trend with duration, cumulative exposure or average exposure to bitumen was present. In a subgroup of road paving workers, the risk of lung cancer increased with average exposure level (P = 0.02)
					Road paving workers	189	1.17 (1.01–1.35)	
					Roofers/ waterproofers	14	1.33 (0.73–2.33)	
					Bitumen workers		*SMR*	
				All causes (001–999)		3987	0.96 (0.93–0.99)	
				All malignant neoplasms (140–180)		1016	0.95 (0.90–1.01)	
				Upper aerodigestive tract (140–150, 161)		92	1.27 (1.02–1.56)	
				Oral cavity and pharynx (140–149)		35	1.21 (0.84–1.68)	
				Oesophagus (150)		37	1.29 (0.91–1.78)	
				Stomach (151)		70	0.99 (0.77–1.25)	
				Colon (153)		55	0.71 (0.54–0.93)	
				Rectum (154)		43	0.89 (0.64–1.20)	
				Liver (155)		17	0.73 (0.43–1.17)	
				Pancreas (157)		43	0.76 (0.55–1.02)	
				Nose and nasal sinuses (160)		160	0.92 (0.25–2.34)	
				Larynx (161)		20	1.34 (0.82–2.07)	
				Lung (162)		330	1.17 (1.04–1.30)	
				Pleura (163)		5	0.72 (0.23–1.68)	
				Connective and other soft tissue (171)		6	1.23 (0.45–2.68)	
				Melanoma of skin (172)		15	0.74 (0.41–1.21)	
				Prostate (185)		82	0.85 (0.68–1.05)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Boffetta *et al.* (2001, 2003a) contd				Testis (186)		4	0.77 (0.21–1.98)	
				Bladder (188)		45	1.05 (0.77–1.41)	
				Kidney (189)		26	0.76 (0.50–1.11)	
				Nervous system (191–192)		22	0.63 (0.40–0.96)	
				lll-defined and unspecified sites (195, 199)		52	0.95 (0.71–1.24)	
				Non-Hodgkin lymphoma (200, 202)		23	0.78 (0.49–1.17)	
				Hodgkin disease (201)		8	1.24 (0.54–2.45)	
				Multiple myeloma (203)		12	0.70 (0.36–1.22)	
				Leukaemia (204–208)		28	0.78 (0.52–1.12)	
				Lymphoid leukaemia (204)		8	0.68 (0.30–1.35)	
				Myeloid leukaemia (205)		13	0.70 (0.37–1.20)	
					Road paver		*SMR*	
				All causes		2411	0.94 (0.90–0.98)	
				All cancers		623	0.96 (0.89–1.04)	
				Head and neck cancer		59	1.30 (0.99–1.68)	
				Lung cancer		189	1.17 (1.01–1.35)	
							RR	
				All causes			1.01 (0.95–1.07)	
				All cancers			1.01 (0.90–1.13)	
				Head and neck cancer			1.24 (0.91–1.68)	
				Lung cancer			1.08 (0.87–1.34)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Boffetta *et al.* (2001, 2003a) contd					*Roofer, waterproofer*		*SMR*	
				All causes		141	0.88 (0.74–1.04)	
				All cancers		44	1.21 (0.88–1.62)	
				Head and neck cancer		4	1.49 (0.40–3.81)	
				Lung cancer		14	1.33 (0.73–2.23)	
							RR	
				All causes			0.93 (0.77–1.14)	
				All cancers			1.27 (0.87–1.84)	
				Head and neck cancer			1.23 (0.45–3.37)	
				Lung cancer			1.34 (0.71–2.53)	
Boffetta *et al.* (2003b)				Lung cancer	Bitumen fume (JEM) (ever)		1.08 (0.92–1.19)	
Burstyn *et al.* (2003b)					*Lag 15 yr: average (mg/m^3)*			
					0	53	1.00 (-)	*P* for trend = 0.0005
					0.71– < 1.21	22	0.37 (0.21–0.66)	
					1.21– < 1.32	20	0.78 (0.42–1.47)	
					1.32– < 1.47	21	1.01 (0.54–1.90)	
					1.47–6.46	19	1.58 (0.79–3.13)	
					Lag 0 yr: average (mg/m^3)			
					0.31– < 1.03	32	1.00 (-)	*P* for trend = 0.0002
					1.03– < 1.23	37	2.72 (1.64–4.53)	
					1.23– < 1.37	33	2.22 (1.22–4.07)	
					1.37–5.38	33	3.02 (1.69–5.39)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Burstyn *et al.* (2003b) contd					*Lag 15 yr: cumulative (mg/m³.yr)*			*P* for trend = 0.2
					0	53	1.00 (-)	
					0.004– < 1.61	20	0.71 (0.40–1.26)	
					1.61– < 3.71	20	0.77 (0.43–1.37)	
					3.71– < 9.57	19	0.50 (0.27–0.91)	
					9.57– < 47.04	23	0.74 (0.38–1.45)	
					Lag 0 yr: cumulative (mg/m³.yr)			
					0.33– < 2.16	34	1.00 (-)	*P* for trend = 0.7
					2.16– < 4.61	31	1.14 (0.70–1.87)	
					4.61– < 9.66	33	0.97 (0.60–1.59)	
					9.66– < 71.96	37	0.55 (0.50–1.44)	
Behrens *et al.* (2009) Germany	7919 asphalt workers employed 1975–97	1975–2004	Exposure groups based on job titles	All causes		835	1.27 (1.19–1.36)	Age, calendar period No data on smoking or alcohol intake available
				All cancers		272	1.37 (1.22–1.55)	
				Oral/pharyngeal cancers		21	1.82 (1.19–2.79)	
				Oesophageal cancer		20	2.43 (1.57–3.76)	
				Laryngeal cancer		14	3.74 (2.21–6.31)	
				Lung cancer		101	1.77 (1.46–2.16)	
				Bladder cancer		14	3.29 (1.95–5.55)	
				Alcoholism		25	1.83 (1.23–2.70)	
				Liver cirrhosis		52	1.39 (1.06–1.83)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Hooiveld *et al.* (2003) the Netherlands	3714 asphalt workers		Semiquantitative exposure assessments of average exposure, exposure duration and cumulative exposure to bitumen	Lung	*Average bitumen exposure (units)*			Age, calendar period, smoking
					Non-exposed	3	1.00	Smoking habits for a subset (~ 1/3) of the cohort were used to assess smoking habits in the cohort
					> 0–1.29	13	0.99	
					≥ 1.29–3.21	52	1.36	
					≥ 3.21–4.76	4	1.57	
					≥ 4.76	0	0	
Burstyn *et al.* (2007) Denmark, Norway, Finland and Israel	7298 male asphalt-paving workers	Varied between countries	BaP was used as a marker of exposure to 4–6-ring PAHs	Bladder	*Average exposure level (ng BaP/m³) unlagged*			Age, country No smoking data available
					0 < – < 65	12	1	*P* for trend = 0.4
					65– < 126	11	1.01 (0.43–2.39)	
					126– < 198	12	1.41 (0.55–3.60)	
					≥ 198	13	1.36 (0.54–3.44)	
				Bladder	*Average exposure level (ng BaP/m³) lagged 15 yr*			
					0 < – < 99	10	1.00 (-)	*P* for trend = 0.15
					99– < 139	9	1.53 (0.54–4.38)	
					139– < 204	10	2.71 (1.01–7.27)	
					≥ 204	10	1.90 (0.66–5.47)	

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases /deaths	Relative risk (95% CI)	Covariates Comments
Olsson *et al.* (2010) Six European countries and Israel 1980–2005	433	1523	Semiquantitative using existing exposure data and expert judgement	Lung	Ever exposed to bitumen fume	303	1.1 (0.8–1.5)	Risk set, age, country, smoking, coal tar in analyses of bitumen fume Response rates: cases, 65%; controls, 58%; Interviews in person: cases, 2%; controls, 66%. Condensate indicates dermal exposure
					Ever exposed to bitumen condensate	309	1.2 (0.9–1.6)	
					Cumulative bitumen fume (unit-yr)			
					0.18–9.55	88	1.31 (0.93–1.85)	
					9.56–28.17	73	0.99 (0.68–1.45)	
					28.2–68.0	82	1.16 (0.78–1.72)	
					68.01–620.48	60	0.77 (0.50–1.19)	
					Duration of bitumen exposure (yr)			
					0.33–7.99	85	1.19 (0.84–1.69)	
					8.00–15.49	82	1.26 (0.87–1.83)	
					15.50–25.99	81	1.23 (0.84–1.79)	
					26.00–54.00	55	0.74 (0.49–1.11)	
					Average bitumen fume (units)			
					0.08–0.97	78	1.20 (0.84–1.71)	
					0.98–2.20	75	1.15 (0.78–1.70)	
					2.21–3.61	65	0.90 (0.60–1.34)	
					3.62–16.67	85	1.16 (0.78–1.73)	
					Cumulative bitumen condensate (unit-yr)			
					0.29–6.62	70	1.10 (0.77–1.57)	
					6.63–13.44	74	1.21 (0.83–1.76)	
					13.45–23.06	80	1.25 (0.84–1.87)	
					23.07–94.11	85	1.23 (0.81–1.88)	

BaP, benzo[*a*]pyrene; JEM, job-exposure matrix; PAH, polycyclic aromatic hydrocarbon; ref., reference; RR, relative risk; SMR, standardized mortality ratio; yr, year

season with a first year of employment between 1910 to 1930, except for Denmark (1953) and Germany (1965), and a last employment between 1992 (Sweden) and 1999 (Germany and the Netherlands). Based on job categories, workers were subdivided into bitumen workers (ever) n = 29820; building- and ground-construction workers (not exposed to bitumens), n = 32245; and other workers, not classifiable as to their exposure to bitumens n = 17757.

Information was retrieved from the companies regarding the calendar periods during which coal tar was used. This information was used to define a "coal-tar free" subcohort comprising 17 443 bitumen workers and 30 273 building- and ground-construction workers, to assess possible confounding from exposure to coal tar.

Start of follow-up for mortality ranged from 1953 (Norway) to 1979 (France) and end of follow-up was between 1995 and 2000. Only 0.7% of the cohort was lost to follow-up. Age, calendar period, country, and sex-specific expected numbers of deaths were calculated from the WHO mortality data bank. Standardized mortality ratios (SMRs) were used to estimate relative risks. Poisson regression was used for a cohort-internal analysis of risk per job title and quantitative and semi-quantitative measures of exposure.

The standardized mortality ratio for cancer of the lung was increased among the bitumen workers, based on 330 deaths (SMR, 1.17; 95% confidence intervals [CI], 1.04–1.30) (Boffetta *et al.*, 2003a). There were 189 deaths from cancer of the lung among road-paving workers (SMR, 1.17; 95% CI, 1.01–1.35). There were 14 deaths from cancer of the lung among roofers/waterproofers (SMR, 1.33; 95% CI, 0.73–2.23). The number of cancers observed was close to the number expected both among building- and ground-construction workers (SMR, 1.01; 95% CI, 0.89–1.15), and in the group of "other workers" (SMR, 1.01; 95% CI, 0.88–1.15). The number of cancers of the head and neck [defined as cancer of the mouth, pharynx, larynx and oesophagus] was significantly increased among the bitumen workers (SMR, 1.27; 95% CI, 1.02–1.56). There was no significant heterogeneity in standardized mortality ratios for cancer of the lung in asphalt workers between countries (P = 0.4), although a large variation in the standardized mortality ratios for cancer of the lung between countries was noted for building- and ground-construction workers who were used as unexposed referents in the internal analysis (P = 0.01). This heterogeneity was partly due to a marked deficit of deaths from lung cancer for this group found in Denmark. [The standardized mortality ratio was < 0.5, which raised concern about methodological problems possibly related to selection bias.] Standardized mortality ratios for cancer of the lung in other specific subgroups of bitumen workers (asphalt pavers, asphalt mixers, unspecified pavers/mixers, and unspecified bitumen workers) were essentially similar to that of the entire group of bitumen workers.

Thirty-three deaths occurred among mastic-asphalt workers, including five deaths from cancer of the lung (SMR, 2.39; 95% CI, 0.78–5.57) (Boffetta *et al.*, 2001). [The Working Group noted that this publication provided no information on a possible overlap with the Danish cohort of mastic-asphalt workers (Hansen, 1989b). However, the authors informed the Working Group during the meeting that there was no overlap, as two of the five deaths were from Germany while the other three were from Norway.]

A cohort-internal analysis based on Poisson regression and using building- and ground-construction workers as referents showed no statistically significantly elevated relative risks for cancer of the lung in any of the specific jobs mentioned above. The relative risk for bitumen workers was 1.09 (95% CI, 0.89–1.34), and was > 1.0 for most subgroups of bitumen workers. There was a marked heterogeneity in relative risk among bitumen workers between countries. [It was unclear to what extent the internal comparisons, based on job categories, reduced

confounding. While the internal comparison may have reduced the potential bias from smoking habits, it may not have provided an advantage over the external comparison group because in the internal group there may have been exposures to other substances, such as coal tar. While the study had an advantage in the large size and detailed data on work history, the lack of data on smoking habits limited the conclusions. The standardized mortality ratio of 1.17 observed with the external comparison group was of a magnitude that could be explained by confounding between the asphalt workers and the external comparison group. The internal analysis gave no firm support for an excess of lung cancer among the bitumen workers.]

Findings regarding non-cancer mortality in this cohort were presented in an IARC internal report (Boffetta *et al.*, 2001). The standardized mortality ratio for circulatory diseases among bitumen workers was 0.93 (95% CI, 0.89–0.98), and that for non-malignant respiratory diseases was 1.08 (95% CI, 0.96–1.22). [While this did not rule out confounding from tobacco smoking either in the internal (Poisson regression) or in the external comparisons (SMR analyses) of lung cancer in relation to bitumen exposure, it indicated that smoking habits in the cohort were not excessive when compared with those of the general population. See below for further data on mortality in the German subcohort.]

A job-exposure matrix was developed for a further analysis of risk of cancer of the lung associated with occupational exposures in the asphalt industry (Burstyn *et al.*, 2000a, 2003a). The matrix was based on questionnaires to the companies, a large database of 2007 industrial hygiene measurements from the asphalt industry, and expert evaluations. The hygiene measurements were mainly collected in the late 1970s and between 1985 and 1997. The matrix contained semiquantitative estimates of exposure to bitumen fume, organic vapours, PAHs, diesel exhaust, asbestos, silica and coal tar. In addition, quantitative assessments of exposure were performed for bitumen fume (mg/m^3), organic vapours (mg/m^3), and benzo[*a*]pyrene (ng/m^3), for jobs entailing exposure to bitumens in road paving. For benzo[*a*]pyrene, exposure levels were modelled to decrease from 322 ng/m^3 before the 1960s to 24 ng/m^3 in the early 1990s (Burstyn *et al.*, 2003a). The exposure assessments were applied to the work histories for all workers in the IARC multicentre cohort (Boffetta *et al.*, 2003b). For each worker, the following indices were derived for all agents assessed: never/ever exposed, duration of exposure, cumulative exposure, and average exposure level. Standardized mortality ratios and internally derived relative risks were estimated using the same methods as in Boffetta *et al.* (2003a).

The standardized mortality ratios for cancer of the lung were similar among workers ever exposed to bitumen fume ($n = 524$; SMR, 1.08; 95% CI, 0.99–1.18) and those unexposed to bitumen ($n = 232$; SMR, 1.05; 95% CI, 0.92–1.19) (Boffetta *et al.*, 2003b). The standardized mortality ratios for cancer of the lung and exposure to known occupational carcinogens (coal tar, asbestos, silica) were approximately equal to one, and no statistically significant trend was noted for cumulative exposure to any of those agents. A regression model simultaneously incorporating indices of all studied exposures showed that the relative risk of cancer of the lung associated with exposure to bitumen tended to be reduced by incorporation of exposure to coal tar in the model. In the "coal-tar free" subcohort, the standardized mortality ratio for cancer of the lung was significantly elevated (SMR, 1.23; 95% CI, 1.02–1.48), but no significant associations were noted with duration, cumulative exposure or average exposure to bitumen. For cancer of the head and neck, standardized mortality ratios were not significantly elevated in any of the exposure groups. No trend in relative risk with semiquantitatively assessed exposure indices (duration, cumulative exposure, average exposure) to bitumens

was found (Boffetta *et al.*, 2003b). [While it is possible that none of the investigated exposures were high enough to present an excess of cancer of the lung, a less intensive effort was made to quantify exposures other than bitumen and the lack of effect for known carcinogens underscored the challenges of developing retrospective exposure assessments.]

Quantitative estimates of exposure to bitumen fume were available for a subset of the cohort exposed during road paving. The subset included 135 cases of cancer of the lung, and a positive association was found between average exposure level of bitumen fume lagged 15 years (P = 0.0005) and average exposure with no lag (P = 0.0002). No association with cumulative exposure was found (P = 0.7) (Burstyn *et al.*, 2003b). [The Working Group noted that the reference categories were defined differently in the analyses lagged by 0 and 15 years. The exposure–effect and shape of the relationship was sensitive to introduction of lagging. Both analyses suggested a trend, but the lagged analysis showed a decline that the Working Group found difficult to interpret.]

A sensitivity analysis of the influence of assumptions made regarding exposure during periods for which no empirical data were available was based on the subset of the cohort for which there were quantitative exposure estimates (de Vocht *et al.*, 2009). This analysis showed that the conclusions presented in Boffetta *et al.* (2003b) were only marginally affected by various assumptions on time trends in exposure. The variability in exposure to bitumen fume between and within working crews was investigated based on the exposure data compiled for the IARC multicentre cohort study (Burstyn *et al.*, 2000a). A substantial variation in exposure between workers was found, while variation within workers was smaller. [The Working Group noted that variation in exposure between workers of the same job in the full cohort was not likely to have been fully accounted for in the exposure classification, which could lead to non-differential measurement error tending to attenuate observed risks.]

[This study had advantages in its very large size and the use of a detailed job-exposure matrix to assess exposures, but a lack of information on smoking habits limited conclusions. In addition, the quality of the data on job categories may have varied between countries. However, the subsequent case–control study nested within this cohort (Olsson *et al.*, 2010) addressed these issues. The applied job-exposure matrix indicated no clear excess of lung cancer in this cohort in association with established lung carcinogens such as asbestos, silica, and coal tar, and there was no effect of exposure to diesel exhaust. Possibly none of these exposures were high enough to cause an excess of lung cancer in the cohort, but it raised some concern for the validity of the exposure classification process for the agent under study, bitumens.]

(b) Additional analyses from the IARC multicentre cohort study

For some of the national cohorts contributing data to the IARC multicentre cohort, there were separate reports presenting additional data, e.g. extension of follow-up, or risk estimates adjusted for smoking. These are presented below.

The German part (n = 7919) of the IARC multicentre cohort was updated by extension of follow-up through 2004 (Behrens *et al.*, 2009). The study showed significantly increased standardized mortality ratios for cancer of the lung, larynx, oesophagus, and for oral/pharyngeal cancers and bladder cancer. However, the study also showed an excess of deaths from alcoholism, non-malignant respiratory diseases and liver cirrhosis, indicating that both alcohol and tobacco habits were in excess in this part of the cohort. [As has been commented above, this was not the case for the multicentre cohort.]

Randem *et al.* (2004) investigated the incidence of cancer in the Nordic part of the IARC

multicentre cohort study. [This study overlapped with studies by Bergdahl & Järvholm (2003), Kauppinen *et al.* (2003), and Randem *et al.* (2003).] The study included 22 362 male bitumen-exposed workers from Denmark, Finland, Norway and Sweden. Cancer cases were identified from the national cancer registries and expected numbers of cases were derived based on national cancer rates. There was a significant excess of lung cancer in the cohort (standardized incidence ratio, SIR, 1.21; 95% CI, 1.07–1.36), as well as among road pavers (SIR, 1.26; 95% CI, 1.08–1.47), but no trend was noted with time since first exposure. No overall excess of cancer of the bladder was noted, but there was a non-statistically significant positive trend of increasing risk with time since first exposure. [This study, based on cancer incidence, may be particularly informative regarding cancer sites with a good survival. There was no adjustment for smoking habits.]

Bergdahl & Järvholm (2003) reported the findings of the Swedish part of the IARC multicentre study, adding data on the incidence of cancer and individual data on smoking habits obtained from health surveys among Swedish construction workers. The incidence of cancer of the lung was not increased compared with the general population. The same findings were obtained using an internal reference group of construction workers not involved in the asphalt trade. Adjustment for smoking did not change the risk estimate.

Hooiveld *et al.* (2003) presented an analysis of the Dutch part of the IARC multicentre cohort study, comprising 3714 workers. Data on smoking habits were available from medical-evaluation records for about one third of the cohort, and were used to assess smoking habits for all workers, assuming that the smoking habits were representative for all workers within each job class. Workers exposed to bitumen were more likely than unexposed workers to have been current or former smokers. A positive trend was noted for cancer of the lung with average exposure to bitumen fume, but this trend was attenuated when adjusted for smoking. [The Working Group noted that the cut-off points for the categorical analysis were chosen to permit direct comparison with the IARC cohort results for the exposure–response analysis.]

Burstyn *et al.* (2007) presented an analysis of the incidence of cancer of the bladder in relation to exposure to PAHs in a subset of the IARC multicentre cohort study. The study included 7298 male asphalt-paving workers from Denmark, Norway, Finland and Israel. Occupational histories were extracted from personnel files. Cancer cases were identified from national cancer registries, follow-up time varied between countries. Benzo[*a*]pyrene was used as a marker of exposure to four- to six-ring PAHs. Relative risks were estimated in an internal analysis using Poisson regression. There were 48 cases of cancer of the bladder. There were indications of a positive trend in risk of cancer of the bladder with increasing average unlagged exposure to benzo[*a*]pyrene (Table 2.1), but the trend was not statistically significant ($P = 0.4$). Lagging of exposure by 15 years gave a P for trend of 0.15. [Risks were not adjusted for smoking or exposure to coal tar.]

(c) IARC multicentre nested case–control study

Olsson *et al.* (2010) conducted a case–control study nested in part of the previously described IARC cohort of 38 296 male asphalt workers in Denmark, Finland, France, Germany, the Netherlands, Norway and Israel, but excluding Sweden (Boffetta *et al.*, 2003a, b). Workers with at least two seasons of employment in an asphalt industry who were aged < 75 years and alive without cancer on 1 January 1980 were eligible. Cases ($n = 433$) were members of the cohort who died of or were diagnosed with cancer of the lung between 1980 and the end of follow-up, which ranged from 2002 to 2005, depending on the country, and was extended relative to the cohort study. Controls ($n = 1253$) were sampled randomly

from members of the cohort without respiratory or ill-defined cancer. Cases and controls, or their next-of-kin if the worker was deceased, were interviewed by telephone to obtain information about demographics, smoking and lifetime work history in asphalt industries and elsewhere. The overall response rate was 65% for cases and 58% for controls, with considerable variation among countries; 2% of cases and 66% of controls were interviewed in person. Further detailed information on asphalt industry jobs was obtained from living subjects and other workers identified from the cohort study or by industry representatives or workers' next-of-kin. Individual-level exposures to bitumen fume, organic vapours and PAHs (four- to six-ring) in air were estimated semiquantitatively from previous estimates and questionnaires from the case–control study. Algorithms were used to combine data on 85 jobs from an exposure database assembled for the cohort study (Agostini *et al.*, 2010) with information about determinants of time worked, such as the length of the working day and the duration of the working season from the case–control study. Dermal exposure to bitumen condensate was assessed based on a relative ranking of jobs. Previous semiquantitative estimates and measurement surveys were used when possible, and expert judgment was used when these were not available. Estimates of dermal exposure were adjusted for time worked and for hygienic behaviours that could reduce exposure. Potentially confounding exposures to asbestos, coal tar, crystalline silica and diesel exhaust both within and outside the asphalt industry were assessed by expert judgment using categories of "no", "low" and "high" exposure. The data were analysed by logistic regression with adjustment for risk set, age, country, and smoking pack-years. Analyses for bitumen fume also adjusted for exposure to coal tar. Results were reported for analyses by unconditional logistic regression with no exposure lag, but the authors reported that analyses using conditional logistic regression and a 15-year lag gave similar results. Fully adjusted odds ratios (OR) for lung cancer were 1.12 (95% CI, 0.84–1.49) for ever exposure to bitumen fume, 1.20 (95% CI, 0.93–1.55) for organic vapour, 1.20 (95% CI, 0.85–1.69) for PAHs in air and 1.17 for dermal exposure to bitumen condensate (95% CI, 0.88–1.56). Adjusted odds ratios for duration of exposure to bitumen fume, average exposure to bitumen fume and cumulative exposure to bitumen fume were generally elevated relative to never exposure (ORs, 1.1–1.3): all odds ratios for bitumen exposure and tests for trend were not statistically significant. Odds ratios with and without adjustment for smoking and coal tar were comparable. For dermal exposure to bitumen condensate classified by duration of exposure, cumulative exposure and average exposure, most adjusted odds ratios were of the order of 1.2 to 1.3 and none was statistically significant; tests for an exposure–effect trend were not statistically significant. The odds ratio for cumulative exposure to coal tar was 1.60 (95% CI, 1.09–2.36) in the highest exposure group and odds ratios for average exposure to coal tar were also elevated.

[This study was nested in a well characterized cohort of exposed workers and included details on smoking, full occupational histories and a more detailed exposure assessment than any other study. Further strengths were the efforts to estimate exposure separately for bitumen and coal tar and to examine inhalation and, for the first time, dermal exposures. Limitations included the relatively low rate of response and the extensive reliance on proxy interviews for cases, which could be a source of differential measurement error. Comparisons between this nested case–control study and the previous analyses of the cohort on which it is based were somewhat hampered by the different inclusion and exclusion criteria, non-response, and differences in the years of entry: subjects of the nested case–control study had typically been employed in later years when exposure was lower and the likelihood of exposure to coal tar was reduced.]

(d) Overall commentary on the results of the IARC multicentre cohort study

[The Working Group noted that the IARC multicentre cohort study (including the cohort study, subsequent analyses of the cohort, and the nested case–control study) was a significant improvement over all the previous studies in that it addressed the key limitations found in the previous reports. This study was large, multicentric, used quantitative exposure assessment of both inhalational and dermal exposure to bitumens, included data on both incidence and mortality, and adjusted for a broad range of confounders (e.g. smoking, coal-tar exposure, silica, and other occupational exposures). The Working Group noted that all the findings in the nested case–control study were based on a complex exposure-assessment model.]

2.2.2 Other cohort studies

See Table 2.2

Hammond *et al.* (1976) published a historical cohort study of roofers and waterproofers exposed to coal-tar pitch and asphalt [bitumen] in the USA. Members of the cohort were likely to have been exposed to both bitumen and coal tar because: "In former years, pitch was used more frequently than asphalt, but today asphalt is more commonly used. Most of the men work with both substances". Filter samples were collected from masks that workers were asked to wear for an entire working shift to analyse their content of benzo[*a*]pyrene. The amount of benzo[*a*]pyrene collected on the filters varied with the type of job, with average values ranging from 1.4 µg (felt layer) and 2.9 µg (mop man) to 51.8 µg (scraper), 53.0 µg (shovelman) and 31.0 µg (kettleman). It was uncommon to wear masks except for work in confined spaces. The authors stated that it was unusual for workers to specialize in a specific job, the custom being to take turns at various jobs. Workers were identified through membership of the United Slate, Tile and Composition Roofers, Damp and Waterproof Workers' Association, excluding locals confined to slate and tile work. The minimum duration of membership was 9 years before the start of the study in 1960. Since the union provided life insurance to both active and retired members, it was possible to obtain copies of the death certificates of all who died "while in good standing". Thus tracing of study subjects was done with assistance of the union. During the follow-up period from 1960 to 1971, 1798 men died. For 4.3% of these, no death certificate could be obtained. Thus the expected number of deaths was adjusted downward accordingly. Mortality of the cohort was compared with mortality of the total male population of the USA. No healthy-worker effect was observed, the standardized mortality ratio for total mortality was 1.02 and 1.09 for 9–19 years and ≥ 20 years since joining the union, respectively. Mortality from cancer of the lung in the cohort was increased after ≥ 20 years since joining the union: SMR for 20–29 years, 1.52 (66 deaths); SMR for 30–39 years, 1.50 (21 deaths); SMR for ≥ 40 years, 2.47 (12 deaths). Duration of membership was considered as a surrogate for exposure duration. Regarding other diseases related to smoking, mortality was elevated for non-malignant respiratory diseases, and was higher 9–19 years after joining the union (SMR, 1.96; 26 deaths; [95% CI, 1.28–2.87]) than ≥ 20 years after joining (SMR, 1.67; 71 deaths; [95% CI, 1.30–2.10]). [The Working Group noted that this study lacked information on tobacco smoking. However, as there was no trend for non-malignant respiratory disease with time since joining the union, it was not likely that confounding by smoking would explain the positive association between risk of cancer of the lung and years since joining the union. Overall, the informativeness of this study was limited since it did not assess bitumen exposure specifically.]

Menck & Henderson (1976) used mortality and morbidity data for white males aged 20–64 years from Los Angeles county (CA, USA) to

Table 2.2 Cohort studies of asphalt workers exposed to bitumen anterior to the IARC multicentre cohort study

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hammond *et al.* (1976) USA 1960–71	5939, men only	Membership of United Slate, Tile and Composition Roofers, Damp and Waterproof Workers Association, excluding locals confined to slate and tile work. Minimum membership duration, 9 yr before 1960	Codes NR	Time since joining the union calculated up to 1960		*SMR*	Age, calendar period Vital status and cause of death traced through death certificates provided by union life insurance. Expected numbers calculated from cause-specific proportions of age- and time period-specific total mortality. CI and *P* values, NR. Time since joining the union used as proxy for exposure duration.
				9–19 yr			
			All cancers		86	1.07 [0.87–1.32]	
			Lung		22	0.92 [0.57–1.39]	
			Oral cavity, pharynx, larynx, oesophagus		7	1.04 [0.42–2.15]	
			Bladder		2	0.82 [0.10–2.97]	
			Skin except melanoma		2	4.65 [0.56–16.80]	
			Stomach		3	0.54 [0.11–1.58]	
			Colon, rectum		14	1.46 [0.86–2.46]	
			Prostate		9	1.87 [0.85–3.54]	
			Leukaemia		5	1.67 [0.54–3.89]	
			Other		22	0.93 [0.61–1.41]	
				≥ 20 yr			
			All cancers		315	1.45 [1.30–1.62]	
			Lung		99	1.59 [1.29–1.94]	
			Oral cavity, pharynx, larynx, oesophagus		31	1.95 [1.32–2.77]	
			Bladder		13	1.68 [0.89–2.87]	
			Skin except melanoma		3	4.00 [0.82–11.69]	
			Stomach		24	1.67 [1.12–2.50]	
			Colon, rectum		37	1.32 [0.95–1.82]	
			Prostate		29	1.38 [0.96–1.99]	
			Leukaemia		13	1.68 [0.98–2.89]	
			Other		66	1.12 [0.88–1.43]	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Menck & Henderson (1976) Los Angeles County, USA 1968–70; 1972–73	1 560 800 (estimated from 1970 census); white males only; age 20–64 yr; 2161 death certificates citing cancer of the lung, trachea and bronchus (1968–70) pooled with 1777 incident cases of lung cancer reported to the Los Angeles CSP (1972–73)	Last occupation and industry as reported on death certificate (1968–70) or hospital admission sheets (1972–73)	Lung (code NR)	Roofers	11	4.96 ($P < 0.05$)	Age Expected numbers calculated for each occupation assuming that age-specific rates were identical for all occupations.
Povarov *et al.* (1988) Estonia 1974–84	1486 men only; age 20–74 yr	Employee in one of 11 plants producing hot-mix asphalt for ≥ 3 yr, in 1974–84	ICD version NR			[Presumably IRR]	Age Cancer incidence in cohort in 1974–84 compared with that in general population, Estonia, 1979–82
			All cancers		51	1.5 ($P < 0.05$)	
			Lung		17	1.5 ($P < 0.5$)	Narrative says NS.
			Stomach		7	1.1 (NS)	
			Skin		4	1.5 (NS)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Maizlish *et al.* (1988) California, USA 1970–83	27 162. Proportional mortality study inc. 1570 decedents (men, 88%; white, 90%), 327 in highway maintenance.	Last job classification of employees of the California Department of Transportation	ICD-8 (1968–78) ICD-9 (1979–83)	Highway maintenance		*PMR*	Age at death, sex, race, year of death
			All cancers		81	1.17 (0.93–1.46)	Proportional mortality for the USA population until 1980 used as reference to estimate standardized PMRs. Analysis for white men only
			Digestive organs		25	1.51 (0.97–2.23)	
			Stomach		6	2.27 (0.83–4.95)	
			Lung		25	0.98 (0.63–1.45)	
			Skin		2	1.22 (0.12–4.39)	
			Prostate		7	2.26 (0.91–4.66)	
			Lymphopoietic (all)		8	1.15 (0.50–2.26)	
Bender *et al.* (1989) Minnesota, USA 1945–84	4849	Highway maintenance workers employed by the Minnesota Department of Transportation for at least 1 yr	Study spanned 5 ICD revisions, transformed to ICD-9 equivalent codes	Highway maintenance		*SMR*	Age at death, year of death White men only; cause of death from death certificates. Expected numbers from white male Minnesota population, divided into urban/rural residence. Stratification by urban/rural may be considered rough adjustment for smoking
			Mouth, pharynx (140–149)		7	0.88 (0.35–1.81)	
			All GI tract (150–159)		90	0.82 (0.66–1.01)	
			Stomach (151)		23	0.91 (0.58–1.37)	
			Intestines (152–153)		30	0.86 (0.58–1.23)	
			Rectum (154)		8	0.66 (0.28–1.30)	
			All respiratory (160–165)		57	0.69 (0.52–0.90)	
			Lung (162)		54	0.69 (0.52–0.90)	
			Bladder (188)		12	1.09 (0.56–1.90)	
			Urinary tract (188–189)		19	0.92 (0.55–1.44)	
			Lymphoreticular (200–208)		34	0.95 (0.66–1.33)	
			Melanoma (172)		0	0 (-)	2.9 expected

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Bender *et al.* (1989) contd				*Highway maintenance* First employment 40–49 yr ago			
			Urinary tract (188–189)		7	2.92 (1.17–6.02)	
				30–39 yr duration			
			Leukaemia (204.0–208.9)		7	4.25 (1.71–8.76)	
Hansen (1989a) Denmark 1970–80	Men only; 1320 unskilled asphalt workers compared with cohort of 43 024 unskilled workers in other trades.	Census data: self-reported occupation and industry at day of census	ICD-8			*SMR*	Age, calendar period 10-yr mortality
			All cancers (140–209)	Mortality 1970–80	37	1.23 (0.87–1.70)	Data from one census
				Mortality 1975–80	29	1.59 (1.06–2.28)	Restricted to occupationally active census population
			Respiratory tract (160–163)	Mortality 1970–80	16	1.43 (0.82–2.32)	
				Mortality 1975–80	11	1.52 (0.76–2.71)	
			Bladder (188)	Mortality 1970–80	5	3.01 (0.98–7.03)	
				Mortality 1975–80	3	2.91 (0.60–8.51)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hansen (1989b) Denmark 1959–84	679 men only	Employment lists of mastic-asphalt plants (n = 400), union files of mastic-asphalt workers (n = 186), benefit society's membership files from one mastic-asphalt plant (n = 93).	ICD-7	Subcohort I (born 1893–1919): likely exposure to coal tar during World War II Subcohort II (born 1920–29): possible exposure to coal tar during World War II Subcohort III (born 1930–60): no exposure to coal tar during World War II			Age, calendar period Ascertainment of incident cases through Danish cancer register. Expected cancer incidence calculated from age-, period-, and site-specific rates in Danish men, 1958–82.
				Age 40–89 yr		*SIR*	
			Mouth (143–144)		2	11.11 (1.35–40.14)	
			Oesophagus (150)		3	6.98 (1.44–20.39)	
			Stomach (151)		4	1.90 (0.52–4.88)	
			Colon (153)		5	1.98 (0.64–4.63)	
			Rectum (154)		7	3.18 (1.28–6.56)	
			Liver (155)		2	4.76 (0.58–17.20)	
			Larynx (161)		3	4.35 (0.90–12.71)	
			Lung (162)		27	3.44 (2.27–5.01)	
			Prostate (177)		4	1.19 (0.32–3.05)	
			Bladder (181)		5	1.55 (0.50–3.61)	
			Skin (191)		3	0.67 (0.14–1.96)	
			Leukaemia		0	0.0 (0.0–4.01)	
			Other		9	1.01 (0.46–1.91)	
			Lung (162)	*Subcohort I*	18	3.02 (1.79–4.77)	Likely exposed to coal tar
			Lung (162)	*Subcohort II*	6	3.92 (1.44–8.54)	Possibly exposed to coal tar
			Lung (162)	*Subcohort III*	3	8.57 (1.77–25.05)	Not likely exposed to coal tar

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hansen (1991) Denmark 1959–86	679 men only	See Hansen (1989b).	ICD-7 and ICD-8	See Hansen (1989b)		*SMR*	Age, calendar period Assessment of vital status through national Danish register, ascertainment of cause of death from death certificates. Expected numbers calculated from national death rates, Danish men, 1960–85.
			Lung (162)		25	2.90 (1.88–4.29)	
			Non-pulmonary		37	2.00 (1.41–2.76)	
Engholm *et al.* (1991) Sweden 1971–85	226 000 construction workers; 2572 road-paving/ asphalt workers; 704 roofers	All workers registered with Bygghälsan, having undergone at least one medical check-up between 1971 and 1979.	ICD version, NR	*Roofers*		*SMR*	Age, calendar period Presumably men. Linkage with Swedish registers: (a) whole living population; (b) deaths; (c) migrants; (d) national cancer registry. Expected numbers calculated from national calendar year, age-, site-specific incidence rates, and from national calendar yr, age-, site- and cause-specific mortality rates. No information on other potentially confounding occupational exposures (coal tar). CI and *P* values NR
			Lung		3	2.79 [0.58–8.16]	
			Stomach		1	2.30 [0.06- 12.81]	
			Lymphatic/ haematopoietic		2	2.68 [0.32–9.68]	
						SIR	
			Lung		4	3.62 [0.99- 9.27]	
			Stomach		1	1.98 [0.05- 11.03]	
			Lymphatic/ haematopoietic		2	1.63 [0.20- 5.89]	
Engholm & Englund (1995) Sweden 1971–88	Same cohort as Engholm *et al.* (1991)	See Engholm *et al.* (1991)	[Probably ICD-8] (ICD version NR)	Asphalt paving workers		*SMR*	Age, calendar period see Engholm *et al.* (1991)
			All cancers (140–209)		42	0.88 (0.63–1.18)	
			Oesophagus (150)		1	0.85 (0.02–4.72)	
			Stomach (151)		6	1.62 (0.60–3.53)	
			Colon (153)		1	0.30 (0.01–1.69)	
			Rectum (154)		0	0.00 (0.00–1.91)	
			Liver (155.0)		1	0.96 (0.02–5.36)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Engholm & Englund (1995) contd			Lung (162.0–162.1)		8	0.79 (0.34–1.55)	
			Malignant melanoma (172)		3	2.08 (0.43–6.09)	
			Prostate (185)		3	0.81 (0.17–2.37)	
			Bladder (188)		0	0.00 (0.00–3.10)	
			Kidney (189)		5	1.98 (0.64–4.61)	
			Leukaemia (204–207)		2	0.98 (0.12–3.54)	
						SIR	
			All cancers (140–209)		72	0.82 (0.64–1.03)	
			Oesophagus (150)		0	0.00 (0.00–3.24)	
			Stomach (151)		8	1.80 (0.78–3.55)	
			Colon (153)		5	0.87 (0.28–2.04)	
			Rectum (154)		0	0.00 (0.00–0.90)	
			Liver (155.0)		1	0.91 (0.02–5.07)	
			Lung (162.0–162.1)		9	0.88 (0.40–1.68)	
			Prostate (185)		12	1.09 (0.56–1.91)	
			Bladder (188)		5	0.81 (0.26–1.90)	
			Kidney (189)		7	1.55 (0.62–3.18)	
			Lymphatic and haematopoietic (200–209)		7	0.77 (0.31–1.58)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Hrubec *et al.* (1992) USA 1954–80	248 046	Mailed questionnaire about occupation, industry of employment and tobacco use (84% response). 170 occupational categories with 50+ respondents or 20+ deaths evaluated.	ICD-7	Roofers and slaters		90% CI	Age, calendar period, type and amount of smoking Mortality study of United States veterans of known smoking status, almost all white men. Death ascertainment through life-insurance claims (96% complete for World War I veterans). Internal analysis using all other occupations as reference (RR, 1) for a given occupation, Poisson regression. Of all roofers and slaters (n = 52) 28 had died.
			All cancers		8	1.2 (0.68–2.20)	Smoking; total group
			All cancers		3	3.7 (1.41–9.47)	Nonsmokers
			Stomach		1	3.3 (-)	Smoking; total group
			Rectum		1	4.3 (-)	Smoking; total group
			Respiratory tract		4	3.0 (1.30–6.75)	Smoking; total group
			Prostate		1	5.5 (1.05–28.37)	Nonsmokers
			Lymphoma		1	2.8 (-)	Smoking; total group
			Respiratory tract		4	3.0 (1.30–6.75)	Smoking; total group
Minder & Beer-Porizek (1992) Switzerland 1979–82	1 378 837	Occupation recorded on death certificate	ICD-8			*PMR*	Age Men aged ≥ 30 yr; death certificates from 1979–82. Census records from 1980 provided population at risk. Only statistically elevated or reduced risks reported.
			Mouth, pharynx (140–149)	Roofers	6	3.30 (1.21–7.20)	
Chiazze *et al.* (1993) Ohio, USA 1940–82	162 cases 363 controls	Personal interview linked with data from company documents, exposure assessment committee	Lung (162)	Asphalt fume (mg/m^3)			Place of birth, education, income, smoking status, year of hire, age at first hire, respirable fibres, asbestos, talc, formaldehyde, respirable silica Nested within a cohort of fibreglass manufacturers (industry sponsors).
				0	51	1.0 (ref.)	
				≥ 0.01	111	0.96 (0.65–1.42)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Pukkala (1992, 1995) Finland 1971–85	Economically active population of Finland, aged 35–64 yr	Occupation recorded on census in 1970, coded in 335 categories	ICD-7			*SIR*	Age and calendar period Census file linked to death records and cancer registry. Sex-, age- and period-specific incidence rates in the Finnish economically active population as referents. Additional adjustment for social class as proxy for smoking and other exposures.
			Lung (162.0–162.1)	"Asphalt roofers" (men only)	18	3.50 (2.07–5.53)	None The Working Group was informed that the term "asphalt roofers" given in the publication was wrong and that the risk estimate refers to "asphalt workers" [bitumen workers] in general.
						3.25 (1.92–5.13)	Social class
			Lung (162.0–162.1)	"Road building hands" (men only)	327	1.61 (1.44–1.79)	None
						1.13 (1.01–1.26)	Social class
Milham (1997) WA, USA 1950–89 (men); 1974–89 (women)	588 090 men; 88 071 women	219 and 68 occupational categories in men and women, respectively. Occupation statements abstracted from death records and manually coded until 1986, computer coded since 1987.	ICD-7 (ICD-8 and ICD-9 codes assigned during late observation period backtranslated to ICD-7)	Road graders, pavers, machine operators & excavators (code 425); roofers & slaters (code 514) *Code 425 (paving)*		*PMR*	Age, year of death Proportionate mortality study considering 161 causes of death incl. all WA resident deaths at age ≥ 20 yr. Female PMRs calculated without housewife category (134 569 deaths). Total deaths in white men, 7266
			All cancers (140–205)		1581	1.02	
			Buccal cavity & pharynx (140–148)		34	0.89	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Milham (1997) contd			Oesophagus (150)		32	0.89	
			Stomach (151)		87	1.12	
			Colon (153)		111	0.83	
			Rectum (154)		40	0.98	
			Liver (155)		19	1.26	
			Larynx (161)		18	1.06	
			Lung (162)		558	1.20 ($P < 0.01$)	
			Prostate (177)		161	1.03	
			Bladder (181)		43	0.83	
			Skin (191)		7	0.99	
			Lymphatic and haematopoietic (200–205)		152	1	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Milham (1997) contd				*Code 514 (roofing)*			Total white male deaths, 1057
			All cancers (140–205)		207	0.99	
			Buccal cavity & pharynx (140–148)		9	1.67	
			Oesophagus (150)		4	0.87	
			Stomach (151)		6	0.58	
			Colon (153)		15	0.87	
			Rectum (154)		6	1.11	
			Liver (155)		2	1	
			Larynx (161)		6	2.59 ($P < 0.05$)	
			Lung (162)		86	1.44 ($P < 0.01$)	
			Prostate (177)		12	0.68	
			Bladder (181)		3	0.49	
			Skin (191)		1	1	
			Lymphatic and haematopoietic (200–205)		11	0.47 ($P < 0.01$)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Stern *et al.* (2000) USA	Proportionate mortality analysis of 11 370 male deaths among unionized roofers and waterproofers.		Lung			*PMR*	Race, age, calendar year
				Entire cohort	1071	1.39 (1.31–1.48)	
				Decade of first membership in union:			
				Before 1935		1.41 (1.08–1.80)	
				1935–44		1.70 (1.49–1.93)	
				1945–54		1.39 (1.26–1.53)	
				1955–64		1.42 (1.24–1.62)	
				1965–74		1.53 (1.26–1.85)	
				After 1975		1.69 (1.16–2.39)	
			All malignant neoplasms (140–208)		2691	1.14 (1.10–1.19)	
			Buccal cavity and pharynx (140–149)		72	1.11 (0.87–1.40)	
			Oesophagus (150)		84	1.34 (1.07–1.16)	
			Stomach (151)		103	0.99 (0.81–1.20)	
			Biliary passages, liver, gall bladder (155.0, 155, 156)		53	1.34 (1.00–1.75)	
			Larynx (161)		46	1.45 (1.06–1.93)	
			Trachea, bronchus, lung (162)		1071	1.39 (1.31–1.48)	
			Bone (170)		15	1.64 (0.92–2.70)	
			Skin (172, 173)		33	0.69 (0.48–0.97)	
			Prostate (185)		181	0.91 (0.78–1.05)	
			Testis (186)		17	1.30 (0.76–2.08)	
			Bladder (188, 189.3–189.9)		89	1.38 (1.11–1.70)	

Table 2.2 (continued)

Reference, study location and follow-up period	Total No. of subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	No. of cases/ deaths	Relative risk (95% CI)	Covariates Comments
Stern *et al.* (2000) USA contd			Kidney (189.0–189.2)		50	0.90 (0.67–1.19)	
			Other and unspecified sites (194–199)		195	1.30 (1.12–1.49)	
			Hodgkin disease (201)		19	0.82 (0.50–1.29)	
			Leukaemia (204–208)		79	0.85 (0.67–1.06)	

Bygghälsan, Swedish Construction Industry's Organization for Working Environment, Safety and Health; CI, confidence interval; CSP, Cancer Surveillance Program; GI, gastrointestinal; incl., including; IRR, incidence rate ratio; NR, not reported; NS, not statistically significant; PMR, proportional mortality ratio; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio; yr, year; WA, Washington state

assess occupation- and industry-specific risks of cancer of the lung. Their database consisted of 2161 death certificates mentioning cancer of the lung, bronchus or trachea for 1968–1970, and 1777 incident cases that were reported to the Los Angeles County Cancer Surveillance Program (CSP) between 1972 and 1973. In addition, pathology records from nearby out-of-county hospitals were searched for inclusion of residents of Los Angeles County who were patients with cancer of the lung. California death certificates included the subject's last occupation, name of last employing firm and type of industry. CSP demographic data included the last occupation and industry of employment that the patient or the next-of-kin reported at admission to hospital. Of all 3938 cases of cancer of the lung, 689 had no reported occupation and 1222 had no reported industry of employment. The 1970 United States Census Industry and Occupation Classification System was used to code the occupation into one of 417 categories and the industry into one of 215 categories. The population at risk by industry and occupation was estimated from two Public Use Samples of the 1970 census, including respondent information on current occupation and industry for 31 216 white males, aged 20–64 years, providing a 2% sample of the corresponding population of Los Angeles county. No data were reported for road pavers. On this basis, the number of expected deaths and incident cases was calculated for each specific occupation. The population at risk was estimated to be 1 560 800, including 2000 roofers. Based on six roofers who died from lung cancer between 1968 and 1970, and five incident cases of lung cancer among roofers reported to the CSP between 1972 and 1973, an almost fivefold risk of lung cancer among roofers was estimated (SMR, 4.96; $P < 0.05$). Cancer risks in road-construction workers were not reported. [The Working Group could not evaluate whether the observed risk may be explained in part by exposure to coal-tar products or asbestos and possible confounding by smoking. The Working Group noted that the population at risk was estimated from a sample of the census and that occupational data were compiled from different sources for the exposed and unexposed populations.]

In 1988, Povarov *et al.* reported the analysis of cancer incidence between 1974 and 1984 in a cohort of male workers employed for at least 3 years during the same period in one of 11 hot-asphalt production plants ("asphalt concrete production") in Estonia. Concentrations of benzo[*a*]pyrene assessed by filtered samples of airborne dust varied between 0.2 and 0.7 μg/100 m^3 in major work areas based on two to three air samples in each of six work areas. The concentration of benzo[*a*]pyrene ranged from 2 to 21 μg/kg in sedimented dust. The cohort comprised 10 369 person-years of observation. The total Estonian population for 1979–82 served as the reference. Cancer cases were identified through the oncological centre of Estonia, which serves as the basis for the Estonian cancer registry. [The Working Group noted that the incidence rates were age-adjusted, but the method for comparison of incidence rates within the cohort to the reference population was not described with clear detail.] Overall, 51 incident cases of cancer were observed, of which 17 were cancer of the lung. The overall incidence of cancer between age 20 and 74 years was reported to be 471.0/100 000 in the cohort as compared with 309.1 in the reference population. The incidence of cancer of the lung in the age group 20–74 years (155.1/100 000) was 1.5 times higher than in the general population (100.8/100 000). [The Working Group noted that in the original manuscript the authors stated that this difference was not statistically significant, but reported a P value of < 0.05. No explanation was given for this discrepancy.] A statistically significant difference for lung cancer was observed in the age group 40–64 years (305.6/100 000 *versus* 144.4/100 000). The age at onset of lung cancer was lower in the cohort than in the general population. Only 44% of cases of cancer of the lung

in the cohort had worked for more than 5 years in the industry. The incidence of cancer of the stomach (7 men) was 77.9/100 000 in the cohort as compared with 69.7/100 000 in the Estonian population. The incidence of skin cancer in the cohort (35/100 000; 4 men) did not differ significantly from that in the general population (23.8/100 000). [The measurement data suggested that there was low exposure to benzo[*a*]pyrene, indicating that coal tar was not a major potential confounder in this cohort. No data on tobacco smoking were reported.]

Maizlish *et al.* (1988) conducted a proportional mortality study among California highway workers who had been employed by the California Department of Transportation (CalTRANS) and died in California between 1 January 1970 and 31 December 1983. Employees included maintenance workers, materials laboratory technicians, engineers, administrators, office workers and secretaries. Exposure to asphalt occurred during maintenance operations, including: paving, crack and joint filling, surface sealing, and repair of concrete roadways using asphalt. Exposure to coal tar was mentioned for surface sealing only. Vital status and cause of death were determined by computerized linkage of the CalTRANS files with the State California death certificate registry. Standardized proportional mortality ratios (PMRs) were calculated in strata of age at death, sex, race and year of death, using the proportional mortalities for the population of the USA through 1980 as the reference. Among men working in highway maintenance, the PMR for all cancers combined was 1.17 (95% CI, 0.93–1.46) based on 81 deaths. The PMR for lung cancer was not elevated (25 deaths; PMR, 0.98; 95% CI, 0.63–1.45). [The Working Group thought this study was not very informative because the confidence intervals were wide, risk estimates may have been confounded by coal tar, and there was no information on smoking.]

Bender *et al.* (1989) conducted a mortality study of 4849 male highway maintenance workers employed for a minimum of 1 year by the Minnesota Department of Transportation (MNDOT) between 1945 and 1984. Work histories were abstracted from personnel records to accommodate discontinuous work histories. No specific exposure data were collected. Expected numbers of deaths were obtained from the mortality experience of all white male Minnesota residents. Patterns of mortality were analysed for urban and rural populations and stratified by duration of employment and time since a person first started work. There were 96 567 person-years of follow-up and 1676 deaths were observed. The overall standardized mortality ratio of 0.91 (95% CI, 0.86–0.96) was due to deficits in heart disease (SMR, 0.93), cerebrovascular disease (SMR, 0.80) and cancer (274 deaths; SMR, 0.83; 95% CI, 0.73–0.94). Rates of cancer of the lung (54 deaths; SMR, 0.69; 95% CI, 0.52–0.90) and of the urinary tract (19 deaths; SMR, 0.92; 95% CI, 0.55–1.44) were not elevated. There were no clear trends in standardized mortality ratio for cancer of the urinary tract with time since first employment or length of employment overall, but an excess of cancers of the urinary tract was observed for workers who were first employed more than 40–49 years previously (seven deaths; SMR, 2.92; 95% CI, 1.17–6.02). The standardized mortality ratio for cancer of the lung did not increase with length of work or year started and did not differ between rural and urban dwellers. [The Working Group noted that this study was informative because it was an industry-based cohort of maintenance workers. However, this study was limited in that the presentation of the results appeared to be selective. The tables did not provide a comprehensive overview of risk in relation to start and duration of employment. It was unclear whether road pavers were included in the study. The Working Group noted that there was no information on smoking, but that no overall excess of lung cancer was observed in this cohort.]

Hansen (1989a) conducted a historical cohort study of men aged 15–74 years identified from the Danish census in 1970. A cohort of 1320 unskilled workers employed in the bitumen industry was selected on the basis of self-reported occupation and industry on the day of census. [The Working Group noted that this cohort may have included an unknown number of pavers.] These men, who had been employed at asphalt plants, roofing-felt plants or one tar plant, were followed for mortality until 1980 and compared with 43 024 men who reported having worked as unskilled workers in other industries, mainly agriculture and forestry. The only exposure information used to classify the cohort members was self-reported occupation at the day of census. Cause of death was ascertained from the Danish Death Certificate Register through an automatic record-linkage system. Mortality ratios standardized for age and calendar period were calculated for men aged 45 years or more. Cancer mortality was elevated overall, in particular for the second half of the observation period. No healthy-worker effect was observed: in total, 104 deaths were observed in men aged 45 years or more (SMR, 1.02; 95% CI, 0.80–1.31), 74 of them occurring in the last 5 years of the observation period (SMR, 1.16; 95% CI, 0.91–1.46). Rates were elevated for respiratory (SMR, 1.43; 95% CI, 0.82–2.32) and bladder cancer (SMR, 3.01; 95% CI, 0.98–7.03). In an analysis of the last 5 years of the observation period, there were elevated risks for cancer of the digestive system, based on six cases (ICD-8 150–159; SMR, 1.57; 95% CI, 0.58–3.43) and for cancer of the brain, based on three cases (SMR, 5.00; 95% CI, 1.03–14.61). [The Working Group noted that this was an indication of latency and a possible indicator of duration.] In addition to the reference cohort of 43 024 unskilled workers from other trades, mortality in the exposed cohort was compared with that in the total economically active, Danish census cohort, which gave similar standardized mortality ratios for cancer and total mortality. [The Working Group noted that a proportion of cohort members were probably exposed to coal-tar products, as workers from a tar plant were included in the exposed group. There were no data on smoking. This study may have overlapped with the IARC multicentre cohort study (see Section 2.1.2).]

In a historical cohort study, Hansen (1989b) analysed the incidence of cancer in 679 male mastic-asphalt workers between 1959 and 1984. Study subjects were identified through historical files covering the period 1959 to 1980 from various sources. Employment lists of four mastic-asphalt companies provided 400 workers; membership files of an organized group of mastic-asphalt workers within the National Union of General Workers provided another 186 men; and 93 subjects were identified from the membership files of a benefit society organized by the workers at one of the mastic-asphalt plants. A subject was enrolled into the study when first identified in one of these historical files. The cohort accumulated a total of 6692 person-years at risk. Incident cases of cancer were identified through linkage with the Danish Cancer Register. No individual exposure assessments were obtained for members of the cohort, but industrial hygiene data were collected by personal samplers during flooring. These data indicated that the median concentration of asphalt fume condensate was about four times higher than the TWA of 5 mg/m^3. Median PAH concentrations were reported to equal 0.183 mg/m^3 for total PAHs (mean, 0.195 mg/m^3) and 0.004 mg/m^3 for benzo[*a*]pyrene (mean, 0.0058 mg/m^3). Concentrations during manual road paving were lower. Hansen (1989b) stated that coal-tar products were not added to the mastic-asphalt mixture except during the Second World War, when a shortage of bitumen initiated the use of coal-tar pitch in the production of asphalt mixes. The author estimated that road paving had made up about two thirds and flooring operations about one third of the working hours of the cohort. In total, 75 new cases of cancer were observed, almost twice as many as expected in

the total male Danish population (SIR, 1.95; 95% CI, 1.53–2.44). The study cohort included men born between 1893 and 1960. As the older men had probably been exposed to tar-containing asphalt mixes during the Second World War, the cohort was divided into three subcohorts: subcohort I (born 1893–1919) with likely exposure to coal tar; subcohort II (born 1920–29) with possible exposure to coal tar; and subcohort III (born 1930–60) not exposed to coal tar. While the overall standardized incidence ratio of lung cancer among men aged 40–89 years was 3.44 (95% CI, 2.27–5.01; 27 cases), the stratified analysis of the incidence of cancer of the lung in the three subcohorts indicated higher risks in the younger cohorts, although based on only three deaths in subcohort III (SMR, 8.57; 95% CI, 1.77–25.05). The extent to which the observed excess of lung cancer could be explained by confounding by smoking was investigated using data from a survey of mastic-asphalt workers, which showed that 22% were non-smokers, 36% were medium smokers and 43% were heavy smokers in 1976, compared with 39%, 24% and 38%, respectively, in the general population of the same age in 1982. It was estimated that these differences in prevalence explained at most a 20% excess of cases of cancer of the lung in the cohort under study. [The Working Group noted that studies among mastic-asphalt workers may be particularly informative in the evaluation of the carcinogenic risk of bitumen fume, since mastic-asphalt work usually entails higher concentrations of fume due to the higher temperature of the asphalt mix compared with asphalt mixes for road paving. Consequently the composition of the fume in mastic-asphalt application differs from the composition of asphalt fume in road paving (see Section 1). Frequent manual working procedures, such as hand floating, and application within buildings such as multistory car parks may further increase exposure. Compared with the other cohort studies, the type of exposure was much more specific and homogeneous.]

The cohort presented in Hansen (1989b) was also followed for mortality until 1986 (Hansen, 1991). This analysis revealed an excess mortality for cancer (SMR, 2.29; 95% CI, 1.75–2.93) based on 62 cases. No healthy-worker effect was observed. Total mortality was elevated (169 deaths; SMR, 1.63; 95% CI, 1.41–1.90), while mortality due to cardiovascular diseases was about equal to that in the male Danish population (48 deaths; SMR, 1.00; 95% CI, 0.74–1.32). Among men aged 40–89 years at death, elevated standardized mortality ratios were observed for lung cancer (SMR, 2.90; 95% CI, 1.88–4.29), non-pulmonary cancer (SMR, 2.00; 95% CI, 1.41–2.76), liver cirrhosis (SMR, 4.67; 95% CI, 1.88–9.62), respiratory diseases (SMR for bronchitis, emphysema, asthma combined, 2.07; 95% CI, 0.95–3.93). Hansen discussed the potential confounding effects of smoking and urban residence; almost all asphalt workers lived in cities as opposed to 40% of the comparison population. Mortality due to the causes of death considered in this study was generally between 5% and 20% higher in urban municipalities in Denmark (137% higher for liver cirrhosis). Using correction factors for urbanization and smoking, Hansen (1991) calculated corrected standardized mortality ratios in a sensitivity analysis. Simultaneous correction for the difference in prevalence of smoking and urban residence gave a standardized mortality ratio of 2.24 for lung cancer in men aged 40–89 years (95% CI, 1.45–3.30). [This sensitivity analysis is an advantage in situations where individual-level smoking adjustment is not possible. However, there is some concern that the author's group-level correction for both smoking and urbanization may result in an over-adjusted estimate of the SMR for lung cancer.]

Wong *et al.* (1992) raised several concerns regarding the studies by Hansen, including: a possible "unhealthy" worker effect; inappropriate adjustment for smoking and urban residence; and possible confounding by exposure to coal-tar products used until 1975 in the Danish asphalt

industry. In response, Hansen (1992) presented further sensitivity analyses using more extreme correction factors to adjust for the confounding effect of smoking and concluded that even in the most extreme case not more than a 21% excess of lung cancer could be explained by differences in smoking habits. The possibility that exposure to coal tar after World War II could explain the observed excess was refuted by Hansen (1992) for three reasons: (1) the Danish asphalt industry always had denied the use of coal-tar products in mastic-asphalt work until the paper by Hansen appeared in 1989; (2) the risk of cancer of the lung observed in the studies by Hansen would require very high levels of exposure to tar, similar to those observed in British gas works until World War II; and (3) no excess risk of skin cancer –considered by Wong *et al.* as a marker of exposure to coal tar –was observed in the Danish mastic-asphalt workers cohort (SMR, 0.78; 95% CI, 0.21–1.99) (Hansen, 1992).

Engholm *et al.* (1991) assessed cancer incidence and mortality in workers registered with the Swedish construction industry's Organization for Working Environment, Safety and Health (Bygghälsan cohort). [The Working Group noted that this cohort was included in the IARC multicentre cohort study (see Section 2.1.2).] The cohort included all workers undergoing at least one medical check-up between 1971 and 1979. These workers were followed until 1984 for cancer incidence and until 1985 for cancer mortality by linkage with Swedish cancer and mortality registries. The average duration of follow-up was 11.5 years and the median age of workers was 42 years. The authors did not mention whether women were included in the cohort. The expected number of cases and of deaths was calculated on the basis of national calendar year, age, site-specific incidence rates, and national calendar year, age, site- and cause-specific mortality rates, respectively. A strong healthy-worker effect was observed, which diminished after 10–12 years of follow-up when the overall standardized mortality ratio was still < 1 (0.80). Based on a small number of cases, the authors observed in roofers an excess of cancer of the stomach (SMR, 2.30; SIR, 1.98), cancer of the lung (SMR, 2.79; SIR, 3.62), and of haematopoietic and lymphatic cancers (SMR, 2.68; SIR, 1.63). Confidence intervals and *P* values were not reported. As the information recorded during medical check-ups included current and previous smoking status, the authors conducted a nested case–control study of the cases of lung cancer with five controls individually matched for year of and age at first check-up. The relative risk of cancer of the lung among roofers, adjusted for smoking and population density of parish of residence, was in the order of six. [The Working Group noted that the adjustment for smoking was a strength of the study; however, the methods for adjustment for smoking habits were not described to usual standards and no confidence intervals were reported. Results for road pavers are not presented here because updated estimates were presented in a subsequent publication (Engholm & Englund, 1995).]

An extended follow-up of the Bygghälsan cohort until 1987 (incidence) and 1988 (mortality) was published by Engholm & Englund (1995). They reported non-statistically significantly elevated risks in asphalt-paving workers for cancer of the stomach (SMR, 1.62; 95% CI, 0.60–3.53; SIR, 1.80; 95% CI, 0.78–3.55) and kidney (SMR, 1.98; 95% CI, 0.64–4.61; SIR, 1.55; 95% CI, 0.62–3.18), but not for cancer of the lung (SMR, 0.79; 95% CI, 0.34–1.55; SIR, 0.88; 95% CI, 0.40–1.68).

Hrubec *et al.* (1992) published a mortality study of United States veterans of known smoking status. Of about 300 000 veterans who had served between 1917 and 1940, 248 046 responded to the mailed questionnaire on smoking. These accumulated 4 530 604 person-years and 164 785 deaths. Relative risks were calculated in an internal analysis by Poisson regression using all other occupations than the one in question as the reference group. Risk estimates were

adjusted for age, time period and smoking (type and amount smoked), and in addition stratified by smoking status at the time of questionnaire (1954–57). Among roofers and slaters, the overall mortality was reduced (smoking-adjusted relative risk, 0.8; [90% CI, 0.58–1.08]; 28 deaths). The healthy-worker effect was more pronounced for all cardiovascular diseases (smoking-adjusted relative risk, 0.6; 90% CI, 0.35–0.91; 12 deaths) and coronary heart disease (smoking-adjusted relative risk, 0.7; 90% CI, 0.39–1.18; nine deaths). Mortality from cancer overall was slightly elevated. Mortality from respiratory cancer was significantly elevated after adjustment for smoking, based on four deaths (RR, 3.0; 95% CI, 1.30–6.75). No cancers of the urinary tract were observed. [The Working Group had doubts that this study was informative regarding bitumen-associated risks of cancer because the proportion of workers potentially exposed to bitumen within the group including roofers may have been small. The information on smoking was a strength of the study. Although there were few deaths, excess risks remained after adjustment for smoking. However, even if a substantial proportion of the group were exposed to bitumen, there was presumably also possible exposure to coal tar and/or asbestos.]

Minder & Beer-Porizek (1992) published a study of mortality in Switzerland in which they screened all occupational groups for excess and deficit mortality from cancer. The database consisted of all death certificates of Swiss men aged 30 years or above who died between 1979 and 1982. The population at risk was obtained from census records for the year 1980. The expected number of deaths was calculated on the basis of these census records, which recorded the occupation of Swiss residents. For most occupations, both the standardized mortality ratios and the standardized proportional mortality ratios were estimated. Only those ratios that significantly differed from unity were reported. For roofers, an elevated proportional mortality ratio for tumours of the mouth and pharynx was observed (PMR, 3.30; 95% CI, 1.21–7.20; six deaths). [The Working Group noted that occupational data were compiled from different sources for the exposed and unexposed populations. There was also no adjustment for alcohol consumption, so it was possible that confounding by alcoholic beverages contributed to the elevated risk of cancer of the mouth and pharynx.]

Pukkala (1992) calculated standardized incidence ratios by social class and occupational group for Finland using the main occupation reported on the 1971 census. In 1995 an update of the original Finnish report was published in English (Pukkala, 1995). The classification of occupations was based on the Nordic Classification of Occupations from 1963 and the International Classification of Occupations published by the International Labour Office in 1958, allowing for 335 specific categories, of which 332 and 324 included men and women, respectively. Subjects were assigned to one of four social classes on the basis of their education, occupation, industrial status and industry socioeconomic grouping. The Population Census file was linked with the annual death files to obtain death certificate information and with the Finnish Cancer Registry to assess cancer incidence. Standardized incidence ratios were calculated using sex-, age- and period-specific incidence rates in the reference population, which was restricted to the economically active population of Finland. Standardized incidence ratios were also adjusted for social class. The cohort was restricted to economically active residents aged 35 to 64 years at the beginning of each follow-up period (1971–75, 1976–80, 1980–85). Overall, the study included 47 178 cases of cancer in men and 46 853 cases in women, of which 15 613 and 1868 were cancers of the lung in men and women, respectively. The social-class pattern of cancer of the lung parallels the corresponding pattern of smoking. Even after adjustment for social class, the risk of cancer of the lung in roofers was substantially elevated

(SIR, 3.25; 95% CI, 1.92–5.13; 18 deaths) and a slightly elevated risk remained in road-building hands [unskilled workers] (SIR, 1.13; 95% CI, 1.01–1.26; 327 deaths). Among women, standardized incidence ratios were reported for none of these occupational categories, either because the expected number of cases was < 5, or because the standardized incidence ratio did not differ statistically significantly from 1 ($P \geq 0.05$). [The Working Group noted that this cohort partially overlapped with the IARC multicentre cohort study (see Section 2.1.2). This study was included here because it adjusted for social class, which may have partly removed the confounding effect of smoking and because the reference population was restricted to the economically active population. The Working Group was aware that potential exposure to wood tar may have occurred in the Finnish cohort. It was unclear what proportion of road-building workers was exposed to bitumens. During the Meeting, information was forwarded to the Working Group from the author of the study that the category labelled as "asphalt roofers" in this publication actually may have referred to "asphalt workers" in general. Thus, the Working Group did not consider it informative for evaluating roofers. The adjustment, the restriction to the economically active reference population, and the good quality and completeness of cancer registration in Finland were considered as strengths of the study.]

Chiazze *et al.* (1993) carried out a nested case–control study of cancer of the lung among employees at a fibreglass manufacturing plant operated by Owens-Corning Fibreglass Corporation in Ohio, USA. The plant environment was described as "very complex", with little information provided about products or production processes. Cases (n = 162) and controls (n = 363) were drawn from a previously enumerated cohort of workers who were employed for at least 1 year between 1940 and 1962 and followed until the end of 1982. Cases and controls were matched on birth year and follow-up time. Information on demographics, occupational and residential history, smoking, hobbies and medical history was obtained by interview. Historical exposures to respirable fibres, TPM, asbestos, talc, formaldehyde, silica and bitumen fume were assessed by an expert committee, which estimated ranges of exposure and assigned numerical scores to the midpoints of one or more categories for each substance. The odds ratio for cancer of the lung among workers with any exposure to bitumen fume was 0.96 with only the matching variables and 1.13 (95% CI, 0.47–2.73) after adjustment for smoking, education, age and year at hire and other exposures. [The Working Group noted that this article provided relatively little information on how the exposure assessment of asphalt fumes was performed, despite an apparently lengthy process involving a wide range of historical documents. The extent to which quantitative data on industrial hygiene were used in the exposure assessment was not clear.]

Milham (1997) analysed the occupational proportionate mortality of residents of Washington State between 1950 and 1989 using occupation and industry data recorded on death records. In women, no deaths were observed in road graders, while six deaths were recorded in roofers. In white men, a significantly elevated risk of cancer of the lung was observed in roofers and slaters (PMR, 1.44; 86 deaths) and in the group of road graders, pavers, machine operators and excavators (PMR, 1.20; 558 deaths). In roofers and slaters, the standardized proportional mortality ratio for cancer of the larynx was also statistically significantly elevated (PMR, 2.59; six deaths). [The Working Group considered the occupational group of road graders, pavers, machine operators and excavators relatively unspecific for exposure to bitumen.]

Stern *et al.* (2000) carried out a proportionate mortality analysis of unionized roofers and waterproofers in the USA. A total of 11 144 men was available for analysis. Standardized

proportional mortality ratios were adjusted for race, age and calendar year. The standardized proportional mortality ratio for cancer of the lung was 1.39 (95% CI, 1.31–1.48; 1071 deaths). Other cancer sites with an excess of cancer risk were: larynx (PMR, 1.45; 95% CI, 1.06–1.93; 46 deaths); urinary bladder (PMR, 1.38; 95% CI, 1.11–1.70; 89 deaths); and oesophagus (PMR, 1.34; 95% CI, 1.07–1.66; 84 deaths). According to a survey among roofers in 1982 and 1983, the prevalence of exposures to various agents was estimated to be as follows: bitumen, 71.6%; asbestos, 47.9%; quartz, 26.4%; and coal-tar pitch, 13.4%. [The Working Group noted that exposure to asbestos, silica dust and coal-tar products may have contributed to the observed excess risks. No data on tobacco or alcohol consumption were available for cohort members.]

2.3 Case–control studies

2.3.1 Cancer of the lung

See Table 2.3

(a) Population- and hospital-based studies

A population-based case–control study by Schoenberg *et al.* (1987) examined associations between cancer of the lung and occupation in New Jersey, USA. The cases (n = 763) were white male residents of the study area, newly diagnosed with cancer of the lung between September 1980 and October 1981. The controls (n = 900) were sampled at random from drivers' licence records for living cases or death-certificate registers for deceased cases. Cases and controls were matched on age, race, area of residence, and date of death, if deceased. Subjects or their next-of-kin were interviewed in person to obtain information on occupational history and other risk factors. Additional questions were asked about specific occupational exposures, but exposure to bitumens or bitumen fume was not specifically assessed. A smoking-adjusted odds ratio of 1.7 (95% CI, 0.68–4.4) was reported for the occupational category of roofers and slaters. No data were reported for other occupation or industry categories with known exposures to bitumens. Employment duration and time since employment were examined, but no data were reported for roofers. [The main limitation of this study was the use of job titles as a surrogate indicator of exposure.]

Vineis *et al.* (1988) pooled data from five case–control studies of cancer of the lung conducted during the 1970s and 1980s in the states of Louisiana, Florida, Pennsylvania, Virginia, and New Jersey, USA. [The Working Group noted that this study included the study by Schoenberg *et al.* (1987), described above.] Information was collected on place and type of work and duration of employment for occupations held for 6 months or more. The pooled data set included 2973 cases in men and 3210 controls. Odds ratios adjusted for age, birth year and smoking were calculated for occupations and occupational exposures that were established or suspected to be associated with cancer. Jobs or exposures not classified as carcinogenic, probably carcinogenic, or possibly carcinogenic, were classified as unexposed. Risk from exposure to bitumen was not specifically assessed, but a smoking-adjusted odds ratio of 1.4 (95% CI, 0.9–2.3) was reported for employment as roofers or asphalt workers. [The Working Group noted that much of the exposure information was reported by next-of-kin.]

Zahm *et al.* (1989) studied associations of smoking and occupation with cancer of the lung of several histological types using data from a cancer registry in Missouri, USA. The cases were diagnosed with cancer of the lung between 1980 and 1985, and controls had diagnoses of other, non-smoking related cancers during the same period. All participants were white men. Occupation at the time of diagnosis and smoking information were abstracted from medical records. Odds ratios were adjusted for age and smoking. The odds ratio for the association of all

Table 2.3 Case–control studies of cancer of the lung and exposure to bitumens

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Schoenberg *et al.* (1987) New Jersey, USA, 1980–81	763 (334 next-of-kin)	900 (336 next-of-kin)	Population (drivers' license files; mortality files)	Face-to-face interview including detailed job history and questions on solvents, fumes, dust and asbestos. 42 job titles and 34 industry categories	Lung (162)	Roofers, slaters	13	1.7 (0.68–4.4)	Smoking (four strata) Response rate: cases, 70.4%; controls, 63.6%. Frequency-matched on race, age, area of residence and data on death (deceased controls). Possible confounding by asbestos and coal tar.
Vineis *et al.* (1988) Five areas in USA 1976–83	2973 men	3210	Hospital and death certificates	Interview (some with next-of-kin), coding of jobs for known and suspected carcinogens	Lung	Roofers or asphalt workers	45	1.4 (0.9–2.3)	Overlaps with Schoenberg *et al.* (1987)
Zahm *et al.* (1989) Missouri USA 1980–85	4431 men	11 326	Cancer registry	Job title from registry	Lung	Pavers, surfacers and material movers Roofers	32 6	0.9 (0.6–1.5) 2.1 (0.6–8.2)	Age, smoking White men only
Morabia *et al.* (1992) Nine areas, USA 1980–89	1793 men	3228	Hospital	Personal interview, checklist of 44 agents, usual occupation, ever employed	Lung (162)	Roofers, slaters	7	2.1 (0.7–6.2)	Age, race, smoking, region, questionnaire version Men only. Response rates not reported. Controls: any non-lung cancer patient; matched on race, age, hospital and smoking. Possible confounding by asbestos and coal tar.

Table 2.3 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Jöckel *et al.* (1992) Western Germany Recruitment period not stated	194 men and women	388	Hospital and population	Job title, JEM and expert assessment	Lung	Road-construction workers	29	2.6 (1.38–4.99)	Age, sex, smoking Hospital and population controls combined for analysis. OR for roofers said to be "not high" but not shown. OR = 1.4 for highest level of PAH exposure
Jöckel *et al.* (1998) Western Germany 1988–93	1004 men and women	1004	Population	Job title, agent checklist, job-specific questionnaire and list of "risk occupations"	Lung	Road-construction, pipe-laying, well-digging and unskilled construction	155	1.02 (0.76–1.36)	Region, sex, age, smoking, asbestos
Brüske-Hohlfeld *et al.* (2000) Germany 1988–96	3498 men	3541	Population	Job title, checklist, job-specific questionnaire, expert assessment of BaP	Lung	Road construction workers and pipe-layers	492	1.2 (1.0–1.5)	Age, region, smoking, asbestos Includes cases and controls from Jöckel *et al.* (1998), men only
Bovenzi *et al.* (1993) Northern Italy 1979–86	756 men	756	Autopsy records	Job title from next-of-kin interview, expert assessment	Lung	Asphalt workers	7	2.3 (0.5–10.3)	Age, death, year, smoking All cases and controls deceased.

Table 2.3 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Watkins *et al.* (2002) USA, 28 plants 1977–97	39	133	Employer records, matched on age, birth year, race	Expert, committee, using historical records	Lung	Ever exposed to bitumen fume	12	1.6 (0.6–4.6)	Matching variables only All cases deceased. Controls deceased or retired. Exposures imputed for year before 1977; no coal tar after 1977; unclear if controls arose from the same study base as cases Sensitivity analyses reflected in different scenarios: *Scenario* 1 based on rate of decline; *Scenario* 2 assumed doubling of exposure
						< 20 yr	7	2.3 (0.7–7.7)	
						≥ 20 yr	5	1.1 (0.3–3.7)	
						Bitumen fume mg/m^3-day			
						Scenario 1	1	2.0 (0.1–17.5)	
						> 0– < 1 000			
						1000–9999	7	1.9 (0.6–6.4)	
						≥ 10 000	4	1.3 (0.4–4.1)	
						Scenario 2			
						> 0– < 1 000	2	2.8 (0.4–18.4)	
						1000–9999	5	1.1 (0.3–4.0)	
						≥ 10 000	5	1.8 (0.6–6.0)	
Richiardi *et al.* (2004) Northern Italy 1990–92	1171 men and woamen	1553	Population	Job title from interview	Lung	Roofers, asphalt workers, insulators and pipe-coverers	9	2.0 (0.6–6.5)	Sex, age, region, smoking, number of jobs Broad definition of exposed group. Confounding by asbestos likely.

Table 2.3 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
McClean *et al.* (2011) USA 1998–2003	422	894	African American and Latin American men and women in San Francisco Bay area	Interview, self-assessed exposure to 21 substances	Lung	Ever exposed to tar and asphalt Cumulative exposure to asphalt and tar	32	1.2 (0.7–2.1) 1.11 (1.01–1.22)	Age, calendar period, race, smoking habits, asbestos, automobile exhaust Broad definition of exposure combining tar and asphalt; OR per exposure year

BaP; benzo[*a*]pyrene; d, day; OR, odds ratio; PAH, polyaromatic hydrocarbon; RR, relative risk; yr, year

types of cancer of the lung with the combined occupational category of pavers, surfacers and material-moving operators was 0.9 (95% CI, 0.6–1.5), based on 32 cases and 64 controls. For roofers, the odds ratio was 2.1 (95% CI, 0.6–8.2), based on six exposed cases and seven controls, and this effect did not differ significantly by histological subtype. Data were not reported for other occupations with known exposure to bitumen. The numbers of subjects with specific combinations of cell type and occupation were small, and no odds ratio for any combination was significantly elevated. [Only white men were included and the use of other cases of cancer as controls may have lead to bias if those cancers were associated (positively or negatively) with exposure. The main limitations of this study were the use of job titles alone as indicators of exposures and the reporting of results for selected occupations only.]

Morabia *et al.* (1992) conducted a case–control study of cancer of the lung and occupation in 24 cities across the USA between 1980 and 1989. The cases were 1793 men with diagnoses of cancer of the lung while controls were 3228 men with other diagnoses, who were matched to cases on age, race, hospital, and smoking history. Information was obtained by interview on smoking, usual occupation and exposure to 44 specific substances on the job or in a hobby. A complete list of the 44 substances was not provided, however, and results were shown only for asbestos and coal dust. Odds ratios were adjusted for the matching factors. An adjusted odds ratio of 2.1 (95% CI, 0.7–6.2) was reported for roofers and slaters. Data were not reported for other occupations known to involve exposure to bitumen or bitumen fume. [The utility of this study was limited by the selective reporting of results for certain occupations and substances.]

Occupational and environmental risk factors for cancer of the lung were analysed by Jöckel *et al.* (1992) in a case–control study in an industrial area of western Germany. Cases were 194 men and women with incident histologically confirmed cancer of the lung recruited from seven hospitals in five cities. Each case was matched by age and sex to one hospital control with a non-smoking related diagnosis and one control selected at random from population registries. Information including job history, occupational exposure and smoking was obtained by structured interview. Occupational exposures were assessed by job-specific questionnaires or responses to a checklist of specific exposures. Exposures to substances regarded as known lung carcinogens, including soot and tars, were assigned numerical weights. Road-construction workers and roofers were given a supplemental questionnaire due to their potential exposure to PAHs. The hospital and population controls were combined for analysis. In analyses restricted to men, the smoking-adjusted odds ratio for road-construction workers was 2.6 (95% CI, 1.38–4.99), while the odds ratio for roofers was reported not to be high, but was not shown. Semiquantitative scores for exposure to PAHs, including fume, coal tar and coal tar products, were not related to risk of cancer of the lung: only the odds ratio for the highest category was greater than unity (OR, 1.40; 95% CI, 0.48–4.20) and a test for trend was not statistically significant. No results were reported for other specific exposures or for exposed occupations among women because of small numbers. [The exposure-assessment approach used in this study was an improvement over the use of job titles alone in earlier studies, but the focus was on PAHs in general, exposure to bitumens were not distinguished specifically, and an effect of coal tar could not be ruled out. There was also some selective reporting, with data not shown for all relevant occupations.]

A subsequent case–control study in the same part of Germany also evaluated occupational risk factors for cancer of the lung (Jöckel *et al.*, 1998). A total of 1004 incident cases of cancer of the lung were recruited between 1988 and 1993 from hospitals; controls matched by region, age

and sex were selected from population registers. Data were collected by interview with similar rates of response (68–69%) for cases and controls. Occupational exposures were assessed by job history, exposure checklist and job-specific questionnaire, including specific questionnaires for roofing and road construction. A subset of occupations including road construction was designated "risk occupations" using lists of jobs with known and suspected exposures (Simonato & Saracci, 1988). Results were tabulated only for men. An odds ratio of 1.02 (95% CI, 0.76–1.36), adjusted for asbestos and smoking, was reported for the occupational category including road construction, pipe-laying, well-digging and unskilled construction work. Odds ratios were not reported for roofing or for exposure to bitumens. [While this study used improved methods of exposure assessment relative to job titles alone, it contributed relatively little to the assessment of bitumen because only results for a single broad job category with potential exposure were reported. The Working Group noted that this study did not replicate the results of the earlier study (Jöckel *et al.*, 1992), which used similar methods.]

Data from the preceding study and another case–control study covering a larger area in Germany were pooled in a study by Brüske-Hohlfeld *et al.* (2000). The pooled study included 3498 cases of cancer of the lung and 3541 matched population controls. Only men were included. The exposure assessment was similar to that used in Jöckel *et al.* (1998), with the addition of a measure of cumulative exposure to benzo[*a*] pyrene that incorporated external exposure data via expert assessment. Matched odds ratios were estimated by conditional logistic regression with adjustment for smoking and exposure to asbestos. Men ever employed as road-construction workers or pipe-layers had an odds ratio of 1.24 (95% CI, 1.04–1.47) with adjustment for smoking and asbestos. Increased risks were seen for roofers and asphalt workers exposed to PAHs, but odds ratios for those occupations were not shown. [This study was comparatively large, but information on specific exposures remained limited, as in the earlier study using similar methods (Jöckel *et al.*, 1998). Notably, exposures to bitumens were combined with exposure to PAHs from sources including coal tar.]

Associations of cancer of the lung with occupational exposure were characterized by Bovenzi *et al.* (1993) in a case–control study of mortality in an industrial area in northern Italy. The cases included 756 men who had died of primary cancer of the lung between 1979 and 1986, identified from a provincial cancer registry. The controls were 756 men who had died of causes other than lung disease or cancers of the aerodigestive, urinary or gastrointestinal tracts, or of the pancreas or liver, sampled at random from autopsy records and matched to cases on time of death and age. Assessment of occupational exposure was based on occupation and industry titles, obtained through interviews with next-of-kin, which were classified as involving exposure to known or suspected carcinogens identified from a review of *IARC Monographs* (Simonato & Saracci, 1988). Odds ratios were adjusted for smoking using information obtained in the interviews. The odds ratio for asphalt workers was 2.27 (95% CI, 0.50–10.3). [It was not clear whether this represented ever being employed in the occupation, the usual occupation or the last occupation.] Data were not reported for other indicators of bitumen exposure.

Associations of cancer of the lung with occupation were also examined in a population-based case–control study in two areas of northern Italy (Richiardi *et al.*, 2004). Cases and controls were enrolled between 1990 and 1992: 1171 men and women with incident, confirmed cancer of the lung were matched by region, sex and age to 1553 controls from population registries. Information on exposure to risk factors for cancer of the lung and a lifetime occupational history were collected by personal interview. Jobs were grouped according to whether exposure to occupational

carcinogens was previously known (list A) or suspected (list B) using an approach similar to that used by Jöckel *et al.* (1998) and Bovenzi *et al.* (1993). Data were analysed by unconditional logistic regression with adjustment for age, region, tobacco use, and total number of jobs. Detailed results were reported only for men. A category of construction occupations on list A that included roofers, asphalt workers, insulators and pipe-coverers had an odds ratio of 2.0 (95% CI, 0.6–6.5), which reduced to 1.5 after further adjustment for education. [The study did not specifically seek to address exposures to bitumen, and the category including potentially bitumen-exposed workers also included occupations with known exposure to asbestos.]

McClean *et al.* (2011) reported a population-based case–control study including 422 incident cases of cancer of the lung and 894 controls identified between 1998 and 2003 among African Americans and Latin Americans in the San Francisco Bay area, California, USA. Both men and women were included. Information on occupational exposure to a list of 21 substances and on smoking habits was obtained in a personal interview. Exposures were self-assessed. Study subjects also provided blood or buccal samples to investigate potential effect modification by cytochrome P450 (CYP) 1A1 type. Refusal rates were about 7% among controls and 14% among referents. The study found an odds ratio of 1.2 (95% CI, 0.7–2.1) for ever having worked with asphalt and tar. Cumulative exposure was investigated and an odds ratio of 1.11 (95% CI, 1.01–1.22) per year of exposure to asphalt and tar was reported. This risk estimate was adjusted for age, sex, race, smoking habits, asbestos and automobile exhaust. In Latin Americans, a higher risk was noted with CYP1A1 wildtype than with the variant type. [The Working Group noted that a broad exposure category combining exposure to asphalt and tar was investigated, and separate effects could not be estimated.]

(b) Industry-based studies

The association of cancer of the lung with exposure to bitumen fume among workers engaged in bitumen-roofing manufacture and bitumen production was examined specifically in a case–control study by Watkins *et al.* (2002). There was no exposure to coal tar in this population from 1977 onward, and there was no information about the presence or absence of exposures to coal tar before that year. The cases were 39 men who had been employed at 28 roofing-manufacture and bitumen-production facilities and had died of cancer of the lung between 1977 and 1997. Twenty-three of the cases had worked at roofing-manufacturing plants. The controls were 133 men employed at the plants who had retired or died and were not cases, matched to cases on age, race and year of birth. Exposures to bitumen fume and respirable crystalline silica were assessed using the same information as in the earlier study by Chiazze *et al.* (1993), but in greater detail. Exposure to bitumen fume was classified in categories of ever/never exposed, duration < 20 years, duration ≥ 20 years, and cumulative exposure of 0, < 1000, 1000–9999 or > 10 000 mg/m^3-days. Since data on industrial hygiene were not available for years (not specified) before 1977, two scenarios were used to extrapolate exposures measured between 1977 and 1989 to the earlier period. Scenario 1 assumed that the average level of exposure had declined at the same rate in the years before 1977 to 1977–82 as in the years from 1977–82 to 1983–89, while scenario 2 assumed that average exposures before 1977 were double those in 1977–82. Only matched, unadjusted odds ratios for the association between cancer of the lung and bitumen fume were reported. The odds ratios for cancer of the lung associated with exposure to bitumen fume were: ever exposure, 1.59 (95% CI, 0.60–4.57); exposure for < 20 years, 2.27 (95% CI, 0.74–7.73); and exposure for ≥ 20 years, 1.06 (95% CI, 0.30–3.65). The highest

odds ratios for quantitative estimates of exposure were observed for workers with exposures between 0 and 1000 mg/m^3-days: scenario 1, 1.99 (95% CI, 0.14–17.52); and scenario 2, 2.84 (95% CI, 0.41–18.42). Odds ratios in the highest exposure category of > 10 000 mg/m^3-days were lower (1.30 and 1.84 for scenarios 1 and 2, respectively). [Exposure assessment in this study focused on bitumen and was more detailed than in any population-based study. The data on exposure duration and estimated cumulative exposure allowed exposure–response relationships to be considered. However, the lack of exposure information for the period before 1977, when exposures were thought to have been higher, is a significant limitation that the authors attempted to address in sensitivity analyses. The number of years without exposure data was not specified, but 85% of workers had worked before 1977. Other notable limitations were the small number of cases and doubts about whether the controls were representative of the base population for the cases.]

2.3.2 Cancer of the urinary bladder

See Table 2.4

Mommsen *et al.* (1983) studied risk factors for cancer of the bladder in a mostly rural region of Denmark. Cases (n = 212) were patients seen between 1977 and 1980 at an oncology service, while controls (n = 259) were matched on sex, age, geographic area and urbanization; the crude odds ratio for work with petroleum or asphalt was 3.78 (95% CI, 1.12–12.81). This was reduced to 2.36 (confidence intervals not given) after adjustment for tobacco, alcohol and other exposures. No other indicators of potential exposure to bitumen were reported. [Although this study suggested a relatively strong association between cancer of the bladder and exposure to petroleum and asphalt, there were several weakness that may compromise validity, including the unknown source of controls, different interviewing methods for cases (face-to-face interview) and controls (telephone interview or mailed questionnaire), and details of the occupational questions and the substances that were queried were not given. In addition, exposures to asphalt and petroleum were aggregated, making the effect of bitumen difficult to gauge.]

Occupational risk factors for cancer of the bladder, including tars and asphalt, were investigated in a population-based case–control study by Risch *et al.* (1988). Cases (n = 826) were residents of two Canadian provinces that were newly diagnosed with histologically confirmed cancer of the bladder during 1979–82. Controls (n = 792) were selected at random from provincial population registers and matched to cases on birth year, sex and area of residence. Information on occupation, exposure to fume, dust, smoke and chemicals, smoking and other risk factors was obtained by face-to-face interview. The method of assessing specific occupational exposures was not reported. The response proportion was 67% for cases and 53% for controls. Associations between incidence of cancer of the bladder and exposure to tars and asphalt were observed for men, but not for women. The smoking-adjusted odds ratio was 1.44 (95% CI, 0.78–2.74) for ever having been exposed to tar and asphalt, and 2.02 (95% CI, 1.08–4.97) for 10 years of exposure. For men exposed 8–28 years in the past, the odds ratio was 3.11 (95% CI, 1.19–9.68). Associations of cancer of the bladder with occupational titles were also reported, but data were not provided for pavers, roofers or other occupations with known exposure to bitumen. [While the effort to identify specific occupational exposures was useful, the grouping of tar and asphalt in the same category prevented their effects from being separated and the lack of information about the exposure assessment methods further hampered interpretation of the results. The low response proportion for controls was also of some concern.]

Some information potentially relevant to the effects of bitumen was reported in

Table 2.4 Case–control studies of cancer of the bladder and exposure to bitumens

Reference, study location and period	Total cases	Total controls	Control source (hospital, Population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Mommsen *et al.* (1983) Denmark 1977–80	212	259	Not specified. Matched on age, sex, area and urbanization	Occupation and chemical exposure in-person interview (cases) and phone interview or mail questionnaire (controls)	Petroleum or asphalt	9	2.36 (95% CI not given)	Tobacco, alcohol, other substances Unspecified source of controls, different interview methods for cases and controls and lack of detail about exposure assessment methods. Crude OR, 3.78 (95% CI, 1.12–12.81)
Risch *et al.* (1988) Canada 1979–82	826	792	Population register. Matched on age, sex, birth year, residence	Personal interview	Tars and asphalt			Smoking Method of assessing exposure to specific substances not specified. Response rate: cases, 67%; controls, 53%. ORs for men only. Exposure to tar and asphalt combined.
					Ever exposed	NR	1.44 (0.78–2.74)	
					Exposed for 10 yr	NR	2.02 (1.08–4.97)	
					Exposed 8–28 yr ago	NR	3.11 (1.19–9.68)	
Bonassi *et al.* (1989) Italy 1972–82	121	342	Population registers. Matched for age, sex and time at risk	Interview and classification of occupations as definitely, possibly or not exposed.	Road menders	2	1.4 (0.3–7.3)	Smoking, aromatic amines Men only

Table 2.4 (continued)

Reference, study location and period	Total cases	Total controls	Control source (hospital, Population)	Exposure assessment	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Kogevinas *et al.* (2003) Pooled data from 11 studies in 6 countries in Europe 1976–96	3346	6840	Hospital (7 studies), population (3 studies) or both (1 study)	Job title, list of high-risk occupational and JEM.	Roofers	13	0.72 (0.36–1.43)	Age, smoking, study centre Relevant data presented only by job title. Men only.
Geller *et al.* (2008) Germany	156 men	336	Prostate cancer cases in same registry as cases.	Postal questionnaire	Frequent exposure to bitumen.	NR	2.92 (1.32–6.48)	Age, smoking, multiple occupational exposures Controls had prostate cancer.

NR, not reported; OR, odds ratio; yr, year

a population-based study of cancer of the bladder and exposure to PAHs in Italy (Bonassi *et al.*, 1989). Cases (n = 121) were identified from hospital records for the years 1972–82 and controls (n = 342) matched by age, sex, and time of case occurrence were sampled at random from population registries. Only men were included in the analysis. Information about risk factors for cancer of the bladder, including occupation, was obtained by interview with subjects or next-of-kin. Occupations were classified as definitely, possibly or not exposed to PAHs using a job-exposure matrix constructed from a review of the literature. Road menders were among the occupations classified as definitely exposed. Odds ratios were adjusted for smoking and exposure to aromatic amines. An odds ratio of 1.4 (95% CI, 0.27–7.28) based on two cases and six controls was reported for work as a road mender. The remaining analyses focused on PAHs in general and were not informative about exposure to bitumens. [The very small numbers of exposed cases and controls limited the information contributed by this study.]

Associations of cancer of the bladder with occupational exposures were considered in a large study (Kogevinas *et al.*, 2003) pooling 11 previous case–control studies of cancer of the bladder in men in six European countries. The pooled analysis included data for 3346 cases and 6840 controls. Occupational exposures were estimated from work histories taken in the original studies (lifetime histories in ten studies and usual occupation in one). Occupations were aggregated according to prior information about risk factors for cancer of the bladder and results were reported only for those with at least 10 subjects. Odds ratios were adjusted for age, smoking and study centre. Roofers were considered to have a high-risk occupation, but had an odds ratio of 0.72 (95% CI, 0.36–1.43) based on 13 cases and 39 controls. No other information relevant to exposure to bitumens was reported. [The sample size for this study was larger than for the other case–control studies of cancer of the urinary bladder and provided a risk estimate with a relatively narrow confidence interval. The exposure assessment was crude, hampering the ability to distinguish specific exposures in roofers.]

Occupational risk factors for cancer of the bladder were also examined in a registry-based case–control study in Germany (Geller *et al.*, 2008). The cases were 156 men with bladder cancer who had applied for cancer treatment, and controls were 336 men with prostate cancer identified from the same database. Information on occupational history, potential exposure to carcinogens, smoking and other factors was obtained by mailed questionnaire. Questions specifically addressed exposure to bitumens, tar and pitch, which could be classified as seldom, often or "permanent." Odds ratios were adjusted for age, smoking and multiple occupational exposures. For frequent exposure to bitumens, the smoking-adjusted odds ratio was 2.92 (95% CI, 1.32–6.48). [This study sought specifically to identify exposure to bitumens, but the Working Group questioned whether it were possible for the workers to differentiate exposures to coal tar, pitch and bitumens. There may be collinearity between these three exposures, but the authors did not attempt to adjust one factor for the other factors in the model.]

2.3.3 Cancer of the kidney

A hospital-based case–control study of cancers of the renal pelvis and ureter in Denmark (Jensen *et al.*, 1988), which was directed primarily towards the effects of smoking, included some information about occupational risk factors. Cases (n = 96) and controls (n = 288) were matched on hospital, sex and age; patients with other diseases of the urinary tract or smoking-related diseases were excluded from the control group. A structured questionnaire was used to obtain information on occupations and occupational exposures. Odds ratios were adjusted for age, sex

and smoking history. Findings for occupational exposure to asphalt and tar were reported only for men: the smoking-adjusted relative risk based on nine exposed cases and six exposed controls was 5.5 (95% CI, 1.6–19.6) [apparently for ever *versus* never exposure.] [The utility of this study for evaluating risks due to bitumens was limited by the lack of detail about the methods and the classification of exposures to asphalt and tar in the same category.]

The relationship between renal cell carcinoma and occupational exposure to chemicals was examined by Hu *et al.* (2002) using data from a national cancer-surveillance system in Canada. Cases were 1279 men and women with incident kidney cancer reported between 1994 and 1997. Controls were 5370 individuals without cancer selected at random from the population of each province, frequency-matched on age and sex. Data on employment, smoking, alcohol use, and other risk factors was collected by mailed questionnaire. Subjects were also asked if they had ever been exposed for > 1 year at work to 17 substances, and, if so, the duration of the exposure. Exposures to coal tar, soot, pitch, creosote and asphalt were combined in the analysis: the odds ratio for ever exposure to this group of substances was 1.4 (95% CI, 1.1–1.8) among men and 1.3 (95% CI, 0.7–2.3) among women with adjustment for age, province, education, body-mass index, smoking, alcohol use and meat consumption. Associations with duration of exposure were reported only for selected substances, and coal tar, soot, pitch, creosote and asphalt were not included. [The large nationally representative sample was a strength of this study. Exposures were assessed by a self-administered checklist of substances, providing more detail than job titles alone. However, the exposure classification did not allow exposures to bitumen, coal tar and other substances to be separated. Selective reporting of results also limited the inferences that could be made.]

2.3.4 Cancer at other sites

The relationship between hepatocellular carcinoma and occupational exposure to chemicals was evaluated as part of a multisite case–control study in the USA that was targeted primarily on the effects of cigarettes, alcohol and hepatitis B virus (Austin *et al.*, 1987). Eighty cases with histologically confirmed cancer and 146 hospital controls were matched on sex, age, race and study centre. Patients with smoking-related diseases, including cancer of the lung, and other liver diseases were not eligible to be controls. Information on all jobs held for 6 months and on ever having been exposed to 26 substances, including tar and asphalt separately, was obtained by interview. Results were tabulated only for jobs and substances reported by at least 10 subjects. Seven cases and five controls reported exposure to asphalt, giving an odds ratio of 3.2 (95% CI, 0.9–11). Data for exposure to tar were not reported. Five cases and two controls had been employed in highway and street construction (OR, 5.0; 95% CI, 1.0–26). [The Working Group observed that this study was noteworthy in that it attempted to differentiate between exposures to tar and asphalt.]

Associations of occupational exposure and cancer of the brain in Canada were investigated by Pan *et al.* (2005) using data sources and methods similar those used by Hu *et al.* (2002) to study cancer of the kidney. The cases were 1009 individuals with incident primary malignant tumours of the brain, including glioblastoma, astrocytoma, oligodendroglioma, ependymoma, and others not specified. The controls were 5039 people selected at random from population registries. Data were obtained by a mailed questionnaire, which had questions about employment history and exposure to 18 substances, including bitumen. The rate of participation was 62% for cases and 67% for controls. Analysis was by logistic regression with adjustment for age, province, sex, education, alcohol, smoking and

energy intake. The odds ratio for ever exposure to bitumen was 1.29 (95% CI, 1.02–1.62) after adjustment. Elevated odds ratios for exposure to bitumen were observed for men (OR, 1.20; 95% CI, 0.93–1.54) and women (OR, 1.85; 95% CI, 1.03–3.34). Monotonically increasing odds ratios and statistically significant (P = 0.33) trends were observed with increasing duration of exposure. The odds ratio for > 10 years exposure was 1.39 (95% CI, 0.97–1.99) with full adjustment as described above. In analyses based on job titles, odds ratios were elevated for ever (OR, 1.16; 95% CI, 0.73–1.85) or usually (OR, 1.22; 95% CI, 0.47–2.19) working in excavating, grading, paving and related occupations. An odds ratio of 1.73 (95% CI, 0.52–5.81) was observed for four individuals who had ever worked as roofers; no result was reported for usual occupation as a roofer. [The large size of this study facilitated a range of analyses. Reasonable precision and the use of an exposure checklist afforded more details about exposure than job titles alone. The study also assessed the trend with duration of exposure.]

Exposure to bitumen and related materials was considered in a study of the relationship between skin cancer and occupational exposure to PAHs among men in Poland (Kubasiewicz *et al.*, 1991). Cases (n = 376) were men with skin cancer enrolled in a cancer registry between 1983 and 1988. Population- and hospital-control groups of 752 men each were randomly sampled from the population at large and hospital services. Information on occupational history was obtained by interview. The analysis of occupational exposure considered substances believed to be risk factors for skin cancer, including pitch, tar, "asphalt", "soft asphalt", and "bituminous mass", but details of how exposure was assessed were not given. The odds ratios for tar, pitch and bituminous mass were 1.09, 0.93 and 2.03, respectively with population controls and 1.00, 0.86 and 2.02, respectively, with hospital controls. No confidence intervals or P values were reported. Results were not reported for asphalt or soft asphalt. [This study was considered to be minimally informative because of its broad focus on PAHs, the lack of detail on the exposure-assessment methods, unclear definitions of the substances of interest, and weak statistical methods that were poorly described.]

2.3.5 Multiple cancer sites

Associations between 11 cancers (oesophagus, stomach, colon, rectum, pancreas, lung, prostate, bladder, kidney, skin melanoma and non-Hodgkin lymphoma) and occupational exposure to bitumen were evaluated in a large, hospital-based case–control study in Canada (Siemiatycki, 1991). A total of 3505 individuals with the cancers of interest were enrolled during 1979–85. Subjects served both as cases and as controls in analyses of other cancers. Occupational exposures were assessed by combining expert assessment with detailed interviews with subjects. Odds ratios were adjusted for potential confounders selected *a priori* for each cancer site. Adjusted odds ratios for any exposure to bitumen were 1.0 or less for all of the cancers evaluated, with the exception of cancer of the colon (OR, 1.6; 90% CI, 1.1–2.5) and non-Hodgkin lymphoma (OR, 1.4; 90% CI, 0.8–2.7). For substantial exposure to bitumen, odds ratios were elevated for cancers of the stomach (OR, 2.0; 90% CI, 1.0–4.1) and prostate (OR, 1.8; 90% CI, 0.8–4.0), but not for other sites. In subgroup analyses, substantial exposure to bitumen was associated with cancer of the prostate (OR, 3.0; 90% CI, 1.0–9.0), bladder (OR, 2.2; 90% CI, 1.0–4.9) and non-Hodgkin lymphoma (OR, 1.5; 90% CI, 0.4–5.1) among French Canadians only. [The Working Group considered the detailed exposure assessment to be a noteworthy strength of this study. Small numbers reduced precision in analyses of some combinations of cancer site and exposure, and this combined with the unusually large number of comparisons made it likely that some associations occurred by chance.]

2.4 Meta-analyses

Several articles have reviewed the epidemiological literature on risk of cancer among bitumen workers or associated with exposure to bitumens and/or bitumen fume; however, only meta-analyses are presented here (Table 2.5).

Partanen & Boffetta (1994) conducted a study of cancer risks among asphalt workers and roofers in a meta-analysis of 19 epidemiological studies (11 cohort, eight case–control) from both Europe and North America. [The authors' text stated that 20 studies were reviewed, but the Working Group counted only 19. All studies are described in Sections 2.1.1 and 2.2.] The timeframe for case ascertainment in these studies ranged from 1945 to 1989. Risk ratios for all bitumen workers were: cancer of the lung, 1.19 (95% CI, 1.08–1.30) (1.21 for cohort and 1.12 for case–control studies); cancer of the stomach, 1.28 (95% CI, 1.03–1.59) (1.33 for cohort and 1.00 for case–control studies); cancer of the bladder, 1.22 (95% CI, 0.95–1.53) (1.38 for cohort and 0.80 for case–control studies); non-melanoma skin cancer, 1.74 (95% CI, 1.07–2.65) (four cohort studies); and leukaemia, 1.41 (95% CI, 1.05–1.85) (four cohort studies). In a subsequent analysis, studies of roofers and pavers/highway-maintenance workers were considered separately for easier understanding of patterns. Risk ratios for cancer of the lung were 0.87 (95% CI, 0.76–1.08) among pavers and highway-maintenance workers and 1.78 (95% CI, 1.50–2.10) among roofers. Some studies included adjustment for smoking. Only one such study was available for pavers and highway-maintenance workers and had a risk ratio of 0.9 (95% CI, 0.6–1.50); among studies of roofers, the smoking-adjusted risk ratio for cancer of the lung was 2.0 (95% CI, 1.3–2.8) based on four studies. [This was one of the most informative reviews because of the details provided by the meta-analysis. Smoking did not appear to confound the risk ratios in these studies. It was not possible to separate the effects of coal tar from those of bitumen.]

Fayerweather (2007) conducted a meta-analysis of the epidemiological literature on bitumen exposures to update the previous meta-analysis (Partanen & Boffetta, 1994) and adjust for possible confounding from exposure to coal tar. Only peer-reviewed, published reports were included. When multiple reports were available on the same study, the most recent version was selected. Also relative risks based on internal referents were selected over those based on external referents. Reported relative risks from exposure to bitumen were adjusted for potential confounding from exposure to coal tar using information from the literature on coal-tar exposure among asphalt workers and referents, and the relative risk for cancer of the lung associated with coal tar. Adjustments were based on concentrations of benzo[*a*]pyrene of 20 μg/m^3 for coal-tar roofing and 10 μg/m^3 for coal-tar paving. Sixteen country-specific epidemiological studies of roofers and eleven studies of pavers were entered into the meta-analysis. The meta-relative risk dropped from 1.67 (95% CI, 1.39–2.02) to 1.10 (95% CI, 0.91–1.33) with adjustment for coal tar in roofing studies, and from 0.98 (95% CI, 0.81–1.18) to 0.96 (95% CI, 0.80–1.16) with adjustment in paving studies. Studies were also stratified by design, with cohort (SMR, SIR, RR) and case–control studies considered to be stronger designs than proportionate mortality, public census, and cross-sectional designs. The point estimates were similar for roofing and paving studies combined: 1.03 (95% CI, 0.85–1.25) for the studies with weaker study designs, *versus* 1.01 (95% CI, 0.88–1.17) for studies with stronger designs. [The Working Group found this meta-analysis noteworthy in its efforts to disentangle exposures to coal tar and bitumen. Group-level adjustments are useful when information is not directly available at the individual level, as in group-level adjustment for smoking differences by occupational category. The interpretation

Table 2.5 Meta-analyses of risk of cancer and exposure to bitumens

Reference	Years covered	Type of analysis	Organ site	Exposure categories	Meta relative risks (95% CI)	Covariates	Comments
Partanen & Boffetta (1994)	1976–93	Meta-analysis of 20 studies		Roofers			Not possible to separate effects of tar and bitumen. Smoking did not appear to confound ORs.
			Lung		1.8		
			Stomach		1.7		
			Non-melanoma skin		4.0		
			Leukaemia		1.7		
				Road pavers			
			Lung		0.9		
			Stomach		1.1		
			Non-melanoma skin		2.2		
			Leukaemia		1.3		
Fayerweather (2007)	Up to 2005	Meta-analysis of 27 studies with adjustment for coal-tar exposure	Lung	Roofers	1.67 (1.39–2.02)	Unadjusted	External adjustment for coal tar. Potential for over-adjustment.
					1.10 (0.91–1.33)	Adjusted for coal-tar exposure	
				Pavers	0.98 (0.81–1.18)	Unadjusted	
					0.96 (0.80–1.16)	Adjusted for coal-tar exposure	

OR, odds ratio

of these group-level adjustments relies on the extent to which the external data, such as historical levels of exposure, prevalence of exposure in the current population, and the risk associated with a given level of exposure, correspond to the experience of the study population. The Working Group noted that some of the studies included in this meta-analysis were already adjusted for coal tar (i.e. the IARC study) and this analysis was therefore over-adjusted for coal tar. Adjusting for coal tar using benzo[*a*]pyrene as a marker could also result in an over-adjustment, because benzo[*a*]pyrene can also be generated by bitumen emissions.]

3. Cancer in Experimental Animals

Several independent studies in mice, rats, guinea-pigs or rabbits, using skin application, subcutaneous injection, intramuscular injection and inhalation were evaluated as inadequate by the Working Group and were not taken into consideration for the evaluation of the carcinogenicity of bitumens in experimental animals (reported in Simmers *et al.*, 1959; Simmers, 1964, 1965a, b, 1966; Hueper & Payne, 1960; Kireeva, 1968). Limitations of these included poor reporting of the study, the small number of animals tested, lack of information on dose and duration of treatment, no information of distribution of "fumes", lack of concurrent vehicle-control group, the use of a carcinogenic agent as vehicle, poor survival, no information on survival, animals lost and replaced in the middle of the experiment, and pathology not provided. These independent studies are not presented in the tables. This section provides a brief summary of each and a more detailed review of the relevant studies.

[To avoid confusion in the nomenclature, the agent administered was always first reported using the name given in the original publication, in quotation marks, followed by the bitumen class, as classified by the Working Group, in square brackets.]

3.1 Mouse

See Table 3.1

3.1.1 Skin application

A group of 32 male and 36 female C57 Black mice (age not reported) were treated with a pooled sample of six "steam-refined and air-blown (oxidized) petroleum asphalts" [bitumens class 1 and 2] dissolved in benzene. A group of 31 male and 32 female C57 Black mice served as controls and were treated with benzene alone. The treated mice received an unspecified dose, applied with a glass rod onto the interscapular skin, twice per week. Twelve epidermoid carcinomas [$P = 0.0002$] appeared at the site of application, with the first tumour appearing during week 54 of the study. No skin tumours were reported in the control mice. [The study was poorly reported, with no indication of the duration of treatment, or the amount of compound applied, or survival. The vehicle used is an IARC Group 1 carcinogen (Simmers *et al.*, 1959).]

Simmers (1965a) treated a group of 25 male and 25 female C57 Black mice (age, approximately 6 weeks) with 75–100 mg of a pooled sample of three "steam-refined petroleum asphalts" [bitumen class 1] heated in a boiling-water bath and applied to the skin with a glass rod, three times per week, for up to 23 months. Because only 15 males and 12 females survived an epidemic of pneumonitis that occurred after 7 weeks, and only 1 male and 5 females were alive after 1 year, 8 males and 5 females of unknown age were added to the group. The total number of applications ranged from 16 to 240. Topical squamous cell carcinomas were found in 3 of the 21 autopsied mice. [The Working Group noted the high mortality in the early part of the study.]

Table 3.1 Studies of carcinogenicity in mice exposed to bitumens

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Skin application				
Mouse, C57 Black (M, F) 92–96 wk Simmers (1965a)	0, 20–30 mg of a 90% solution of an "air-refined (oxidized) petroleum asphalt" (bitumen class 2) in toluene; 3 ×/wk to skin with glass rod 20 mice; 15 toluene controls.	Topical squamous-cell carcinoma: 0/15, 9/20 (45%)	[$P = 0.002$, Fischer's exact test]	No information on survival.
Mouse, Swiss albino (M, F) Duration NR Wallcave *et al.* (1971)	2.5 mg of eight different "road-paving-grade asphalts" (bitumen class 1) produced by vacuum distillation from well-defined crude sources dissolved in benzene (10% solutions); 2 ×/wk to the skin of the back 24–32 treated; 30 control mice treated with benzene only.	Skin tumours: Carcinoma: 1/218, 0/26 Papilloma: 5/218 (2%), 1/26 (4%) Combined: 6/218 (3%), 1/26 (4%)	[NS] [NS] [NS]	No indication of duration of treatment, vehicle used is an IARC Group 1 carcinogen.
Mouse, C3H/HeJ (M) 80 wk Emmett *et al.* (1981)	0, 50 mg of a solution of a standard "roofing petroleum asphalt" (bitumen class 2) dissolved in toluene (1:1 w/w); 2 ×/wk on the intrascapular skin 50 mice/group.	No skin tumours	[NS]	Skin tumours were observed in 31/39 (79%) of a benzo[*a*]pyrene (0.1% toluene solution, 50 mg of solution/application) positive control.
Mouse, Sencar (F) 52 wk Robinson *et al.* (1984), Bull *et al.* (1985)	0%, 89% (asphalt A), 98% (asphalt B) 97% (asphalt C), 97% (asphalt D) "asphalt cutbacks" (class 3 bitumen, a solid petroleum asphalt material cut back to 64% solid with mineral spirits) diluted with xylene and/or mineral spirits to give a dosing volume of 0.2 mL; a single application of 200 µL applied to the shaved dorsal surface followed in 2 wk by topical application of 1.0 µg of TPA in 0.2 mL acetone 3 ×/wk for 20 wk 30 or 40 mice/group.	Squamous cell carcinoma: 2/36 (6%; asphalt A), 0/31 (asphalt B), 5/31 (16%; asphalt C), 6/33 (18%; asphalt D) Acetone control: 0/23	$P \leq 0.05$ for asphalts C and D	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Mouse, Sencar (F) 52 wk Robinson *et al.* (1984), Bull *et al.* (1985) contd	0%, 89% (asphalt A), 98% (asphalt B) 97% (asphalt C), 97% (asphalt D) "asphalt cutbacks" (class 3 bitumen, solid petroleum bitumen material cut back to 64% solid with mineral spirits) diluted with xylene and/or mineral spirits to give a dosing volume of 0.2 mL; 3 weekly applications of 200 µL applied to the shaved dorsal surface followed in 2 wk by topical application of 1.0 µg of TPA in 0.2 mL acetone 3 ×/wk for 20 wk 30 or 40 mice/group.	Squamous cell tumours: 10/38 (26%; asphalt A), 6/35 (17%; asphalt B), 13/36 (36%; asphalt C), 4/35 (11%; asphalt D) Mineral-spirits control: 1/37	$P \leq 0.05$ for asphalts A, B, and C	
Mouse, CD1 & C3H/HeJ (M) 78 wk Niemeier *et al.* (1988), NIOSH (2001a)	Fumes generated by heating "type I or type III asphalts" (produced by distillation and air blowing of Arabian crude) or type I or type III coal tar pitch at either 232 °C or 316 °C were collected and diluted in 1/1 cyclohexane/acetone to an unspecified concentration and then applied to the clipped interscapular area. Thirty-two groups of mice for the primary factorial experiment, i.e. 2 strains × 4 materials × 2 generation temperatures × 2 light exposure conditions (presence or absence of simulated sunlight); each animal was dosed 2×/wk with 50 µL of the appropriate test material; four groups for a combination treatment of bitumen and coal-tar pitch fume condensate; eight groups for controls; and four groups for positive control with benz[*a*]pyrene 50 mice/group.	Specific tumour data not provided. The average latent period in the groups treated with the condensed fume of the roofing materials ranged from 39.5 to 56.1 wk among the C3H/HeJ groups, and from 47.4 to 76.5 wk among the CD-l. Skin tumours – see Tables 3.2 and 3.3.		

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Mouse, C3H/HeJ and Sencar (M) 96 wk Sivak *et al.* (1997)	Exposure to a 'type III "steep" asphalt' (class 2), produced by distillation and air-blowing Arabian crude, heated at 316 °C and the fume condensates collected, fractionated, and then applied dermally at doses of 1.6–25 mg bitumen, bitumen + fume or fume alone in 0.05 mL cyclohexane/acetone (1/1); 2 ×/wk for 104 wk 39 treated and 2 control groups of 30.	Skin tumours – see Table 3.4	NR	
Mouse, C3H/HeNCrl (M) 104 wk Clark *et al.* (2011)	Exposed to a "field-matched" BURA [class 2 bitumen] fume condensate (collected at 199 °C) or a "lab-generated" BURA fume condensate (collected at 232 °C) applied 2×/wk in a volume of 37.5 µL (25 mg) mineral oil for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and benzo[*a*]pyrene (BaP 0.05% in 37.5 µL toluene, applied 2 ×/wk) were used as negative and positive controls 2 ×/wk for 104 wk 80 treated and 2 control groups of 50 and 80 mice.	*Skin tumours* Squamous cell carcinoma: 0/80 (control), 8/62 (13%), 35/64 (55%), 34/49 (69%) (BaP) Squamous cell papilloma: 0/80 (control), 4/62 (6%), 3/64 (5%), 2/49 (4%) (BaP)	$P < 0.0001$, Fisher's exact test $P < 0.0001$ for BaP	
	Exposed to a "field-matched" paving [class 1 bitumen] fume condensate (collected at 148 °C) applied daily in a volume of 37.5 µL (7.14 mg) mineral oil for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and BaP (0.05% in 37.5 µL toluene, applied 2×/wk) were used as negative and positive controls 2×/wk for 104 wk 80 treated and 2 control groups of 50 and 80 mice	*Skin tumours* Squamous cell carcinoma: 0/80 (control), 0/80, 37/50 (74%) (BaP) Squamous cell papilloma: 0/80 (control), 1/80 (1%), 1/50 (2%) (BaP)	$P < 0.0001$ for BaP	
Mouse, C_3H/HeNCr1BR (M) 96 wk Goyak *et al.* (2011)	An "asphalt cement 20" and a "coastal residuum" (both class 1) were diluted with mineral oil and applied at the limits of solubility in mineral oil, 30% and 75% ([w/w]), respectively, as 37.5 µL doses. BaP was applied at a 0.05% (w/v) dilution in toluene; 2×/wk to clipped back. 50 mice/group	*Skin tumours* Carcinoma: 0/50 (oil), 0/50 (toluene), 46/50 (92%) (BaP), 0/50, 0/50.	[NS]	Mineral oil should have been used as vehicle for the positive control group. Significant for BaP.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance	Comments
Mouse, Crl:CD1 (M) 28 wk Freeman *et al.* (2011)	Initiation/promotion A "field-matched" roofing (BURA) fume condensate (collected at 148 °C) was tested as an initiator by applying it in a volume of 37.5 µL (25 mg) mineral oil 2 ×/wk (total weekly dose, 50 mg) for 2 wk followed by administration of TPA (5 µg, 0.01% in acetone) 2×/wk for 25 wk and as a promoter by applying it in a volume of 37.5 µL (25 mg) mineral oil 2 ×/wk for 28 wk after a single 50 µg dose of DMBA. Mineral oil, TPA and DMBA controls were included. 30 mice/group	*Skin tumours* Squamous cell papilloma: 0/30 (control), 0/30 (BURA), 1/30 (3%) (TPA) 5/30 (17%) (BURA/TPA), 0/30 (DMBA), 2/30 (7%) (DMBA/BURA), 27/30 (90%) (DMBA/TPA).	$P < 0.01$	
Subcutaneous or intramuscular injection				
Mouse, C57 Black (M, F) 54 wk Simmers *et al.* (1959)	Subcutaneous injection 0, unspecified dose of a pooled sample of six steam- and air-blown (oxidized) petroleum asphalts (bitumens class 1 and 2) suspended in olive oil (1%). 0.2 mL of 2 ×/wk for 41 wk and then 1×/wk for unspecified time in the interscapular region 63 or 68 mice/group	Injection site sarcoma: 0/63, 8/68 (12%)	$P = 0.0035$, one-tailed Fisher's exact test	Study poorly reported, no indication of duration of treatment, author indicates "Thus far distant metastasis has not been seen …" implying this is a preliminary report of an unfinished study, no indication of the number of tumour bearing animals, no statistical analysis applied.

BaP: benzo[*a*]pyrene; BURA, built-up roofing asphalt; d, day; DMBA, 7,12-dimethylbenz[*a*]anthracene; h, hour; mo, month; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; wk, week; yr, year.

In a second experiment, a group of 25 male and 25 female C57 Black mice (age, approximately 6 weeks) was treated with 75–100 mg of "air-refined (oxidized) petroleum asphalt" [bitumen class 2] heated in a boiling-water bath and applied to the skin with a glass rod, one to three times per week for up to 7 weeks. No carcinomas were observed at the site of treatment in the 10 mice autopsied. [The Working Group noted the absence of concurrent controls for both experiments.]

In a third experiment, a group of 10 male and 10 female mice were treated with the same "air-refined asphalt" [bitumen class 2], diluted in toluene (10% toluene : 90% asphalt) that was applied to the skin with a glass rod, three times per week for up to 2 years. Squamous cell carcinoma of the skin developed in 9 of the 20 mice autopsied [$P = 0.002$]. No squamous cell carcinomas were observed in the 15 control mice treated with toluene only; one mouse developed a skin papilloma (Simmers, 1965a).

A group of 25 male and 25 female C57 Black mice (age, 20–22 weeks) was treated with a mixture of "aromatics" and "saturates" [a fraction of a class 1 bitumen], isolated by fractionation of a "steam-refined asphalt" [bitumen class 1] from a California crude petroleum. The steam-refined asphalt had been separated into four fractions: asphaltenes, aromatics, saturates and resins. The thick oily liquid was applied with a glass rod three times per week (about 33.4 mg per application) to the intrascapular non-shaved skin (duration of study not given). The number of applications ranged from 72 to 242 because of differential survival. Forty mice (18 males, 22 females) survived to be autopsied. Thirty of the autopsied mice had gross evidence of neoplastic pathology and were studied microscopically: 13 skin papillomas, 7 epidermoid skin carcinomas, 5 baso-squamous cell cancers and 1 sebaceous-gland carcinoma were observed. Other tumours found included one epidermoid carcinoma of the anus and two leiomyosarcomas (one subcutaneous and one intestinal) (Simmers, 1965b). [The study was poorly reported, with no indication of the duration of treatment, and no controls were used.]

Groups of 25 male and 25 female C57 Black mice were exposed to "road petroleum asphalts" [bitumen class 1] obtained by steam distillation of crudes from Mississippi and California, USA, Venezuela, or by steam-vacuum distillation of one Oklahoma crude, respectively. Each mouse received one drop of an unspecified dose of bitumen, liquefied with acetone, applied to the neck skin, twice per week for up to 2 years. One skin carcinoma was observed in the group treated with the Mississippi sample, and one skin papilloma was observed in the groups treated with the Oklahoma and the Mississippi samples. No skin tumours were found in the groups treated with the samples from Venezuela or California or in 100 male and 100 female untreated mice (Hueper & Payne, 1960). [The study was poorly reported, and there were no vehicle controls.]

A group of 25 male and 25 female C57 Black mice received an unspecified dose of a sample (heated to liquefy) of an "air-blown asphalt" [bitumen class 2] used for roofing purposes, applied to the skin of the nape of the neck, twice weekly, for up to 2 years. One skin carcinoma was reported (Hueper & Payne, 1960). [The study was very poorly reported, no controls were used, and survival data were not provided.]

Different sized groups of SS-57 white mice (sex and age unspecified) were exposed to two cracking-residue [destructive thermal distillation] bitumens (BN-5 and BN-4) (bitumen class 6) and four residual bitumens [straight distillation] (BN-5, BN-4, BN-3 and BN-2) (class 1). Carcinogenicity was tested by skin painting with each bitumen in a 40% solution in benzene, once per week for 19 months (70 applications) (Kireeva, 1968).

The cracking-residue bitumen BN-5 study started with 52 mice, and 49 survived to the time of appearance of the first tumour (month 9). Nine

animals developed skin tumours at the treatment site [$P < 0.05$, *versus* untreated controls]: five cornified squamous cell carcinomas, one fibrosarcoma and three papillomas. In addition, seven mice developed pulmonary adenoma and adenocarcinoma, and one developed a squamous cell carcinoma of the forestomach.

The cracking-residue bitumen BN-4 study started with 47 mice, and 42 survived to the time of appearance of the first tumour (month 10). Four mice had skin tumours (one cornified carcinoma, one noncornified carcinoma, and two papillomas), and all four also had pulmonary adenoma.

There were initially 50 animals in the residual bitumen BN-5 study, 37 animals in the BN-4 study, 50 animals in the BN-3 study, and 40 animals in the BN-2 study. Skin tumours were reported in two (one cornified squamous cell carcinoma and one sebaceous carcinoma) of 43 (BN-5), none of 30 (BN-4), two (one fibrosarcoma and one papilloma) of 43 (BN-3) and none of 30 (BN-2) mice surviving 9 months, respectively. In addition, tumours of the lung were observed in 5 out of 43 (12%), 1 out of 30 (3%), 1 out of 43 (2%) and 1 out of 30 (3%) mice, respectively. In 23 control mice painted with benzene only, no skin tumours were seen; one mouse developed lung adenomas (Kireeva, 1968). [The study was poorly reported, survival data were lacking, and the vehicle used is an IARC Group 1 carcinogen.]

Groups of 24–32 male and female random-bred Swiss albino mice (age, 7–11 weeks) were exposed to samples of eight "road-paving-grade asphalts" [bitumen class 1] produced by vacuum distillation from well-defined crude sources. The different bitumens were dissolved in benzene (10% solution) and applied twice per week to the skin of the back with a calibrated dropper delivering 2.5 mg of bitumen per application. An additional group of 15 males and 15 females were painted with benzene only and served as controls. Mean survival times were 81 weeks for bitumen-treated mice and 82 weeks for benzene-treated mice. At the end of the experiment, 6 out of 218 animals treated with the different bitumens developed skin tumours: one was a carcinoma and there were five papillomas. In 26 control mice treated with benzene only, one papilloma was observed (Wallcave *et al.*, 1971). [There was no indication of duration of treatment. The vehicle used is an IARC Group 1 carcinogen.]

A group of 50 male C3H/HeJ mice (age, 6 weeks) were treated with "standard roofing petroleum asphalt" [bitumen class 2] dissolved in toluene (1:1 w/w). Each mouse received 50 mg of the solution on the intrascapular skin, twice per week for 80 weeks. A group of 50 mice were treated with toluene alone and served as controls. No skin tumours were observed in 26 treated mice that survived 60 or more weeks, or in 37 mice of the control group. An additional group of 50 mice served as positive controls and were treated with benzo[*a*]pyrene (0.1% toluene solution, 50 mg of solution per application). Skin tumours were observed in 31 out of 39 (79%) mice surviving at the time of appearance of the first skin tumour (24 malignant, 7 papillomas; average latent period of papilloma, 32 weeks) (Emmett *et al.*, 1981).

Groups of 40 female Sencar mice (age, 6 weeks) were exposed to an "asphalt cutback"[class 3 bitumen], a solid petroleum asphalt material, cut back to 64% solid with mineral spirits), designated "asphalt D", diluted with xylene to 97% asphalt D, 3% xylene, and 200 µL of the resultant solution was applied to the shaved dorsal surface of the mice, once per week for 30 weeks. An additional group of 40 mice were treated with mineral spirits alone and served as controls. All surviving mice were euthanized at week 52. There was one papilloma observed in the treated group and three papillomas in the controls. No carcinomas were observed in either the treated or control group (Robinson *et al.*, 1984).

A study of initiation-promotion was also conducted in groups of 40 Sencar mice given a single initiation dose of 200 µL of four "asphalt

cutbacks" [class 3 bitumen], solid petroleum asphalt materials cut back to 64% solid with mineral spirits): 89% asphalt A, 1% xylene, and 10% mineral spirits; 98% asphalt B and 2% xylene; 97% asphalt C and 3% xylene; and 97% asphalt D and 3% xylene. This was followed 2 weeks later by topical application of 1.0 µg of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 mL of acetone, three times per week for 20 weeks. All surviving mice were euthanized at week 52. Squamous cell tumours were observed in 4 out of 23 (17%; acetone control), 6 out of 36 (17%; asphalt A), 5 out of 31 (16%; asphalt B), 8 out of 31 (26%; asphalt C), and 9 out of 33 (27%; asphalt D) mice. Squamous cell carcinomas were observed in 0 out of 23 (acetone control), 2 out of 36 (asphalt A), 0 out of 31 (asphalt B), 5 out of 31 (16%; asphalt C), and 6 out of 33 (18%; asphalt D) mice. The incidence of squamous cell carcinoma in the groups receiving asphalt C and asphalt D was significantly different from the control group [$P \leq 0.05$; one-tailed Fisher exact test] (Robinson *et al.*, 1984; Bull *et al.*, 1985).

An additional study of initiation–promotion was conducted in four groups of 40 mice given 200 µL of the four "asphalt cutbacks" [class 3 bitumen] used in the previous experiment, once per week for 3 weeks. Mice in the control group received 600 µL of mineral spirit. This was followed 2 weeks later by topical application of 1.0 µg of TPA in 0.2 mL of acetone, three times per week for 20 weeks. All surviving mice were euthanized at week 40. Squamous cell tumours (papilloma and/or carcinoma) were observed in 1 out of 37 (3%; acetone control), 10 out of 38 (26%; asphalt A), 6 out of 35 (17%; asphalt B), 13 out of 36 (36%; asphalt C), and 4 out of 35 (11%; asphalt D) mice. The incidence of squamous cell tumours in mice treated with asphalts A, B, and C was significantly different from that in the control group [$P \leq 0.05$; one-tailed Fisher exact test]. Squamous cell carcinoma was only observed in mice treated with the asphalt preparations (Robinson *et al.*, 1984; Bull *et al.*, 1985).

Groups of 50 CD1 and C3H/HeJ male mice (age, 12–15 weeks) were exposed to condensed fumes from "type I or type III asphalts" [class 2 bitumen], produced by distillation and air blowing of Arabian crude) generated by heating at either 232 °C or 316 °C. Fumes were collected and diluted in 1/1 cyclohexane/acetone and applied to the clipped interscapular area [final dose could not be calculated from reported data]. For the primary factorial experiment, there were two strains × two materials × two generation temperatures × two light-exposure conditions (presence or absence of simulated sunlight); each mouse was dosed twice per week with 50 µL of the appropriate test material. Four groups of 50 mice of each strain served as vehicle controls, while two groups of 50 mice of each strain treated with benzo[*a*]pyrene served as positive controls. Tumours were induced in both strains given condensed fumes from both types of bitumen. Tumour incidence and histology are provided in Table 3.2 for CD-1 mice and in Table 3.3 for C3H/HeJ mice. Condensed neat bitumen fume produced similar and statistically increased tumour yields of papilloma and carcinoma in both strains compared with respective vehicle controls. Recombination of all fractions resulted in a tumour response similar to neat bitumen fume. Raw unheated bitumen produced few tumours in C3H mice, but no tumours were seen when raw bitumen heated to 316 °C, with the fume permitted to escape, was applied. In the C3H/HeJ mice, there was a significant increase in the incidence of malignant and benign tumours with all of the bitumen samples at all temperatures, both with and without simulated sunlight. In the CD1 mice, the response was lower but there was a statistically significant increase of benign tumours in all samples and at all temperatures in the absence of simulated sunlight (Niemeier *et al.*, 1988; NIOSH, 2001a).

Groups of 30 male C3H/HeJ and Sencar mice (age, 8 weeks) were exposed dermally to 'type III "steep" asphalt' [class 2 bitumen], produced

Table 3.2 Histopathology of tumours induced in CD-1 mice treated dermally with roofing bitumen-fume condensates, with or without the presence of sunlight

Material tested	Sunlight	Number of tumour-bearing animals	Number of tumours			
		Benign	Malignant	Papilloma	Squamous cell carcinoma	Total[a]
Type I bitumen @ 232 °C[b]	–	6[c]	0	12	0	12
	+	2	0	3	0	3
Type I bitumen @ 316 °C[b]	–	13[c]	1	18	0	19 [a]
	+	3	0	3	0	3
Type III bitumen @ 232 °C[b]	–	9[c]	1	11	1	13 [a]
	+	5[c]	2	5	1	7 [a]
Type III bitumen @ 316 °C[b]	–	13[c]	3	17	1	20 [a]
	+	4	1	5	1	6
Benzo[*a*]pyrene[d]	–	24[c]	11[c]	43	10	58[a]
	+	9[c]	3	11	1	18[a]
Cyclohexane/acetone[e]	–	0	0	0	0	0
	+	0	0	0	0	0

[a] Other tumours observed included fibrosarcoma, keratoacanthoma, fibroma, and unclassified benign epithelioma
[b] 25 mg of total solid per application
[c] Significantly different ($P \leq 0.05$; one-tailed Fisher's exact test) from the appropriate cyclohexane/acetone control group
[d] 5 µg per application
[e] 50 µL of a 1:1 solution
Adapted from NIOSH (2001a)

Table 3.3 Histopathology of tumours induced in C_3H/HeJ mice treated dermally with roofing bitumen-fume condensates, with or without the presence of sunlight

Material tested	Sunlight	Number of tumour-bearing animals		Number of tumours		
		Benign	Malignant	Papilloma	Squamous cell carcinoma	Total[a]
Type I bitumen at 232 °C[b]	–	24[c]	22[d]	34	26	76
	+	14[c]	27[d]	22	25	62
Type I bitumen at 316 °C[b]	–	13[c]	31[d]	27	31	78
	+	18[c]	26[d]	36	26	73
Type III bitumen at 232 °C[b]	–	15[c]	25[d]	32	19	66
	+	11[c]	20[d]	14	19	54
Type III bitumen at 316 °C[b]	–	12[c]	28[d]	24	36	82
	+	20[c]	18[d]	34	20	65
Benzo[*a*]pyrene[e]	–	11[c]	27[d]	12	29	53
	+	7[c]	27[d]	11	22	43
Cyclohexane/acetone[f]	–	0	0	0	0	0
	+	1	0	2	2	4

[a] Other tumours observed included fibrosarcoma, keratoacanthoma, fibroma, and unclassified benign epithelioma
[b] 25 mg of total solid per application
[c] Significantly different ($P \leq 0.03$; one-tailed Fisher's exact test) from the appropriate cyclohexane/acetone control group
[d] Significantly different ($P \leq 0.001$; one-tailed Fisher's exact test) from the appropriate cyclohexane/acetone control group
[e] 5 µg per application
[f] 50 µL of a 1:1 solution
Adapted from NIOSH (2001a)

by distillation and air-blowing Arabian crude, heated at 316 °C, with the fume condensate collected and fractionated, for up to 24 months. The aims of this study were: (a) to examine the co-carcinogenic and tumour-promoting activities of three bitumen-fume fractions with benzo[*a*]pyrene; (b) to evaluate the direct tumorigenic activity of the five fractions individually and in a variety of combinations; (c) to assess the proportion of tumorigenic activity in the fume and heated residue; and (d) to compare the tumorigenic responses of neat bitumen fume in male C3H/HeJ and Sencar mice. The C3H/HeJ mice were given the test materials in a 50 µL volume of cyclohexane/acetone (1 : 1), applied to the shaved dorsal skin, twice per week. The Sencar mice were treated with the neat bitumen fume only. The tumorigenic responses are presented in Table 3.4. The composition of the bitumen-fume fractions by chemical class determined by GC-MS is presented in Table 3.5. Condensed neat bitumen fume produced similar and statistically significant increased incidence of papilloma in both strains [$P < 0.05$ for Sencar and $P < 0.002$ for C3H/HeJ mice] and of carcinoma in the C3H/HeJ mice [$P < 0.0001$] compared with respective vehicle controls. Among individual fractions, fraction C was most potent, followed by B. The other single fractions were without significant tumorigenic activity. Raw unheated bitumen produced a few tumours in C3H/HeJ mice, but no tumours were seen when raw bitumen heated to 316 °C, with the fumes permitted to escape, was applied (Sivak *et al.*, 1997).

Groups of 80 male C3H/HeNCrl mice (age, 8 weeks) were exposed to a "field-matched" built-up roofing asphalt (BURA) (CASRN 64742-93-4) type III [class 2 bitumen] fume condensate (collected at 199 °C in tank and matched to the chemistry of field fumes collected at higher

Table 3.4 Mortality analysis and tumorigenic response in mice exposed dermally to 'type III "steep" asphalt' and its fume, and fractions thereof

Group	Treatment	Bitumen dose[a]	Mean survival (days)	Median survival (days)[b]	No. of deaths[c]	Total no. of tumours per group[d]		No. of tumour-bearing mice	Multiplicity
						Papilloma	Carcinoma		
C3H/HeJ mice									
1	Raw bitumen	25	610	698	15	1	3	4	1.0
2	Heated bitumen (less fume)	25	655	–	12				
3	Heated bitumen (plus fume)	25	692	–	9				
4	Neat bitumen fume	25	526	573	28	12[e]	25[e]	21	1.8
5	Solvent control	0	607	629	19				
6	Fraction A	16	690	–	11				
7	Fraction B	2.3	643	678	18	2	10[e]	11	1.1
8	Fraction C	2.6	610	659	24	4	18[e]	20	1.1
9	Fraction D	2.3	572	588	23				
10	Fraction E	1.6	629	675	17				
11	Fractions ABCDE	24.8	555	533	29	30[e]	23[e]	25	2.1
24	0.01% BaP [f]	0	449	464	30	1	28[e]	27	1.1
25	0.001% BaP [f]	0	666	732	15	2	3	5	1.0
26	0.0001% BaP [f]	0	630	727	15				
Sencar mice									
41	Neat bitumen fume	25	571	592	25	21[e]	18[e]	20	2.0
42	Solvent control	0	672	–	12				

[a] mg bitumen, bitumen plus fume, or bitumen fume alone/50µL application
[b] For certain groups with low mortality, this percentile could not be estimated
[c] Number of mice that died before the final euthanasia
[d] Only histologically confirmed skin tumours are presented.
[e] Significantly more tumours or earlier onset or both in this group compared with the respective control
[f] 5, 0.5 and 0.05 µg BaP/50 µL application/group, respectively
From Sivak *et al.* (1997)

Table 3.5 Composition of bitumen-fume fractions by chemical class, as determined by gas chromatography-mass spectrometry

Fraction	Composition
A	Alkanes, alkenes and/or cycloalkanes, alkylated benzenes, naphthalenes, benzothiophenes, biphenyls, fluorenes, indanes and indenes
B	Alkylated benzothiophenes, dibenzo- and/or naphthothiophenes, anthracenes and/or phenanthrenes, fluorenes, pyrenes and/or fluoranthenes, benzo- and dibenzofurans and fluorenones
C	Cycloalkenones and/or alkadienones, alkylated phenylethanones, dihydrofuranones, dihydroindenones, isobenzofuranones, hydroxybenzenethiols, pyrenes and/or fluoranthenes, chrysenes and tricarbocyclic fused-ring thiophenes
D	Alkanones, cycloalkenones and/or alkadienones, alkanoic acids, alkylated carbazoles, dihydrofuranones, furanones, isobenzofuranones, naphthols and phenols
E	Alkanones, alkanoic acids and alkylated benzoic acids

From Sivak *et al.* (1997)

temperatures in the kettle and on the rooftop) or a "laboratory-generated" BURA fume condensate (collected at 232 °C) applied twice per week in a volume of 37.5 µL (25 mg) of mineral oil for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and benzo[*a*]pyrene (0.05% in 37.5 µL of toluene, applied twice per week) were used as negative and positive controls, respectively. The incidence of squamous cell carcinoma was significantly increased (35 out of 64; 55%; $P < 0.0001$) in the mice treated with "laboratory-generated" BURA fume condensate. Squamous cell carcinoma was also observed in "field-matched" BURA fume condensate (8 out of 62; 13%; $P = 0.0063$) and in the positive-control group receiving benzo[*a*]pyrene (34 out of 49; 69%; $P < 0.0001$). No tumours were observed in the negative-control group receiving mineral oil (Clark *et al.*, 2011).

In a parallel study, groups of 80 male C3H/HeNCrl mice (age, 8 weeks) were exposed to a "field-matched" paving fume condensate (CASRN 8052-42-4) [class 1 bitumen] (collected at 148 °C), applied daily in a volume of 37.5 µL (7.14 mg) of mineral oil, for a total weekly dose of 50 mg. Mineral oil (37.5 µL per application) and benzo[*a*]pyrene (0.05% in 37.5 µL of toluene, applied twice per week) were used as negative and positive controls, respectively. No squamous cell carcinomas were observed in either the treated or vehicle-control mice and a single squamous cell papilloma was observed in the treated group. The incidence of squamous cell carcinoma was significantly increased in the group receiving benzo[*a*]pyrene (37 out of 50; 74%; $P < 0.0001$) (Clark *et al.*, 2011).

Groups of 50 male C3H/HeNCr1BR mice (age, 8–10 weeks) were treated with an "asphalt cement 20" (CASRN 8052-42-4) and a "coastal residuum" (CASRN 64741-56-6), both produced from a naphthenic crude [both class 1 bitumens], which were diluted with mineral oil (USP grade) and applied at the limits of solubility in mineral oil, 30% and 75% (w/w), respectively. A dose of 37.5 µL was applied twice per week to the clipped back of the mice. Benzo[*a*]pyrene was used as a positive control and was applied at a 0.05% (w/v) dilution in toluene. Control groups received mineral oil or toluene. No skin tumours were observed in mice treated with asphalt cement 20, the coastal residuum or the vehicle controls (toluene and mineral oil). Treatment with benzo[*a*]pyrene produced histopathologically confirmed tumours (all but one being carcinoma) in 92% of the mice (Goyak *et al.*, 2011).

Groups of 30 male Crl:CD1 mice (age, 8 weeks) were exposed to a "field-matched" "BURA type III" (CASRN 64742-93-4) [class 2

bitumen] fume condensate (collected at 199 °C) in an initiation-promotion study. The BURA condensate was tested as an initiator by applying it in a volume of 37.5 µL (25 mg) of mineral oil twice per week (total weekly dose, 50 mg) for 2 weeks, followed by 5 µg of TPA (0.01% in acetone), twice per week for 25 weeks. Squamous cell skin papillomas were observed in 5 out of 30 (17%; $P < 0.01$) mice in the group treated with BURA/TPA compared with 1 out of 30 (3%) in the group receiving TPA. The BURA condensate was also tested as a promoter by applying it in a volume of 37.5 µL (25 mg) mineral oil, twice per week for 28 weeks after a single treatment with 7,12-dimethylbenz[*a*]anthracene (DMBA) at a dose of 50 µg. The BURA condensate did not act as a promoter when tested with DMBA (Freeman *et al.*, 2011).

3.1.2 Subcutaneous and/or intramuscular injection

A group of 33 male and 29 female C57 Black mice (age not reported) were treated with a pooled sample of six "steam-refined and air-blown (oxidized) petroleum asphalts" [bitumens class 1 and 2] suspended in olive oil (1%). A group of 32 male and 28 female mice served as controls and were treated with olive oil only. The treated mice were injected subcutaneously in the interscapular region with 0.2 mL of the suspension twice per week for 41 weeks and then once per week. Eight sarcomas ($P = 0.0035$) appeared at the site of injection, with the first tumour appearing during week 36 of the study. No tumours were reported in mice in the control group (Simmers *et al.*, 1959). [The study was poorly reported, the age of the mice was not given, and there was no indication of duration of treatment.]

Two groups of 25 male and 25 female C57 Black mice received a single subcutaneous injection of 200 µg of "steam-refined asphalt" [bitumen class 1] heated to 70 °C, or to "air-refined (oxidized) asphalt" [bitumen class 2] heated to 100 °C in the interscapular region. After 111 days, all mice without palpable deposits of steam-refined bitumen (9 males and 4 females) were re-injected with an additional 200 µg of bitumen. After 4 months, those mice without palpable deposits of air-refined bitumen (11 males and 7 females) were re-injected with an additional 200 µg of bitumen . The mice were maintained for a total of up to 23 months. No skin tumours were observed in 32 autopsied mice from the group receiving steam-refined bitumen. Five malignant tumours (two rhabdomyosarcomas, one sebaceous-gland carcinoma, two not described) were found in the 38 autopsied mice treated with air-refined asphalt (Simmers, 1965a). [There was no information on survival or use of controls in this study. The author reported that study material was found at the intended site of injection in only a small percentage of the treated mice.]

Groups of 12–26 male and 16–27 female C57 Black mice (age, 9–12 weeks) were exposed to a mixture of "aromatics" and "saturates" [a fraction of a class 1 bitumen] isolated by fractionation of a steam-refined bitumen from California crude petroleum by injecting subcutaneously either a single injection of 0.5 µL, 8 injections of 0.25 µL over a 16-week period or 9–11 injections of 1.0 µL over their lifetime. Tumours of the lung and "skin accessory organs" were observed in all groups (Simmers, 1966). [The study was poorly reported, mice were lost and replaced in the middle of the experiment, and no controls were used.]

Groups of 50 C57 Black mice (sex not reported) were exposed to one of four "road petroleum asphalts" [bitumen class 1] obtained by steam distillation of crudes from Mississippi or California, USA, or Venezuela, and by steam-vacuum distillation of one Oklahoma crude. The mice received six injections (twice per week) of 0.1 mL of the respective bitumens, diluted with equal parts of tricaprylin, into the right thigh muscle, and were maintained for 2 years. Another group of 144 mice similarly received six injections

Table 3.6 Study of carcinogenicity in rats exposed to bitumens by inhalation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours (%)	Significance
Rat, SPF-Wistar (M, F) 96 wk Fuhst *et al.* (2007)	0, 4, 20, or 100 mg/m^3 bitumen-fume condensate collected at 175 °C comprising a majority (70% mass) of air-rectified bitumen (CAS 64 742-93-4, class 2 bitumen) with the remainder being straight-run vacuum residue (CAS 64 741-56-6, class 1 bitumen) (representative of exposure of workers during road paving) Nose-only exposure, 6 h/d for up to 24 mo 50 males and 50 females/group	See Table 3.7	[NS]

d, day; F, female; h, hour; M, male; mo, month; NS, not significant; wk, week

(twice per week) of 0.1 mL of tricaprylin alone and served as controls. After 2 years, sarcoma at the injection site were noted in one mouse in each of the groups treated with samples from crudes from Mississippi, California and Venezuela. No sarcomas were observed in the group treated with the sample from Oklahoma crude, or in the controls treated with tricaprylin (Hueper & Payne, 1960). [The study was poorly reported and there was no information on survival.]

3.1.3 Inhalation

Groups of 10 male and 10 female C57 Black mice (age not reported) were exposed in a nose-only apparatus to an aqueous aerosol of "petroleum asphalt" droplets suspended in moist air for 30 minutes per day, 5 days per week, for 72 weeks. The aerosol was a pooled sample from six different California refineries and contained both steam- and air-blown samples [class 1 and 2 bitumens]. Seventeen mice were autopsied and the tracheo-bronchial tree and lungs were examined microscopically. One papillary adenoma was observed.

In another experiment, a group of 30 C57 Black mice (sex and age not reported) was exposed to "smoke" generated by heating the pooled petroleum-bitumen sample at ~250 °F [121 °C] for 6–7.5 hours per day, 5 days per week, for 21 months. Twenty-one mice were autopsied and the tracheo-bronchial tree and lungs were examined microscopically. No tumours of the respiratory tract were observed. The author reported epithelial hyperplasia occurred occasionally (Simmers, 1964). [The study was poorly reported, especially the information about aerosol and "smoke" generation and dose. There was limited histopathology, a small number of animals, and no controls were used.]

3.2 Rat

See Table 3.6

3.2.1 Intramuscular injection

Groups of 30 Bethesda Black rats (sex not reported) were exposed to one of four "road petroleum asphalts" [bitumens class 1] obtained by steam distillation of crudes from Mississippi or California, USA, or Venezuela, and by steam-vacuum distillation of one Oklahoma crude. The rats received 12 biweekly injections of 0.2 mL of the respective bitumens, diluted with equal parts of tricaprylin, into the right thigh muscle and held for 2 years. Another group of 60 rats served as untreated controls. After 2 years of observation, injection-site sarcomas were noted in two rats in the Venezuela group, 2 rats in the Mississippi group, 4 rats in the Oklahoma group [P = 0.01] and 6 rats in the California

group [P = 0.001]. No sarcomas were observed in the untreated control group (Hueper & Payne, 1960). [The study was poorly reported. While a significant increase in the incidence of injection-site sarcoma was observed in treated rats compared with the untreated controls, the study was considered inadequate because of the lack of vehicle controls.]

3.2.2 Inhalation

A group of 65 female Bethesda black rats (age, 2 months) were exposed to "fumes" of a "blown-petroleum roofing asphalt" [bitumen class 2] for 5 hours per day, 4 days per week, for 2 years. The "fumes" were generated inside the exposure chamber by placing the bitumen in a large evaporating dish that was heated to ~250–275 °F [121–135 °C]. The authors reported that there were no cancers of the lung observed (Hueper & Payne, 1960). [The study was poorly designed and reported. No controls were used.]

Using a rat model that had been demonstrated to be sensitive to PAH-mediated effects on the respiratory tract, Fuhst *et al.* (2007) exposed groups of 50 male and 50 female SPF-Wistar [WU] rats (age, 8 weeks) to atmospheres containing bitumen-fume condensate collected at 175 °C comprising a majority (~70% mass) of air-rectified bitumen [class 2] (CAS 64742-93-4) with the remainder (~30% mass) being straight-run vacuum residue (class 1) (CAS 64741-56-6). The study material was representative of that to which workers are exposed to during road paving The rats were exposed to concentrations of 0, 4, 20, or 100 mg/m^3 by nose-only exposure, 6 hours per day, 5 days per week for up to 24 months. Target concentrations using Berufsgenossenschaftliches Institut für Arbeitssicherheit (BIA) guidelines were 4, 20, and 100 mg/m^3. Actual mean concentrations were 4.1, 20.7, and 103.9 mg/m^3. Taking into account the conversion factor (1.66) between the absolute concentration of bitumen fume determined using the BIA method, the concentrations were 6.8, 34.4, and 172.5 mg/m^3. Mortality was comparable in all groups, but slightly higher in females than in males. Both males and females exposed to 100 mg/m^3 had a statistically significant increase in bronchiolo-alveolar hyperplasia. However, there was no increase in the number of tumour-bearing animals in any of the bitumen-exposed groups compared with the clean-air control group after an exposure of 24 months (see Table 3.7). One of the males at the highest dose had a nasal adenocarcinoma. There were no statistically significant increases in total or organ-specific tumour incidence observed between the clean-air control and the bitumen exposure groups (Fuhst *et al.*, 2007).

3.3 Rabbit

Skin application

Groups of six New Zealand rabbits (sex not reported) were exposed to one of four "road petroleum asphalts" [bitumen class 1] obtained by steam distillation of crudes from Mississippi and California, USA, and Venezuela, and by steam-vacuum distillation of one Oklahoma crude, respectively. Each rabbit received a skin application of undiluted, heated test material painted on the inside of both ears and on a shaved (2 cm^2) area of the back twice per week for up to 2 years. No results were reported (Hueper & Payne, 1960). [The study was very poorly reported, the age of the rabbits was not reported, the number of treated animals was small, and there were no controls.]

A group of six New Zealand rabbits (sex not reported) received an unspecified dose of heated sample of an "air-blown asphalt" [bitumen class 2] used for roofing purposes twice per week on the inside of both ears and on a shaved area (2 cm^2) of the back for up to 2 years. No tumours were observed (Hueper & Payne, 1960). [The study was very poorly reported, the age of the

Table 3.7 Incidence of tumours in rats exposed to bitumens by inhalation

Number of rats	Concentration of bitumen (mg/m³)							
	Males				Females			
	0 (control)	4	20	100	0 (control)	4	20	100
Total number of rats	50	50	50	50	50	50	50	50
With tumours	28	30	27	30	42	34	39	33
With single tumours	19	21	15	23	24	21	25	18
With multiple tumours	9	9	12	7	18	13	14	15
With benign tumours	25	27	27	25	39	32	36	32
With malignant tumours	7	5	5	6	6	8	5	4
With metastasizing tumours	0	0	0	0	1	0	0	2

From Fuhst *et al.* (2007)

rabbits was not reported, the number of treated animals was small, no controls were used, and survival data were not provided.]

3.4 Guinea-pig

Inhalation

A group of 30 Strain 13 guinea-pigs (age, 2 months; sex not reported) were exposed to "fumes" of a "blown petroleum roofing asphalt" [bitumen class 2] for 5 hours per day, 4 days per week, for 2 years. The "fumes" were generated inside the exposure chamber by placing the bitumen in a large evaporating dish that was heated to ~250–275 °F [121–135 °C]. No tumours were observed (Hueper & Payne, 1960). [The study was poorly designed and reported. No controls were used. No dose concentration was provided.]

4. Mechanistic and Other Relevant Data

4.1 Overview of the mechanisms of carcinogenesis of PAHs

This chapter is a short summary of data relevant to the mechanisms of carcinogenesis of PAHs from *IARC Monograph* 92 (IARC, 2010), with a focus on the PAHs detected in bitumens and bitumen emissions.

4.1.1 Introduction

The toxicokinetics of PAHs have been reviewed by the Agency for Toxic Substances and Disease Registry (ATSDR, 1995) and the International Programme on Chemical Safety (IPCS, 1998), while Conney (1982), Cooper *et al.* (1983), Shaw & Connell (1994), Penning *et al.* (1999), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) and Xue & Warshawsky (2005) have also reviewed the metabolism and bioactivation of PAHs. Little is known about the toxicokinetics of individual PAHs, or mixtures of PAHs, in humans. Multiple studies have been conducted to monitor urinary metabolites of PAHs and PAH–DNA adducts in

the lymphocytes of workers exposed to mixtures of PAHs. However, most of the available data on toxicokinetic parameters for PAHs are derived from studies of benzo[*a*]pyrene in animals.

Because of their lipophilicity, PAHs dissolve into and are transported by diffusion across lipid/lipoprotein membranes of mammalian cells, thus facilitating their absorption by the respiratory tract, gastrointestinal tract and skin. PAHs with two or three rings can be absorbed more rapidly and extensively than those with five or six rings. Once absorbed, PAHs are widely distributed throughout the body, with some preferential distribution to or retention in fatty tissues. They are rapidly metabolized to more soluble metabolites (epoxides, phenols, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, quinones and tetrols), and conjugates of these metabolites are formed with sulfate, glutathione (GSH) or glucuronic acid. The covalent binding of reactive PAH metabolites to form DNA adducts may represent a key molecular event in the development of mutations and the initiation of cancer. From the structures of the DNA adducts that are formed, the precursor metabolites may be inferred. PAHs are eliminated from the body principally as conjugated metabolites in the faeces, via biliary excretion, and in the urine.

Most PAHs with potential biological activity range in size from two to six fused aromatic rings. Because of this vast range in relative molecular mass, several of the physicochemical properties that are critical to their biological activity vary greatly. Five properties in particular have a decisive influence on the bioavailablilty of PAHs: vapour pressure; adsorption onto surfaces of solid carrier particles; absorption into liquid carriers; lipid/aqueous partition coefficient in tissues; and limits of solubility in the lipid and aqueous phases of tissues. These properties are intrinsically linked to the metabolic activation of the most toxic PAHs, and an understanding of the nature of this interaction may facilitate the interpretation of studies on their deposition and disposition that are occasionally conflicting.

4.1.2 Absorption

PAHs can be absorbed via the respiratory tract, the gastrointestinal tract and the skin.

(a) Absorption via the respiratory tract

Respiratory absorption depends on the vapour pressure of the PAH between the particulate and gaseous phase of the aerosol by which the substance is emitted into the atmosphere. The vapour pressure of PAHs decreases drastically with increasing molecular mass (Lohmann & Lammel, 2004), so that two-ring naphthalenes are mostly found in the gas phase, whereas five-ring PAHs such as benzo[*a*]pyrene are mostly adsorbed on airborne particles at room temperature (Lane & Gundel, 1996). Strong sorption of a PAH onto particles can further increase the particle-bound fraction of that substance (Lohmann & Lammel, 2004). Gas/particle partitioning is also of great importance during exposure by inhalation, to determine the probable sites of deposition within the respiratory tract. The smaller gaseous PAHs are deposited mostly as soluble vapours, whereas five- to six-ring aromatic compounds are mostly particle-associated at ambient temperatures and can be expected to be deposited with the carrier particles. The rate and extent of absorption by the respiratory tract of PAHs from PAH-containing particles are dependent on particle size (i.e. aerodynamic diameter, which influences regional deposition in the respiratory tract) and the rate of release of PAHs from the particle. Because the release of PAHs is extraneous in exposure to vapours, the rate and extent of absorption of inhaled vapour-phase PAHs are different from those of particle-bound PAHs.

After deposition in the respiratory tract, the sorptive properties of PAHs are a major determinant of the bioavailability of the substance in the organism. For solid particles, the major

determinant for the release is the rate of desorption of the hydrocarbons from the surface, whereas for liquid aerosols, either the dissolution of the entire particle or desorption from insoluble carrier particles is a decisive factor. Substantial fractions of inhaled PAHs deposited in the tracheobronchial region and upper airways can be redistributed by the mucociliary escalator to the gastrointestinal tract, which thereby changes the exposure route from inhalation to ingestion (Sun *et al.*, 1982).

After deposition and desorption from their carrier particles, PAHs are absorbed through the epithelial barriers onto which they are deposited. Highly lipophilic PAHs that are released from particles deposited in the conducting and bronchial airways are retained for several hours and absorbed slowly by a diffusion-limited process, whereas PAHs that are released from particles in alveolar airways are absorbed within minutes (Gerde *et al.*, 1991a, b; Gerde & Scott, 2001). A major effect of the metabolic conversion of PAHs of lower molecular mass is to decrease their lipophilicity and thus accelerate their mobility in tissues (Gerde *et al.*, 1997). Phase I metabolites are slightly more mobile and phase II metabolites are considerably more mobile than the parent compound. As a result, the overall effect of metabolism in the epithelium at the site of entry is to accelerate transport of a lipophilic substrate into the circulation and thereby directly decrease high, acute exposures to this particular epithelial cell population. This local metabolism in airway epithelium probably explains the high levels of benzo[*a*]pyrene-related DNA adducts that have been measured in pure preparations of bronchial epithelial cells from patients with cancer of the lung (Rojas *et al.*, 2004).

(b) Absorption via the gastrointestinal tract

PAHs are absorbed via the gastrointestinal tract through diffusion across cellular membranes, based on their lipophilicity, and through normal absorption of dietary lipids (O'Neill *et al.*, 1991). Absorption of specific PAHs, such as benzo[*a*]pyrene, has been demonstrated after oral administration of radiolabelled compounds to laboratory animals (for review, see ATSDR, 1995; IPCS, 1998). Results from studies in animals have indicated that absorption is rapid (Rees *et al.*, 1971; Modica *et al.*, 1983), that fractional absorption of PAHs of lower relative molecular mass, such as two-ring naphthalene, may be more complete than that of PAHs of higher relative molecular mass, such as five-ring benzo[*a*]pyrene (Chang, 1943; Modica *et al.*, 1983), and that the presence of other materials, such as bile salts or components of the diet, can influence the rate or extent of absorption of PAHs from the intestine (Rahman *et al.*, 1986).

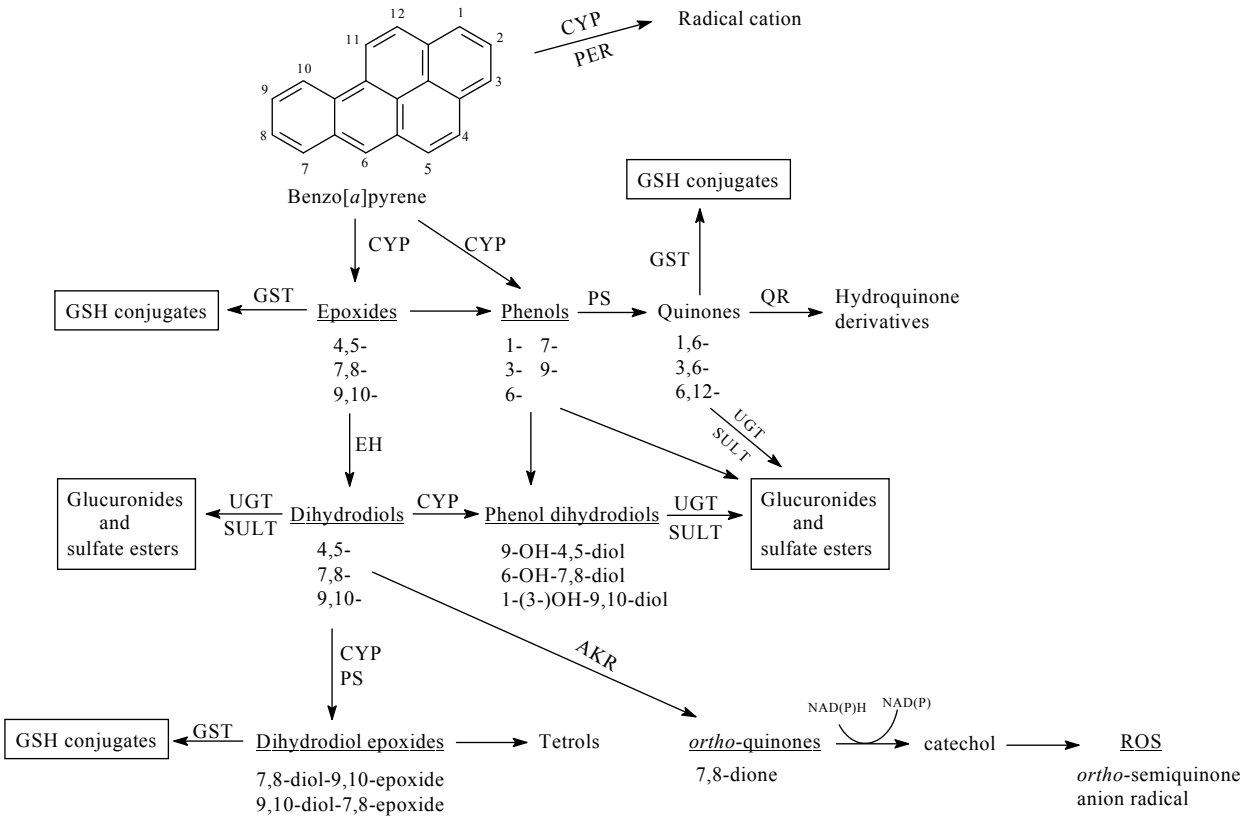
(c) Absorption via the skin

Evidence for the dermal absorption of PAHs includes the detection of elevated levels of PAH metabolites, such as 1-hydroxypyrene, in the urine of humans exposed dermally to complex mixtures of PAHs, such as coke-oven emissions or creosote mixtures in the workplace (Van Rooij *et al.*, 1993a, b), or coal-tar ointments (Godschalk *et al.*, 1998). Results from studies in animals have indicated that dermal absorption of PAHs can be rapid and extensive (Withey *et al.*, 1993a).

4.1.3 Distribution

The data on distribution of PAHs are based mainly on studies in rats, and indicate that: (i) absorbed PAHs are widely distributed to most organs and tissues; (ii) fatty tissues can serve as storage sites to which PAHs may be gradually absorbed and from which they are then released; and (iii) the gastrointestinal tract can contain high concentrations of PAHs and their metabolites after exposure (by any route), due to mucociliary clearance from the respiratory tract and hepatobiliary excretion of metabolites (Mitchell & Tu, 1979; Mitchell, 1982, 1983; Sun *et al.*, 1984; Withey *et al.*, 1991, 1993b). The results from

Fig. 4.1 Metabolic schema for benzo[*a*]pyrene



AKR, aldo-keto reductase; CYP, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; GST, glutathione *S*-transferase; NAD(P)H, nicotinamide adenine dinucleotide (with or without phosphate); PER, peroxidases; PS, prostaglandin H synthase; QR, quinone reductase; ROS, reactive oxygen species; SULT, sulfotransferase; UGT, Uridine 5′-diphosphate-glucuronosyltransferase
Adapted from Cooper *et al.* (1983), ATSDR (1995), IPCS (1998).

these studies are consistent with the concept that PAHs are, in general, cleared rapidly from the initial sites of deposition in the respiratory tract and distributed to a significant extent in the gastrointestinal tract, liver and kidney; the kinetics and patterns of distribution, however, can be influenced by size and compositional characteristics of the particulate matter, as well as by the chemical properties of the PAHs themselves (IARC, 2010).

4.1.4 Metabolism

The metabolism of benzo[*a*]pyrene has been studied extensively in human and animal tissues, and generally serves as a model for the metabolism of other PAHs (for review, see ATSDR, 1995; IPCS, 1998). A metabolic scheme for benzo[*a*]pyrene is presented in Fig. 4.1, which shows pathways to the formation of epoxides, phenols, quinones, hydroquinones, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, tetrols and other potentially reactive intermediates.

Benzo[*a*]pyrene is initially metabolized by cytochrome P450 (CYP) monooxygenases to several epoxides. CYP1A1 can metabolize a wide range of PAHs, but other CYPs, including CYP1A2 and members of the CYP1B, CYP2B, CYP2C and CYP3A families of enzymes, have been demonstrated to catalyse the initial oxidation of benzo[*a*]pyrene and other PAHs to varying extents (for review, see IPCS, 1998; Xue

& Warshawsky, 2005). PAHs are recognized inducers or inhibitors (Shimada & Guengerich, 2006) of CYP enzymes, and exposure to PAHs can therefore influence the balance of phase I and phase II enzymes, which can determine whether or not a toxic cellular response occurs. The mammalian CYP genes that encode CYP1A1, 1A2 and 1B1 are regulated in part by the aryl hydrocarbon receptor (AhR). Differences in AhR affinities in inbred mice correlate with variations in the inducibility of CYP and may be associated with differences in the risk for cancer from PAHs (Nebert *et al.*, 2004). A correlation between the variability in AhR affinity in humans and differences in cancer risk remains unproven. Therefore, the role of CYP in activation *versus* detoxification probably depends on multiple factors such as the subcellular content and location, the degree of phase II metabolism and the pharmacokinetics of the chemical.

Epoxides may rearrange spontaneously to phenols, be hydrated via epoxide hydrolase catalysis to dihydrodiols or be conjugated with GSH, either spontaneously or via glutathione-*S*-transferase (GST) catalysis. It has been proposed that the formation of 1-, 3- and 6-hydroxybenzo[*a*]pyrene from benzo[*a*]pyrene and their subsequent conversion to quinones involve CYP isoforms (Cavalieri *et al.*, 1988) and 6-hydroxybenzo[*a*]pyrene can also be formed by prostaglandin H synthase (Cooper *et al.*, 1983; for a review, see IPCS, 1998). Quinones can be converted to hydroquinone derivatives by quinone reductase or be conjugated with GSH, sulfate or glucuronic acid.

Dihydrodiol derivatives can be further oxidized by CYPs to form phenol dihydrodiols or dihydrodiol epoxides. Phenols, phenol dihydrodiols and dihydrodiols can be conjugated with glucuronic acid or sulfate. Dihydrodiol epoxides may also be formed from dihydrodiols by reaction with peroxyl radicals generated from the oxidative biosynthesis of prostaglandins from fatty acids via prostaglandin H synthase (Marnett, 1981, 1987; Reed *et al.*, 1988; Eling *et al.*, 1990). The metabolic fate of dihydrodiol epoxides includes conjugation with GSH or covalent modification of cellular macromolecules that possibly lead to mutagenic and carcinogenic responses.

Dihydrodiols may also be metabolized to *ortho*-quinones by aldo-keto reductases (AKR1C1–AKR1C4, AKR1A1). *ortho*-Quinone derivatives have been demonstrated *in vitro* to produce, via redox cycling with nicotinamide adenine dinucleotide (with or without phosphate) (NAD(P)H) and copper, reactive oxygen species that cause DNA fragmentation and mutation of *TP53* (Flowers *et al.*, 1996, 1997; Penning *et al.*, 1999; Yu *et al.*, 2002). PAH *ortho*-quinones produced by this pathway are also ligands for AhR (Burczynski & Penning, 2000). This effect of *ortho*-quinones may play a role in the mutagenicity and carcinogenicity of benzo[*a*]pyrene and other PAHs.

The stereochemistry of the dihydrodiol epoxide derivatives of benzo[*a*]pyrene is important in the toxicity of benzo[*a*]pyrene and other PAHs (Conney, 1982; Shaw & Connell, 1994; for a review, see IPCS, 1998). Of the four possible stereoisomers of the 7,8-dihydrodiol-9,10-epoxide benzo[*a*]pyrene derivative, the predominant one formed in mammaliam systems, (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, has been shown to have the highest tumour-initiation activity and to be the predominant metabolite that forms DNA adducts in mammalian tissues exposed to benzo[*a*]pyrene. The formation of DNA adducts may be a first step in the initiation of carcinogenesis by PAHs.

4.1.5 Elimination

Results from studies of animals exposed to PAHs indicate that their metabolites are largely excreted as conjugates of GSH, glucuronic acid or sulfate in the faeces, via biliary excretion and in the urine (for review, see ATSDR, 1995; IPCS, 1998).

4.1.6 Mechanisms of metabolic activation and carcinogenesis

(a) Bay- and fjord-region PAH diol epoxide

In benzo[*a*]pyrene, the bay region encompasses four carbons (carbons 10, 10a, 10b and 11) and three carbon–carbon bonds. In the case of benzo[*a*]pyrene, metabolism by CYP isozymes at the C7–C8 aromatic double bond creates an arene oxide, benzo[*a*]pyrene-7,8-oxide. Benzo[*a*]pyrene-7,8-oxide is hydrated by epoxide hydrolase to form a dihydrodiol (diol), benzo[*a*]pyrene-7,8-diol. Benzo[*a*]pyrene-7,8-diol is further metabolized (epoxidized) by the CYP isozymes at the C9–C10 double bond to give the bay-region diol epoxide, benzo[*a*]pyrene-7,8-diol-9,10-oxide. This diol epoxide possesses the inherent ability to undergo carbon–oxygen bond scission or ring opening, to form a carbonium ion (i.e. a positively charged carbon atom) on carbon 10. Carbonium ions are highly reactive species that react with nucleophiles, such as DNA and proteins, to form covalent adducts. One of the postulated quantitative measures of the reactivity of diol epoxides is carbonium ion delocalization energy ($\Delta E_{deloc}/\beta$), which is based on perturbational molecular orbital calculations that predict the ease of carbonium-ion formation. The greater the $\Delta E_{deloc}/\beta$ value, the more reactive the carbonium ion; greater values were associated with PAHs that exhibited higher tumorigenic activities (Jerina *et al.*, 1976). This theory was expanded to include PAH structures with deeper peripheral indentations in their structure – those that contain a fjord region (e.g. dibenzo[*a*,*l*]pyrene). The fjord region encompasses five carbons and four carbon–carbon bonds; in some cases, the steric interactions between atoms within the fjord region of the PAH forces the PAH ring system out of planarity (Katz *et al.*, 1998). Some PAH fjord-region diol epoxides are non-planar (Lewis-Bevan *et al.*, 1995), and these non-planar PAH diol epoxides possess even higher reactivities than those predicted by $\Delta E_{deloc}/\beta$ alone. The enzymes primarily responsible for phase I metabolism of PAHs are CYP1A1, CYP1A2 and CYP1B1 and NADPH CYP reductase, which convert PAHs to different arene oxides, and epoxide hydrolase that catalyses the addition of water to the arene oxides to form *trans*-diols. PAH phenols are also formed either by rearrangement of arene oxides or by direct oxygen insertion into a carbon–hydrogen bond. Quinones are formed by further oxidation of phenols or by the enzymatic action of aldo-keto reductases (AKRs) on PAH diols. The phase II enzymes, uridine 5′-diphosphate (UDP)-glucuronosyltransferase (UGT), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), sulfotransferase (SULT) and GST, conjugate PAH diols, phenols and epoxides to glucuronic acid, sulfate and GSH, respectively.

The stereochemistry of the metabolic transformation of PAHs to diols and diol epoxides is an important component of this mechanism of action and affects the biological activities of these metabolites. CYPs can be regio- and stereospecific in their action. The stereospecific metabolizing activity of each CYP, in combination with the capacity of many PAH carbons to form chiral centres through metabolism, can create multiple forms of many PAH metabolites. For example, benz[*a*]anthracene is metabolized in a stereospecific manner at the C3–C4 bond to give two benz[*a*]anthracene-3,4-oxides (benz[*a*]anthracene-3*S*,4*R*-oxide and benzo[*a*]anthracene-3*R*,4*S*-oxide) in different amounts (Yang, 1988), which are then hydrated in a stereospecific manner by epoxide hydrolase to give two benzo[*a*]anthracene-3,4-diols (benzo[*a*]anthracene-3*R*,4*R*-diol and benzo[*a*]anthracene-3*S*,4*S*-diol) in different amounts (Yang, 1988). Each diol can form two diol epoxides that vary depending on the relative position of epoxide function in relation to one of the diol hydroxyls – a *syn*-benzo[*a*]anthracene diol epoxide and an *anti*-benz[*a*]anthracene diol epoxide – for a total of four benz[*a*]anthracene diol epoxides. While diol epoxides are not subject to enzymatic hydrolysis

by epoxide hydrolase (Thakker *et al.*, 1976; Wood *et al.*, 1976), they are non-enzymatically hydrolysed to tetrols (Jankowiak *et al.*, 1997) and are enzymatically detoxified by GSTs (Dreij *et al.*, 2002). Therefore, the formation and degradation of stereochemically specific diol epoxides is dependent on species, strain, sex, organ, tissue, type of CYP and phase II enzymes.

Bay-region and fjord-region diol epoxides possess many biological activities; one of the most important of these is the formation of stable covalent adducts with DNA. The nature and sequence specificity of these DNA adducts is based, in part, on the absolute configuration, molecular conformation and stereochemistry of the diol epoxide, the specific purine (or pyrimidine base) that is adducted, the site of adduction and the nature and sequence of the DNA that is adducted (Jerina *et al.*, 1976). As described previously, each PAH diol can form four diastereomeric *syn*- and *anti*-diol epoxides. When diol epoxides react with DNA (mainly at the purines, i.e. deoxyguanosine and deoxyadenosine), each can form both *cis* and *trans* adducts, thus giving a total of 16 possible DNA adducts. However, in most cases, far fewer DNA adducts are actually observed. While PAH–DNA adducts represent a type of DNA damage, they can be converted into heritable mutations by misrepair or faulty DNA synthesis (Watanabe *et al.*, 1985; Rodriguez & Loechler, 1995). Bay- or fjord-region diol epoxide–DNA adducts are repaired by nucleotide-excision repair (Geacintov *et al.*, 2002). Numerous examples have shown that bay- and fjord-region diol epoxides of PAHs are mutagenic in bacteria, cause damage to DNA or induce chromosomal damage in human and mammalian cells in culture, and induce skin, lung or liver tumours in mice, similarly to the parent PAH. Furthermore, PAHs or their bay- or fjord-region diol epoxides induced mutations in critical genes associated with chemical carcinogenesis such as proto-oncogenes (Prahalad *et al.*, 1997; Chakravarti *et al.*, 1998) and tumour-suppressor genes (Ruggeri *et al.*, 1993; Rämet *et al.*, 1995). A strong relationship exists between the nature of the DNA adducts of the diol epoxide and the type of *ras* proto-oncogene mutations observed in DNA from tumours induced by PAHs. In general, PAHs that form DNA adducts at deoxyguanosine primarily induce mutations in the *ras* gene at codons 12 or 13, while those that form DNA adducts at deoxyadenosine induce mutations in the *ras* gene at codon 61. PAHs that induce adducts at both purine bases induced both types of mutations (Ross & Nesnow, 1999). In addition to their genotoxic effects, some bay- or fjord-region diol epoxides are reported to induce apoptosis and cell-cycle arrest in mammalian cells (Chramostová *et al.*, 2004).

The diol epoxide–DNA adducts of PAHs have also been identified in populations exposed to complex mixtures that contain PAHs, i.e. foundry workers (Hemminki *et al.*, 1988; Perera *et al.*, 1988), coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999), cigarette smokers (Rojas *et al.*, 1995; Lodovici *et al.*, 1998), chimney sweeps (Pavanello *et al.*, 1999) and people exposed to mixtures in smoke emissions from coal combustion (Mumford *et al.*, 1993). Some bay- or fjord-region diol epoxides form DNA adducts in the human *TP53* tumour-suppressor gene at sites that are hotspots for cancer of the lung (Smith *et al.*, 2000).

(b) Cyclopenta-ring oxidation

The cyclopenta-ring oxidation mechanism involves the formation of the arene oxide at a highly electron-rich isolated double bond that is located at a five-membered ring within a PAH. The cyclopenta ring is an external five-membered carbocyclic ring that is situated on a carbocyclic hexameric fused-ring system. For example, a cyclopenta-ring derivative of pyrene is cyclopenta[*cd*]pyrene. Since the cyclopenta ring is usually the region of highest electron density, it is a major site of oxidation by the CYP isozymes (Nesnow *et al.*, 1984, 1988). Preparations of rat

and mouse liver, human and rodent cells in culture, human CYP1A1, CYP1A2 and CYP3A4, human liver microsomes and rats *in vivo*, metabolize cyclopenta-fused PAHs at the cyclopenta-ring double bond to give cyclopenta-ring oxides and diols (Gold & Eisenstadt, 1980; Mohapatra *et al.*, 1987; Kwon *et al.*, 1992; Nyholm *et al.*, 1996; Johnsen *et al.*, 1998a, b; Hegstad *et al.*, 1999). Cyclopenta-ring oxides are reactive intermediates and bind to DNA to form DNA adducts *in vitro* and *in vivo* mainly at deoxyguanosine (Surh *et al.*, 1993; Beach & Gupta, 1994; Hsu *et al.*, 1997, 1999). Cyclopenta-ring oxides are hydrated by epoxide hydrolase to diols. Some cyclopenta-ring diols are conjugated to sulfate esters by PAPS SULT. Cyclopenta-ring oxides, like their parent cyclopenta-PAHs, are mutagenic in bacteria and mammalian cells and can morphologically transform immortalized cells in culture (Bartczak *et al.*, 1987; Nesnow *et al.*, 1991). In general, cyclopenta-ring derivatives of PAHs are more mutagenic and more carcinogenic than their unsubstituted counterparts (e.g. pyrene is not carcinogenic, while cyclopenta[*cd*]pyrene has been shown to induce mutations at the K_i-*Ras* proto-oncogene in lung tumours of treated mice and is highly carcinogenic) (Nesnow *et al.*, 1994, 1998).

(c) *Formation of radical cations*

Removal of one electron from the π system of a PAH generates a radical cation, in which the positive charge is usually localized at an unsubstituted carbon atom or adjacent to a methyl group. Nucleophilic attack at the position of highest charge density at an unsubstituted carbon atom produces an intermediate radical that is further oxidized to an arenium ion to complete the substitution reaction. Development of the chemistry of PAH radical cations has provided evidence that these intermediates can play a role in the process of tumour initiation by several potent PAHs (Cavalieri & Rogan, 1985, 1992; for a review see IARC, 2010).

(d) *Formation of* ortho*-quinones and generation of reactive oxygen species*

As seen above and in Fig. 4.1, $NAD(P)^+$-dependent dehydrogenation of PAH dihydrodiols, which is catalysed by monomeric cytosolic oxidoreductases of the AKR superfamily, yield ketols, which spontaneously rearrange to catechols. Catechols are extremely air-sensitive and undergo two sequential one-electron auto-oxidation steps to yield the corresponding reactive PAH *ortho*-quinones (Smithgall *et al.*, 1986, 1988). An intermediate in this auto-oxidation is the corresponding *ortho*-semiquinone anion radical. Each one-electron oxidation event (either catechol → *ortho*-semiquinone anion radical or *ortho*-semiquinone anion radical → *ortho*-quinone) yields reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl radical). This leads to oxidative stress and a pro-oxidant state. For benzo[*a*]pyrene, this reaction sequence would comprise dehydrogenation of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to form 7,8-dihydroxybenzo[*a*]pyrene (catechol) and auto-oxidation to yield benzo[*a*]pyrene-7,8-dione (Penning *et al.*, 1996, 1999).

The resulting PAH *ortho*-quinone is a highly reactive Michael acceptor that can undergo 1,4- or 1,6-Michael addition reactions with cellular nucleophiles (e.g. L-cysteine, GSH) to yield conjugates (Murty & Penning, 1992a, b; Sridhar *et al.*, 2001) or with macromolecules (e.g. protein, RNA and DNA) to yield adducts (Shou *et al.*, 1993; McCoull *et al.*, 1999; Balu *et al.*, 2004). The PAH *ortho*-quinones and the reactive oxygen species that they generate may form mutagenic lesions in DNA (initiation) or act as electrophilic and pro-oxidant signals that may affect cell growth. In this manner, the pathway may contribute to the complete carcinogenicity of the parent PAH (for a review, see IARC, 2010).

4.1.7 Activation of AhR and carcinogenesis

Several of the biological effects of PAHs, such as induction of xenobiotic metabolizing enzymes, immunosuppression, teratogenesis and carcinogenicity, are thought to be mediated by activation of AhR signalling. This receptor is widely distributed and has been detected in most cells and tissues.

AhR is a ligand-activated transcription factor that mediates responses to a variety of toxins; PAHs and halogenated aromatic toxins such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD) are among the best characterized, high-affinity, exogenous AhR ligands (Stejskalova *et al.*, 2011).

This receptor plays an essential role in the regulation of the metabolism of xenobiotics (phase I/phase II enzymes; also termed AhR signalling in the adaptive-response pathway) and in the initiation of homeostatic responses (also termed AhR signalling in endogenous pathways) upon exposure to xenobiotics.

There is also evidence that AhR signals act through a variety of pathways, and more recently cross-talk with other nuclear receptors has been demonstrated to enable cell-type- and tissue-specific control of gene expression (Puga *et al.*, 2009; Stevens *et al.*, 2009).

Different high-affinity ligands for AhR have been shown to differ in their biological responses. Furthermore, translocation of activated AhR may require threshold concentrations of the ligand and involves a variety of cellular response. Altered AhR-signalling responses may therefore be designated as adaptive or toxic, and/or as perturbations of endogenous pathways. Among these effects, alteration of xenobiotic enzymes and alteration of immunological mechanisms are most relevant to PAH-induced carcinogenesis.

(a) Alteration of xenobiotic enzymes and carcinogenicity

AhR-induced expression of CYP1 enzymes impacts the metabolism of PAHs and results in genotoxicity, mutations and tumour initiation (Nebert *et al.*, 2000). Individual risk for cancer may be attributed to metabolic activation of PAHs, but the balance between detoxification and metabolic potentiation depends on many factors (Nebert *et al.*, 2004), and loosely or tightly coupled phase I and phase II metabolic reactions may be influential factors for risk of toxicity and cancer (see Sections 4.1.6 and 4.3 in this *Monograph*).

(b) Alteration of immunological mechanisms

Carcinogenic PAHs have been found to suppress the immune system of animals (White & Holsapple, 1984; Wojdani & Alfred, 1984; Wojdani *et al.*, 1984).

Generally, a positive correlation is seen between the carcinogenicity and immunotoxicity of a PAH. This correlation probably exists because both carcinogenicity and immunotoxicity are largely dependent on AhR binding, increased CYP expression and the formation of bioactive metabolites (White *et al.*, 1985; Burchiel & Luster, 2001).

PAHs exert many important effects on the immune system of many species. The dose and route of exposure determine the nature of the effect on specific and adaptive immune responses. Studies with pure PAHs suggest that AhRs play a critical role in the activation of immunotoxic PAHs, such as benzo[*a*]pyrene, via the diol epoxide mechanism that leads to DNA interactions that cause genotoxicity and suppress immunity by TP53-dependent pathways. Benzo[*a*]pyrene diol epoxide may also affect protein targets and modulate lymphocyte signalling pathways via non-genotoxic (epigenetic) mechanisms. Certain oxidative PAHs, such as benzo[*a*]pyrene quinones, may be formed

via CYP-dependent and -independent (peroxidase) pathways. Redox-cycling PAHs quinones may exert oxidative stress in lymphoid cells.

4.2 Absorption, distribution, metabolism, and excretion of bitumens and bitumen fume

4.2.1 Introduction

Bitumen fume comprises a complex mixture of constituents, which is strongly dependent on how the fume is generated. The pharmacokinetics of these individual components will depend on their physicochemical properties and on the biological interactions with different tissues and organs. Notably, to become available systemically, components of bitumen fume and condensate need to cross barriers such as the alveolar barrier in the case of inhalation, or the intestinal epithelial barrier after ingestion, or the skin after dermal exposure. Upon entry into the systemic circulation many of the constituents of bitumen are subject to extensive and tissue-specific metabolism that modifies highly lipophilic molecules to facilitate their clearance from the body through excretion into urine and faeces (see the Overview in Section 4.1).

Several studies in humans and animals *in vitro* have demonstrated that PAHs present in condensates of bitumen fume are subject to extensive metabolism. As summarized in Section 4.1, PAHs undergo metabolic activation by cytochrome P450 (CYP450) enzymes, primarily CYP1A1, CYP1A2 and CYP1B1. However, there are considerable differences in the activity of CYP monooxygenase between animal species and between animals and humans. An interpretation of animal data for assessing human absorption, distribution, metabolism and excretion is therefore confounded by several factors, most notably the significant interspecies differences in metabolism and metabolic clearance. While there is overwhelming evidence that aliphatic, aromatic and/or polycyclic aromatic hydrocarbons within bitumen are substrates for CYP monooxygenases, the carcinogenic potential of hydrocarbons is primarily caused by their metabolic activation to proximate and ultimate carcinogens. In an initial step, aromatic hydrocarbons are metabolically activated to epoxides that either isomerize to phenols or upon addition of water form *trans*-dihydrodiols. Further oxidative metabolism leads to the production of potentially genotoxic, DNA-reactive metabolites (ultimate carcinogens).

Naphthalene, phenanthrene and pyrene are PAHs of low relative molecular mass that are found in bitumen fume at various concentrations, depending on the temperature at which the fume is generated (see Section 1).

Naphthalene is metabolized in humans and rodents to reactive intermediates by hepatic and extrahepatic CYP enzymes; these reactive metabolites deplete GSH and bind covalently to proteins to cause necrosis in Clara cells of the lung. Naphthalene is metabolized by CYP1A1, 1A2, 2A1, 2E1, 2F (Waidyanatha & Rappaport, 2008) and 2S1 (Karlgren *et al.*, 2005) to its 1,2-epoxide, which may undergo non-enzymatic isomerization to 1- and 2-hydroxynaphthalene (1- and 2-naphthol), and is further metabolized by microsomal epoxide hydrolase EPXH1 to 1,2-dihydro-1,2-dihydroxy-naphthalene (*trans*-1,2-dihydrodiol). Both 1- and 2-naphthol are further oxidized to 1,2- and 1,4-naphthoquinone (Waidyanatha & Rappaport, 2008). The main route for excretion of naphthalene metabolites in humans and rodents is via the urine (Buckpitt *et al.*, 2002).

Another significant component of bitumen fume is phenanthrene, which is initially converted to three different isomeric epoxides. This non-enzymatic isomerization process results in five different isomeric phenols, whereas three different *trans*-dihydrodiols are formed by the action of epoxide hydrolase. The regio-selective oxidation differs among species and according

to the CYP enzyme involved, as was demonstrated by Jacob & Grimmer (1996). In rats, the 9,10-position of phenanthrene is mainly oxidized by CYP1A1, 1A2, and 2B1 (84–100%), whereas in humans the positions 1,2-, 3,4-, and 9,10- of phenanthrene are oxidized by the enzymes CYP1A1, 1A2, 3A4, 2A6 and 2E1 (Jacob *et al.*, 1996a, b). In rats, phenanthrene-9,10-epoxide is further conjugated by GST and excreted predominantly via the mercapturic-acid pathway (Boyland & Sims, 1962a, b, c; Lertratanangkoon *et al.*, 1982). In humans, phenanthrene is metabolized at the 9,10-position to 9-phenanthrol and *trans*-9,10-dihydrodiol.

The phase-II enzymes catalyse conjugation reactions, resulting in conversion of xenobiotic substances into water-soluble metabolites that can be excreted via the urine or bile. Such conjugation reactions include glucuronidation, sulfation, and GSH and amino-acid conjugation.

A fine balance exists between activation and detoxification of constituents of bitumen fume and condensate, whereby inadequate or saturated detoxification pathways may result in accumulation of DNA-reactive metabolites. Information on profiles of absorption, distribution, metabolism and excretion associated with exposure to bitumen is therefore of critical importance for understanding its toxicological properties. Moreover, while metabolic activation of constituents of bitumen fume and condensate may lead to DNA damage, this damage is also subject to DNA repair, which is not always error-free. As a consequence, single cells bearing DNA adducts may undergo mutation during replication, which can result in malignant transformation leading to tumour formation by clonal expansion.

It is therefore important to study the capacity of target tissues to metabolically activate individual constituents of bitumen fume. In addition, a wide range of PAHs are capable of activating nuclear transcription factors, including the AhR, which control transcriptional activation of genes encoding metabolic enzymes. This may also influence pharmacokinetics and toxicity of the constituents of bitumen fume and condensate.

It should be noted that many of the laboratory-generated bitumen fumes or condensates used in experimental studies do not necessarily represent field-generated fumes. Due to the complexity of the problem, models specific for bitumen-fume exposures have not yet been proposed. Nonetheless, urinary 1-hydroxypyrene (1-OHP) and other PAH metabolites have been used as markers to monitor exposure to bitumen fume and PAHs (see Section 4.2.3).

4.2.2 Toxicokinetics of bitumens and bitumen emissions

(a) Humans

Toxicokinetic information in humans was obtained from a study designed to investigate the potential for percutaneous absorption of aerosols and vapours of bitumen (Walter & Knecht, 2007). Ten male non-smoking volunteers were exposed in an experimental chamber to bitumen emissions generated from commercial bitumen B65 for 8 hours. To ensure that there was no exposure by inhalation, the subjects used a powered air-purifying respirator (PAPR) for the entire period of exposure and wore only shorts and shoes. Under the same conditions, two other volunteers did not use a PAPR, so that a comparison could be made between inhalation of PAH from bitumen emissions and dermal uptake. Urinary PAH metabolites of phenanthrene, pyrene and chrysene were determined.

The bitumen emissions in the chamber were measured to be ~20 mg/m^3 with a vapour content of about 88%. The components of higher relative molecular mass were present predominantly in the aerosol phase, whereas lighter polycyclics remained in the vapour phase. The proportion of PAHs absorbed via the skin was between 50% and 60% of the total amount incorporated for pyrene, chrysene and phenanthrene.

Table 4.1 Half-lives of urinary metabolites of polycylic aromatic hydrocarbons (PAHs) after exposure of male non-smoker volunteers to bitumen emissions

PAH metabolites	With PAPR (n = 10 volunteers)[a]	Without PAPR (n = 2 volunteers)[a]
1-OH-phenanthrene	5.96 ± 1.70	5.43 ± 2.70
4-OH-phenanthrene	8.49 ± 1.65	4.83 ± 1.55
Total phenanthrene	8.63 ± 2.01	5.22 ± 3.07
6-OH-chrysene	7.61 ± 1.49	7.74 ± 3.34
1-OH-pyrene	8.16 ± 1.54	5.65 ± 0.99

[a] Values after percutaneous only or combined inhaled/percutaneous exposure were obtained from subjects with or without powered air-purifying respirator (PAPR), respectively. Values are given in hours
Adapted from Walter & Knecht (2007)

Biomonitoring of the main urinary metabolites of phenanthrene, pyrene and chrysene was shown to be an objective measure of the PAH contribution to exposure to bitumen emissions. Under this experiment setting, the concentration of 6-OH-chrysene in urine was markedly higher than concentration of other PAH metabolites examined, e.g. 1-OHP, the most often used marker of exposure to PAHs (Walter & Knecht, 2007).

The values for the biological half-life for PAH metabolites examined varied only slightly and were about 5–8 hours. Detailed results after percutaneous only or combined inhaled/percutaneous exposure to bitumen emission are shown in Table 4.1.

(b) Rodents

The animal pharmacokinetic profile of individual components of bitumen and bitumen emissions after inhalation, ingestion or dermal uptake was studied in considerable detail by Syracuse Research Corporation (1985). Data indicated that, after inhalation, hydrocarbons with 9–16 carbons were distributed into the blood, brain, liver, kidneys and fat of rats (ATSDR, 1999). Aliphatic hydrocarbons may be oxidized to alcohols, ketones and carboxylic as well as fatty acid derivatives, and some of these compounds are slowly eliminated in the urine and faeces. Detectable concentrations of PAHs occured in almost all internal organs and accumulate in fatty tissues to be eliminated only via urinary or biliary excretion of metabolites.

To determine the bioavailability of genotoxic compounds in bitumen and viscous oils, samples were spiked with radiolabelled benzo[*a*]pyrene and were then applied to mouse skin *in vivo* and to human skin biopsies in short-term culture (Potter *et al.*, 1999). High-viscosity oils and bitumens caused 10 times less binding of benzo[*a*]pyrene to skin DNA, relative to a low-viscosity oil.

The levels of representative metabolites of benzo[*a*]pyrene were measured by mass spectrometry in the urine of rats (16 female Sprague-Dawley) exposed to fume from hot-performance grade bitumen (PG 64–22) in a whole-body inhalation chamber (4 hours per day for 10 days) (Wang *et al.*, 2003a). Eight other rats were controls. The fume was generated by heating the bitumen to 170 °C, then blowing hot air (150 °C) over it. The fume had a concentration of 76–117 mg/m^3. Benzo[*a*]pyrene was detected in the urine of the exposed rats at 21.9 ± 4.9 ng/L, 3-hydroxybenzo[*a*]pyrene at 161.7 ± 3 ng/L, benzo[*a*]pyrene-7,8-dihydrodiol at 62.8 ± 3.6 ng/L and (±)benzo[*a*]pyrene-7,8,9,10-tetrahydrotetrol at 293.5 ± 2.6 ng/L. Only the tetrol metabolite was detectable in the urine of the control rats, at the significantly lower concentration of 1.9 ng/L.

The urinary excretion of metabolites of PAHs was determined after exposure of SPF-Wistar rats to bitumen fume (Halter *et al.*, 2007).

Here, an exposure atmosphere was generated using an evaporation condensation generator. The hot vapour issued through a nozzle into a slowly flowing cool air stream surrounding the jet. The fume, diluted with clean air to achieve the intended concentration, was directed to the nose-only inhalation chambers. The rats were exposed for 4 hours per day, 5 days per week, for the required period. Target exposure concentrations were 0, 4, 20, or 100 mg/m^3 total hydrocarbon (THC). Upon exposure to bitumen fume at 4 mg/m^3, urinary excretion of naphthols, phenanthrene, phenanthrene-premercapturic acid and phenanthrols was similar to that in air-exposed controls. A clear time-dependent increase in excretion of naphthols, phenanthrene, phenanthrene-premercapturic acid, 1-hydroxy-phenanthrene and phenanthrene-1,2-dihydrodiol was observed in male and female rats in the groups receiving the intermediate and highest dose after 12 months of exposure. In this study, CYP1A1 gene and protein expression was dose-dependently induced in lung tissue and nasal epithelium, as confirmed by RT-PCR and Western blotting, and this enzyme induction agreed well with the observed production of 1-OH-phenanthrene. In the study, Halter *et al.* (2007) noted that diet was an unexpected source of exposure to naphthalene and phenanthrene. It is well known that diet can be a source of PAHs (Phillips, 1999).

4.2.3 Urinary PAH metabolites in workers exposed to bitumen emissions

Hydroxylated PAHs (OH-PAHs) have been measured in the urine of bitumen-exposed workers (roofers, pavers and mastic workers). Metabolites typically found were 1-OHP, naphthalene, phenanthrene and fluorene metabolites.

(a) 1-Hydroxypyrene (1-OHP)

Urinary levels of 1-OHP measured in road-pavers (0.20 ng/mL) were shown to be significantly ($P < 0.05$) higher than in controls (0.11 ng/mL). Although there was no difference between the road-pavers samples post- and pre-shift (0.21 ng/mL *versus* 0.20 ng/mL), urine samples collected on Monday morning had significantly ($P < 0.05$) lower concentrations of 1-OHP (0.15 ng/mL) than samples collected on other weekday mornings (0.30 ng/mL), indicating occupational exposure to PAHs. Controls and road pavers were non-smokers (Levin *et al.*, 1995).

Toraason *et al.* (2001) examined urinary concentrations of 1-OHP at the beginning and end of the same working week (4 days later) of 26 workers who applied hot bitumen products in the USA. Urinary concentrations of 1-OHP were significantly increased at the end of the working week. [The Working Group noted potential confounding by exposure to coal tar and smoking.]

Heikkilä *et al.* (2002) measured pre- and post-shift urinary concentrations of 1-OHP in 32 road pavers at 13 paving sites. The workers had been exposed to 11 different asphalt mixtures. The results showed that concentrations of 1-OHP were significantly higher among pavers than among controls, and twice as high among pavers who were smokers than in non-smokers.

Campo *et al.* (2006) monitored asphalt workers ($n = 100$) in Italy, exposed to bitumen fume and diesel exhausts, and road-construction workers ($n = 47$) exposed to diesel exhausts only. Concentrations of 1-OHP were determined in spot samples of urine collected after 2 days of vacation (baseline), before and at the end of the monitored work-shift, in the second part of the working week. Median airborne concentration of the sum of 15 PAHs (in both vapour and particulate phases) during the working shift was 607 ng/m^3, with values for individual PAHs

ranging from < 0.1 to 426 ng/m^3. Median excretion values of 1-OHP in baseline, before- and end-shift samples were 228, 402, and 690 ng/L for the asphalt workers and 260, 304 and 378 ng/L for the road-construction workers. Lower values were found in non-smokers than in smokers (e.g. for asphalt workers, 565 and 781 ng/L *versus* 252 and 506 ng/L in before-shift and end-shift samples, respectively). These results showed that asphalt workers experienced occupational exposure to airborne PAHs, resulting in a significant increase of urinary concentrations of 1-OHP during the working day and the working week. The contribution of working activities to internal dose was in the same order of magnitude as the contribution from cigarette smoking.

Several other studies performed in different countries – Turkey (Burgaz *et al.*, 1998), the United Kingdom (Hatjian *et al.*, 1995), Sweden (Järvholm *et al.*, 1999), and Germany (Marczynski *et al.*, 2006, 2007) – reported the same increase in urinary 1-OHP concentrations in workers exposed to bitumen.

Increases in urinary concentrations of 1-OHP were also reported for asphalt workers who wore gloves, safety shoes and disposable respirators in post-shift measurements (Karaman & Pirim, 2009).

McClean *et al.* (2004b) designed a study to evaluate the total effect of exposure by inhalation and dermal exposure to PAHs among road-paving workers. Urinary concentration of 1-OHP was used as a measure of total absorbed dose in a study population that included two groups of highway-construction workers: 20 paving workers who worked with hot-mix bitumen, and six milling workers who did not. During multiple consecutive working shifts, personal air and dermal samples were collected from each worker and analysed for pyrene. During the same working week, urine samples were collected pre-shift, post-shift and at bedtime each day and analysed for 1-OHP. The paving workers had inhalation (mean, 0.3 μg/m^3) and dermal (mean, 5.7 ng/cm^2) exposures to pyrene that were significantly higher than those of the milling workers. At pre-shift on Monday morning, after a weekend away from work, the pavers and millers had the same mean baseline urinary concentration of 1-OHP of 0.4 μg/g creatinine. The mean urinary 1-OHP concentrations among pavers increased significantly from pre-shift to post-shift during each working day, while it varied little among millers. Among pavers there was a clear increase in the pre-shift levels during the working week, such that the average pre-shift levels on day 4 were 3.5 times higher than those on day 1. The impact of dermal exposure was approximately eight times that of inhalation exposure. Furthermore, dermal exposure that occurred during the preceding 32 hours had a statistically significant effect on urinary 1-OHP, while the effect of inhalation exposure was not significant.

In a further study, McClean *et al.* (2007a) investigated dermal exposure to PAHs among bitumen-roofing workers and used urinary concentrations of 1-OHP as a measurement of dermal exposure for total absorbed dose. The study population included 26 roofing workers who performed three primary tasks: tearing off old roofs (tear-off), putting down new roofs (put-down), and operating the kettle at ground level (kettle). During multiple consecutive work shifts (90 working days), dermal patch samples were collected from the underside of each worker's wrists and were analysed for PAHs, pyrene and benzo[*a*]pyrene. During the same working week, urine samples were collected at pre-shift, post-shift, and bedtime each day and were analysed for 1-OHP (205 urine samples). Dermal exposures were found to vary significantly by roofing task (tear-off > put-down > kettle) and by the presence of an old coal-tar pitch roof (pitch > no pitch). For each of the three analytes, the adjusted mean dermal exposures associated with tear-off were approximately four times higher than exposures associated with operating the kettle. The pyrene measurements obtained during the working shift

were found to be strongly correlated with urinary 1-OHP measurements obtained at the end of that shift as well as at bedtime. The task-based differences that were observed while controlling for coal-tar pitch suggested that exposure to bitumen contributes to dermal exposures.

(b) Other metabolites

In asphalt-mastic workers, the increase in concentrations of 1-, 2+9-, 3-, 4-hydroxyphenanthrene (OHPhe) in post-shift urine samples was greater than those of 1-OHP, compared with pre-shift samples. It was noted that the presence of urinary 1-OHP and OHPhe reflects recent exposure (Marczynski *et al.*, 2006, 2007).

The recent study of Raulf-Heimsoth *et al.* (2011a) reported urinary PAHs metabolites of six bitumen workers handling mastic and mastic asphalt in two consecutive weeks at the same construction site in a tunnel. Median personal shift concentration of vapours and aerosols of bitumen was 1.8 mg/m^3 (range, 0.9–2.4 mg/m^3) during the application of rolled asphalt and 7.9 mg/m^3 (range, 4.9–11.9 mg/m^3) when mastic asphalt was applied. Area measurement of vapours and aerosols of bitumen revealed higher concentrations than the personal measurements of mastic asphalt (mastic asphalt, 34.9 mg/m^3; rolled asphalt, 1.8 mg/m^3). Processing mastic asphalt was also associated with higher concentrations of PAH. Urinary 1-hydroxypyrene and the sum of 1-, 2+9-, 3- and 4-hydroxyphenanthrene increased slightly during the shift, without clear differences between mastic and rolled-asphalt applications. However, the post-shift urinary concentrations of PAH metabolites did not reflect the different levels of PAH exposure during mastic and rolled-asphalt applications. Individual workers could be identified by their spirometry results, indicating that these data reflected long-term rather than acute effects.

In the study by Buratti *et al.* (2007), the urinary excretion of OH-PAHs was measured among asphalt workers (road pavers). Total PAHs and 15 individual PAHs in inhaled air were measured by personal sampling. In addition, the OH-PAHs 2-naphthol, 2-hydroxyfluorene, 3-hydroxyphenanthrene, and 1-OHP were quantified in urine samples collected at three different time-points during the week. Specifically, the median cencentrations of vapour-phase polycyclic aromatic compounds, PACs (5.5 µg/m^3), PAHs (≤ 50 ng/m^3) and OH-PAHs (0.08–1.11 µg/L) were significantly higher in asphalt workers than in controls, except in the case of naphthalene and 2-naphthol. The urinary concentrations of OH-PAHs increased with time: median concentrations for 2-hydroxyfluorene, 3-hydroxyphenanthrene and 1-OHP were 0.29, 0.08 and 0.18 µg/L at baseline; 0.50, 0.18 and 0.29 µg/L pre-shift; and 1.11, 0.44 and 0.44 µg/L post-shift, respectively. Each OH-PAH showed a characteristic profile of increase, reflecting differences in half-lives of individual constituents of bitumen emissions. In non-smoking subjects, positive correlations were found between vapour-phase PACs or PAHs and OH-PAHs, both in pre- and post-shift samples. Smokers had concentrations of OH-PAHs that were two to five times higher than those of non-smokers.

Väänänen *et al.* (2006) investigated the occupational exposure of road pavers to asphalt that contained waste plastic and tall oil pitch (WPT). Traffic controllers distant from the paving site served as controls. Exposure was monitored over one working day at four paving sites among 16 road pavers who used mixtures of conventional asphalt, stone-mastic asphalt (SMA) and asphalt concrete (AC), or mixtures containing waste material (SMA-WPT, AC-WPT). The concentrations of 11 aldehydes in air at the SMA-WPT and AC-WPT worksites were 3 and 13 times greater than at the corresponding worksites where conventional asphalt was used. Eight OH-PAH biomarkers were quantified in pre- and post-shift urine, as a measure of exposure to naphthalene, phenanthrene and pyrene. The post-shift concentrations (mean ± SD, µmol/mol

creatinine) of 1- and 2-naphthol, combined 1-,2-,3-,4-, 9-phenanthrol and 1-OHP were: 6.0 ± 2.3, 1.70 ± 0.72 and 0.27 ± 0.15 µmol/mol for conventional asphalt workers (non-smokers), respectively. For WPT-asphalt workers (non-smokers), the concentrations were 6.8 ± 2.6, 2.35 ± 0.69 and 0.46 ± 0.13 µmol/mol. As noted in other studies, concentrations of PAH metabolites were significantly higher in smokers than in non-smokers.

Pasquini *et al.* (1989) measured the urinary excretion of thioethers and D-glucaric acid in road workers exposed to bitumen emissions from a hot mixture of cracked rocks and petroleum bitumen. Urinary excretion of D-glucaric acid was also determined to investigate the potential of bitumens to induce enzymes. Thio-ethers were higher only in subjects exposed simultaneously to bitumens and cigarette smoke. Excretion of D-glucaric acid did not increase significantly.

4.2.4 Effect on pulmonary cytochrome P450 in rats

The effects of exposure to bitumen-fume condensate (BFC) on pulmonary cytochrome P450 (Cyp450) were studied by Ma *et al.* (2002). Male Sprague-Dawley rats were treated intratracheally with saline or with BFC at 0.45, 2.22 or 8.88 mg/kg for three consecutive days and euthanized the following day. Lung microsomes were isolated and microsomal protein levels, NADPH cytochrome-c reductase activity, and the activities and protein levels of the isozymes Cyp1a1 and Cyp2b1 were quantified.

Exposure of rats to BFC did not significantly affect total Cyp450 content or cytochrome-c reductase activity in the lung. The amount and activity of Cyp2b1 were not significantly affected by exposure to BFC. In contrast, Cyp1a1 levels and activity were significantly increased in microsomes isolated from BFC-exposed lungs. Exposure to bitumen emissions may alter the metabolism of PACs by the Cyp system in the rat lung, which may contribute to BFC-induced genotoxic effects (see Section 4.3).

4.3 Genetic and related effects

4.3.1 Studies in humans

Studies of genotoxicity in workers exposed to bitumen are presented in Table 4.2.

(a) Urinary mutagenicity

A comparison of urinary mutagenicity was carried out in 17 bitumen road pavers (exposed to emissions from a hot mixture of cracked rocks and petroleum bitumen at a concentration of 0.6–0.8 mg/m^3) and 27 control subjects (Pasquini *et al.*, 1989). Five workers were also exposed to diesel exhaust. All 15 smokers (six exposed to bitumens, nine non-exposed) had mutagenic urine. Among the non-smokers, 9 out of 11 exposed workers (82%) had mutagenic urine, compared with 5 out of 18 (28%) non-exposed controls. This difference was statistically significant ($P < 0.025$). Urinary mutagenicity was detectable only with *S. typhimurium* TA98 with S9 fraction, but not with TA100.

(b) DNA damage

DNA damage, as measured by alkaline elution, in peripheral mononuclear blood cells of bitumen-exposed workers (roofers exposed to class 2 bitumens; pavers exposed to class 1 bitumens; bitumen painters exposed to class 1 bitumens) was measured at the end of the working week and again at the beginning of the subsequent week (Fuchs *et al.*, 1996). For roofers ($n = 7$; all smokers), levels of alkaline DNA strand breaks were 43% higher than in the 34 controls ($P < 0.002$); for road pavers ($n = 18$; 12 smokers) and bitumen painters ($n = 9$; eight smokers), DNA damage was similar to that in controls. Nevertheless, for pavers there was more DNA damage observed in samples collected on Friday

Table 4.2 Studies of genotoxicity in workers exposed to bitumen

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Mutagens in urine						
Urinary mutagenicity *S. typhimurium*, TA98 + S9	Pavers (cracked rocks, petroleum bitumen)	17 (6)	27 (9)	+	Among non-smokers, 82% of exposed and 28% of non-exposed had mutagens in urine ($P < 0.025$). Five workers were also exposed to diesel exhaust.	Pasquini *et al.* (1989) Italy
DNA damage						
DNA damage, mononuclear blood cells (alkaline elution)	Pavers Roofers Bitumen painters	18 (12) 7 (7) 9 (8)	34 (> 50%)	– + –	Samples taken on Monday and Friday. Roofers had higher levels of DNA-damage on Friday ($P < 0.05$) and higher levels *vs* control smokers ($P < 0.002$)	Fuchs *et al.* (1996) Germany
DNA damage, leukocytes (comet assay)	Roofers (bitumen ± coal-tar pitch)	26 (16)	15 (3)	+	Significant comet results for seven roofers who had no coal-tar exposure.	Toraason *et al.* (2001) USA
DNA damage in buccal leukocytes (comet assay)	Pavers (asphalt concrete and stone-mastic asphalt with or without waste plastic and tall-oil pitch)	15 (10)	5 (0)	–	No statistically significant differences in pre-shift *vs* post-shift samples, or in exposed vs controls. Nonetheless, DNA damage and urinary metabolites of PAHs (naphtol and 1-OHP) were correlated	Lindberg *et al.* (2008) Finland
DNA damage, blood lymphocytes, and leukocytes in sputum (comet assay)	Pavers (mastic asphalt)	42 (27)	NA	–	Pre-shift samples served as controls. No change in sputum leukocytes; decrease in lymphocytes.	Marczynski *et al.* (2010) Germany
DNA damage, lymphocytes (comet assay)	Pavers (mastic asphalt)	320 (199)	118 (61)	–	DNA damage decreased during shift in exposed and controls. No effect of smoking.	Marczynski *et al.* (2011) Germany
DNA damage, whole blood (comet assay)	Pavers (mixing/ paving)	36 (20)	37 (19)	+	Significant increase in exposed *vs* controls, in smokers (exposed *vs* controls) and non-smokers (exposed *vs* controls).	Sellappa *et al.* (2011) India

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Oxidative DNA damage						
Oxidative DNA damage (HPLC-electrochemical)	Roofers (bitumen ± coal-tar pitch)	26 (16)	15 (3)	–	Increase in oxidative damage was not significant; no effect of smoking.	Toraason *et al.* (2001) USA
Oxidative DNA damage in lymphocytes (comet assay, Fpg-modified)	Pavers (concrete asphalt; 160 °C)	19 (9)	22 (11)	+	$P = 0.008$, exposed *vs* controls; 7/19 (37%) had oxidative DNA damage.	Cavallo *et al.* (2006) Italy
Oxidative DNA damage (HPLC-electrochemical)	Pavers (mastic-asphalt) (exposure to coal-tar pitch was excluded)	320 (199)	118 (61)	+	Oxidative DNA damage (8-OH-dG) higher in exposed *vs* controls before shift ($P = 0.0001$) and after shift ($P = 0.0001$). No effect of smoking.	Marczynski *et al.* (2011) Germany
DNA/protein adducts						
DNA adducts, leukocytes (USERIA)	Roofers	28 (NR)	9 (2)	+	Seven of the roofers (four smokers) and two controls (both smokers) showed measurable BPDE-DNA adducts. No exposure information given.	Shamsuddin *et al.* (1985) USA
DNA adducts, leukocyte (^{32}P-postlabelling)	Roofers, incl. removal of old roofs	12 (8)	12 (8)	+	Detectable adducts in 10 roofers (83%), in 2 controls (17%), in the 4 exposed non-smokers, and in none of the control non-smokers. Removal of old roofs may involve coal-tar exposure.	Herbert et al. (1990a, b) USA
DNA adducts, mononuclear blood cells (^{32}P-postlabelling)	Pavers Roofers Bitumen painters	18 (12) 7 (7) 9 (8)	34 (> 50%)	±	Samples taken from 12 pavers and 2 painters; adduct-related spots detectable in 4 samples (3 pavers, 1 painter). No data given for other exposed or controls.	Fuchs *et al.* (1996) Germany
DNA adducts, mononuclear blood cells (^{32}P-postlabelling)	Pavers (hot-mix asphalt)	49 (11)	36 (13)	±	Samples taken in four seasons. No difference in pavers *vs* non-pavers. Adduct levels in pavers increased by weekday during work-season, not in off-season. Such increase was not seen in non-pavers.	McClean *et al.* (2007b) USA

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
DNA adducts, leukocytes (HPLC-fluorescence)	Pavers (mastic asphalt) (exposure to coal-tar pitch was excluded).	202 (133)	55 (23)	–	Detectable adducts in only 42% (pre-shift) and 40% (post-shift) of samples of 154 workers. No difference. No data on controls.	Marczynski *et al.* (2007) Germany
DNA adducts, leukocytes (HPLC-fluorescence)	Pavers (mastic asphalt) (exposure to coal-tar pitch was excluded).	320 (199)	118 (61)	–	Adducts measured in 227 exposed and 66 controls, of whom 110 and 27, resp., had detectable BPDE-adducts. No difference in exposed *vs* controls, or pre-shift *vs* post-shift.	Marczynski *et al.* (2011) Germany
Protein (albumin) adducts, blood plasma (ELISA)	Roofers	12 (NR)	12 (NR)	±	Monoclonal antibody recognizes PAH/protein adducts of BPDE; crossreacts with chrysene adducts.	Lee *et al.* (1991) USA
Sister-chromatid exchange						
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers and roofers	14 (3)	8 (0)	±	Pavers/roofers *vs* controls, $P < 0.05$; sister-chromatid exchange frequencies in pavers/roofers and 13 manual workers were similar.	Hatjian *et al.* (1995) United Kingdom of Great Britain and Northern Ireland
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (raking, bitumen production)	28 (16)	19 (12)	+	Exposed *vs* controls, $P < 0.05$ For non-smokers: exposed *vs* control, $P < 0.001$	Burgaz *et al.* (1998) Turkey
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers ("ordinary" road paving)	28 (0)	30 (0)	–		Järvholm *et al.* (1999) Sweden
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (hand-pavers, finishers, road pavers)	46 (26)	87 (46)	+	Hand-pavers and finishers *vs* industrial controls: $P < 0.05$ in 1996; sister-chromatid exchange frequency decreased in 1997 to 1999	Major *et al.* (2001) Hungary
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (concrete asphalt; 160 °C)	19 (9)	22 (11)	–		Cavallo *et al.* (2005, 2006) Italy

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Sister-chromatid exchange, lymphocytes (BrdU staining of metaphase)	Pavers (concrete asphalt; 170 °C)	26 (1)	24 (1)	+	Samples taken just after 2-wk holiday and post-shift at 14 days. Exposed *vs* controls, $P < 0.001$. Positive correlation with duration of exposure. All workers wore gloves, safety shoes, respirators.	Karaman & Pirim (2009) Turkey
Micronucleus formation						
Micronucleus formation, blood lymphocytes	Pavers (raking, asphalt production)	28 (16)	28 (18)	+	Exposed *vs* controls, $P < 0.0001$ For non-smokers: exposed *vs* control, $P < 0.01$.	Burgaz *et al.* (1998) Turkey
Micronucleus formation, blood lymphocytes	Pavers (road paving)	28 (0)	30 (0)	–		Järvholm *et al.* (1999) Sweden
Micronucleus formation, exfoliated urothelial cells, blood lymphocytes	Pavers	12 (6)	18 (6)	+	For both cell types: MN/1 000 cells, $P < 0.01$. No effect of smoking.	Murray & Edwards (2005) Australia
Micronucleus formation, blood lymphocytes	Pavers (mastic asphalt) (exposure to coal-tar pitch was excluded)	202 (133)	55 (23)	–	MN data given for 34 exposed and 14 controls.	Marczynski *et al.* (2007) Germany
Micronucleus formation, blood lymphocytes	Pavers (concrete asphalt; 170 °C)	26 (1)	24 (1)	+	Samples taken just after 2-wk holiday and post-shift at 14 days. Exposed *vs* controls, $P < 0.001$. Positive correlation with duration of exposure. All workers wore gloves, safety shoes, respirators.	Karaman & Pirim (2009) Turkey
Micronucleus formation, blood leukocytes	Pavers (mixing/ paving)	36 (20)	37 (19)	+	Significant increase in exposed *vs* controls, in smokers and non-smokers; also exposed to coal tar.	Sellappa *et al.* (2011) India
Micronucleus formation, blood lymphocytes	Mastic-asphalt workers	225 (139)	69 (41)	–		Welge *et al.* (2011) Germany

Table 4.2 (continued)

End-point (test system used)	Occupational group	Exposed (smokers)	Controls (smokers)	Result	Comments	Reference, country
Chromosomal aberrations						
Chromosomal aberrations, blood lymphocytes	Pavers (hand-pavers, finishers, road pavers) *vs* industrial controls	46 (26)	87 (46)	+	$P < 0.05$ in 1996 and 1997. Frequency decreased in 1998–99; also exposed to diesel exhaust	Major *et al.* (2001) Hungary
Chromosomal aberrations, blood lymphocytes	Pavers (hand-pavers, finishers, mixers) *vs* industrial controls	66 (45)	56 (24)	+	$P < 0.05$; also exposed to diesel exhaust	Tompa *et al.* (2007) Hungary

1-OHP, 1-hydroxypyrene; BPDE, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; BrdU, bromodeoxyuridine; 8-OH-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; d, day; ELISA, enzyme-linked immunosorbent assay; Fpg, formamido-pyrimidine-glycosylase; HPLC, high-pressure liquid chromatography; MN, micronuclei; NR, not reported; S9, 9000 × *g* rat liver supernatant; USERIA, ultrasensitive enzymatic radioimmunoassay; *vs*, *versus*; wk, week

than on Monday, while the contrary was evident for the bitumen painters.

DNA damage, measured as alkali-labile lesions with the comet assay, were determined in peripheral blood leukocytes of 26 roofers (16 smokers) exposed to roofing bitumen [class 2], and 15 construction workers (three smokers), not exposed to bitumen during the past 5 years. A subgroup of 19 roofers (12 smokers) was exposed to coal tar during removal of existing roofs. There was statistically significantly more DNA damage in end-of-week samples from workers exposed to bitumens only, compared with start-of-week samples. This group also had elevated 1-OHP levels in the urine (Toraason *et al.*, 2001).

Comet-assay analysis of pavers (n = 19; nine smokers) and controls (n = 22; 11 smokers) in Italy, showed higher levels of oxidative DNA damage in the pavers (Cavallo *et al.*, 2006). Additional use of formamido-pyrimidine-glycosylase (Fpg) in the comet assay indicated that oxidative damage to DNA contributed in about one third of the samples from the pavers (7 out of 19), but in none of the 22 controls.

Buccal leukocytes collected from 15 pavers (10 smokers) were collected pre- and post-shift, analysed by the comet assay, and compared with 5 controls (all non-smokers). The pavers were exposed to asphalt concrete (without or with WPT, produced at 145–165 °C; [class 1, class 5]), and to stone-mastic asphalt (without or with WPT, produced at 151–157 °C; [class 1, class 5]) (Lindberg *et al.*, 2008). No significant differences were found between pre- and post-shift samples, or between the workers and the unexposed controls.

An analysis of 202 mastic-asphalt [class 1] workers exposed during high-temperature application at 240–260 °C, mainly indoors and in basement garages (exposure to coal-tar pitch was excluded) and 55 non-exposed construction workers (controls) found that DNA-strand breaks post-shift (mid-week), relative to pre-shift, were statistically significantly decreased in these larger groups ($P < 0.05$) (Marczynski *et al.*, 2007). Levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OH-dG) were significantly higher post-shift in both the exposed workers and the non-exposed controls ($P < 0.0001$), but significantly higher levels of 8-OH-dG and alkali-labile DNA lesions were found in the exposed workers than in the controls both before and after the working shifts.

This study has been expanded further to include 320 bitumen [class 1]-exposed workers and 118 non-exposed construction workers (Marczynski *et al.*, 2011). Blood lymphocytes were tested in the alkaline comet assay. Midweek pre-shift levels of DNA damage were significantly higher than post-shift in both the exposed group and the control group ($P < 0.0001$ and $P = 0.012$, respectively); the levels of damage in the exposed workers were significantly higher than in the controls at both sampling times ($P < 0.0001$). Levels of 8-OH-dG in lymphocytes were significantly higher in the exposed group at both sampling times ($P < 0.0001$) and the levels increased from pre- to post-shift in both groups ($P < 0.0001$ for exposed workers; $P = 0.0002$ for controls).

In a pilot study, the same investigators compared leukocytes from induced sputum with peripheral blood lymphocytes from 42 bitumen-exposed workers before and after shift (Marczynski *et al.*, 2010). There was no correlation between DNA damage in the two cell types, as measured by the comet assay (Spearman rank correlation coefficient: $r_s = -0.04$, $P = 0.802$ before shift; and $r_s = 0.27$, $P = 0.088$ after shift).

In a study of 36 bitumen-exposed road pavers in India (no protective equipment worn other than safety shoes) and 37 controls, DNA damage in peripheral blood leukocytes, analysed by the comet assay, was significantly greater ($P < 0.05$) in the exposed workers than in the controls (Sellappa *et al.*, 2011). In both the exposed and the control group, smokers and alcohol-drinkers had higher levels of damage than did non-smokers

and non-drinkers. [The Working Group noted that these workers were also exposed to coal tar.]

(c) DNA adducts and protein adducts

Antibodies raised against DNA modified by 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) were used to develop an ultrasensitive enzymatic radioimmunoassay (USERIA) to analyse leukocyte DNA from 28 roofers (no other information on occupational exposures was given) and nine controls (Shamsuddin *et al.*, 1985). With a limit of detection of one adduct per 7.5×10^7 nucleotides, adducts were detected in seven of the roofers (three of these were smokers, three were non-smokers and one unspecified; no information was given on the smoking status of the other 21 roofers in whom adducts were not detected). Among the controls, two of nine samples were positive, both of them from smokers (the remaining seven individuals in the control group were non-smokers).

An analysis by ^{32}P-postlabelling of DNA isolated from leukocytes of roofers (n = 12; eight smokers) revealed detectable levels of bulky/aromatic adducts in ten roofers, compared with detectable levels in only two of twelve control subjects (eight smokers) (Herbert *et al.*, 1990b). The roofers were involved in the removal of old pitch roofs and replacement of each section with a new asphalt roof. Among the roofers, all four non-smokers had detectable levels of DNA adducts; among the controls, none of the non-smokers showed DNA adducts. Among the roofers, skin wipes taken post-shift contained concentrations of PAHs that correlated with the levels of DNA adducts.

In a subsequent study, serum samples from the same workers and controls were analysed for PAH–albumin adducts using an enzyme-linked immunosorbent assay (ELISA): the roofers had higher levels of adducts (5.19 fmol/μg versus 3.28 fmol/μg; marginally statistically significant, $0.1 > P > 0.05$) (Lee *et al.*, 1991). There was also a weak correlation between levels of PAH–albumin adducts and levels of DNA adducts measured in the earlier study ($0.1 > P > 0.05$).

Analysis of peripheral mononuclear cells from bitumen-exposed workers (twelve road-paving workers and two bitumen painters; nine smokers) revealed the presence of DNA adducts detected by ^{32}P-postlabelling analysis in ten workers (Fuchs *et al.*, 1996). [The Working Group noted that no results were reported on DNA adducts in control subjects.]

Forty-nine asphalt-paving workers [bitumen, class 1] were monitored for formation of DNA adducts over a 12-month period and compared with 36 non-paving construction workers, both during the working season and during the off-season (winter) (McClean *et al.*, 2007b). Although levels of DNA adducts – measured by ^{32}P-postlabelling – were increased in the lymphocytes of the exposed workers through the working week, they were not higher overall than in the non-exposed workers, and seasonal variations were such that levels were higher in the off-season.

A modified HPLC-FD method was used to determine adducts of benzo[*a*]pyrene diol-epoxide in leukocytes of 154 mastic-asphalt workers, and in road-construction workers not exposed to bitumens (Marczynski *et al.*, 2007). The method was based on determination of benzo[*a*]pyrene tetrol after acidic hydrolysis of DNA. A low level of adducts was reported in the workers, with no significant difference between pre- and post-shift samples. [The Working Group noted that no results were reported on DNA adducts in control subjects.] This study was expanded further to include 320 bitumen-exposed workers and 118 non-exposed construction workers (Marczynski *et al.*, 2011). Levels of DNA adducts were not significantly different between the two groups, both pre- and post-shift, with no change in levels during the shift.

(d) Sister-chromatid exchange

In a study from the United Kingdom, pavers and roofers (n = 14; three smokers) had significantly higher frequencies of sister-chromatid exchange in peripheral blood lymphocytes than did administrative staff (n = 8; all non-smokers) (P < 0.05), but so also did manual workers (n = 13; three smokers) with no known exposure to PAHs, and there was no significant difference between the pavers/roofers and the manual workers (Hatjian *et al.*, 1995).

In a Turkish study, sister-chromatid exchange was measured in lymphocytes from 28 bitumen-exposed workers (16 smokers), 21 of whom were employed as rakermen in road-paving operations, while 7 worked in an asphalt plant. The control group for sister-chromatid exchange analysis (n = 19; 12 smokers) was recruited from university and hospital staff (Burgaz *et al.*, 1998). The frequency of sister-chromatid exchange in the exposed group overall was statistically significantly higher than that in the control group (P < 0.05). The non-smokers in the exposed group had significantly higher sister-chromatid exchange levels than the non-smokers in the control group (P < 0.001).

Another study from Turkey included 26 asphalt workers and a matched control group of 24 administrative workers. The asphalt workers were involved in paving with concrete asphalt (170 °C), and they all wore personal protection devices. Blood samples were collected from the asphalt workers immediately after a 2-week holiday and before starting work, and after the Friday working shift 2 weeks later. The exposed workers had a significantly increased frequency of sister-chromatid exchange compared with controls (P < 0.001). The frequencies of sister-chromatid exchange in the two samples taken from the workers before and after the 14-day period of work were not statistically significantly different (Karaman & Pirim, 2009).

A study from Hungary in 1996–99 included eight road pavers (five smokers; working close to the fresh pavement as "hand-pavers"), 14 drivers of paving equipment (nine smokers; working in closed cabins as "finishers"), 24 other road pavers (12 smokers), eight workers (six smokers) in bitumen production, six non-exposed white-collar workers (four smokers), and a historical control group of 87 industrial workers (46 smokers). Frequencies of sister-chromatid exchange were initially (in 1996) higher (P < 0.05) in the exposed workers (hand-pavers, finishers) than in the controls, although subsequently they decreased to control levels (Major *et al.*, 2001), possibly as a result of changes in work practice and in the composition of asphalts.

In a Swedish study, no difference in the frequency of sister-chromatid exchange in peripheral blood lymphocytes was found between 28 non-smoking workers involved in "ordinary" road-paving operations, with exposure to PAHs at an average concentration of 2.3 μg/m^3 (range, 0.2–23.8 μg/m^3), and 30 non-smoking controls (Järvholm *et al.*, 1999). Likewise, no difference was observed in an Italian study between the frequency of sister-chromatid exchange in blood lymphocytes of 19 pavers (9 smokers) working with concrete asphalt (at 160 °C) and that in the 22 controls (11 smokers) (Cavallo *et al.*, 2005, 2006).

(e) Micronucleus formation

Studies on the frequency of micronucleus formation in bitumen and asphalt workers showed a mixture of positive and negative results.

In the comparison of 28 workers in Sweden workers [bitumen class 1] with 30 controls (see above), no difference in frequency of micronucleus formation in peripheral lymphocytes was observed (Järvholm *et al.*, 1999). Similarly there was no difference in micronucleus formation in 34 exposed German workers compared with 14 controls, except in "before-shift" comparisons (Marczynski *et al.*, 2007). Expansion of this study

group to include analysis of 225 exposed workers and 69 unexposed construction workers did not reveal any association between micronucleus frequency and exposure to bitumen emissions (Welge *et al.*, 2011). However, in the two Turkish studies mentioned above, frequencies of micronucleus formation were significantly higher in lymphocytes of the exposed workers compared with controls (Burgaz *et al.*, 1998; Karaman & Pirim, 2009). Also, in an Australian study that included 12 bitumen road-layers (6 smokers; [bitumen class 1]) and 18 non-exposed controls (6 smokers), the workers had statistically significantly higher ($P < 0.01$) frequencies of micronucleus formation in both peripheral lymphocytes and in exfoliated urothelial cells (Murray & Edwards, 2005). In an Indonesian study, micronucleus frequencies in the blood cells of bitumen-exposed road pavers ($n = 36$) were statistically significantly higher ($P < 0.05$) than in controls ($n = 37$) (Sellappa *et al.*, 2011). In both groups, higher frequencies were found in smokers and alcohol-drinkers than in non-smokers and non-drinkers (statistically significant in the exposed workers, $P < 0.05$).

(f) Chromosomal aberrations

In monitoring studies carried out in 1996–99 among road pavers and workers with related occupations in Hungary (see above), the frequencies of chromosomal aberrations were initially higher in the road pavers than in the controls, but the levels declined over time in the pavers until they were the same in both groups (Major *et al.*, 2001; Tompa *et al.*, 2007); this may have been the consequence of changes in work practices and composition of asphalts. [The Working Group agreed with the authors who declared that "increase in chromosomal aberrations yields can be attributed to the use of genotoxic agents other than 'asphalt fumes', mainly diesel exhausts, and crude oil (frequently used for cleaning the equipment in Hungary)."]

(g) HPRT mutations

The frequencies of *HPRT* variants in peripheral blood lymphocytes of "tar-free" asphalt road-pavers were not higher than in controls (Major *et al.*, 2001).

(h) Unscheduled DNA synthesis

Peripheral blood lymphocytes from "tar-free" asphalt road-pavers and other bitumen-exposed workers ($n = 111$) did not differ in their response to ultraviolet-induced unscheduled DNA synthesis compared with control workers ($n = 93$) (Major *et al.*, 2001).

4.3.2 Mutagenicity in bacteria

Studies of mutagenicity in *Salmonella typhimurium* exposed to bitumen and bitumen emissions are presented in Table 4.3.

In the Ames test, using *S. typhimurium* strains TA98 and TA100 in the presence or absence of metabolic activation from S9, petroleum bitumen paints were not mutagenic, while coal-tar paints gave positive results after metabolic activation with S9. Nonetheless, both types had tumour-initiating activity on mouse skin (coal tar being more active than bitumen) (Robinson *et al.*, 1984).

DMSO-extracted oils, including bitumen vacuum residue, were also found to be mutagenic in *S. typhimurium* TA98 (Booth *et al.*, 1998).

Bitumen-fume condensate [derived from bitumen class 1] was mutagenic in *S. typhimurium* TA98, TA100, YG1041 and YG1042 in the presence of metabolic activation from S9 from rat liver, but 15–600 times less active than coal-tar fume condensate (De Méo *et al.*, 1996).

Bitumen and asphalt particulates and fumes were tested in *S. typhimurium* TA98 and YG1024 (Heikkilä *et al.*, 2003). Bitumens containing coal-fly ash or waste plastics [bitumen class 5] were heated to paving temperatures in the laboratory. The vapour fractions were negative for mutagenicity, but the particulate fractions were mutagenic

Table 4.3 Studies of mutagenicity in *Salmonella typhimurium* treated with bitumen, bitumen fume or their condensates

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium, TA98, TA100, TA1535, TA1537, TA1538	Petroleum-bitumen paints (n = 4): bitumen cut-backs (64% solid, diluted with 1–3% xylene); 0.005–10 µL/plate.	–	–	No toxicity. Some of these paints were mouse-skin carcinogens	Robinson *et al.* (1984)
	Coal-tar paints (n = 4): coal-tar pitch/xylene (67/33); coal-tar pitch/clay talc/solvent (47/16/37 or 37/42/21 or 39/30/30); 0.005–10 µL/plate.	–	+ positive in all strains, except in TA1535	Toxicity at high dose; all paints were mouse-skin carcinogens	
S. typhimurium, TA98 (pre-incubation assay)	Petroleum oils (n = 13), DMSO-extracted; 20% solution in EGDE	NT	+ (n = 6) – (n = 7)	Mutagenicity correlated with skin carcinogenicity	Blackburn *et al.* (1984)
S. typhimurium, TA98	Bitumen, vacuum residue; various petroleum-derived oils	NT	± bitumen	Mutagenicity index correlated with DNA adducts in mouse skin	Booth *et al.* (1998)
S. typhimurium, TA98, TA100, YG1041, YG1042 (plate-incorporation assay)	Condensates of bitumen fume generated at 160 °C or 200 °C	–	+ condensates positive in all strains	Mutagenicity 15–600 times lower than for coal-tar condensates (110 °C/160 °C).	De Méo *et al.* (1996)
S. typhimurium, TA98, YG1024	Bitumen B120; bitumen B80 with 66% coal fly ash; bitumen B120 with 10% waste plastics. Samples taken at 170–180°C; C_2Cl_4 extract of filters; 0.03–0.5 mg/plate tested.			Note: samples correspond to class 5 bitumen.	Heikkilä *et al.* (2003)
	Vapour fractions of the different samples	–	–		
	Particle fractions of the different samples	+	+		
	Stone-mastic asphalt fume collected during paving and remixing, +/– coal fly ash (10%) or lime (10%); C_2Cl_4 extract of Teflon filters; only particles tested.	+	+	Sampling temperature 160–210 °C (paved asphalt) and 150–350 °C (remixed asphalt)	

Table 4.3 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
S. typhimurium, TA98, YG1024	*Laboratory samples:* Fumes from stone-mastic asphalt ± waste plastics and tall oil pitch, produced in the laboratory at 150 °C. *Collected during paving:* Asphalt concrete ± waste plastics and tall oil pitch, produced at 145–165 °C; Stone-mastic asphalt ± waste plastics and tall oil pitch, produced at 151–157 °C (filters all extracted with C_2Cl_4/DMSO).	–	–	Samples also contain coal fly ash and lime. Note: samples corresponded to class 1 and 5 bitumens	Lindberg *et al.* (2008)
S. typhimurium, TA98 (pre-incubation assay)	Fumes from roofing (n = 4) and paving bitumens (n = 18) were generated at 232–316 °C and 163 °C, respectively. The oil phase of the fume condensates was extracted with DMSO.	NT	+	For some samples, the mutagenic response correlated with the 3- to 7-ring PAH content.	Machado *et al.* (1993)
S. typhimurium, TA98 (pre-incubation assay)	Condensate of bitumen fume, drawn from a storage tank at 147–157 °C	NT	–		Reinke *et al.* (2000)
	Condensate of bitumen fume from a laboratory generator at 149 °C and 316 °C.	NT	+	More 3–4-ring *S*-heterocyclic PAH in high-temperature samples	
S. typhimurium, TA98 (ASTM Standard Method E 1 687–95)	Copenhagen mastic-asphalt core samples 1952–91; DMSO extracts (n = 11)	NT	++ early samples (before 1970) + later samples	Higher mutagenicity in early samples was consistent with the presence of coal tar.	Kriech *et al.* (1999a)
S. typhimurium, TA98 (ASTM Standard Method E 1 687–95)	Laboratory-generated roofing bitumen-fume condensates, various fractions; DMSO extracts	NT	+	Mutagenic effects correlated with skin carcinogenicity in the mouse.	Kriech *et al.* (1999b)

C_2Cl_4, tetrachloroethylene; DMSO, dimethyl sulfoxide; EGDE, ethylene glycol dimethyl ether; NT, not tested

in the presence and absence of S9 in both strains. In addition, the particulate fractions of bitumen fumes collected in the field during paving with stone-mastic asphalts (with either lime or coal-fly ash as filler) and during remixing of stone-mastic asphalt or asphalt concrete, were tested. These were also mutagenic in the presence and absence of S9 in both strains. The field samples were more mutagenic than the laboratory-generated fumes without S9, and the remixing fumes were more potent than the normal paving fumes and the laboratory-generated fumes with S9.

Another study of field samples of fumes from asphalt concrete (without or with WPT, produced at 145–165 °C; [bitumen class 1, class 5]) and from stone-mastic asphalt (without or with WPT, produced at 151–157 °C; [bitumen class 1, class 5]) found the materials to be non-mutagenic in *S. typhimurium* strains TA98 and YG1024 with or without metabolic activation (Lindberg *et al.*, 2008).

Asphalt-fume condensate (derived from class 2 bitumen) was reported to be mutagenic in the modified Ames test with *S. typhimurium* TA98 in the presence of S9, but was about 100 times less mutagenic than coal-tar pitch fume condensate (Machado *et al.*, 1993). The weak-to-moderate potency observed in this study broadly correlated with PAH content, in particular for three- to seven-ring PAHs.

The mutagenic activity in the modified Ames assay of several fume condensates from bitumens K and E [class 1] was found to correlate with the total content of three- to six-ring PACs, as determined by extraction with DMSO followed by GC (Brandt, 1994). This was found to be a better measure of mutagenic potential than comparisons with either the concentrations of single compounds or with the sum of the concentrations of 14 compounds.

In another study, condensate of laboratory-generated fumes of 85/100 grade paving bitumen [class 1] was tested with the modified Ames test; the sample generated at 316 °C was more mutagenic than the material generated at 149 °C, reflecting higher three- and four-ring *S*-heterocyclic PAH content (Reinke *et al.*, 2000).

When roofing-bitumen [class 2] fume condensate ("NIOSH fumes") were fractionated by HPLC, the two subfractions that were carcinogenic on mouse skin were also the fractions that were mutagenic in the modified Ames assay. These subfractions showed relatively high fluorescence intensities at 415 mn, consistent with the presence of four- to six-ring PACs (Kriech *et al.*, 1999b). Furthermore, there was good correlation between the biological activity of the subfractions, or various combinations of subfractions, and their PAC content, as determined by fluorescence emissions.

In an Ames test that included S9 prepared from the lungs of rats exposed to high concentrations of bitumen fumes ([class 1]; 1150 ± 63 mg.h/m^3; generated at 170 °C), the mutagenic activity of 2-aminoanthracene, but not of benzo[*a*]pyrene, was statistically significantly enhanced compared with when S9 from control (non-exposed) rats was used (Zhao *et al.*, 2004).

4.3.3 Genotoxicity in mammalian systems

Genotoxicity data in mammalian systems *in vivo* and *in vitro* are presented in Table 4.4.

(a) DNA damage

Exposure of plasmid DNA to bitumen (100 µg/mL) plus ultraviolet A (UVA) did not reveal the formation of single- or double-strand breaks, but reactive oxygen formation was demonstrated by incubation with deoxyguanosine, resulting in the formation of 8-OH-dG (Hong & Lee, 1999). However, the combination of bitumen (10 µg/mL; obtained from distillation of crude oil) and UVA (1.5, 3.0 or 6.0 mJ/cm^2) on the human promyelocytic leukaemia cell-line HL 60 showed a significant increase in DNA–protein crosslinks over the modest increase produced by either exposure alone (Hong & Lee, 1999).

Table 4.4 Studies of genotoxicity in mammalian systems *in vitro* and *in vivo* treated with bitumen or bitumen fume

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
In vitro					
DNA strand-breaks, λ DNA	Bitumen (100 µg/mL) + UVA (24 mJ/cm^2)	–	NT		Hong & Lee (1999)
DNA adducts (^{32}P-postlabelling), calf thymus DNA	Condensates of bitumen fume generated at 160 °C or 200 °C	NT	+	DNA-adduct formation correlated with mutagenicity in *S. typhimurium*	De Méo *et al.* (1996)
DNA adducts (^{32}P-postlabelling, ^{32}P-HPLC), calf thymus DNA	Bitumen fume sampled at hot storage tanks	NT	+	Activation by rat or human liver microsomes	Akkineni *et al.* (2001)
Formation of oxidative DNA damage (8-OH-dG), λ DNA	Bitumen (100 µg/mL) + UVA (6 mJ/cm^2)	+	NT		Hong & Lee (1999)
	Bitumen (only) 10 µg/mL	±	NT		
Micronucleus formation, Chinese hamster lung V79 cells	Fume of type I and III bitumen, generated at 316 ± 10 °C. Fume condensates were tested at 0–250 µg/mL.	+	NT	Immunostaining for kinetochores suggests activity as aneuploidogen	Qian *et al.* (1996)
Micronucleus formation, Chinese hamster lung V79 cells	Fume of type III bitumen, generated at 316 ± 10 °C. HPLC of condensates: five fractions tested up to 250 µg/mL[a]	+	NT	The four most polar of the five fractions were positive	Qian *et al.* (1999)
Chromosomal aberrations, Chinese hamster ovary cells	Condensate of bitumen fume, drawn from a storage tank at 147–157 °C (5–120 µg/mL for up to 18 h).	–	–		Reinke *et al.* (2000)
	Condensate of bitumen fume from a laboratory generator at 149 °C and 316 °C (5–120 µg/mL for up to 18 h).	–	–		
DNA–protein cross-links, HL60 human promyelocytic leukaemia cell-line	Bitumen (10 µg/mL) combined with UVA (1.5 mJ/cm^2)	+	NT		Hong & Lee (1999)
	Bitumen (only) 10 µg/mL	±	NT		
DNA strand-breaks (comet assay), BEAS 2B human bronchial epithelial cells	Laboratory-generated SMA-WPT fume	+	–		Lindberg *et al.* (2008)

Table 4.4 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
DNA adducts (^{32}P-postlabelling), adult and fetal human skin samples	Bitumen paint, applied topically on the epidermis: 4% or 20% in THF (3 or 15 mg bitumen); 24-h treatment	+	NT	Similar adduct pattern found in skin of mice treated with bitumen, coal-tar or creosote	Schoket *et al.* (1988b)
Micronucleus formation, BEAS 2B human bronchial epithelial cells	*Laboratory samples:*			Sightly toxic at concentrations > 10 µg/ml	Lindberg *et al.* (2008)
	Fumes from SMA produced at 150 °C (40 µg/mL)	+	–		
	Fumes from SMA-WPT produced at 150 °C (10 µg/mL)	+	–		
	Field samples collected during paving:			Slightly toxic at 40 µg/ml	
	Asphalt concrete ± WPT, produced at 145–165 °C	–	–		
	SMA produced at 151–157 °C (20 µg/mL)	+	–		
	SMA-WPT produced at 151–157 °C (10 µg/mL)	+	–		
In vivo					
DNA strand-breaks (comet assay), rat (female Sprague-Dawley) alveolar macrophages	Bitumen fume (class 1) generated at 170 °C; inhalation exposure (353, 641 and 1150 mg.h/m^3)	+	NT	Dose-dependent increase	Zhao *et al.* (2004)
DNA adducts (^{32}P-postlabelling), male Parkes mouse skin and lung	Bitumen paint (57% bitumen) applied to mouse skin (15 mg). Killed after 24 h	+	NT	Adducts found in the treated epidermis and lungs	Schoket *et al.* (1988a)
DNA adducts (^{32}P-postlabelling), BD4 rat (age, 7–8 wk), skin, lung and lymphocytes	Bitumen-fume (160 °C, 200 °C) condensate (class 1) trapped on glass-fibre filter and XAD-2 resin, extracted with benzene and diethylether, respectively. Applied topically on skin, twice.	+	NT	Bitumen-specific DNA adduct found in skin, lung and lymphocytes	Genevois *et al.* (1996)

Table 4.4 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
DNA adducts (^{32}P-postlabelling), female CD1 mouse (age, 8–11 wk), skin	Bitumen, vacuum residue; dermal application	+	NT	Adduct levels correlated with mutagenicity in *S. typhimunium*	Booth *et al.* (1998)
DNA adduct (^{32}P-postlabelling), male CD rat lung cells and blood leukocytes	Fumes of type I and III bitumen, generated at 316 ± 10 °C. Fume condensates were intratracheally instilled, 3×/24 h, at 250–2000 mg/kg bw. Killed 6 h after last administration.	+	NT	Adducts found in lung cells but not in leukocytes	Qian *et al.* (1998)
DNA adducts (^{32}P-postlabelling), Sprague-Dawley BD6 rat (age, 8 wk), lung	Bitumen fume, generated at 200 °C; particles at 5 or 50 mg/m^3; nose-only inhalation, 6 h/d for 5 d	+	NT	Single DNA adduct detected in high-particle sample (50 mg/m^3).	Genevois-Charmeau *et al.* (2001)
DNA adducts, (^{32}P-postlabelling), Big Blue mouse, lung	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (particles, 100 mg/m^3); 6 h/d, 5 d; expression period, 30 d	–	NT		Micillino *et al.* (2002)
DNA adduct (^{32}P-postlabelling), B6C3F$_1$ mouse, lung	Bitumen-fume condensates generated at 180 °C; inhalation (whole-body exposure) 4 h/d for 10 days, 152–198 mg/m^3.	+	NT	BPDE-dG, -dA, and -dC adducts identified by nanoflow-LC/Q-TOF-MS.	Wang *et al.* (2003b)
DNA adducts, two-months old Big Blue® male rat	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (100 mg/m^3 particles); 6 h/d, 5 d.	±	NT	Bitumen-specific DNA adduct.	Bottin *et al.* (2006), Gate *et al.* (2007)
DNA adducts (^{32}P-postlabelling), (young adult SPF-Wistar) rat lung, nasal, and alveolar epithelium	Bitumen-fume condensate, 4, 20, 100 mg/m^3; 6 h/d, 5 d, 30 d, 12 mo inhalation.	+	NT	Adducts found in lung, nasal epithelium and alveoli. Highest adduct level in nasal epithelium.	Halter *et al.* (2007)
cII and *lacI* mutation in lung DNA, Big Blue mouse	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (100 mg/m^3 particles); 6 h/d, 5 d; expression period 30 d.	–	NT		Micillino *et al.* (2002)
cII mutation in lung DNA, Big Blue® male rats (age, 2 mo)	Bitumen fume generated at 170 °C (class 1); inhalation, nose-only (particles, 100 mg/m^3); 6 h/d, 5 d	–	NT	Mutation spectrum associated with increase of G:C → T:A and A:T to C:G	Bottin *et al.* (2006), Gate *et al.* (2007)

Table 4.4 (continued)

Test system	Substance tested	Result		Comment	Reference
		Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, male Sprague-Dawley rat bone-marrow polychromatic erythrocytes	Asphalt-fume condensate, collected at 160 °C; intratracheal instillation, 0.45–8.88 mg/kg bw, 3 d, killed after 24 h	+	NT	Significant increase in micronuclei at the highest dose	Ma *et al.* (2002)
Micronucleus formation, rat (female Sprague-Dawley) bone-marrow polychromatic erythrocytes	Bitumen fume (class 1) generated at 170 °C; inhalation, higher exposure, 1733 mg.h/m^3.	–	NT		Zhao *et al.* (2004)
Micronucleus formation, young adult SPF-Wistar rat, peripheral blood erythrocytes and bone-marrow polychromatic erythrocytes	Bitumen-fume condensate, 4, 20, 100 mg/m^3; 6 h/d, 5 d, 30 d, 12 mo inhalation.	–	NT		Halter *et al.* (2007)
Micronucleus formation, Wistar rat bone-marrow erythrocytes	Roofing bitumen-fume condensate (class 3); nose-only, inhalation, 30, 100 and 300 mg/m^3, 28 d	–	NT		Parker *et al.* (2011)

[a] There is a confusion throughout the article with the units of concentration; the most plausible unit (µg/mL) is given in the Table.

8-OH-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; bw, body weight; d, day; h, hour; LC/Q-TOF-MS, liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry; mo, month; NT, not tested; SMA, stone-mastic asphalt; THF, tetrahydrofuran; WPT, waste plastics and tall oil pitch
[The exact names of the three adducts are as follows: dA-BPDE, N^6-deoxyadenosine-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; dC-BPDE, N^4-deoxycytidine-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide; dG-BPDE, N^2-deoxyguanosine-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide.]

Laboratory-generated stone-mastic asphalt fumes, with WPT, produced at 150 °C [class 5] induced DNA damage in the cultured human bronchial epithelial cells BEAS 2B without metabolic activation from rat liver S9, but not after incubation with S9 mix. The laboratory-generated stone-mastic asphalt fumes alone [bitumen class 1], however, gave negative results in the comet assay (alkali-labile damage) with and without metabolic activation. None of the asphalt fumes collected in the field at the paving sites induced DNA damage in BEAS 2B cells with or without metabolic activation (Lindberg *et al.*, 2008).

In an experiment *in vivo* in which rats were exposed by inhalation to bitumen fumes generated at 170 °C under simulated road-paving conditions for 1 hour or 6 hours, the comet assay revealed DNA damage in alveolar macrophages obtained from the rats by bronchoalveolar lavage (Zhao *et al.*, 2004).

(b) DNA adducts

The adduct-forming ability of condensates of bitumen fume generated at 160 °C or 200 °C, when incubated with calf-thymus DNA and rat liver S9, correlated with their bacterial mutagenicity (De Méo *et al.*, 1996). There was also a correlation between DNA adduct-forming ability in female CD1 mouse skin and mutagenicity in *S. typhimurium* for a series of petroleum products, including bitumens (Booth *et al.*, 1998).

Application of black bitumen paint (57% bitumen) to the skin of male Parkes mice resulted in the formation of DNA adducts (detected by ^{32}P-postlabelling analysis) in the treated epidermis and in the lungs of the animals (Schoket *et al.*, 1988b). The pattern of adducts indicated a complex mixture of different species and was similar to the profile produced by coal tar and creosote, although the levels of adduct formation were lower for bitumen. Similar patterns, and levels, of adducts were formed in samples of adult and fetal human skin maintained in short-term organ culture and treated topically with bitumen paint (Schoket *et al.*, 1988a).

Bitumen fumes (160 °C, 200 °C) were trapped on glass-fibre filters and XAD-2 resin, extracted with benzene and diethylether, and the condensates applied twice to the skin of BD4 rats aged 7–8 weeks. After this treatment, complex patterns of DNA adducts were detected in the skin, lung and lymphocytes of the rats. It was noted that the patterns of adducts obtained with bitumen fume were qualitatively different to those induced by coal-tar fume condensate (Genevois *et al.*, 1996).

In another study, microsomes from rat liver and human microsomes both activated components of bitumen to form DNA adducts *in vitro* (Akkineni *et al.*, 2001). Studies with liver microsomes from different strains of mice and with yeast microsomes expressing human CYPs were conducted to investigate mechanisms of metabolic activation of the active components of bitumen (45/60 PEN hardness, derived from heavy Venezuelan crude oil; bitumen fume produced at 160 °C and 200 °C) (Genevois *et al.*, 1998). The findings demonstrated that CYP1A isoforms were partially, but not wholly, responsible for activating the genotoxic components of bitumen; other enzymes under the control of AhR may also play a role.

Several studies have investigated the formation of DNA adducts in mouse or rat lung after exposure to bitumen and bitumen emissions by inhalation. One study did not detect gene mutations at the *cII* and *lacI* loci, or DNA adducts, in the lungs of Big Blue mice exposed to bitumen fume (100 mg/m^3) for 6 hours per day for 5 days, followed by a 30-day fixation period (Micillino *et al.*, 2002). In Big Blue rats treated under the same regimen, and from which there was some evidence of exposure-related mutations, there was also evidence for DNA-adduct formation that appeared to be bitumen-specific (Bottin *et al.*, 2006; Gate *et al.*, 2007). This bitumen-specific DNA adduct was similar to the adduct found in skin, lung and lymphocytes after bitumen

skin-painting of BD4 rats (Genevois *et al.*, 1996) and shown to be different from both major and minor benzo[*a*]pyrene adducts (Bottin *et al.*, 2006; Gate *et al.*, 2007).

DNA adducts were detected in the lungs and in nasal and alveolar epithelium of rats exposed to inhaled bitumen-fume condensate, which increased in a time- and dose-dependent manner (Halter *et al.*, 2007). Nose-only inhalation of bitumen fume by rats also resulted in DNA-adduct formation in the lung at a high level of exposure (50 mg/m^3, 6 hours per day, for 5 days) but not at a lower dose (5 mg/m^3) (Genevois-Charmeau *et al.*, 2001).

Similarly, inhalation by rats of bitumen-fume condensate resulted in DNA-adduct formation in the lung. The presence of adducts formed by benzo[*a*]pyrene was shown by MS (Wang *et al.*, 2003b).

When bitumen-fume condensate was given to rats by intratracheal instillation, DNA adducts were formed in lung tissue, but not in leukocytes (Qian *et al.*, 1998).

(c) Mutagenicity in vivo

In a study in which Big Blue mice were exposed to bitumen fume (100 mg/m^3; CAS No. 8052-42-4; generated at 170 °C; [class 1]) for 6 hours per day for 5 days, followed by a 30-day fixation period, no increase in either the frequency of *cII* mutation, or in the level of bulky DNA adducts detected by ^{32}P-postlabelling, was found in exposed mice compared with non-exposed mice (Micillino *et al.*, 2002). In an analogous experiment in Big Blue rats, the frequency of *cII* mutation was also similar in exposed and non-exposed lungs, although the exposed rats had a slight, non-statistically significant, increase in G:C to T:A and A:T to C:G transversions (Bottin *et al.*, 2006; Gate *et al.*, 2007).

(d) Micronucleus formation

Whole fume condensates of two types of roofing bitumen (type I and type III; fume generated at 316 °C) caused a significant (> five–six times) increase in the frequency of micronucleus formation in Chinese hamster lung fibroblasts (V79 cells). About 70% of the micronucleated cells induced by bitumen-fume condensate carried kinetochore-positive micronuclei, which indicated that the cytogenetic damage caused by the condensates was primarily at the level of the spindle apparatus of the exposed cultured cells (Qian *et al.*, 1996). In a subsequent study, one of the condensates (type III) was separated – on the basis of polarity – into five fractions, four of which showed activity (Qian *et al.*, 1999). The two most active (most polar) fractions contained alkylated benzo- and dibenzothiophenes, and alkylated benzonaphthothiophenes in one case, and alkylated phenylethanones and dihydrofuranones in the other.

In another study, field samples of bitumen fume collected from a paving site (asphalt concrete without or with WPT, produced at 145–165 °C; [class 1, class 5]; stone-mastic asphalt without or with WPT, produced at 151–157 °C; [class 1, class 5] were tested for induction of micronuclei in BEAS 2B human bronchial epithelial cells, with positive results for both types of bitumen, without metabolic activation from S9 (Lindberg *et al.*, 2008).

Male Sprague-Dawley rats were intratracheally instilled with bitumen-fume condensate at 0.45–8.88 mg/kg bw, collected at the top of a paving-bitumen storage tank, in sterile saline for three consecutive days and killed on the next day (Ma *et al.*, 2002). The frequency of micronucleated polychromatic erythrocytes in bone marrow was determined. The mean numbers of cells with micronuclei per 1000 polychromatic erythrocytes was statistically significantly increased at the highest dose (2.9 ± 0.6 *versus* 1.5 ± 0.4 for saline controls, $P < 0.05$).

In another study *in vivo*, exposure of rats to high total levels of bitumen fume by inhalation (1733 mg h/m^3) did not result in detectable micronucleus formation in bone-marrow polychromatic erythrocytes (Zhao *et al.*, 2004). No micronuclei were detected in erythrocytes or polychromatic erythrocytes of the bone marrow of rats exposed to bitumen fume at up to 100 mg/m^3 for 6 hours per day, for 5 days, 30 days, or 12 months (Halter *et al.*, 2007).

Micronucleus formation was not detected in bone-marrow polychromatic erythrocytes of Wistar rats given [class 3] roofing bitumen-fume condensates by inhalation (nose-only) with up to 300 mg/m^3 (the highest concentration tested) for 28 days (Parker *et al.*, 2011).

(e) Chromosomal aberrations

No chromosomal aberrations were induced in Chinese hamster ovary (CHO) cells exposed, with or without exogenous metabolic systems, to condensate of asphalt fumes collected from the head space of an operating hot-mix asphalt storage tank or to laboratory-generated bitumen-fume condensate (Reinke *et al.*, 2000).

4.4 Other effects relevant to carcinogenesis

4.4.1 Activation of AhR-associated pathways

Besides the direct genotoxic effects of the parent compound or of their metabolites, PAHs that are present in bitumen fume and condensate may have more pleiotropic effects that may lead to carcinogenesis by acting through activation of AhR (Schmidt & Bradfield, 1996; Yamaguchi *et al.*, 1997; Puga *et al.*, 2009). The basic information on AhR-mediated mechanisms in relation to the biochemical and toxicological effects of PAHs, some of which also are present in bitumen fume and condensate, is briefly summarized at the beginning of this chapter, and was previously developed in Volume 92 of the *IARC Monographs* (IARC, 2010).

PAHs are indeed among the best characterized high-affinity exogenous AhR ligands, which include a variety of toxic and hydrophobic chemicals (Stejskalova *et al.*, 2011).

AhR is a ligand-dependent transcription factor that regulates a wide range of biological and toxical effects in many species and tissues. In addition to the regulation of the CYP1 family of xenobiotic metabolizing enzymes by AhR via exogenous ligands, the recent recognition of endogenous AhR ligands has helped to understand that AhR also plays a role in many physiological functions, such as regulation of the cell cycle and proliferation, immune response, circadian rhythm, expression of enzymes involved in lipid metabolism, and tumour promotion (Puga *et al.*, 2009; Stevens *et al.*, 2009).

Activation of AhR by high-affinity PAH ligands results in a wide range of cell-cycle perturbations, including G0/G1 and G2/M arrest, diminished capacity for DNA replication, and inhibition of cell proliferation. At present, all available evidence indicates that AhR can trigger signal-transduction pathways involved in proliferation, differentiation and apoptosis by mechanisms dependent on xenobiotic ligands or on endogenous activities that may be ligand-mediated or completely ligand-independent. These functions of AhR coexist with its well characterized toxicological functions involving the induction of phase I and phase II genes for detoxification of foreign compounds (Puga *et al.*, 2009).

Transcriptional activation of targeted genes in response to AhR is not only species- and tissue-specific, but also ligand-specific. Interaction between a ligand and a receptor is characterized by several variables and the final cellular response is dependent on the combination of these variables. In other words, activation of the receptor by two different compounds will result in different quantitative and qualitative outcomes

in terms of the cellular response. It was shown that the majority of exogenous AhR ligands are partial agonists that never elicit maximal response, even if all receptors are occupied, and importantly some partial agonists can behave as functional antagonists, i.e. when combining full agonist with partial agonist, the effect of the full agonist is diminished by partial agonist, hence, displaying antagonistic behaviour (Stejskalova *et al.*, 2011).

The ligand-specificity of AhR responses is an important notion to consider for complex PAH mixtures such as bitumen fume and condensate. As inducers of CYP xenobiotic-metabolizing and conjugating enzymes, individual PAHs present in bitumen fume and condensate that are AhR partial agonists normally stimulate their own metabolism and that of other carcinogens (Stejskalova *et al.*, 2011). Nonetheless, several other carcinogenic PAHs (e.g. benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, dibenzo[*a,c*]anthracene) were also found to be potent inhibitors of *CYP1A2* and *CYP1B1* (Shimada & Guengerich, 2006).

CYP monooxygenases, which are expressed in basically all tissues, albeit at different levels, play an essential role in the metabolic activation of the many constituents of bitumen, including aliphatic, aromatic, and PACs. Their metabolites may also interact with cellular processes and interfere with cellular functions, resulting in cellular stress and/or dysregulation of biological processes (Shimada & Guengerich, 2006).

4.4.2 Changes in gene and protein expression

(a) Workers exposed to bitumen

Changes in protein expression were investigated in skin punch biopsies from 16 bitumen-exposed road pavers (non-smokers; [exposure to bitumen class 1]) and of 10 age-matched controls. Overexpression of BAX and underexpression of BCL2 were observed in skin that had been exposed to bitumen in the long term, suggesting that bitumen fume induces activation of apoptosis (Loreto *et al.*, 2007). Moreover, the overexpression of the proteins TRAIL, DR5 and CASP3, also involved in apoptosis, was also observed as detected by immunohistochemistry in the skin of the same 16 workers – who reported having worn protective gloves, shoes and clothing (Rapisarda *et al.*, 2009). Furthermore, the activation of programmed cell death was demonstrated in the skin by enhanced terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL) positivity.

Skin punch biopsies from 16 road pavers, daily exposed to asphalt and bitumen, were investigated by immunohistochemistry for levels of HSP27, a member of the heat-shock protein family of chaperone proteins, which are involved in cellular defence mechanisms (Fenga *et al.*, 2000). A total of 25 biopsies from the 16 workers were compared with 5 biopsies from unexposed controls. In the worker samples, immunostaining for HSP27 was homogeneously detected in the whole epidermis, including the basal cell layer, and more intense than in the control samples, indicating that HSP27 was upregulated in the workers' skin.

(b) Experimental animals

Microarray analysis of gene-expression changes was performed on tissue from the lungs of rats exposed by nose-only inhalation to bitumen fume generated at 170 °C for 5 days, 6 hours per day. The analysis identified increased expression of many genes involved in lung inflammatory and immune responses (see Section 4.4.3), as well as genes encoding enzymes involved in the metabolism of PAHs and other xenobiotics. The PAH-inducible genes *Cyp1a1* and *Cyp1b1* genes were the most overexpressed in exposed rat lungs. In contrast, another phase I metabolism enzyme, *Cyp2f2*, was downregulated in bitumen-exposed lungs. Other inducible genes with AhR binding site in their promoter, including the antioxidant genes *Nqo1*, *Aldh3a1* and *Gsta5*

were also significantly upregulated in exposed lungs. Moreover, various genes involved in the cellular response to oxidative stress, including superoxide dismutase 2 (*Sod2*) heat-shock 70 kDa protein 1A (*Hspa1a*), haemo oxygenase 1 (*Hmox1*), glutathione peroxidase 1 (*Gpx1*) and metallothionein 1a (*Mt1a*) have been found to be overexpressed in treated animals. Furthermore, genes associated with protease activity and inhibition were differentially modulated in bitumen-exposed and non-exposed rats. In total, 363 out of the 20 500 probes were differentially expressed (Gate *et al.*, 2006).

Analysis by RT-PCR of the expression of selected genes was carried out on lung tissue from rats exposed to bitumen fume by inhalation for up to 12 months. *Cyp1a1* and *Cyp2g1* were up- and downregulated, respectively. Also significantly modulated, although not in a dose-dependent manner, were genes encoding cathepsin K and D, cadherin 22 and the regulator of G-protein signalling. With the CYP monooxygenases *Cyp1a1* and *Cyp2g1*, a dose-dependent regulation in respiratory epithelium of the upper (nasal) and lower (lung) airways was observed. In addition, there was a dose-dependent (not statistically significant) regulation of genes associated with immune response, inflammation and extracellular matrix remodelling, albeit at different levels when lung and nasal epithelium were compared (Halter *et al.*, 2007).

Exposure to an extract of bitumen fume (generated at 150 °C; typical for paving asphalt) of JB6 P^+ cells (mouse epidermal cell line), and in cultured primary keratinocytes (from AP-1-luciferase reporter transgenic mice) resulted in statistically significant increases in the activity of AP-1, a transcription factor that regulates many genes including some involved in cell growth, proliferation and transformation (Ma *et al.*, 2003a). Downstream effects included activation of the PI3K/Akt pathway, which has been shown to play a critical role in tumour promotion. Furthermore, topical application of bitumen fume by painting the tail skin of mice increased AP-1 activity by 14 times (Ma *et al.*, 2003a).

4.4.3 Alteration of the immune system, inflammation, and risk of cancer

Chronic inflammation increases the risk for cancer, in part as a result of enhanced production of reactive oxygen species, inflammatory mediators, and proteolytic enzymes that can both damage DNA and lead to increases in reparative cell proliferation rates (Grivennikov *et al.*, 2010).

Exposure of the immune system to bitumen and bitumen emissions results in a complicated interplay between individual constituents that have the potential to bind to endogenous AhR of immune competent cells and to metabolize as part of the detoxification programme; this results in induction of oxidative stress and/or the removal of reactive metabolites via secondary metabolic processes. As with many tissues, the immunotoxicity of PAHs present in bitumen emissions is dependent on exposure levels to circulating parent compounds and metabolites, cell type-specific expression of AhR and the balance between bioactive *versus* detoxified metabolites. The dose and route of exposure to PAHs present in bitumen and bitumen emissions are important determinants of immunotoxicity in animals and possibly humans. In general, the total cumulative dose of exposure to PAHs correlates well with immunotoxicity in mice. For individual PAHs (e.g. benzo[*a*]pyrene), a biphasic dose–response curve has been reported, whereby low doses stimulated immune responses and high doses caused inhibition (Burchiel & Luster, 2001; Booker & White, 2005).

As summarized in Volume 92 of the *IARC Monographs* (IARC, 2010), the overall effects of PAHs on the immune and haematopoietic systems result from activation of both genotoxic and non-genotoxic (epigenetic) pathways. Because of the heterogeneity of lymphoid and myeloid cell populations and the complex interplay between

different types of cells and secreted products, the mechanisms of action of individual constituents of bitumen fume and condensate have not been assessed as yet. Nonetheless, many of the PAHs clearly exert effects on the developing as well as the mature immune system and some correlation exists between the carcinogenicity of PAHs and their ability to produce immunosuppression.

There is evidence that immunosuppressive PAHs, some of which are present in bitumen fume, function as AhR ligands and this receptor plays an important role in the development of the immune system in mice (Fernandez-Salguero *et al.*, 1995). The precise mechanism whereby the activation of AhR leads to immunotoxicity is not known. Furthermore, as many of the PAHs and their metabolites are moderate to strong (high-affinity) AhR ligands, it is difficult to distinguish between the action of a parent compound, such as benzo[*a*]pyrene, and that of metabolites that are formed in response to AhR binding and the induction of metabolic enzymes. Ligand-dependent AhR activation can lead to immune effects via interaction of AhR with regulatory sequences, i.e. xenobiotic response element/xenobiotic dioxide response element (XRE/XDRE), which are also found in genes coding for innate and adaptive immune response (Esser *et al.*, 2009). Thus, regulation via AhR activation of genes containing XRE/XDREs may well be correlated with immunotoxicity, as observed for several halogenated aromatic hydrocarbons and many PAHs (for review, see IARC, 2010).

Lung tissue from rats exposed to bitumen fume by inhalation had increased expression of many genes involved in the inflammatory and immune response, as shown using microarray technology (Gate *et al.*, 2006). The inflammatory cytokines (interleukins *IL6* and *IL8*) and chemokines (*CCL2*/*MCP1*, *CXCL1*/*CINC1* and *CXCL2*/*M1P2*) were among the most strongly upregulated genes in exposed lungs. In addition, among the 363 differentially regulated genes, about two dozen were associated with the inflammatory process. Furthermore, bronchiolar lavage cells from exposed rats showed increased expression of pulmonary inflammatory-response genes (*TNFα, IL2β, MIP2*) (Gate *et al.*, 2006). While short-term exposure of rats to bitumen fume by inhalation did not produce acute lung damage or inflammation (Ma *et al.*, 2003b), long-term exposure for 1 year produced inflammatory responses (Fuhst *et al.*, 2007).

As bitumen fume and condensate consist of mixtures of PAHs and other PACs, it is difficult to attribute the relative contributions of individual PAHs to the overall immunotoxic effects.

Data concerning the irritative effects of exposure to bitumen emissions on the airways in humans are limited. Raulf-Heimsoth *et al.* (2007) investigated the irritant effects of bitumen on the airways by monitoring inflammatory processes in the upper and lower airways of 74 mastic-asphalt workers exposed to bitumen emissions and of workers in a reference group. All workers were examined immediately before and after shift. At both time-points, nasal lavage fluid (NALF), induced sputum and spot urine were collected and analysed. Exposure to bitumen emissions was monitored by personal air sampling. Significantly higher concentrations of IL-8, IL-6, nitrogen oxide (NO) derivatives, and total protein were determined in sputum collected before and after shift in exposed workers, especially in those that were highly exposed. Thus, irritative effects in response to exposure to fumes and aerosols of bitumen on the upper and lower airways were demonstrated, especially in mastic-asphalt workers with exposure > 10 mg/m^3.

In a further study by the same investigators, the irritative effects caused by vapours and aerosols of bitumen were assessed in a cross-shift study comparing 320 bitumen-exposed mastic-asphalt workers, with 118 road-construction workers as the reference group (Raulf-Heimsoth *et al.*, 2011b). The induced sputum concentrations of IL-8, matrix metalloproteinase-9, and total protein were significantly higher in

bitumen-exposed workers than in the reference group, suggesting potentially (sub)chronic irritative inflammatory effects in the lower airways of bitumen-exposed workers. These investigators also reported an association between genotoxic and inflammatory effects in the lower airways, which they had compared simultaneously with DNA-strand breaks in induced sputum and blood of bitumen-exposed workers (Marczynski *et al.*, 2010).

In the study of Ellingsen *et al.* (2010), several biomarkers of systemic inflammation and endothelial activation were studied during a working season in 72 pavers, 32 asphalt-plant operators and 19 asphalt engineers. Among the bitumen-exposed workers, smokers had lower concentrations of Clara cell protein (CC-16) and surfactant protein A, but higher concentrations of surfactant protein D, IL-6, C-reactive protein, fibrinogen and intercellular adhesion molecule (ICAM)-1 than non-smokers. [The Working Group noted that in this study no evidence for increased systemic inflammation and endothelial activation in bitumen-exposed workers throughout the season could be determined and that several identified confounders such as smoking habits, body mass index and the level of physical activity needed to be considered.]

In lung and nasal respiratory epithelium of rats exposed to bitumen fume, the expression of chitinase – a candidate gene associated with asthma – as well as other genes coding for immune response and of chronic obstructive pulmonary disease was significantly altered (Halter *et al.*, 2007).

4.4.4 Inhibition of gap-junction intercellular communication

In the study of Sivak *et al.* (1997), different laboratory-generated bitumen-roofing fume fractions [bitumen class 2] were produced and tested for inhibition of gap-junction intercellular communication (Toraason *et al.*, 1991; Wey *et al.*, 1992). All fractions inhibited intercellular communication in Chinese hamster lung fibroblasts (V79 cells) as defined by inhibition of the transfer of toxic phosphorylated metabolites of 6-thioguanine (6TG) from wildtype 6TG cells to 6TG-resistant cells via gap junctions. Induction of 6TG-resistant colonies was considered to be inhibitory of intracellular communication (Toraason *et al.*, 1991). Similarly, Wey *et al.* (1992) examined the effect of these fractions on gap-junction intercellular communication in human epidermal keratinocytes. All fractions inhibited gap-junction intercellular communication in a concentration-dependent fashion. Modulation of gap junctions in functional intercellular communication has been implicated in the promotion of tumour growth. The inhibition of gap-junction intercellular communication may disconnect preneoplastic cells from the regulatory signals of surrounding cells, leading to the development of neoplasms.

4.5 Mechanistic considerations

Bitumens, like many petroleum-based products, are complex mixtures that contain a variety of different chemical compounds that can contain carbon, hydrogen, sulfur, nitrogen and oxygen (IARC, 1985). PAHs containing two to seven aromatic, fused-ring systems have been detected in bitumen-fume samples. Several of these PAHs and related agents are known to be genotoxic and/or carcinogenic in experimental systems. These include benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,i*]pyrene, indeno[1,2,3-*cd*]pyrene and naphthalene. Benzo[*a*]pyrene, an IARC Group 1 carcinogen, is also found in some bitumen-fume samples (IARC, 2010). Non-substituted and substituted thiaarenes containing two to four rings, including dibenzothiophene, benzo[*b*]naphtho[2,1-*d*]thiophene, benzo[*b*]naphtho[1,2-*d*]thiophene and benzo[*b*]naphtho[2,3-*d*]thiophene were also detected in

bitumen fumes (Brandt *et al.*, 2000; Reinke *et al.*, 2000; Binet *et al.*, 2002). The azaarene quinoline, and the oxyarene dibenzofuran, were also detected (Brandt *et al.*, 2000).

Studies in experimental systems on the genotoxic activities of bitumen-fume samples have given mixed results that have been ascribed to the differing compositions of the bitumen samples used, the temperature at which fumes are generated, and experimental conditions used to collect the fume condensates. These genotoxic activities included DNA-adduct formation, bacterial mutagenicity, DNA damage and chromosomal effects. Many of these studies used laboratory-generated bitumen-fume samples and a group of these studies showed positive results in tests for genotoxicity. Field-simulated samples were generally negative for genotoxicity. One study used field-collected samples and gave positive results for bacterial mutagenicity (Heikkilä *et al.*, 2003). Bitumen-fume condensate was mutagenic in bacterial assays in the presence of metabolic activation (Machado *et al.*, 1993; De Méo *et al.*, 1996; Kriech *et al.*, 1999a; Reinke *et al.*, 2000; Heikkilä *et al.*, 2003). Bitumen-fume condensate and several of its fractions induced micronucleus formation in mammalian cells (Qian *et al.*, 1996, 1999). Bitumen fume induced DNA damage (comet assay) in rat lung and pulmonary alveolar macrophages after inhalation exposure (Zhao *et al.*, 2004) and induced micronucleus formation in polychromatic erythrocytes from rats treated by intratracheal instillation with bitumen-fume condensate (Ma *et al.*, 2002).

Lung tissue from rats exposed to bitumen fume and condensates by inhalation had increased expression of *Cyp1a1, Cyp1b1, Cyp2g1* genes and other genes involved in PAH metabolism (Gate *et al.*, 2006; Halter *et al.*, 2007). Nasal tissues showed increased expression of *Cyp1a1* (Halter *et al.*, 2007). These results suggested that bitumen-fume exposure activated AhR, a ligand-dependent transcription factor that regulates a wide range of biological and toxical effects. Increases in rat microsomal lung Cyp1a1 protein levels and enzymatic activity were also observed (Ma *et al.*, 2002).

Many studies using ^{32}P-postlabelling techniques have shown the presence of bulky aromatic DNA adducts in mammalian cells, isolated tissues or tissue samples from experimental animals treated dermally with bitumen-fume condensate or by inhalation of bitumen fume (Table 4.4). In a study of inhalation of bitumen fume in mice, MS of DNA isolated from lung tissue identified three specific benzo[*a*]pyrene–DNA adducts that were derived from *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide: *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-deoxyguanosine; *anti*-benzo[*a*]-pyrene-7,8-dihydrodiol-9,10-epoxide-deoxyadenosine and *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-deoxycytosine. Rats exposed under similar experimental conditions produced detectable urinary levels of benzo[*a*]pyrene-7,8-dihydrodiol, benzo[a]pyrene-7,8,9,10-tetrol and 3-hydroxybenzo[a]pyrene (Wang *et al.*, 2003a, b).

Lung tissue from rats exposed to bitumen fume by inhalation showed increased expression of many genes involved in the inflammatory and immune responses, as shown using microarray technology (Gate *et al.*, 2006). Bronchiolar lavage cells from exposed rats showed increased expression of genes involved in the pulmonary inflammatory response (*Tnfα, Il2β, Mip2*) (Gate *et al.*, 2006). While short-term exposure of rats to bitumen fume by inhalation did not produce acute lung damage or inflammation (Ma *et al.*, 2003b), long-term exposure for 1 year produced inflammatory responses (Fuhst *et al.*, 2007).

Humans are exposed to bitumen emissions dermally and by inhalation. Over the years, conflicting results have been reported in studies on biomarkers of exposure and effects using populations of bitumen-exposed workers. Confounding variables such as differences in population characteristics (e.g. roofers *versus*

pavers), population size, changes in exposure levels due to improved safety practices and potential confounding factors such as age, smoking, nationality, time of measurement, diet, and exposures from other sources may have contributed to the disparate results reported. Overall, there was evidence from studies of occupationally exposed populations that workers exposed to bitumen fume have been exposed to a group of PACs, some of which are genotoxic and carcinogenic. This conclusion was based on studies that measured the mutagenicity of urine samples in bacteria (Pasquini *et al.*, 1989), urinary 1-OHP and other OH-PAHs (Burgaz *et al.*, 1992; Toraason *et al.*, 2001; Campo *et al.*, 2006; Buratti *et al.*, 2007; Raulf-Heimsoth *et al.*, 2007; Pesch *et al.*, 2011), and bulky aromatic DNA adducts and PAH–albumin adducts in peripheral blood (Herbert *et al.*, 1990a, b; Lee *et al.*, 1991). Workers exposed to bitumen had higher levels of oxidative DNA damage measured as 8-OH-dG adducts in peripheral blood lymphocytes and increased levels of DNA damage, sister-chromatid exchange, micronucleus formation and chromosomal aberrations in leukocytes (see Table 4.2). The qualitative and quantitative analyses of bulky aromatic DNA adducts in leukocytes of bitumen-exposed populations established that these populations had been exposed to PACs.

Constituents of bitumen fume also interfere with intercellular gap-junctional communication. Inhibition of intercellular communication may disconnect a pre-neoplastic cell from the regulatory signals of surrounding cells to possibly foster tumour development (see Section 4.4.4).

In summary, bitumen fume contains PAHs and heterocyclic PACs. Many of the PAHs are mutagenic and carcinogenic and have produced many of the same genotoxic activities as those reported for bitumen-fume condensates (IARC, 2010). One of these PAHs, benzo[*a*]pyrene, has been detected in bitumen-fume condensates and in the lungs (as benzo[*a*]pyrene-diol-epoxide–DNA adducts) of mice and in the urine (as benzo[*a*]pyrene metabolites) of rats exposed to bitumen fume. It is noted that while bitumen-fume condensates induced skin tumours in mice after dermal application (Sivak *et al.*, 1997; Clark *et al.*, 2011; Freeman *et al.*, 2011), there are no adequate studies of bitumen fume and cancer in mice exposed by inhalation, and a study of bitumen fume and cancer in rats exposed by inhalation was considered negative, even though a nasal tumour, defined as an adenocarcinoma, was reported (Fuhst *et al.*, 2007). While there was evidence for the role of benzo[*a*]pyrene in the genotoxicity of some bitumen fumes in experimental systems, the lack of definitive studies linking the genotoxic effects induced by exposure to bitumen fume to other specific PAHs or heterocyclic PACs prevents the identification of a clear role for these agents in the mechanism of genotoxic or carcinogenic action of bitumen fume.

There is conclusive evidence that bitumen fume and condensate cause cellular stress and disrupt cellular defence programmes. This leads to overproduction of reactive oxygen species, which perpetuates inflammatory signalling as a result of AhR-mediated or AhR-independent signalling. An imbalance in the detoxification of reactive oxygen species stimulates the immune response. Inflammation affects immune surveillance and immune cells infiltrate tumours to engage in an extensive and dynamic crosstalk with cancer cells. Bitumen fume and condensate induce inflammatory signalling, but may also function as immunosuppressants, possibly via AhR-mediated immunotoxicity (Burchiel & Luster, 2001; Gate *et al.*, 2006; Puga *et al.*, 2009; Stevens *et al.*, 2009).

On the basis of the weight of evidence from studies in experimental systems, it is highly probable that a mechanism involving genotoxicity is responsible for the tumorigenic effects of exposure to bitumen in mouse skin.

In studies in humans, exposure to bitumen fume resulted in more mutagenic urine, 8-OH-dG in DNA – a measure of reactive oxygen species – DNA damage, unidentified bulky aromatic DNA adducts, PAH–albumin adducts, sister-chromatid exchange, micronucleus formation, and chromosomal aberrations. Associations have been reported between genotoxicity and inflammatory effects in the lower airways of humans.

These positive findings in humans are consistent with a mechanism involving genotoxicity that is responsible for the tumorigenic effects of exposure to bitumen.

5. Summary of Data Reported

5.1 Exposure data

Bitumens are a complex mixture of organic compounds of high relative molecular mass that are manufactured in large quantities as residuum of crude oil in petroleum refineries, and that also occur naturally in petroleum-rich regions of the world. The major classes of bitumen are straight-run bitumens (class 1), oxidized bitumens (class 2) cutback bitumens (class 3), bitumen emulsions (class 4), modified bitumens (class 5) and thermally cracked bitumens (class 6). Oxidized bitumens are further divided into semi-blown (or air-rectified) bitumens, with applications similar to class 1 bitumens, and fully oxidized bitumens. Global annual consumption of bitumens is estimated to be more than 100 million tonnes, the vast majority of which is used for road paving (85%) and roofing (10%). Straight-run bitumens mixed with mineral aggregates are used in road paving, generally at 110–160 °C. Oxidized bitumens are used in hot-roofing applications (180–230 °C). Mastic asphalt, a subclass of class 1 bitumens, is used in specialized applications at higher temperatures (200–250 °C). These applications result in emissions of fume (the aerosolized fraction of total emissions, i.e. solid particulate matter, condensed vapour, and liquid petroleum droplets), vapours and gases. Although most polycyclic aromatic compounds are removed during the manufacturing process, these fumes and vapours contain a mixture of two- to seven-ring polycyclic aromatic compounds varying in composition and concentration by application temperature. There is strong evidence that higher application temperatures are associated with higher exposures.

More than one million workers in road paving, roofing, manufacture of bitumen and bitumen products and other specialized applications may be exposed to bitumen by dermal contact and to bitumen emissions by inhalation. The levels of exposure to bitumen and bitumen emissions vary by type of application, job, type of bitumen used and over time. The highest concentrations of bitumen fumes and vapours have been measured during mastic-asphalt application and for roofers applying hot bitumen, while asphalt-mixing plant workers and pavers are exposed to lower concentrations.

Both pavers and roofers may be coexposed to coal tar. Exposure to coal tar among roofers was associated with a 35-times increase in dermal exposure to benzo[*a*]pyrene and a 6-times increase in dermal exposure to polycyclic aromatic compounds. Similarly, exposure by inhalation to benzo[*a*]pyrene among road pavers was estimated to be a factor of 5 higher when coal tar was present. Both roofers and road-paving workers may also have coexposure to engine exhausts, mineral dusts, diluents of bitumens, and thermal degradation products of modified bitumens.

Time-trend data are primarily available for road paving in Europe, where exposures to bitumen fume, bitumen vapour and benzo[*a*]pyrene have decreased by a factor of 2–3 each decade since 1970. In the USA and Europe, the recent introduction of warm-mix asphalts with a lower application temperature (100–140 °C) will further lower exposures to bitumen fumes and

vapours for road pavers. Similarly, cold and soft applications have been developed to lower exposure to bitumen emissions in roofers.

5.2 Human carcinogenicity data

The Working Group reviewed all the available studies to evaluate the risks of cancer in workers occupationally exposed to bitumens. The Working Group summary of the findings emphasized a meta-analysis published in 1994 that included eight case–control and eleven cohort studies. Of those studies not included in the meta-analysis, the IARC multicentre cohort study was considered to be the most informative because of its large size and detailed evaluations of exposure to bitumen and potentially confounding exposures. The risk ratios from the 1994 meta-analysis provided the starting point for the evaluation of exposure to bitumens and risk of cancer. Next, results from studies not included in the meta-analysis were considered. Finally, findings from the IARC multicentre study were considered. The meta-risk ratio from the more recent meta-analysis of the literature was not used for the evaluation because it included the IARC multicentre study. When making its assessments, the Working Group took into consideration that there was some overlap in the populations included in some of the individual studies, the meta-analysis and the IARC multicentre cohort study. Four major occupational categories exposed to bitumens were identified, namely, road paving, roofing, mastic-asphalt applications and diverse occupations involving exposure to bitumens, including asphalt products manufacturing, mixing plants , and unspecified occupations.

5.2.1 Road-paving workers

(a) Cancer of the lung

Several studies have assessed the risk of cancer of the lung among road-paving workers. A meta-analysis published in 1994 calculated a meta-risk ratio of 0.87 (95% CI, 0.76–1.08) for lung cancer based on four studies (three case–control and one cohort) of pavers and road-maintenance workers.

Two independent case–control studies were not included in the meta-analysis. A study from Northern Germany reported an unstable odds ratio of 3.7 (95% CI, 1.06–13.20), while the other, which pooled two other German case–control studies, found only a small excess (odds ratio, 1.20; 95% CI, 1.0–1.5). The occupational group examined in the German studies combined road pavers with excavating workers and pipe-layers and therefore probably involved exposure to asbestos and silica dust in addition to bitumen, and therefore the effect of bitumens may have been diluted. Risk ratios among road pavers varied between 0.8 and 1.6 in the cohort studies published after 1994.

The IARC multicentre cohort study, by far the largest study reporting data for road-paving workers, observed increased mortality from cancer of the lung among road-paving workers in comparison to the general population (SMR, 1.17; 95% CI, 1.01–1.35), which was attenuated when an internal group of ground and building construction workers was used as the referent (RR, 1.08; 95% CI, 0.89–1.34). While internal referents may give risk estimates that are less likely to be confounded by tobacco smoking, they may be exposed to other occupational carcinogens (e.g. quartz, asbestos) that may negatively bias the risk estimates for bitumen and thus underestimate an association. A large variation in the risk of cancer of the lung was present for the internal controls between countries, which calls for additional caution when comparing risks obtained with the external *versus* internal referents.

Exposure response was investigated within the group of road-paving workers in the IARC multicentre cohort study, using quantitative exposure estimates (cumulative exposure, average exposure, and exposure duration) for bitumen fume, organic vapours and benzo[*a*] pyrene. In a comparison of different quantitative measures of bitumen exposure in road-paving workers, average exposure to bitumen fume improved the model fit compared with cumulative exposure and duration in both lagged and unlagged analyses and was significantly associated with mortality from cancer of the lung. In the case–control study nested within a part of the IARC cohort that excluded earlier workers exposed to coal tar but exposed to higher levels of bitumens, there were no indications of a positive trend in risk of cancer of the lung with average or cumulative exposure to bitumen.

The Working Group evaluated a group of studies to focus on exposure to bitumens and bitumen emissions in road pavers. These studies partly overlapped with those reviewed for the evaluation of road paving with coal-tar pitch, which has previously been classified by IARC as a Group 1 carcinogen. Overall, the evidence for an increased risk of cancer of the lung among road pavers and road-maintenance workers exposed to bitumens was inconsistent and observed relative risks were – if anything – only marginally elevated.

(b) Cancer of the urinary bladder

The 1994 meta-analysis reported a meta-risk ratio of 1.20 (95% CI, 0.74–1.83). Among the four additional studies not included in this meta-analysis, two reported slight deficits for bladder cancer, and one a slight excess, while a twofold relative risk was reported in the extended German part of the IARC multicentre cohort study. The IARC multicentre cohort study reported an increased risk of bladder cancer among pavers associated with exposure to benzo[*a*]pyrene, but not with bitumens specifically. Although there was a suggestion of an association between bladder cancer and bitumen exposure among road pavers in some studies, the data were inconclusive.

(c) Cancer of the upper airway and upper digestive tract

A study of proportionate mortality in the USA found a deficit for cancers of the buccal cavity and pharynx, and of the oesophagus among road pavers. Mortality for cancer of the larynx was about as expected. In the IARC multicentre cohort study, the standardized mortality ratio for cancer of the head and neck was 1.30 (95% CI, 0.99–1.68), and a risk ratio of 1.24 (95% CI, 0.91–1.68) was found when using an internal control group. Overall, the data regarding these cancers and bitumen exposure among pavers were inconclusive.

(d) Other cancer sites

An association between cancer at other sites (e.g. stomach, kidney, leukaemia and skin) and exposure to bitumens as a road-paving worker was investigated in several other studies. The data were inconclusive.

5.2.2 Roofing workers exposed to bitumens

When assessing risk of cancer associated with roofing, it should be noted that the proportion of roofers actually applying or removing bitumen roofs or involved in waterproofing with bitumens varies between studies and between countries. The occupational category "roofer" may be a poor proxy for exposure to bitumen in population-based studies conducted in countries where most roofs are covered with shingles or clay-based roof tiles. Industry-based studies have a better ability to restrict cohorts to the exposure of more specific interest. Roofers are more likely to be exposed to coal tar than are road pavers, since a common task for roofers on a gentle slope or flat roofs is to remove old roofs that may contain coal tar – a procedure that

involves high exposure to coal-tar dusts. Roofers may be exposed to other agents such as asbestos, e.g. while removing corrugated asbestos cement plates. Roofers are typically exposed to higher emissions of bitumen than are road pavers due to the higher application temperatures in use.

(a) Cancer of the lung

Twelve publications (eight cohort studies and four case–control studies) provided information on risks of cancer of the lung among roofers. A meta-analysis published in 1994 that included four cohort studies and three case–control studies showed a meta-risk ratio of 1.78 (95% CI, 1.50–2.10), with virtually no difference between cohort and case–control studies, although the latter studies generally adjusted for smoking.

Four additional publications of cohort studies and one case–control study from Italy, all published after 1994, provided additional information on risk of cancer of the lung among roofers. Statistically significantly increased risks of cancer of the lung were observed among roofers in two studies of proportionate mortality from the USA, one based on the population in Washington State and the other on unionized roofers and waterproofers. In the IARC multicentre cohort study, a standardized mortality ratio of 1.33 (95% CI, 0.73–2.23) was observed among roofing and waterproofing workers.

The Working Group evaluated a group of studies to focus on exposure to bitumens and bitumen emissions among roofers. These studies overlapped with those reviewed for the evaluation of roofing with coal-tar pitch, which has previously been classified by IARC as a Group 1 carcinogen. There was a clear association between roofing and lung cancer in the studies reviewed here that was not likely to be a result of chance. Risk estimates were as high in the case–control studies that were adjusted for smoking as in the cohort studies, which suggested that the observed excess was not likely to be explained by uncontrolled confounding from smoking. Roofers have been exposed to other human lung carcinogens such as coal tar, and few studies assessed the magnitude of this potential confounding. Although it was unlikely that the excess risks are entirely explained by coal-tar exposure, confounding could not be ruled out with reasonable certainty.

(b) Cancer of the urinary bladder

Two independent cohort studies of roofers found excesses for bladder cancer, while a study of proportionate mortality reported deficits. The association of bladder cancer with employment as a roofer was assessed in a large multicentre case–control study in Europe, showing an odds ratio of 0.72 (95% confidence interval, 0.36–1.43) for roofers, after adjustment for smoking. A subsequent follow-up of the Scandinavian part of the IARC multicentre cohort showed no overall excess incidence of cancer of the bladder among roofers, but indicated a non-statistically significant association with time since follow-up. Overall, cancer of the bladder did not appear to be associated with exposure to bitumens in these studies of roofers.

(c) Cancer of the upper airway and upper digestive tract

Four cohort studies that assessed the risk of cancers of the upper aerodigestive tract among roofers all showed elevated risks. These studies showed relative risks ranging from 1.3 to 3.3 for cancers of the buccal cavity, pharynx, larynx and oesophagus, although possible confounding by tobacco, alcohol or other occupational exposures could not be ruled out.

(d) Other cancer sites

The Working Group considered several other cancer sites (e.g. stomach, kidney, leukaemia and skin) associated with occupation as a roofer, with some studies showing excesses, but in general the results were inconsistent.

5.2.3 Mastic-asphalt workers

The highest reported exposures to bitumen emissions occured among mastic-asphalt workers, and it is noteworthy that the temperature at which this material is applied is generally 200–250 °C. Informative data about the association of cancer with mastic-asphalt work were provided by a cohort study of mastic-asphalt workers in Denmark who were followed for cancer incidence and mortality, showing a standardized incidence ratio of 3.44 (95% CI, 2.27–5.01) for cancer of the lung. The risk for cancer of the lung was higher (SIR, 8.57; 95% CI, 1.77–25.05), although imprecise when the cohort was restricted to time periods during which no coal tar was used. Results similar to the incidence study were found in a subsequent mortality study of the same cohort, and remained substantially elevated after group-level adjustment for smoking. This study was noteworthy because of the distinctive exposures among mastic-asphalt workers and the efforts to control confounding by coal tar and tobacco smoking. Excess risks were noted for several other cancers, notably cancers of the upper aerodigestive tract (mouth, larynx, oesophagus).

The IARC multicentre cohort study also showed increased risks of cancer of the lung among workers identified as mastic-asphalt workers (SMR, 2.39; 95% CI, 0.78–5.57).

5.2.4 Other occupational groups

Exposure to bitumens has been studied in several occupations other than roofing, paving and mastic-asphalt work, namely during asphalt-product manufacturing and fibreglass manufacturing. In addition, case–control studies often reported information on self-reported or assigned exposures integrated across a wide range of occupations that were not specifically reported (potentially including exposures encountered through employment in paving, roofing and mastic-asphalt work). Few studies have reported on individual cancer sites and the findings were inconsistent. The Working Group considered studies of these diverse occupational groups to be uninformative regarding the carcinogenicity of exposure to bitumens.

5.3 Animal carcinogenicity data

Straight-run bitumens (class 1), oxidized bitumens (class 2), pooled mixtures of class 1 and class 2 bitumens, fumes generated from class 1, class 2 or pooled mixtures of class 1 and class 2 bitumens, cutback bitumens (class 3), and thermally cracked bitumens (class 6) have been tested for carcinogenicity in experimental animals.

Straight-run bitumens (class 1) have been studied as the neat material in one skin-painting study in mice, one skin-painting study in rabbits and one study of subcutaneous injection in mice. All three of these studies were considered to be inadequate for the evaluation of carcinogenicity. Straight-run bitumen (class 1) has been studied by application in a vehicle solvent in four skin-painting studies in mice, one study of intramuscular injection in mice and one study of intramuscular injection in rats. Two of the studies of skin-painting in mice were considered to be inadequate for the evaluation of carcinogenicity. Eight different "road-grade asphalts" (bitumens class 1) applied in benzene in one of the skin-painting studies in mice did not produce an increase in the incidence of skin tumours. Another of the skin-painting studies of two different class 1 bitumens applied in mineral oil to mice did not produce skin tumours. The study of intramuscular injection in mice of four different "road petroleum asphalts" (bitumen class 1) diluted in tricaprylin was considered to be inadequate for the evaluation of carcinogenicity. Straight-run bitumen (class 1) fume condensates have been studied in two skin-painting studies and one study of subcutaneous injection in mice. One of the skin painting studies and the study

of subcutaneous injection were considered to be inadequate for the evaluation of carcinogenicity. A "field matched" paving bitumen (class 1) fume condensate collected at 148 °C applied in mineral oil in one skin-application study in mice did not produce an increase in the incidence of skin tumours.

Oxidized bitumen (class 2) has been studied as the neat material in three skin-painting studies in mice, one skin-painting study in rabbits, and one study of subcutaneous injection in mice. Two of the skin-painting studies in mice, the skin-painting study in rabbits and the subcutaneous-injection study in mice were all considered to be inadequate for the evaluation of carcinogenicity. Application of a neat "built-up type III roofing 'steep' asphalt" (bitumen class 2) did not produce an increase in the incidence of skin tumours in mice. Oxidized bitumen (class 2) has been studied by application in a vehicle solvent in two skin-painting studies in mice. An "air-refined petroleum asphalt" (bitumen class 2) applied in toluene in one of the skin-application studies in mice produced a significant increase in the incidence of malignant skin tumours. In the other skin-painting study in mice, a "standard roofing-petroleum asphalt" (bitumen class 2) applied in toluene did not produce skin tumours. Oxidized bitumen (class 2) fume or fume condensates have been studied in seven skin-painting studies in mice, and three inhalation studies in rats and guinea-pigs. One of the skin-painting studies in mice and the studies of inhalation in rats and guinea-pigs were considered to be inadequate for the evaluation of carcinogenicity. Six of the skin-painting studies in mice were conducted with oxidized bitumen (class 2) fume condensates generated by heating bitumen at temperatures ranging from 199 °C to 316 °C, collecting the resultant fume condensate and applying it either neat or in a vehicle (mineral oil). Significant increases in the incidence of skin tumours in treated animals were observed in these six studies. For example, type I and type III "built-up roofing 'steep' asphalts" (bitumen class 2) were heated to 232 °C or 316 °C, and the laboratory-generated fume condensate collected, applied in cyclohexane/acetone (1:1) to two strains of mice and caused a significant increase in the incidence of malignant and benign skin tumours in both strains. A "field-matched" type III "built-up roofing asphalt" (bitumen class 2) fume condensate collected at 199 °C and applied in mineral oil in a skin-application study in mice produced an increase in the incidence of malignant skin tumours. A laboratory-generated type III "built-up roofing asphalt" (bitumen class 2) fume condensate collected at 232 °C also applied in mineral oil in a skin-application study in mice, produced a significant increase in the incidence of malignant skin tumours. A "field-matched" type II built-up roofing asphalt (bitumen class 2) fume condensate collected at 199 °C and applied in mineral oil gave positive results as an initiator in a skin-painting initiation–promotion study in mice.

Pooled samples of straight-run bitumens (class 1) and oxidized bitumens (class 2) applied in solvents have been studied in one skin-painting study and one subcutaneous-injection study in mice. Pooled samples of bitumens of class 1 and class 2 have also been used to generate fumes for two studies of inhalation in mice and one study of inhalation in rats. The skin-painting study in mice was considered to be inadequate for the evaluation of carcinogenicity. Subcutaneous injection of a pooled sample of six class 1 and class 2 bitumens suspended in olive oil caused a significant increase in the incidence of injection-site sarcoma. The studies of inhalation of pooled samples of straight-run bitumens (class 1) and oxidized bitumens (class 2) in mice were considered to be inadequate for the evaluation of carcinogenicity. A study of inhalation in rats of a bitumen-fume condensate generated at 175 °C and comprised of a majority (> 70% mass) of air-rectified bitumen (bitumen class 2) with the

remainder being straight-run vacuum residue (bitumen class 1) gave negative results.

Cutback bitumens (class 3) have been studied in two skin-painting studies in mice. An "asphalt cutback" (a solid petroleum-bitumen material cut back to 64% solid with mineral spirits; bitumen class 3) applied in mineral spirits in one skin-application study in mice did not produce an increase in skin tumours. In a skin-painting initiation–promotion study in mice that was conducted on four different cutback materials (bitumen class 3), two of the samples promoted the formation of skin tumours.

Thermally cracked bitumens (class 6) have been studied by applying them in a vehicle solvent in a skin-painting study in mice that was considered to be inadequate for the evaluation of carcinogenicity.

5.4 Mechanistic and other relevant data

Bitumen fume contains PAHs and heterocyclic polycyclic aromatic compounds. Many of the PAHs are mutagenic and carcinogenic and have produced many of the same genotoxic activities as those reported using bitumen-fume condensates. One of these PAHs, benzo[*a*]pyrene, has been detected in bitumen-fume condensates and in the lungs (as benzo[*a*]pyrene-diol-epoxide–DNA adducts) of mice and in the urine (as benzo[*a*] pyrene metabolites) of rats exposed to bitumen fume. It is noted that while bitumen fume induced skin tumours in mice treated dermally, there were no adequate studies of cancer in mice exposed to bitumen fume by inhalation. A study of carcinogenicity in rats exposed to bitumen fume by inhalation was considered negative; even though a nasal tumour, defined as an adenocarcinoma, was reported. While there was evidence for the role of benzo[a]pyrene in the genotoxicity of some bitumen fumes in experimental systems, the lack of definitive studies linking the genotoxic effects induced by bitumen-fume exposures to other specific PAHs or heterocyclic polycyclic aromatic compounds prevented the identification of a clear role for those agents in the mechanism of genotoxic or carcinogenic action of bitumen fume.

In experimental studies, exposure to bitumen fume produced bulky aromatic DNA adducts and specific benzo[*a*]pyrene–DNA adducts related to *anti*-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide. Bitumen fume was mutagenic in bacteria, induced DNA damage and induced cytogenic alterations (micronucleus formation and sister-chromatid exchange).

There was conclusive evidence that bitumen fume and condensate cause cellular stress and disrupt cellular defence programmes. This leads to overproduction of reactive oxygen species, which perpetuates inflammatory signalling as a result of AhR-mediated or AhR-independent signalling. An imbalance in the detoxification of reactive oxygen species stimulates the immune response. Inflammation affects immune surveillance and immune cells that infiltrate tumours to engage in an extensive and dynamic cross-talk with cancer cells. Bitumen fume and condensate induce inflammatory signalling, but may also function as immunosuppressant, possibly via AhR-mediated immunotoxicity.

On the basis of the weight of evidence from studies in experimental systems, it is highly probable that a mechanism involving genotoxicity is responsible for the tumorigenic effects of exposure to bitumen in mouse skin.

In studies in humans, higher levels of mutagenic urine, 8-oxo-deoxyguanosine in DNA (a measure of reactive oxygen species), DNA damage, unidentified bulky aromatic DNA adducts, PAH–albumin adducts, sister-chromatid exchange, micronucleus formation and chromosomal aberrations were observed in workers exposed to bitumen emmissions compared with unexposed workers. Associations have been reported

between genotoxicity and inflammatory effects in the lower airways of humans.

These positive findings in humans are consistent with a genotoxic mechanism for the tumorigenic effects of exposure to bitumens.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of occupational exposures to bitumens and bitumen emissions during roofing and mastic-asphalt work. A positive association has been observed between occupational exposures to bitumens and bitumen emissions during roofing and mastic-asphalt work and cancers of the lung and the upper aerodigestive tract (buccal cavity, pharynx, oesophagus and larynx).

There is *inadequate evidence* in humans for the carcinogenicity of occupational exposures to bitumens and bitumen emissions during road paving.

6.2 Cancer in experimental animals

There is *inadequate evidence* in experimental animals for the carcinogenicity of straight-run bitumens class 1 and fume condensates generated from straight-run bitumens class 1.

There is *limited evidence* in experimental animals for the carcinogenicity of oxidized bitumens class 2.

There is *sufficient evidence* in experimental animals for carcinogenicity of fume condensates generated from oxidized bitumens class 2.

There is *limited evidence* in experimental animals for the carcinogenicity of pooled samples of straight-run bitumens class 1 and oxidized bitumens class 2.

There is *inadequate evidence* in experimental animals for the carcinogenicity of fume condensates generated from pooled samples of straight-run bitumens class 1 and air-rectified bitumens class 2.

There is *limited evidence* in experimental animals for the carcinogenicity of cutback bitumens class 3.

There is *inadequate evidence* in experimental animals for the carcinogenicity of thermally cracked bitumens class 6.

6.3 Mechanistic and other relevant data

6.3.1 Pavers

In studies of pavers, bitumen emissions produced higher levels of mutagenic urine, increased DNA damage, and increased levels of sister-chromatid exchange, micronucleus formation and chromosomal aberrations in human lymphocytes compared with control populations. These positive genotoxic findings in pavers provided strong evidence for a genotoxic mechanism for a tumorigenic effect of exposures to bitumens and bitumen emissions in pavers.

6.3.2 Roofers and mastic-asphalt workers

In studies of roofers exposed to bitumens and bitumen emissions, there was increased DNA damage compared with control populations. In mastic-asphalt workers, there was increased DNA damage and higher levels of 8-OH-dG in lymphocyte DNA – a measure of reactive oxygen species. These positive genotoxic findings in roofers and in mastic-asphalt workers provide weak evidence for a genotoxic mechanism for the tumorigenic effects of exposures to bitumens and bitumen emissions. There was also an association between genotoxic and inflammatory effects in the lower airways in mastic-asphalt workers.

6.4 Overall evaluation

Occupational exposures to oxidized bitumens and their emissions during roofing are *probably carcinogenic to humans (Group 2A)*.

Occupational exposures to hard bitumens and their emissions during mastic-asphalt work are *possibly carcinogenic to humans (Group 2B)*.

Occupational exposures to straight-run bitumens and their emissions during road paving are *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

In making the overall evaluation for occupational exposures to straight-run bitumens and their emissions during road paving, the Working Group considered the following mechanistic results and other relevant data from independent studies in exposed workers:

- Increased levels of DNA damage
- Increased levels of sister-chromatid exchange
- Increased levels of micronucleus formation
- Increased levels of chromosomal aberration.

Many of these events are known to be associated with human neoplasia. In addition, data in experimental systems *in vitro* and *in vivo* support these findings.

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SOME *N*- AND *S*-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS

The nine agents under review can be divided into two broad categories: *N*-heterocyclic polycyclic aromatic hydrocarbons (PAHs) – also known as azaarenes – including five acridines and two carbazoles; and *S*-heterocyclic PAHs – also known as thiaarenes – including two thiophenes [*S*-substituted cyclopentadiene moiety].

Benz[*a*]acridine was considered by previous IARC Working Groups in 1983 and 1987 (IARC, 1983, 1987). Since that time, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

Benz[*c*]acridine was considered by previous Working Groups in 1972, 1983, and 1987 (IARC, 1973a, 1983, 1987). Since that time, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

Dibenz[*a,h*]acridine was considered by previous Working Groups in 1972, 1983, and 1987 (IARC, 1973a, 1983, 1987). Since that time, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

Dibenz[*a,j*]acridine was considered by previous Working Groups in 1972, 1983, and 1987 (IARC, 1973a, 1983, 1987). Since that time, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

Dibenz[*c,h*]acridine has not previously been considered by an IARC Working Group.

Carbazole was considered by previous Working Groups in 1983, 1987, and 1998 (IARC, 1983, 1987, 1999). Since that time, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

7*H*-Dibenzo[*c,g*]carbazole (DBC) was considered by previous Working Groups in 1972, 1983, and 1987 (IARC, 1973b, 1983, 1987). Since that time, new data have become available; these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

Dibenzothiophene has not previously been considered by an IARC Working Group.

Benzo[*b*]naphtho[2,1-*d*]thiophene has not previously been considered by an IARC Working Group.

1. Exposure Data

1.1 Identification of the agents

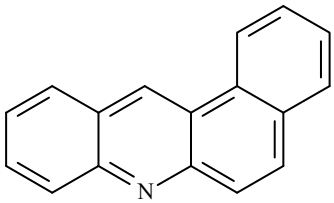
From Santa Cruz Biotechnology (2008), PubChem (2011a) and Sigma-Aldrich (2012a).

1.1.1 Benz[a]acridine

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 225-11-6
RTECS No.: CU2700000
Synonyms: 7-Azabenz[*a*]anthracene; 1,2-benzacridine

(b) Structural and molecular formulae and relative molecular mass



$C_{17}H_{11}N$
Relative molecular mass: 229.29

(c) Chemical and physical properties of the pure substance

Description: Solid powder
Melting-point: 130 °C
Boiling-point: 446.2 °C at 760 mm Hg
Flash-point: 201.4 °C
Density: 1.239 g/cm^3
Solubility: Soluble in water (0.000 034 g/100 mL); soluble in ethanol, ether and acetone

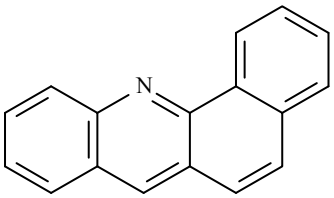
1.1.2 Benz[c]acridine

From HSDB (2003) and PubChem (2011b).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 225-51-4
RTECS No.: CU2975000
Synonyms: B[c]AC; 3,4-benzacridine; α-chrysidine; α-naphthacridine

(b) Structural and molecular formulae and relative molecular mass



$C_{17}H_{11}N$
Relative molecular mass: 229.29

(c) Chemical and physical properties of the pure substance

Description: Yellow needles
Melting-point: 108 °C (needles from aqueous ethanol)
Boiling-point: 446 °C at 760 mm Hg
Flash-point: 201 °C
Density: 1.2 g/cm^3
Solubility: Soluble in water (< 0.000035 g/100 mL at 25 °C); soluble in ethanol, ether and acetone

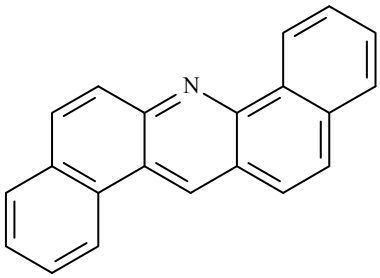
1.1.3 Dibenz[a,h]acridine

From ChemNet (2011), Royal Society of Chemistry (2011a) and Sigma-Aldrich (2012b).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 226-36-8
RTECS No.: HN0875000
Synonyms: 7-Azadibenz[*a*,*h*]anthracene; DB[a,h]AC; 1,2,5,6-dibenzacridine; 1,2,5,6-dinaphthacridine; former nomenclature: 1,2:5,6-dibenzacridine; dibenz[*a*,*d*]acridine

(b) Structural and molecular formulae and relative molecular mass



$C_{21}H_{13}N$

Relative molecular mass: 279.35

(c) Chemical and physical properties of the pure substance

Description: Yellow crystalline solid
Melting-point: 223–224 °C
Boiling-point: 524 °C at 760 mm Hg
Flash-point: 240 °C
Density: 1.3 g/cm^3
Solubility: Soluble in water (0.00016 g/100 mL); soluble in acetone and cyclohexane

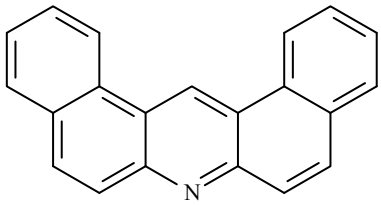
1.1.4 Dibenz[a,j]acridine

From CSST (2000), GSI Environmental (2010), ALS (2011a) and Sigma-Aldrich (2012c).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 224-42-0
RTECS No.: HN1050000
Synonyms: 7-Azadibenz[*a*,*j*]anthracene; DB[*a*,*j*]AC; 1,2,7,8-dibenzacridine; 3,4,6,7-dinaphthacridine; former nomenclature: dibenz[*a*,*f*]acridine; 3,4,5,6-dibenzacridine (may correspond to dibenz[*c*,*h*]acridine)

(b) Structural and molecular formulae and relative molecular mass



$C_{21}H_{13}N$

Relative molecular mass: 279.35

(c) Chemical and physical properties of the pure substance

Description: Yellow crystalline solid
Melting-point: 219.2 °C
Boiling-point: 534 °C at 760 mm Hg
Solubility: Insoluble in water; soluble in ethanol and acetone

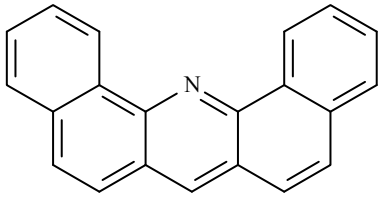
1.1.5 Dibenz[c,h]acridine

From Santa Cruz Biotechnology (2007a), LookChem (2008a), Royal Society of Chemistry (2011b) and Sigma-Aldrich (2012d).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 224-53-3
RTECS No.: HN1225000
Synonyms: 14-Azadibenz[*a*,*j*]anthracene; 3,4,5,6-dibenzacridine; former nomenclature: 3:4:5:6-dibenzacridine; 3,4:5,6 dibenzacridine

(b) Structural and molecular formulae and relative molecular mass



$C_{21}H_{13}N$

Relative molecular mass: 279.35

(c) Chemical and physical properties of the pure substance

Description: Solid
Melting-point: 190.6 °C
Boiling-point: 534 °C at 760 mm Hg
Flash-point: 240.3 °C
Density: 1.274 g/cm^3
Solubility: Soluble in water (0.00040 g/100 mL); soluble in ethanol and acetone

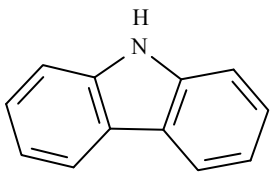
1.1.6 Carbazole

From PubChem (2011c), Sigma-Aldrich (2012e) and TCI America (2012a).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 86-74-8
RTECS No.: FE3150000
Synonyms: 9-Azafluorene; dibenzopyrrole; diphenylenimide; diphenylenimine

(b) Structural and molecular formulae and relative molecular mass



$C_{12}H_9N$
Relative molecular mass: 167.21

(c) Chemical and physical properties of the pure substance

Description: Yellow solid
Melting-point: 240–246 °C
Boiling-point: 355 °C at 760 mm Hg
Flash-point: 220 °C
Density: 1.1 g/cm^3
Solubility: Insoluble in water; soluble in benzene, chloroform and toluene

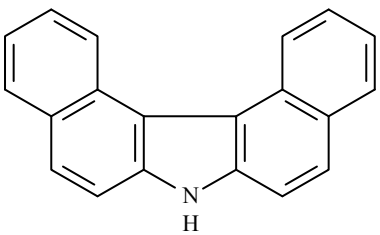
1.1.7 7H-Dibenzo[c,g]carbazole

From LookChem (2008b), ALS (2011b), Cambridge Isotope Laboratories (2012) and Sigma-Aldrich (2012f).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 194-59-2
RTECS No.: HO5600000
Synonyms: 7-DB[*c*,*g*]C; 3,4,5,6-dibenz carbazole; 3,4:5,6-dibenzocarbazole; 3,4,5,6-dibenzocarbazole; dibenzo[*c*,*g*] carbazole; 3,4,5,6-dinaphthacarbazole

(b) Structural and molecular formulae and relative molecular mass



$C_{20}H_{13}N$
Relative molecular mass: 267.32

(c) Chemical and physical properties of the pure substance

Description: Yellow crystalline solid
Melting-point: 156 °C
Boiling-point: 544.1 °C at 760 mm Hg
Flash-point: 246.5 °C
Density: 1.308 g/cm^3
Solubility: Soluble in water (0.0063 g/100 mL); soluble in benzene, chloroform and toluene

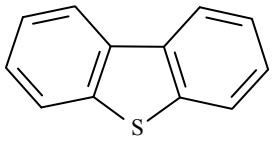
1.1.8 Dibenzothiophene

From Royal Society of Chemistry (2011c), Sigma-Aldrich (2012 g) and TCI America (2012b).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 132-65-0
RTECS No.: HQ3490550

(b) Structural and molecular formulae and relative molecular mass



$C_{12}H_8S$

Relative molecular mass: 184.26

(c) Chemical and physical properties of the pure substance

Description: Colourless crystals
Melting-point: 97–100 °C
Boiling-point: 332–333 °C at 760 mm Hg
Density: 1.252 g/cm^3
Solubility: Insoluble in water; soluble in benzene and related solvents

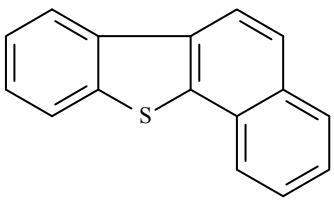
*1.1.9 Benzo[*b*]naphtho[2,1-*d*]thiophene*

From Santa Cruz Biotechnology (2007b) and Chemexper (2012).

(a) Nomenclature

Chem. Abstr. Serv. Reg. No.: 239-35-0
Synonyms: Benzo[*a*]dibenzothiophene; 3,4-benzodibenzothiophene; benzonaphtho[2,1-*d*]thiophene; 1,2-benzodiphenylene sulfide; 1,2-benzo-9-thiafluorene; naphtha[1,2:2,3]thionaphthen; naphtho[1,2-b]thianaphthene; 11-thiabenzo[*a*]fluorene

(b) Structural and molecular formulae and relative molecular mass



$C_{16}H_{10}S$

Relative molecular mass: 234.32

(c) Chemical and physical properties of the pure substance

Description: Solid
Melting-point: 188–190 °C
Boiling-point: 434.3 °C at 760 mm Hg
Flash-point: 163 °C
Density: 1.292 g/cm^3
Solubility: Insoluble in water; soluble in benzene and related solvents

1.2 Analysis

Various techniques have been described for the separation, identification and quantitative determination of *N*- and *S*-heterocyclic PAHs.

Improved isolation of benz[*a*]acridine, benz[*c*]acridine, dibenz[*a*,*j*]acridine, dibenzo[*c*,*h*]acridine and carbazole by gas chromatography from tobacco-smoke condensate has been reported (Rothwell &Whitehead, 1969).

Methods for the identification and quantitation of benz[*a*]acridine and its methyl-substituted congeners have been reviewed (Motohashi *et al.*, 1991, 1993).

High-performance liquid chromatography (HPLC) with fluorescence, and gas chromatography with mass spectrometry (GC-MS), were compared for the determination of 20 azaarenes in atmospheric particulate matter, including benz[*a*]acridine, benz[*c*]acridine, dibenz[*a*,*h*]acridine, dibenz[*a*,*j*]acridine and dibenzo[*c*,*h*]acridine (Delhomme & Millet, 2008). Although HPLC was proven to be the most sensitive method, GC-MS was selected in particular for the efficiency of the separation of the azaarenes.

More recently, a liquid chromatography-atmospheric pressure photoionization tandem mass-spectrometric method (LC-MS/MS) was proposed for the determination of azaarenes, including benz[*a*]acridine, benz[*c*]acridine, dibenz[*a*,*j*]acridine, dibenz[*a*,*h*]acridine and dibenz[*c*,*h*]acridine in atmospheric particulate matter (Lintelmann *et al.*, 2010).

De Voogt & Laane (2009) developed a method to determine the contents of azaarenes (including benz[*a*]acridine and benz[*c*]acridine) and azaarones (oxidized azaarene derivatives) simultaneously by GC-MS in sediment.

Liquid-chromatography tandem mass spectrometry was also used to determine *N*-heterocyclic PAHs in soil (Švábenský *et al.*, 2007).

Chen & Preston (2004) described analytical procedures for the simultaneous determination of both gas- and particle-phase azaarenes of two, three and four rings. Samples of particulate material were collected on the glass-fibre filters and gas-phase material on polyurethane foam plugs. Isolated azaarene compounds were analysed by GC-MS.

1.3 Production and use

None of the heterocyclic PAHs under review are produced for commercial use (IARC, 1983; HSDB, 2009).

1.4 Occurrence and exposure

1.4.1 Occurrence

N-heterocyclic PAHs and *S*-heterocyclic PAHs generally occur as products of incomplete combustion of nitrogen- and sulfur-containing organic matter. Thermal degradation of nitrogen-containing polymers may produce *N*-heterocyclic PAHs (Wilhelm *et al.*, 2000).

(a) Natural sources

The natural sources of *N*-heterocyclic PAHs and *S*-heterocyclic PAHs are largely analogous to those of other PAHs, namely volcanic activities, wildfires, storm events and fossil fuels (Moustafa & Andersson, 2011).

(b) Air

N-heterocyclic PAHs and *S*-heterocyclic PAHs enter the environment as a result of natural oil seeps, oil spills, atmospheric deposition, and industrial effluents, or from incinerators (Nito & Ishizaki, 1997). Other sources are automobile exhausts (Yamauchi & Handa, 1987), coal burning, bitumen spreading and tobacco smoke (Rogge *et al.*, 1994).

The mainstream smoke of cigarettes contains dibenz[*a*,*h*]acridine at up to 0.1 ng per cigarette, dibenz[*a*,*j*]acridine at up to 10 ng per cigarette and 7*H*-dibenzo[*c*,*g*]carbazole (DBC) at 700 ng per cigarette (IARC, 2004). The airborne particulate Standard Reference Material (SRM 1649, NIST) contains benz[*c*]acridine at 0.26 µg/g (Durant *et al.*, 1998).

Azaarene compounds have been documented in air (Nielsen *et al.* 1986; Adams *et al.*, 1982; Cautreels *et al.*, 1977; Yamauchi & Handa, 1987; Chen & Preston, 1997, 1998, 2004), but are rarely characterized individually. One study (Delhomme & Millet, 2012) measured mean concentrations of total four-ring azaarenes, including benz[*a*]acridine and benz[*c*]acridine, of 0.007–0.72 ng/m^3 in the urban atmosphere. A seasonal variation was observed, in which the maximum concentration occurred in the winter and the minimum in the summer months. [The Working Group noted that this study had sampling issues. There was an important effect of gas/particle partitioning on seasonal variability; the sampling of particulate matter (glass-fibre filter only at high sampling volume, without absorbent or foam) may have introduced some bias. A better approach is the quantitation of azaarenes (two, three and four rings) both in gas phase and particle phase (see Section 1.2; Chen & Preston, 1997, 2004).]

Table 1.1 Concentrations of selected heterocyclic polycyclic aromatic hydrocarbons in soil samples from two creosote-contaminated sites

Compound	Mean concentration ± standard error (mg/kg)	
	Site A (n = 3)	Site B (n = 3)
S-heterocyclic PAHs		
Dibenzothiophene	11.2 ± 0.15	12.6 ± 0.33
Benzo[*b*]naphtho[2,1-*d*]thiophene	15.8 ± 0.48	33.3 ± 0.01
Benzo[*b*]naphtho[2,3-*d*]thiophene	5.2 ± 0.13	13.4 ± 0.48
N-heterocyclic PAHs		
Benz[*a*]acridine	1.6 ± 0.03	4.3 ± 0.97
Benz[*c*]acridine	7.3 ± 0.42	13.3 ± 1.20
Dibenz[*a*,*c*]acridine	0.4 ± 0.01	0.7 ± 0.02
Carbazole	1.0 ± 0.15	0.9 ± 0.05
Dibenzo[*a*,*i*]carbazole	0.4 ± 0.02	< 0.2

PAH, polycyclic aromatic hydrocarbons
Adapted from Meyer *et al.* (1999)

(c) Soil and sediment

N-heterocyclic PAHs were found in soils (Kočí *et al.*, 2007; Švábenský *et al.*, 2009) and lake sediments (Wakeham, 1979); *S*-heterocyclic PAHs have been detected in fossil fuels, coal and bitumen (Vu-Duc *et al.*, 2007).

The presence of azaarenes in the Dutch surface coastal zone of the North Sea was reported by de Voogt & Laane (2009). The concentrations of acridine in the sediments varied between 9.97 and 63.5 ng/g dry weight (mean, 30.3 ± 15.2 ng/g; n = 48). The concentrations of the sum of benz[*a*]acridine and benz[*c*]acridine ranged between 7 and 36.1 ng/g dry weight (mean, 15.4 ng/g).

The concentrations of selected heterocyclic PAHs and metabolites at two creosote-contaminated sites are shown in Table 1.1.

(d) Water

Azaarenes and thiaarenes can be mobilized from land during storm events, transported into the aquatic environment, and contaminate drinking-water, recreation waters, fisheries, and wildlife (US EPA, 2010).

Azaarenes have been shown to dissolve more rapidly in water than homocyclic PAHs (Pearlman *et al.*, 1984). Azaarenes have been reported in rainwater (Chen & Preston, 1998).

Benzothiophene was found in one sample of stormwater runoff samples in California, at a concentration of 110 ± 13 ng/L (Zeng *et al.*, 2004).

Hamilton Harbour, located on Lake Ontario, Canada, is representative of a lake heavily polluted by industrial chemicals (Marvin *et al.*, 2000). Thiaarene profiles of reference and sediment samples showed that harbour contamination could be distinguished as arising from two primary sources of contamination: mobile emissions and emissions related to steel manufacturing.

Detailed investigation in Germany showed that the distribution of non-polar compounds (such as homocyclic PAHs) can only be detected close to the source of contamination, whereas the distribution of more polar compounds (such as azaarenes and thiaarenes) and degradation products is more widespread downstream of an aquifer (Schlanges *et al.*, 2008). Table 1.2 shows the range of concentrations of some *N*-heterocyclic PAHs and *S*-heterocyclic PAHs in groundwater samples analysed at four tar-contaminated sites in Germany (Schlanges *et al.*, 2008).

Table 1.2 Concentrations of selected heterocyclic polycyclic aromatic hydrocarbons in groundwater samples from four tar-contaminated sites in Germany

Compound	Concentration, range of means (μg/L)			
	Castrop Rauxel (*n* = 61)	Wülknitz (*n* = 8)	Stuttgart (*n* = 5)	Lünen (*n* = 14)
Carbazole	ND–101	ND–51	ND–19	ND–135
1-Benzothiophene	ND–1420	8–947	< 1–1	ND–1572
Dibenzothiophene	ND–4	ND–15	ND–2	ND–4

ND, not detected or below limit of detection (0.2–30 ng/L)
Adapted from Schlanges *et al.* (2008)

(e) Food

Hydroxydibenzothiophenes, including C1–C3-substituted dibenzothiophenes, were detected by GC-MS in considerable amounts in fish bile sampled in Alaska after the Exxon Valdez oil spill (Krahn *et al.*, 1992).

The concentrations of benz[*c*]acridine and of dibenzacridine isomers in grilled meat were found to be in the range of 0.2 to 2.9 ng/g meat (Janoszka, 2007). Table 1.3 provides information about content of acridines in cooked meat (Blaszczyk & Janoszka, 2008).

(f) Bitumens and bitumen fume

The *S*-heterocyclic PAHs dibenzothiophene, benzo[*b*]naphtho[1,2-*d*]thiophene and benzo[*b*]naphtho[2,1-*d*]thiophene were detected in raw bitumen samples and in laboratory-generated bitumen fume at concentrations of 1.3–7.6 μg/g and 15–384 μg/g, respectively, as shown in Table 1.4 (Vu-Duc *et al.*, 2007).

(g) Crude oil

Nitrogen compounds are frequently present in fossil fuels, generally associated with the organic portion of crude material. The presence of dibenz[*a*,*j*]acridine at concentrations of 1–6.3 μg/L was reported in aviation kerosene (Rocha da Luz *et al.*, 2009).

The *S*-heterocyclic PAHs dibenzothiophene, benzo[*b*]naphtho[1,2-*d*]thiophene and benzo[*b*]naphtho[2,1-*d*]thiophene were detected in petroleum crude oil (SRM 1582) at concentrations of 34, 11.4 and 3.8 ppm, respectively (Mössner & Wise, 1999).

Table 1.3 Concentrations of selected *N*-heterocyclic polycyclic aromatic hydrocarbons in cooked meat (pork joint)

Compound	Mean concentration ± standard error (ng/g)			
	Collar		Chop	
	Meat	Gravy	Meat	Gravy
Benz[*c*]acridine	0.83 ± 0.37	0.09 ± 0.01	0.99 ± 0.29	0.21 ± 0.01
Benz[*a*]acridine	0.54 ± 0.24	0.07 ± 0.01	0.13 ± 0.05	0.13 ± 0.03
Dibenz[*a*,*j*]acridine	0.36 ± 0.17	0.07 ± 0.01	0.09 ± 0.03	0.08 ± 0.03
Dibenz[*a*,*h*]acridine	0.52 ± 0.21	0.06 ± 0.01	0.17 ± 0.02	0.11 ± 0.04

Adapted from Blaszczyk & Janoszka (2008)

Table 1.4 Concentrations of *S*-heterocyclic polycyclic aromatic hydrocarbons in raw bitumen and in bitumen fume generated in a laboratory at 170 °C

Compound	Mean concentration ± standard error (μg/g)	
	Bitumen (n = 6)	Bitumen fume[a] (n = 6)
Dibenzothiophene	3.6 ± 0.02	384.1 ± 38
Benzo[*b*]naphtho[1,2-*d*]thiophene	1.3 ± 0.6	15.0 ± 0.9
Benzo[*b*]naphtho[2,1-*d*]thiophene	7.6 ± 0.5	54.4 ± 3.6

[a] Concentration in μg/g of collected fumes
Adapted from Vu-Duc *et al.* (2007)

(h) Coal tar

Carbazole has been reported to be a major active component of coal tar that is responsible for its antipsoriatic activity (Arbiser *et al.*, 2006).

1.4.2 Occupational exposure

No occupational exposure data concerning *N*-heterocyclic PAHs and *S*-heterocyclic PAHs specifically were available for the Working Group, except for some *S*-heterocyclic PAHs detected while generating bitumen fume in a laboratory (Binet *et al.*, 2002). However, it must be noted that *S*-heterocyclic PAHs occur in many of the same occupational settings in which exposure to other PAHs occurs. For example, *S*-heterocyclic PAHs, including dibenzothiophene, benzo[*b*]naphtha[2,1-*d*]thiophene and benzo[*b*]naphtha[1,2-*d*]thiophene, were detected in bitumen and bitumen emissions at concentrations of 1.3–7.6 and 15–384 μg/g respectively, as shown in Table 1.4.

1.5 Regulations and guidelines

No data specifically concerning *N*- or *S*-heterocyclic PAHs were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Benz[*a*]acridine

One study in mice treated by skin application was evaluated as inadequate by the Working Group and was not taken into consideration for the evaluation (Lacassagne *et al.*, 1956). This study is not presented in the tables.

3.1.1 Mouse

Skin application

Twelve XVII mice (age and sex not specified) were each given one drop of a 0.3% solution of benz[*a*]acridine (purity not reported) in acetone, applied to the nape of the neck, twice per week for up to 54 weeks (Lacassagne *et al.*, 1956). Six of the mice did not survive past day 90 of treatment and the remaining mice were removed from the study between days 165 and 379. None of the mice developed skin tumours. [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the lack of concurrent control group, the lack of information on the age and sex of the mice, on the purity and amount of benz[*a*]acridine administered, on

Table 3.1 Study of carcinogenicity in rats given benz[*a*]acridine by intrapulmonary injection

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance
Rat, Osborne-Mendel (F) At least 111 wk Deutsch-Wenzel *et al.* (1983)	A single pulmonary implantation of 0, 0.2, 1.0, or 5.0 mg (purity, 99.8%) in 50 µL of a 1 : 1 mixture of beeswax and tricaprylin. An additional group was untreated. Positive control groups received benzo[*a*]pyrene at 0.1, 0.3, or 1 mg. 35 rats/group	*Pleomorphic sarcoma* Not observed in untreated or benz[*a*]acridine-treated groups. Benzo[*a*]pyrene: 3/35 (9%), 0/35, 0/35 *Epidermoid carcinoma* Not observed in untreated or groups treated with benz[*a*]acridine. Benzo[*a*]pyrene: 5/35 (14%), 24/35 (69%), 27/35 (77%)	[NS]

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

the histological procedures employed, and on the poor survival of the dosed mice.]

3.1.2 Rat

See Table 3.1

Intrapulmonary injection

Groups of 35 female Osborne-Mendel rats (age, 3 months; mean body weight, 247 g) were given benz[*a*]acridine as a single pulmonary implantation of 0.0, 0.2, 1.0, or 5.0 mg (purity, 99.8%) in 50 µL of a 1 : 1 mixture of beeswax and tricaprylin that had been preheated to 60 °C (Deutsch-Wenzel *et al.*, 1983). One group of 35 rats was not treated. Positive control groups were also included, consisting of groups of 35 rats that were given benzo[*a*]pyrene as a pulmonary implantation of 0.1, 0.3, or 1.0 mg in beeswax and tricaprylin.

All rats survived the surgical procedure. Mean survival in rats given benz[*a*]acridine (105–111 weeks) was similar to that in rats in the control groups (103 and 110 weeks). The lungs and any other organs showing abnormalities were examined by histopathology. Lung tumours were not detected in rats given benz[*a*]acridine or in the control groups. In comparison, rats given benzo[*a*]pyrene had a dose-dependent increase in the incidence of lung epidermoid carcinoma, with incidence being 5 out of 35 (14%) at 0.1 mg, 24 out of 35 (69%) at 0.3 mg, and 27 out of 35 (77%) at 1.0 mg.

3.2 Benz[*c*]acridine

Two studies using skin application in mice or lung implantation in rats were evaluated as inadequate by the Working Group and were not taken into consideration for the evaluation (Lacassagne *et al.*, 1956; Hakim, 1968). The limitations of these studies included the small number of mice tested, the lack of a concurrent vehicle-control group, lack of information on the strain, age and sex of the animals, lack of information on the purity and total amount of benz[*c*]acridine administered, and absence of any description of the histological procedures employed. These studies are not presented in the tables.

3.2.1 Mouse

See Table 3.2

(a) Skin application

Twelve XVII mice (age and sex not reported) were each given one drop of a 0.3% solution of benz[*c*]acridine (purity not reported) in acetone, applied to the nape of the neck, twice per week, for up to 54 weeks (Lacassagne *et al.*, 1956). Five

Table 3.2 Studies of carcinogenicity in mice given benz[c]acridine

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and multiplicity of tumours	Significance	Comments
Skin application – initiation–promotion				
Mouse, CD-1 (F) 27 wk Levin *et al.* (1983)	Single topical application of 0, 0.4, 1.0 or 2.5 µmol of benz[*c*]acridine in 200 µL of 5% DMSO in acetone to the shaved dorsal surface. After 12 days, topical application of 16 nmol of TPA in 200 µL of acetone, twice/wk 30 mice/group	*Skin papilloma* No. of tumour-bearing animals 15 wk: 3%, 10%, 13%, 30% 25 wk: 7%, 23%, 16%, 37% Multiplicity 15 wk: 0.03 ± 0.03, 0.10 ± 0.06, 0.19 ± 0.10, 0.77 ± 0.26 25 wk: 0.10 ± 0.06, 0.30 ± 0.12, 0.27 ± 0.17, 1.33 ± 0.38	Incidence: $P < 0.05$ for 2.5 µmol of benz[*c*] acridine *vs* control at 15 and 25 wk Multiplicity: $P < 0.05$ for 2.5 µmol of benz[*c*] acridine *vs* control at 15 and 25 wk	Purity of benz[*c*]acridine, NR
Mouse, CD-1 (F) 21 wk Chang *et al.* (1986)	Single topical application of 2.5 µmol of benz[*c*]acridine, benz[*a*]anthracene or 7-methylbenz[*c*]acridine (purity, ≥ 97%) in 200 µL of 5% DMSO in acetone to the shaved dorsal surface. Control group treated with 5% DMSO in acetone. After 9 days, all groups treated with topical applications of 16 nmol of TPA in 200 µl of acetone twice/wk for 20 wk 30 mice/group	*Skin papilloma* Incidence Benz[*c*]acridine: 16/30 (54%) Benz[*a*]anthracene: 11/30 (37%) 7-Methylbenz[*c*]acridine: 23/30 (77%) Control: 2/30 (7%) Multiplicity Benz[*c*]acridine: 0.89 ± 0.20 Benz[*a*]anthracene: 0.50 ± 0.14 7-Methylbenz[*c*]acridine: 4.47 ± 0.94 Control: 0.07 ± 0.05	Incidence: $P < 0.05$ for all three compounds; method, NR Multiplicity: $P < 0.05$ for all three compounds; method, NR	
Intraperitoneal injection				
Mouse, Swiss-Webster [Blu:Ha (ICR)] newborn (M, F) 37 wk Chang *et al.* (1984)	Injections on postnatal days 1, 8, and 15 with 150, 300 and 600 nmol (total dose, 1 050 nmol) of benz[*c*]acridine ("pure") in 5, 10, and 20 µl DMSO A control group was treated in similar manner with vehicle only 30 M and 30 F/group	*Lung tumours* (primarily adenoma) M: 9/13 (69%), 4/24 (17%) F: 12/20 (60%), 2/16 (12%) *Liver tumours* (mostly neoplastic nodules) M: 2/13 (15%), 0/24.	$P < 0.05$, Fisher 2 × K exact test for M and F combined	

DMSO, dimethylsulfoxide; F, female; M, male; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; *vs, versus*; wk, week

of the mice did not survive past day 90 of treatment; the remaining mice were removed from the study between days 230 and 394. None of the mice developed skin tumours. [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the lack of data concerning the concurrent control group, the age and sex of the mice, the purity and amount of benz[*c*]acridine administered, the histopathological procedures employed, and the poor survival of the dosed mice.]

As part of a study investigating the carcinogenicity of the alkaloid sanguinarine (Hakim, 1968), 64 Swiss mice (Haffkine, or their hybrids) (sex and age not specified) were treated by placing a drop of a 0.3% solution of benz[*c*]acridine (purity not reported) in benzene applied to the skin between the ears, three times per week for up to 67 weeks. Fifty mice survived 180 days and 19 survived 400 days. Five epitheliomas (squamous cell carcinoma) were found in the 19 mice surviving beyond 400 days (26%).

A second experiment was conducted in which 24 mice were treated in a manner identical to the first experiment and, in addition, were given 0.5% croton oil in acetone (volume not specified) once per week. Eighteen mice survived 180 days and only three survived until the first tumour was detected (time not specified). Two epitheliomas (squamous cell carcinoma) were found in the three surviving mice.

In a third experiment, 24 mice were treated topically twice with benz[*c*]acridine (the interval between treatments and amount of benz[*c*]acridine was not specified). After 1 month, they were treated with croton oil (amount not specified) once per week. Sixteen mice survived 180 days and four survived 400 days. No tumours were detected.

As a control, 12 mice were given croton oil once per week (Hakim, 1968). Four mice survived 180 days and two survived 400 days. No tumours were detected. [The Working Group noted several deficiencies, including the lack of a concurrent vehicle-control group for the first experiment, the lack of information on the strain, age and sex of the mice, on the purity of the benz[*c*]acridine, on the amount of benz[*c*]acridine administered, and on the histopathological procedures employed, the poor survival of the test mice, and the use of benzene, which is classified as a carcinogen (IARC Group 1), as a vehicle.]

As part of a study to determine the tumour-initiating ability of oxidized derivatives of benz[*c*]acridine, groups of 30 female CD-1 mice (age, 7 weeks) received a single topical application of benz[*c*]acridine at 0.4, 1.0, or 2.5 µmol (purity not reported), in 200 µL of 5% dimethyl sulfoxide (DMSO) in acetone, applied to the shaved dorsal surface (Levin *et al.*, 1983). A control group of 30 mice received the solvent only. Twelve days later, all rats received topical applications of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) at 16 nmol in 200 µL of acetone, twice per week for 25 weeks. The formation of papillomas was monitored every 2 weeks; those papillomas of 2 mm or greater in diameter and persisting more than 2 weeks were included in the final total. The tumours were not examined by histopathology. At least 28 mice in each group survived until the end of the experiment.

After 15 weeks of treatment with TPA, the percentage of tumour-bearing mice and the multiplicity of tumours were 3%, 10%, 13%, and 30%, and 0.03 ± 0.03, 0.10 ± 0.06, 0.19 ± 0.10, and 0.77 ± 0.26 (mean ± standard error) for the groups at 0.0, 0.4, 1.0, and 2.5 µmol benz[*c*]acridine, respectively. The comparable values after 25 weeks of treatment with TPA were 7%, 23%, 16%, and 37%, and 0.10 ± 0.06, 0.30 ± 0.12, 0.27 ± 0.17, and 1.33 ± 0.38. Compared with the control group, the incidence and multiplicity of tumours was significantly increased in the groups receiving benz[*c*]acridine at 2.5 µmol (fourfold contingency test and Student's *t*-test, respectively) at both time-points. In the same study, benz[*c*]acridine-3,4-dihydrodiol and benz[*c*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide were

potent initiators of skin tumours in mice and induced lung and liver tumours when administered to newborn mice.

As part of a study to compare the tumour-initiating ability of benz[*c*]acridine with that of benz[*a*]anthracene and 7-methylbenz[*c*]acridine, groups of 30 female CD-1 mice (age, 7 weeks) were given a single dose of 2.5 µmol of each compound (purity of benz[*c*]acridine, ≥ 97%) in 200 µL of 5% DMSO in acetone, applied topically to the shaved dorsal surface (Chang *et al.*, 1986). A control of 30 mice received the solvent only. Nine days later, all mice received 16 nmol of TPA in 200 µL of acetone, applied twice per week for 20 weeks. The formation of papillomas was monitored every 2 weeks; those papillomas of 2 mm or greater in diameter and persisting more than 2 weeks were included in the final total. The tumours were not examined by histopathology. The number of mice surviving until the end of the study was not indicated.

The percentage of mice with papilloma and the multiplicity of papillomas in mice treated with benz[*c*]acridine were 54% [16 out of 30] and 0.89 ± 0.20 (mean ± standard error of the mean), which were significantly greater ($P < 0.05$; statistical tests not specified) than the values observed in the control group (7% [2 out of 30] and 0.07 ± 0.05, respectively). In mice treated with benz[*a*]anthracene, the incidence and multiplicity of tumours were 37% [11 out of 30] and 0.50 ± 0.14, while in mice treated with 7-methylbenz[*c*]acridine, these values were 77% [23 out of 30] and 4.47 ± 0.94, respectively.

(b) Intraperitoneal injection

As part of an investigation to compare the tumorigenicity of suspected benz[*c*]acridine metabolites, groups of 20–40 male and 20–40 female newborn Swiss-Webster [Blu:Ha (ICR)] mice were given intraperitoneal injections of benz[*c*]acridine, benz[*c*]acridine-1,2-dihydrodiol, benz[*c*]acridine-3,4-dihydrodiol, benz[*c*]acridine-5,6-dihydrodiol, benz[*c*]acridine-8,9-dihydrodiol, benz[*c*]acridine-10,11-dihydrodiol, benz[*c*]acridine-5,6-oxide, benz[*c*]acridine-*syn*-3,4-dihydrodiol-1,2-epoxide, benz[*c*]acridine-*syn*-8,9-dihydrodiol-10,11-epoxide, or benz[*c*]acridine-*anti*-8,9-dihydrodiol-10,11-epoxide at a dose of 150, 300, or 600 nmol (total dose, 1050 nmol), or benz[*c*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide at a dose of 70, 140, or 280 nmol (total dose, 490 nmol) in 5, 10, and 20 µL of DMSO on postnatal days 1, 8, and 15 (Chang *et al.*, 1984). All compounds were pure as determined by nuclear magnetic resonance spectroscopy. A control group consisting of 30 male and 30 female mice was treated in an identical manner with the vehicle only. Forty-six of the mice in the control group and 30–66 of the experimental mice survived until weaning at postnatal day 25. The experiment was terminated when the mice were aged 33–37 weeks. A gross necropsy was performed, and selected lung and all liver tumours were examined histologically.

The incidence of lung tumours (primarily adenoma) in female and male mice treated with benz[*c*]acridine was 60.0% [12 out of 20] and 69.2% [9 out of 13], respectively, with a multiplicity of 3.15 and 1.86 tumours per mouse. The comparable incidence values in control female and male mice were 12.5% [2 out of 16] and 16.7% [4 out of 24], with a multiplicity of 0.13 and 0.17 tumours per mouse. The incidence [63.6%; 21 out of 33] and multiplicity [2.64 tumour per mouse] of lung tumours in combined male and female mice treated with benz[*c*]acridine were statistically significantly different than the incidence ([15.0%; 6 out of 40]; $P < 0.05$, Fisher $2 \times K$ exact test) and multiplicity ([0.15 tumours per mouse]; $P < 0.05$, statistical test not specified) in combined male and female mice in the control group. The incidence of lung tumours in mice treated with benz[*c*]acridine-3,4-dihydrodiol, benz[*c*]acridine-8,9-dihydrodiol, benz[*c*]acridine-10,11-dihydrodiol, benz[*c*]acridine-*syn*-3,4-dihydrodiol-1,2-epoxide, and benz[*c*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide was

Table 3.3 Study of carcinogenicity in rats given benz[*c*]acridine by intrapulmonary implantation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours
Rat, Osborne-Mendel (F) At least 116 wk Deutsch-Wenzel *et al.* (1983)	A single pulmonary implantation of 0, 0.2, 1.0, or 5.0 mg of benz[*c*]acridine (purity, 99.8%) in 50 µl of a 1 : 1 mixture of beeswax and tricaprylin. An additional group was untreated. Positive-control groups received benzo[*a*]pyrene at 0.1, 0.3, or 1.0 mg 35 rats/group	*Pleomorphic sarcoma* Untreated, 0, 0.1, 0.3, and 1 mg benz[*c*]acridine: 0/35, 0/35, 0/35, 0/35, 1/35 (3%) Benzo[*a*]pyrene: 3/35 (9%), 0/35, 0/35 *Epidermoid carcinoma* Untreated or benz[*c*]acridine-treated groups: no tumours observed Benzo[*a*]pyrene: 5/35 (14%), 24/35 (69%), 27/35 (77%)-

F, female; NR, not reported; wk, week

statistically significantly different from that in the control group, with values of 82.0%, 37.4%, 41.5%, 46.6%, and 100%, respectively.

Male mice treated with benz[*c*]acridine also developed liver tumours ("mostly type A or neoplastic nodules"), with an incidence of 15.4% [2 out of 13] and a multiplicity of 0.15 tumours per mouse. Liver tumours were not found in control male mice [0 out of 24]. The incidence of liver tumours in male mice treated with benz[*c*]acridine-3,4-dihydrodiol and benz[*c*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide was 58.6% [17 out of 29] and 81.3% [13 out of 16], values that were significantly different from those in the control group [$P < 0.0001$; one-tailed Fisher exact test]. None of the other benz[*c*]acridine dihydrodiols or dihydrodiol epoxides caused a significant increase in the incidence of liver tumours.

3.2.2 Rat

See Table 3.3

(a) Intrapulmonary implantation

Groups of 35 female Osborne-Mendel rats (age, 3 months; mean body weight, 247 g) received a single pulmonary implantation of benz[*c*]acridine (purity, 99.8%) at a dose of 0.0, 0.2, 1.0, or 5.0 mg in 50 µL of a 1 : 1 mixture of beeswax and tricaprylin that had been preheated to 60 °C (Deutsch-Wenzel *et al.*, 1983). Another group of 35 rats was not treated. Positive controls were also included, comprising groups of 35 rats receiving a pulmonary implantation of benzo[*a*]pyrene of 0.1, 0.3, or 1.0 mg in a 1 : 1 mixture of beeswax and tricaprylin.

All rats survived the surgical procedure. The mean survival in rats given benz[*c*]acridine (112–116 weeks) was similar to that in the control groups (103 and 110 weeks). The lungs and any other organs showing abnormalities were examined by histopathology. One rat given 1.0 mg of benz[*c*]acridine developed a pleomorphic sarcoma at the implantation site. None of the other rats treated with benz[*c*]acridine and none of the rats in either of the control groups developed lung tumours. In comparison, rats given benzo[*a*]pyrene had a dose-dependent increase in lung epidermoid carcinoma, with the incidence being 5 out of 35 (14%) at 0.1 mg, 24 out of 34 (69%) at 0.3 mg, and 27 out of 35 (77%) at 1.0 mg.

(b) Bladder implantation

As part of a study investigating the carcinogenicity of the alkaloid sanguinarine, 58 rats (strain, age, and sex not specified) received a paraffin pellet (~15 mg) containing an unspecified amount of benz[*c*]acridine (the pellets were prepared by dissolving benz[*c*]acridine in chloroform and mixing with paraffin in a weight ratio of 1 : 3) implanted into the bladder (Hakim, 1968).

An additional group of 64 rats was implanted with pellets not containing benz[*c*]acridine. The experiment was terminated after 16 months. In the rats implanted with benz[*c*]acridine pellets, there were 29 bladder papillomas, of which 8 were "cancers." In the control rats, there were two bladder papillomas [$P < 0.0001$; one-tailed Fisher exact test]. [The Working Group noted several deficiencies in this study, including the lack of information on the strain, age, and sex of the rats, and on the purity and amount of benz[*c*]acridine administered, the inadequate description of the histopathological procedures, and the use of chloroform, which is classified as a possible carcinogen (IARC Group 2B), to dissolve the benz[*c*]acridine.]

3.3 Dibenz[*a,h*]acridine

Several studies in mice given dibenz[*a,h*]acridine by oral administration, skin application or subcutaneous injection were evalauated as inadequate by the Working Group and were not taken into consideration for the final evaluation (Barry *et al.*, 1935; Bachmann *et al.*, 1937; Orr, 1938; Andervont & Shimkin, 1940; Badger *et al.*, 1940; Lacassagne *et al.*, 1956). The limitations of these studies included the small number of mice tested, lack of concurrent vehicle control group, lack of information on strain, age and sex of the animals, lack of information on the purity and total amount of dibenz[*a,h*]acridine administered, absence of description of the histological procedures employed, lack of description of the tumours, and the use of benzene as a vehicle. These studies are not presented in the tables.

3.3.1 Mouse

See Table 3.4

(a) Oral administration

In one experiment, a group of 10 mice (age, sex, and strain not specified) was fed dibenz[*a,h*]acridine ("pure") as a 2% or 4% solution in olive oil (volume not reported) mixed with their food (Badger *et al.*, 1940). The last mouse died 627 days after the initiation of dosing. Two of the mice developed multiple sebaceous adenomas and other tumours. In another experiment, an unspecified number of mice (age, sex, and stain not specified) was given dibenz[*a,h*]acridine ("pure") orally, either as in the previous experiment or by gavage (1 mg in 250 µL of butter or margarine, 5 days per week). Of the 12 mice that were examined from day 100 onward, 5 developed papilloma and epithelioma [squamous cell carcinoma] of the stomach. [The Working Group noted that the experimental details and results were poorly presented. The Working Group also noted several deficiencies in both experiments, including the limited number of mice tested, the lack of a concurrent control group, and the lack of information on the age, sex, and strain of the mice, on the purity and total amount of the dibenz[*a,h*]acridine administered, on the precise route of administration, and on the histopathological procedures employed.]

(b) Skin application

A group of 10 mice (age, sex, and strain not specified) was given an unspecified amount of dibenz[*a,h*]acridine (purity not reported) as a 0.3% solution in benzene applied to the interscapular region, twice per week (Barry *et al.*, 1935). Seven of the mice survived 6 months and the last mouse died after 349 days of treatment. One mouse developed an epithelioma [squamous cell carcinoma]. In a second experiment, a group of 30 mice was treated in a manner identical to the first experiment. Twenty-seven of the mice survived 6 months, 12 of the mice survived 12 months, and the last mouse died 482 days after the initiation of treatment. Four

Table 3.4 Studies of carcinogenicity in mice given dibenz[*a,h*]acridine

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and multiplicity of tumours	Significance	Comments
Skin application – initiation–promotion				
Mouse, CD-1 (F) 26 wk Kumar *et al.* (2001)	Single topical application of 500 nmol of dibenz[*a,h*]acridine (purity, > 98%) in 200 µL of acetone to the shaved dorsal surface. After 9 days, treated with 16 nmol of TPA in 200 µL of acetone, twice/wk for 25 wk. Control group was treated with acetone only. 30 mice/group	*Papilloma* Dibenz[*a,h*]acridine: 24/30 (80%) Control: 0/30 Multiplicity: 3.33 ± 0.57	Incidence: $P < 0.0001$; Fisher exact test Multiplicity: $P < 0.01$; Student's *t*-test	No histopathological examination was made.
Mouse, CD-1 (F) 21 wk Kumar *et al.* (2001)	Single topical application of 50 or 175 nmol of dibenz[*a,h*]acridine (purity, > 98%) in 200 µL of acetone to the shaved dorsal surface. After 9 days, treated with 16 nmol of TPA in 200 µL of acetone, twice/wk for 20 wk. Control group was treated with acetone only. 30 mice/group	*Papilloma* 10 wk TPA 175 nmol: 6/30 (20%) Control: 0/30 Multiplicity: 0.2 ± 0.09 20 wk TPA 50 nmol: 18/30 (60%) 175 nmol: 24/30 (80%); Control: 1/30 (3%) Multiplicity: 0.04 ± 0.04; 1.6 ± 0.33, 2.3 ± 0.37	Multiplicity: $P < 0.05$; Fisher exact test (compared with controls) Multiplicity: $P < 0.01$ for both dose groups; Fisher exact test (compared with controls)	No histopathological examination was made.
Intravenous injection				
Mouse, Strain A (M, F) 20 wk Andervont & Shimkin (1940)	0, 250 µg in 250 µL of water. Controls received water only	*Pulmonary tumours* 8 wk: 1/20 (5%), 3/10 (30%) (multiplicity: 1.0, 1.3) 14 wk: 3/20 (15%), 9/13 (69%) (multiplicity, 1.0; 2.9) 20 wk: 4/19 (21%), 11/12 (92%) (multiplicity, 1.0, 2.2)	[$P = 0.0025$; one-tailed Fisher exact test] [$P = 0.0002$; one-tailed Fisher exact test]	Number at start, NR

F, female; M, male; NR, not reported; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week

(13%) mice developed epithelioma [squamous cell carcinoma] and two (7%) developed skin papilloma. [The Working Group noted that the experimental details and results were poorly presented, and there were several deficiencies in both experiments, including the limited number of mice tested in the first experiment, the lack of a concurrent control group, the lack of information on the age, sex, and strain of the mice, on the purity and amount of dibenz[*a,h*]acridine administered, and the use of benzene, which is classified as a carcinogen (IARC Group 1), as the vehicle.]

A group of 10 mice (age, sex, and strain not specified) was given "a few drops" of a saturated solution (concentration not specified) of dibenz[*a,h*]acridine (purity not reported) in acetone, applied topically to the interscapular region at weekly intervals (Orr, 1938). Two mice survived 28 weeks of treatment and one of these mice developed a skin tumour. [The Working Group noted several deficiencies including the lack of a concurrent control group, the lack of information on the age, sex and strain of the mice, and on the purity and amount of dibenz[*a,h*]acridine administered, and the poor survival of the dosed mice.]

A group of 40 mice (age, sex, and strain not specified) was given an unspecified amount of dibenz[*a,h*]acridine (purity not reported) as a 0.3% solution in benzene, applied topically twice per week (Badger *et al.*, 1940). The last mouse died after 482 days of treatment. Two mice developed papilloma and five developed epithelioma [squamous cell carcinoma]. [The Working Group noted several deficiencies in this study, including the lack of a concurrent control group, lack of information on the age, sex and strain of the mice, or on the purity and amount of dibenz[*a,h*]acridine administered, and the use of benzene, which is classified as a carcinogen (IARC Group 1), as the vehicle.]

A group of 12 XVII mice (age and sex not specified) was given one drop of dibenz[*a,h*]acridine (purity not reported) as a 0.3% solution in acetone applied to the nape of the neck, twice per week, for up to 416 days (Lacassagne *et al.*, 1956). Six of the mice did not survive 90 days of treatment; the remaining mice were removed from the study between days 93 and 416. One mouse developed an epithelioma [squamous cell carcinoma]; at this time, three mice were still alive. [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the lack of a concurrent control group, lack of information on the age and sex of the mice, or on the purity and amount of dibenz[*a,h*]acridine administered, and the poor survival of the mice tested.]

As part of a study to determine the tumour-initiating ability of a series of oxidized dibenz[*a,h*]acridine derivatives, a group of 30 female CD-1 mice (age, 7 weeks) received a single topical application of 500 nmol of dibenz[*a,h*]acridine, dibenz[*a,h*]acridine-1,2-dihydrodiol, dibenz[*a,h*]acridine-3,4-dihydrodiol,dibenz[*a,h*]acridine-8,9-dihydrodiol, dibenz[*a,h*]acridine-10,11-dihydrodiol, dibenz[*a,h*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide, dibenz[*a,h*]acridine-*syn*-3,4-dihydrodiol-1,2-epoxide, dibenz[*a,h*]acridine-*anti*-10,11-dihydrodiol-8,9-epoxide, dibenz[*a,h*]acridine-*syn*-10,11-dihydrodiol-8,9-epoxide (purity, > 98%) in 200 µL of acetone applied to the shaved dorsal surface (Kumar *et al.*, 2001). A control group of 30 mice received only the solvent. Nine days later, all mice received applications of 16 nmol of TPA in 200 µL of acetone, twice per week for 25 weeks. The formation of skin papillomas was monitored macroscopically every 2 weeks; those papillomas of 2 mm or greater in diameter and persisting more than 2 weeks were included in the final total. The tumours were not examined by histopathology. The number of mice surviving until the end of the study was not indicated.

The incidence of papilloma in mice treated with dibenz[*a,h*]acridine was 80%, with a multiplicity of 3.33 ± 0.57 tumours per mouse

(mean ± standard error). There were no tumours in the control group. Based upon the number of mice initially treated, the incidence of papillomas (24 out of 30) in the group receiving dibenz[*a,h*]acridine was statistically significantly different [$P < 0.0001$; Fisher exact test] compared with that in the control group (0 out of 30). The tumour multiplicity in the group receiving dibenz[*a,h*]acridine was also statistically significantly different compared with that in the control group ($P < 0.01$; Student's *t*-test). Mice treated with dibenz[*a,h*]acridine-3,4-dihydrodiol and dibenz[*a,h*]acridine-10,11-dihydrodiol showed significant increases in the incidence [5 out of 30, and 17 out of 30, respectively; $P \leq 0.03$] and multiplicity (0.17 ± 0.07, and 1.23 ± 0.31, respectively; $P < 0.01$) of skin tumours compared with the control group. Mice given dibenz[*a,h*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide, dibenz[*a,h*]acridine-*anti*-10,11-dihydrodiol-8,9-epoxide, and dibenz[*a,h*]acridine-*syn*-10,11-dihydrodiol-8,9-epoxide showed significant increases in the incidence [5 out of 30, 13 out of 30, and 6 out of 30, respectively; $P \leq 0.03$] and multiplicity (0.21 ± 0.09, 1.56 ± 0.55, and 0.20 ± 0.07, respectively; $P < 0.05$) of skin tumours compared with the control group.

In a subsequent experiment, Kumar *et al.* (2001) treated mice in an identical manner to the first experiment with 50 or 175 nmol of dibenz[*a,h*]acridine, (+)-dibenz[*a,h*]acridine-10*S*,11*S*-dihydrodiol, (–)-dibenz[*a,h*]acridine-10*R*,11*R*-dihydrodiol, (+)-dibenz[*a,h*]acridine-*syn*-10*R*,11*S*-dihydrodiol-8*R*,9*S*-epoxide, (–)-dibenz[*a,h*]acridine-*syn*-10*S*,11*R*-dihydrodiol-8*S*,9*R*-epoxide, (+)-dibenz[*a,h*]acridine-*anti*-10*S*,11*R*-dihydrodiol-8*R*,9*S*-epoxide, and (–)-dibenz[*a,h*]acridine-*anti*-10*R*,11*S*-dihydrodiol-8*S*,9*R*-epoxide. (+)-Dibenz[*a,h*]acridine-*anti*-10*S*,11*R*-dihydrodiol-8*R*,9*S*-epoxide was also given at a dose of 10 nmol. The control group (30 mice) received the solvent only. At least 26 mice in each group survived until the termination of the study after 20 weeks of treatment with TPA. When assessed after 10 weeks of promotion with TPA, there was a statistically significant increase in the incidence [6 out of 30; $P < 0.01$] and multiplicity (0.2 ± 0.09; $P < 0.05$) of skin tumours in mice receiving 175 nmol of dibenz[*a,h*]acridine compared with the control group [0 out of 30]. Likewise, after 20 weeks of promotion with TPA, 50 and 175 nmol of dibenz[*a,h*]acridine caused a significant increase in the incidence of tumours [18 out of 30 and 24 out of 30; $P < 0.001$] and multiplicity (1.6 ± 0.33 and 2.3 ± 0.37; $P < 0.01$) compared with the control group (1 out of 30 and 0.04 ± 0.04). Of all the compounds tested, the highest tumourgenicity was observed with (+)-dibenz[*a,h*]acridine-*anti*-10*S*,11*R*-dihydrodiol-8*R*,9*S*-epoxide after 10 weeks of promotion with TPA: incidence [5 out of 30, 14 out of 30, and 26 out of 30, at 10, 50 and 175 nmol] and multiplicity (0.2 ± 0.09, 0.7 ± 0.14, and 2.3 ± 0.30 at 10, 50 and 175 nmol). A similar trend ($P < 0.01$) occurred after 20 weeks of promotion with TPA.

(c) Subcutaneous administration

A group of 19 mice (age, sex, and strain not specified) received 0.9 mg of dibenz[*a,h*]acridine (purity not reported) in 300 µL of sesame oil, administered subcutaneously, every 2 weeks, for 34 weeks (Bachmann *et al.*, 1937). Thirteen mice survived more than 168 days, and of these, eight developed sarcomas at the injection site before the end of the experiment, 240 days after the initiation of treatment. [The Working Group noted several deficiencies in this study, including the lack of a concurrent control group, and the lack of information on the age, sex, and strain of the mice, on the purity and total amount of dibenz[*a,h*]acridine administered, and on the histopathological procedures employed.]

A group of 10 mice (age, sex, and strain not specified) received repeated doses of 5 mg of dibenz[*a,h*]acridine ("pure") in 200 µL of sesame oil applied subcutaneously at intervals of a few (3–5) weeks (Badger *et al.*, 1940). The last mouse

died 246 days after the initiation of treatment. Three of the mice developed sarcoma. [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the lack of a concurrent control group, and the lack of information on the age, sex, and strain of the mice, on the purity and total amount of dibenz[*a,h*]acridine administered, and on the histopathological procedures employed.]

Andervont & Shimkin (1940) gave a group of male and female strain A mice (age, 2–3 months; total number, and number of each sex not specified) a single subcutaneous injection of 500 µg of dibenz[*a,h*]acridine dissolved in 100 µL of tricaprylin. Fourteen weeks after the injection, the mice were killed and their lungs were examined for pulmonary nodules; representative samples were characterized histologically as adenoma. The incidence of pulmonary tumours was 20 out of 20, with a multiplicity of 3.0 tumours per tumour-bearing mouse. There were no tumours at the injection sites.

In a subsequent experiment, strain A mice (age, 2–3 months; sex and total number not specified) were given a single subcutaneous injection of 1.0 mg of dibenz[*a,h*]acridine dissolved in 300 µL of sesame oil (Andervont & Shimkin, 1940). The mice were killed 22 weeks and 40 weeks after injection and the number of pulmonary nodules was determined. At 22 weeks, 6 out of 6 mice had pulmonary tumours. The corresponding value at 40 weeks was 14 out of 14, with a multiplicity of 70 tumours per tumour-bearing mouse. There were no tumours (0 out of 14) at the injection site. [The Working Group noted several deficiencies in this study, including the lack of a concurrent control group and the lack of information on the sex and initial number of mice treated.]

(d) Intravenous injection

Equal numbers of male and female strain A mice (age, 2–3 months) [total number not specified] were given a single intravenous injection 0.25 mg of dibenz[*a,h*]acridine suspended in 250 µL of water (Andervont & Shimkin, 1940). A control group was injected with water only. The injections resulted in "almost no mortality," and all mice surviving the injections survived until the scheduled terminations at 8, 14, and 20 weeks. The lungs were examined for pulmonary nodules; representative samples were characterized histologically as adenoma. At 8 weeks, the incidence of lung tumours in mice receiving dibenz[*a,h*]acridine was 3 out of 10 (30%), with a multiplicity of 1.3 tumours per tumour-bearing mouse, while the incidence in the control group was 1 out of 20 (5%), with a multiplicity of 1.0 tumours per tumour-bearing mouse. At 14 weeks, the incidence of lung tumours in mice receiving dibenz[*a,h*]acridine was 9 out of 13 (69%), with a multiplicity of 2.9 tumours per tumour-bearing mouse, while the incidence in the control group was 3 out of 20 [15%; $P = 0.0025$; one-tailed Fisher exact test], with a multiplicity of 1.0 tumours per tumour-bearing mouse. At 20 weeks, the incidence of lung tumours in mice receiving dibenz[*a,h*]acridine was 11 out of 12 (92%), with a multiplicity of 2.2 tumours per tumour-bearing mouse, while the incidence in the control group was 4 out of 19 [21%; $P = 0.0002$; one-tailed Fisher exact test], with a multiplicity of 1.0 tumour per tumour-bearing mouse.

3.3.2 Rat

See Table 3.5

(a) Subcutaneous administration

A group of 30 random-bred female Wistar albino rats (body weight, 100–110 g) were given 10 mg of dibenz[*a,h*]acridine (purity not reported) dissolved in a 3 × 10 mm disk of paraffin, as a single subcutaneous implantation to the right side of the chest (Bahna *et al.*, 1978). As a control, the rats were implanted on the left side of the chest with a paraffin disk not containing dibenz[*a,h*]acridine. The rats were monitored for 21 months, at which time 12 rats were still alive.

Table 3.5 Studies of carcinogenicity in rats given dibenz[*a,h*]acridine

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Subcutaneous administration				
Rat, Wistar (F) 84 wk Bahna *et al.* (1978)	Dibenz[*a,h*]acridine dissolved in paraffin implanted on right side of chest. As a control, the rats were implanted on left side with paraffin only 30 rats/group	*Sarcoma* Dibenz[*a,h*]acridine: 5/30 (17%) Control: 0/30	[$P < 0.03$; one-tailed Fisher exact test]	Amount of dibenz[*a,h*]acridine, not reported
Pulmonary implantation				
Rat, Osborne-Mendel (F) 113 wk Deutsch-Wenzel *et al.* (1983)	A single dose of dibenz[*a,h*]acridine at 0, 0.1, 0.3, or 1.0 mg (purity, 99.8%) in 50 µl of a 1 : 1 mixture of beeswax and tricaprylin. An additional group was untreated. Positive-control groups received benzo[*a*]pyrene at 0.1, 0.3, or 1.0 mg 35 rats/group	*Epidermoid carcinoma* Untreated, 0, 0.1, 0.03 and 1.0 mg Dibenz[*a,h*]acridine: 0/35, 0/35, 0/35, 3/35 (9%), 9/31 (29%) Benzo[*a*]pyrene: 5/35 (14%), 24/35 (69%), 27/35 (77%)	$P = 0.0005$ for 1.0 mg dibenz[*a,h*]acridine; Fisher exact test	Osteosarcoma at implantation site in one animal given 0.3 mg of dibenz[*a,h*]acridine

F, female; wk, week

Five of the 30 rats (17%) developed histologically confirmed sarcoma at the site of implantation of the dibenz[*a*,*h*]acridine-containing disk, with the first being diagnosed 14 months after implantation. There were no sarcomas at the site of implantation of the paraffin-only disk [$P < 0.03$ for dibenz[*a*,*h*]acridine implantation site *versus* paraffin-only implantation site; one-tailed Fisher exact test].

(b) Pulmonary implantation

Groups of 35 female Osborne-Mendel rats (age, 3 months) received a single pulmonary implantation of 0.0, 0.1, 0.3, or 1.0 mg of dibenz[*a*,*h*]acridine (purity, 99.8%) in 50 µL of a 1 : 1 mixture of beeswax and tricaprylin that had been preheated to 60 °C (Deutsch-Wenzel *et al.*, 1983). Another group of 35 rats was not treated. Positive controls were also included, comprising groups of 35 rats receiving a pulmonary implantation of 0.1, 0.3, or 1.0 mg of benzo[*a*]pyrene in beeswax and tricaprylin.

All rats, except four that were treated with 1.0 mg of dibenz[*a*,*h*]acridine, survived and were evaluated. Mean survival in rats given dibenz[*a*,*h*]acridine (99–113 weeks) was similar to that in the negative-control groups (103 and 110 weeks). The lungs and any other organs showing abnormalities were examined by histopathology. At the end of the experiment, the incidence of lung epidermoid carcinoma was 0 out of 35 in the group receiving 0.1 mg of dibenz[*a*,*h*]acridine, 3 out of 35 [9%] in the group receiving 0.3 mg of dibenz[*a*,*h*]acridine, and 9 out of 31 [29%] in the group receiving 1.0 mg of dibenz[*a*,*h*]acridine. There were no tumours in either of the two control groups (0 out of 35 in each group). The incidence of epidermoid carcinoma in the group receiving 1.0 mg of dibenz[*a*,*h*]acridine was statistically significantly different [$P = 0.0005$; Fisher exact test] from that in either control group. One rat given 0.3 mg of dibenz[*a*,*h*]acridine developed an osteosarcoma at the implantation site. Rats given benzo[*a*]pyrene had a dose-dependent increase in the incidence of lung epidermoid carcinoma, which was 5 out of 35 (14%) at 0.1 mg, 24 out of 35 (69%) at 0.3 mg, and 27 out of 35 [77%] at 1.0 mg.

3.4 Dibenz[*a*,*j*]acridine

Several studies using oral administration, skin application or subcutaneous injection were evaluated as inadequate by the Working Group and were not taken into consideration for the final evaluation (Barry *et al.*, 1935; Bachmann *et al.*, 1937; Andervont & Shimkin, 1940; Badger *et al.*, 1940; Lacassagne *et al.*, 1955a, b, 1956; Wynder & Hoffmann, 1964). Limitations of these studies included the small number of mice tested, the lack of concurrent vehicle control group, lack of information on strain, age and sex of the animals, lack of information on the purity and total amount of dibenz[*a*,*j*]acridine administered, and no description of the histological procedures employed. These studies are not presented in the tables.

3.4.1 Mouse

See Table 3.6

(a) Oral administration

A group of 10 mice (age, sex, and strain not specified) was fed dibenz[*a*,*j*]acridine ("pure") as a 2% or 4% solution in olive oil (specific amount not specified) mixed with the food, or 1 mg of dibenz[*a*,*j*]acridine in 260 µL of butter, by stomach tube, for 5 days per week for up to 82 weeks (Badger *et al.*, 1940). The last mouse died 572 days after the initiation of dosing. No tumours were observed. [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the lack of concurrent control group, and the lack of information on the age, sex, and strain of the mice, the purity and total amount of dibenz[*a*,*j*]acridine

Table 3.6 Studies of carcinogenicity in mice given dibenz[*a,j*]acridine

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Skin application				
Mouse, Hsd:(ICR)BR (F) 99 wk Warshawsky *et al.* (1994; 1996a)	Treated topically with 50 nmol (13.95 µg) of dibenz[*a,j*]acridine (purity, 99%) in 50 µL of acetone, or 50 µL of acetone only, or untreated; twice/wk on shaved interscapular region 50 mice/group	*Skin tumours* Untreated control, acetone control, dibenz[*a,j*]acridine 2/11 (18%), 3/11 (27%), 27/40 (68%) Squamous cell carcinoma 0/11, 1/11 (9%), 15/40 (38%) Papilloma 0/11, 2/11 (18%), 7/40 (18%) Basal cell carcinoma 0/11, 0/11, 3/40 (8%) Keratoacanthoma 0/11, 0/11, 1/40 (3%) Undifferentiated carcinoma 0/11, 0/11, 1/40 (3%)	[$P \leq 0.02$ treated *vs* either control; one-tailed Fisher exact test]	Histopathology conducted on a limited number of mice.
Mouse, C3H/Hej (M) 99 wk Warshawsky & Barkley (1987), Warshawsky *et al.* (1996a)	Treated topically with 12.5 µg dibenz[*a,j*]acridine (purity, 99%) in 50 µL of acetone, or 50 µL of acetone only, or untreated; twice/wk, in interscapular region 50 mice/group	*Skin tumours (papilloma and carcinoma combined)* Dibenz[*a,j*]acridine: 25/50 (50%) Acetone control: 0/50 Untreated control: 0/50 *Carcinoma*: 22/25 (88%)	[$P < 0.001$, for combined papilloma and carcinoma, and for malignant skin tumours only; one-tailed Fisher exact test]	
Skin application – initiation–promotion				
Mouse, Hsd(ICR)BR (F) 25 wk Warshawsky *et al.* (1992, 1996a)	Treated topically with a single dose of 200 nmol (55.8 µg) of dibenz[*a,j*] acridine (purity, 99%) in 50 µL of acetone. After 2 wk, treated with 2 µg of TPA in 50 µL of acetone. Control groups treated with acetone only, TPA only, dibenz[*a,j*]acridine only, or not treated; twice/wk, on shaved interscapular region 30 mice/group	*Skin papilloma* Dibenz[*a,j*]acridine + TPA: 17/30 (57%) Acetone: 0/30 TPA: 0/30 Dibenz[*a,j*]acridine: 0/30 Untreated: 0/30	[$P < 0.0001$; dibenz[*a,j*]acridine *vs* each control group; one-tailed Fisher exact test]	

F, female; M, male; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; *vs, versus*; wk, week

administered, the precise route of administration, and the histopathological procedures employed.]

(b) Skin application

Barry *et al.* (1935) treated a group of 10 mice (age, sex, and strain not specified) with an unspecified amount of dibenz[*a,j*]acridine (purity not reported) as a 0.3% solution in benzene, applied to the interscapular region, twice per week. Six of the mice survived 6 months, three survived 12 months, and the last mouse died after 597 days of treatment. Two mice developed epithelioma [squamous cell carcinoma].

In a second experiment, Barry *et al.* (1935) treated a group of 30 mice in a manner identical to the first experiment. Twenty-eight of the mice survived 6 months, eighteen survived 1 year, and the last mouse died 551 days after the initiation of treatment. Nine mice developed epithelioma (squamous cell carcinoma) and two developed papilloma. [The Working Group noted several deficiencies in both experiments, including the limited number of mice tested in the first experiment, the lack of a concurrent control group, the lack of information on the age, sex, and strain of the mice, and on the purity and amount of dibenz[*a,j*]acridine administered, and the use of benzene, which is classified as a carcinogen (IARC Group 1), as the vehicle.]

A group of 40 mice (age, sex, and strain not specified) was given an unspecified amount of dibenz[*a,j*]acridine (purity not reported) as a 0.3% solution in benzene, applied topically twice per week (Badger *et al.*, 1940). The last mouse died after 597 days of treatment. Two mice developed papillomas and eleven developed epitheliomas (squamous cell carcinoma). [The Working Group noted several deficiencies in the study, including the lack of a concurrent control group, the lack of information on the age, sex and strain of the mice, and on the purity and amount of dibenz[*a,j*] acridine administered, and the use of benzene, which is classified as a carcinogen (IARC Group 1), as the vehicle.]

A group of 20 XVII mice (age and sex not specified) was given one drop of a 0.3% solution of dibenz[*a,j*]acridine (purity not reported) in acetone, applied to the nape of the neck, twice per week (Lacassagne *et al.*, 1955a, 1956). Six of the mice did not survive the 90 days of treatment; the remaining mice were removed from the study between days 139 and 450. None of the mice developed epithelioma (squamous cell carcinoma). [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the lack of a concurrent control group, the lack of information on the age and sex of the mice, and on the purity and amount of dibenz[*a,j*]acridine administered, and the poor survival of the dosed mice.]

Groups of 20 female Swiss mice (age not specified) were treated topically with dibenz[*a,j*] acridine (purity not reported) as a 0.5% or 1.0% solution in acetone (volume not reported) three times per week (Wynder & Hoffmann, 1964). After 12–14 months, 16 of the mice treated with 0.5% dibenz[*a,j*]acridine and 15 of the mice treated with 1.0% dibenz[*a,j*]acridine developed tumours; in both groups 60% of the tumours were carcinoma. [The Working Group noted several deficiencies in this study, including the lack of a concurrent control group, the lack of information on the age of the mice, survival, histopathology procedures, and the purity and amount of dibenz[*a,j*]acridine administered.]

Groups of 50 female carcinogen-sensitive Hsd:(ICR)BR mice (age, 7–8 weeks) were treated with 0 or 50 nmol (13.95 μg) of dibenz[*a,j*]acridine (purity, 99%) in 50 μL of acetone, applied topically on the shaved interscapular region twice per week, or were not treated (Warshawsky *et al.*, 1994, 1996a). The treatment was continued for 99 weeks. Histopathology was conducted. In mice treated with dibenz[*a,j*]acridine, the incidence of skin tumours was 27 out of 40 (68%), with the tumours being characterized as squamous cell

carcinoma (15 out of 40; 38%), squamous cell papilloma (7 out of 40; 18%), basal cell carcinoma (3 out of 40; 8%), keratoacanthoma (1 out of 40; 3%), and undifferentiated carcinoma (1 out of 40; 3%). In untreated mice, the incidence of skin tumours was 2 out of 11 (18%), while in mice treated with acetone only, the incidence was 3 out of 11 (27%). The incidence of skin tumours in mice treated with dibenz[*a,j*]acridine was statistically significantly different from both control groups [$P \leq 0.02$; one-tailed Fisher exact test]. [The Working Group noted that histopathology was conducted only on a limited number of animals.]

Groups of 50 male C3H/Hej mice (age, 8–10 weeks) were treated with 0 or 12.5 µg of dibenz[*a,j*] acridine (purity, 99%) in 50 µL of acetone, applied topically in the interscapular region, twice per week, or were not treated (Warshawsky & Barkley, 1987; Warshawsky *et al.*, 1996a). The treatment was continued for 99 weeks. Lesions with a minimum volume of 1 mm^3 and persisting for at least 1 week were classified as papilloma. Histopathology was conducted. Twenty-five of the mice treated with dibenz[*a,j*]acridine developed skin tumours, with an average latency of 80.3 weeks. Malignant skin tumours (carcinomas) occurred in 22 of the 25 (88%) mice. There were no skin tumours in either of the control groups (0 out of 50). [$P < 0.001$, for combined papilloma and carcinoma, and for malignant skin tumours only; one-tailed Fisher exact test.]

(c) Skin application: initiation–promotion

A group of 30 female carcinogen-sensitive Hsd:(ICR)BR mice (age, 7–8 weeks) received a single treatment with 200 nmol (55.8 µg) of dibenz[*a,j*]acridine (purity, 99%) in 50 µL of acetone, applied topically to the shaved interscapular region (Warshawsky *et al.*, 1992, 1996a). Two weeks later, the group was treated topically twice per week with 2 µg of TPA in 50 µL of acetone. Control groups consisted of 30 mice that were not treated, 30 mice treated with acetone only, 30 mice treated with TPA only, and 30 mice treated with dibenz[*a,j*]acridine only. The last mouse was removed from the study 23 weeks after the start of treatment with TPA. Histopathology was conducted. Mice given dibenz[*a,j*]acridine followed by TPA had an incidence of skin papilloma of 17 out of 30 (57%), with a multiplicity of 1.8 papillomas per tumour-bearing mouse and a mean latency of 14.5 weeks. There were no papillomas detected in any of the control groups. The incidence of skin papilloma in the groups receiving dibenz[*a,j*]acridine and TPA was statistically significantly different from the negative groups [$P < 0.0001$; one-tailed Fisher exact test].

(d) Subcutaneous administration

Two groups of 10 mice (age, sex, and strain not specified) were given 300 µg of dibenz[*a,j*] acridine (purity not reported) in 900 µL of sesame oil by subcutaneous administration (Bachmann *et al.*, 1937). The application was repeated fortnightly [every 2 weeks]. The last mouse in the first group died 310 days after the initiation of treatment, while the last mouse in the second group died after 266 days. There were no tumours in either group. [The Working Group noted several deficiencies in this study, including the limited number of mice, the lack of a concurrent control group, the lack of information on age, sex, and strain, or on the purity and total amount of dibenz[*a,j*]acridine administered, or on the histopathological procedures employed.]

A group of 10 mice (age, sex, and strain not specified) was given 5 mg of dibenz[*a,j*]acridine (purity not reported) in 200 µL of sesame oil by subcutaneous injection (Badger *et al.*, 1940). The application was repeated at intervals of a few (3–5) weeks. The last mouse died after 583 days of treatment. Two mice developed sarcoma. [The Working Group noted several deficiencies, including the limited number of mice tested, lack of a concurrent control group, the lack of information on the age, sex and strain of the mice, the

Table 3.7 Study of carcinogenicity in rats given dibenz[*a,j*]acridine by pulmonary implantation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance
Rat, Osborne-Mendel (F) 111 wk Deutsch-Wenzel *et al.* (1983)	A single pulmonary implantation of dibenz[*a,j*]acridine (purity, 99.3%) of 0, 0.1, 0.3, or 1.0 mg in 50 µL of a 1 : 1 mixture of beeswax and tricaprylin. An additional group was untreated. Positive-control groups received 0.1, 0.3, or 1.0 mg of benzo[*a*]pyrene 35 rats/group	*Pleomorphic sarcoma* Untreated, 0, 0.1, 0.3, or 1.0 mg of dibenz[*a,j*]acridine: 0/35, 0/35, 1/35 (3%), 0/35, 0/35 Benzo[*a*]pyrene: 3/35 (9%), 0/35, 0/35 *Epidermoid carcinoma* Untreated or treated with dibenz[*a,j*]acridine: no tumours reported 0.1, 0.3, and 1.0 mg of benzo[*a*]pyrene: 5/35 (14%), 24/35 (69%), 27/35 (77%)	[NS] - -

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

purity and total amount of dibenz[*a,j*]acridine administered, and the histopathological procedures employed.]

Strain A mice (age, 2–3 months; sex and total number not specified) were given 1.0 mg of dibenz[*a,j*]acridine dissolved in 300 µL of sesame oil as a single subcutaneous injection (Andervont & Shimkin, 1940). Mice were killed 22 weeks and 40 weeks after injection to determine the number of pulmonary nodules; representative samples were characterized histologically as adenoma. At 22 weeks, six out of six mice had pulmonary tumours. The corresponding value at 40 weeks was 13 out of 13, with a multiplicity of 20 tumours per tumour-bearing mouse. There were no tumours (0 out of 13) at the injection site. [The Working Group noted several deficiencies in this study, including the lack of a concurrent control group and the lack of information on the sex and initial number of mice treated.]

Ten XVII mice (age and sex not specified) received a subcutaneous injection of 1 mg of dibenz[*a,j*]acridine (purity not reported) in 200 µL of peanut oil, three times at monthly intervals (Lacassagne *et al.*, 1955a, 1956). Five of the mice did not survive 90 days of treatment; the remaining mice were removed from the study between day 139 and day 590. None of the mice developed sarcoma. [The Working Group noted several deficiencies in this study, including the limited number of mice tested, the poor survival of the mice, the lack of a concurrent control group, and the lack of information on the age and sex of the mice and the purity of the dibenz[*a,j*]acridine.]

3.4.2 Rat

See Table 3.7

Pulmonary implantation

Groups of 35 female Osborne-Mendel rats (age, 3 months) were given a single pulmonary implantation of 0.0, 0.1, 0.3, or 1.0 mg of dibenz[*a,j*]acridine (purity, 99.3%) in 50 µL of a 1 : 1 mixture of beeswax and tricaprylin that had been preheated to 60 °C (Deutsch-Wenzel *et al.*, 1983). Another group of 35 rats was not treated. Positive controls were also included, consisting of groups of 35 rats that were given a pulmonary implantation of 0.1, 0.3, or 1.0 mg of benzo[*a*]pyrene. The mean survival in rats given

dibenz[*a,j*]acridine (102–111 weeks) was similar to the mean survival in the negative-control groups (103 and 110 weeks). The lungs and any other organs showing abnormalities were examined by histopathology. At the end the experiment, a single pleomorphic sarcoma (1 out of 35; 3%) was observed in the group receiving 0.1 mg of dibenz[*a,j*]acridine. There were no tumours in the groups receiving 0.3 or 1.0 mg of dibenz[*a,j*] acridine, or in either of the two negative-control groups. In the groups of rats receiving benzo[*a*] pyrene, there was a dose-dependent increase in the incidence of lung epidermoid carcinoma, with the incidence being 5 out of 35 (14%) at 0.1 mg, 24 out of 35 (69%) at 0.3 mg, and 27 out of 35 (77%) at 1.0 mg.

3.5 Dibenz[*c,h*]acridine

3.5.1 Mouse

See Table 3.8

(a) Skin application

As part of a study to determine tumour initiation by a series of oxidized derivatives of dibenz[*c,h*]acridine (Chang *et al.*, 2000), groups of 30 female CD-1 mice (age, 7 weeks) were given a single topical application of 50 or 200 nmol of dibenz[*c,h*]acridine, (+)-dibenz[*c,h*] acridine-1,2-dihydrodiol, (+)-dibenz[*c,h*] acridine-3*S*,4*S*-dihydrodiol, (–)-dibenz[*c,h*] acridine-3*R*,4*R*-dihydrodiol, (+)-dibenz[*c,h*] acridine-5,6-dihydrodiol, (+)-dibenz[*c,h*]acridine-*syn*-3*S*,4*R*-dihydrodiol-1*S*,2*R*-epoxide, (–)-dibenz[*c,h*]acridine-*syn*-3*R*,4*S*-dihydrodiol-1*R*,2*S*-epoxide, (+)-dibenz[*c,h*]acridine-*anti*-3*S*,4*R*-dihydrodiol-1*R*,2*S*-epoxide, (–)-dibenz[*c,h*]acridine-*anti*-3*R*,4*S*-dihydrodiol-1*S*,2*R*-epoxide (purity of dibenz[*c,h*]acridine not reported; purity of all other compounds, > 99%) in 200 µL of acetone, applied to the shaved dorsal surface. A control group of 30 mice received the solvent only. Nine days later, all mice received 16 nmol of TPA in 200 µL of acetone, applied twice per week for 20 weeks. The formation of papillomas was monitored every 2 weeks; those papillomas of 2 mm or greater in diameter and persisting more than 2 weeks were included in the final total. The tumours were not examined by histopathology. At least 28 mice in each group survived until the end of the study.

In the group of mice treated with 50 nmol of dibenz[*c,h*]acridine, the incidence of papilloma was 33% [10 out of 30], with a multiplicity of 0.50 ± 0.15 tumours per mouse (mean ± standard error of the mean); in the group of mice treated with 200 nmol of dibenz[*c,h*]acridine, the incidence of papilloma was 60% [18 out of 30], with a multiplicity of 1.83 ± 0.43 tumours per mouse. The incidence of papilloma in the control group was 3% [1 out of 30], with a multiplicity of 0.03 ± 0.03 tumours per mouse. The incidence and multiplicity of tumours in both groups of mice treated with dibenz[*c,h*]acridine were statistically significantly different ($P < 0.05$) from the control group (fourfold contingency test and Student's *t*-test, respectively). A significant increase in tumour incidence and multiplicity was also observed after treatment with (–)-dibenz[*c,h*]acridine-3*R*,4*R*-dihydrodiol and with each of the dibenz[*c,h*]acridine dihydrodiol epoxides.

(b) Intraperitoneal injection

As part of an investigation to evaluate the tumorigenicity of a series of oxidized dibenz[*c,h*]acridine metabolites, groups of 80 newborn CD-1 mice (presumably 40 males and 40 females) were given intraperitoneal injections of 25, 50, and 100 nmol (total dose, 175 nmol) of dibenz[*c,h*]acridine, (+)-dibenz[*c,h*] acridine-1,2-dihydrodiol, (+)-dibenz[*c,h*] acridine-3*S*,4*S*-dihydrodiol, (–)-dibenz[*c,h*] acridine-3*R*,4*R*-dihydrodiol, (+)-dibenz[*c,h*] acridine-5,6-dihydrodiol, (+)-dibenz[*c,h*] acridine-*syn*-3*S*,4*R*-dihydrodiol-1*S*,2*R*-epoxide, (–)-dibenz[*c,h*]acridine-*syn*-3*R*,4*S*-

Table 3.8 Studies of carcinogenicity in mice given dibenz[*c,h*]acridine

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and multiplicity of tumours	Significance	Comments
Skin application – initiation–promotion				
Mouse, CD-1 (F) 21 wk Chang *et al.* (2000)	A single topical application of 0, 50 or 200 nmol of dibenz[*c,h*]acridine in 200 µL of acetone, to the shaved dorsal surface. After 9 days, treated with 16 nmol of TPA in 200 µL of acetone, twice/wk. 30 mice/group	*Papilloma* Incidence 1/30 (3%), 10/30 (33%), 18/30 (60%) Multiplicity: 0.03 ± 0.03, 0.50 ± 0.15, 1.83 ± 0.43	$P < 0.05$ for both treated groups *vs* control; fourfold contingency test $P < 0.05$ for both treated groups *vs* control; Student's *t*-test	Purity of dibenz[*c,h*]acridine, NR
Intraperitoneal injection				
Mouse, CD-1 (newborn, M, F) 39 wk Chang *et al.* (2000)	Injections on postnatal days 1, 8, and 15 with 25, 50, and 100 nmol (total dose, 175 nmol) of dibenz[*c,h*]acridine (purity NR) in 5, 10, and 20 µL DMSO respectively. A control group treated in similar manner with vehicle only 80 mice/group [presumably 40 M and 40 F]	*Lung tumours (primarily adenoma)* M: 13/26 (50%), 2/33 (6%) F: 7/24 (29%), 2/36 (6%) for dibenz[*c,h*]acridine and control, respectively *Liver tumours (mostly type A or neoplastic nodules)* M: 12/26 (46%), 1/33 (3%) F: 0/24, 0/36 for dibenz[*c,h*]acridine and control, respectively	$P < 0.02$, for M, F, and M+F; one-tailed Fisher exact test $P = 0.0001$ for M, one-tailed Fisher exact test	Number of animals each sex, NR

d, day; DMSO, dimethyl sulfoxide; F, female; M, male; NR, not reported; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; *vs, versus*; wk, week

dihydrodiol-1*R*,2*S*-epoxide, (+)-dibenz[*c*,*h*]acridine-*anti*-3*S*,4*R*-dihydrodiol-1*R*,2*S*-epoxide, or (–)-dibenz[*c*,*h*]acridine-*anti*-3*R*,4*S*-dihydrodiol-1*S*,2*R*-epoxide (purity of dibenz[*c*,*h*]acridine not reported; purity of all other compounds, > 99%) in 5, 10, and 20 µL of DMSO, respectively, on postnatal days 1, 8, and 15 (Chang *et al.*, 2000). An additional group of 80 mice was given 10, 20, and 40 nmol (total dose, 70 nmol) of (+)-dibenz[*c*,*h*]acridine-*anti*-3*S*,4*R*-dihydrodiol-1*R*,2*S*-epoxide. A control group of 80 mice (presumably 40 males and 40 females) was treated in an identical manner with 5, 10, and 20 µL of the DMSO vehicle. The number of mice surviving until weaning at postnatal day 25 was 72 in the control group, and 31–71 in the treated group. The experiment was terminated when the mice were aged 36–39 weeks. A gross necropsy was performed, and selected lung and all liver tumours were examined histologically.

The incidence of lung tumours (primarily adenoma) in female, male, and combined female and male mice in the control group was 6% [2 out of 36], 6% [2 out of 33], and 6% [4 out of 69], with a multiplicity of 0.14, 0.12, and 0.13 tumours per mouse. The comparable incidence values in female, male, and combined male and female mice treated with dibenz[*c*,*h*]acridine were 29% [7 out of 24], 50% [13 out of 26], and 40% [20 out of 50], with a multiplicity of 3.25, 3.42, and 3.34 tumours per mouse. The incidence of lung tumours in female, male, and combined male and female mice given dibenz[*c*,*h*]acridine was statistically significantly different from that in the control mice [$P < 0.02$; one-tailed Fisher exact test]. Treatment with (+)-dibenz[*c*,*h*]acridine-3*S*,4*S*-dihydrodiol, (–)-dibenz[*c*,*h*]acridine-3*R*,4*R*-dihydrodiol, (+)-dibenz[*c*,*h*]acridine-*syn*-3*S*,4R-dihydrodiol-1*S*,2*R*-epoxide, and (+)-dibenz[*c*,*h*]acridine-*anti*-3*S*,4R-dihydrodiol-1*R*,2*S*-epoxide also increased the incidence of lung tumours.

In male mice treated with dibenz[*c*,*h*]acridine, liver tumours ("mostly type A or neoplastic nodules") developed, with an incidence of 46% [12 out of 26], and a multiplicity of 1.96 tumours per mouse. The incidence of liver tumours in male mice in the control group was 3% [1 out of 33], a difference that was statistically significant [$P = 0.0001$; one-tailed Fisher exact test], with a multiplicity of 0.63 tumours per mouse. Liver tumours were not detected in the control group [0 out of 36] or in female mice treated with dibenz[*c*,*h*]acridine [0 out of 24]. Treatment with (+)-dibenz[*c*,*h*]acridine-3*S*,4*S*-dihydrodiol, (–)-dibenz[*c*,*h*]acridine-3*R*,4*R*-dihydrodiol, (+)-dibenz[*c*,*h*]acridine-*syn*-3*S*,4*R*-dihydrodiol-1*S*,2*R*-epoxide, or (+)-dibenz[*c*,*h*]acridine-*anti*-3*S*,4*R*-dihydrodiol-1*R*,2*S*-epoxide also increased the incidence of liver tumours (Chang *et al.*, 2000).

3.6 Carbazole

Three studies in mice given carbazole by skin application or by subcutaneous injection were evaluated as inadequate (Kennaway, 1924; Maisin *et al.*, 1927; Schürch & Winterstein, 1935; Shear & Leiter, 1941). The limitations of these studies included the small number of mice tested, lack of concurrent vehicle control group, lack of information on strain, age and sex, lack of information on the purity and total amount of carbazole administered, and absence of description of the histological procedures employed. These studies are not presented in the tables.

3.6.1 Mouse

See Table 3.9

(a) Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were fed a pellet diet containing technical-grade carbazole (purity, 96%) at a concentration of 0%, 0.15%, 0.3% or 0.6% (Tsuda *et al.*, 1982). The treatment was continued for 96 weeks, after which the mice were maintained

Table 3.9 Studies of carcinogenicity in mice given carbazole

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and multiplicity of tumours	Significance	Comments
Oral administration				
Mouse, B6C3F$_1$ (M, F) 104 wk Tsuda *et al.* (1982)	Fed diet containing carbazole (purity, 96%) at 0%, 0.15%, 0.3%, or 0.6% for 96 wk, followed by 8 wk of basal diet 50 M and 50 F/group	*Hepatocellular carcinoma* M: 9/46 (20%), 12/42 (29%), 20/42 (48%), 37/48 (77%) F: 2/45 (4%), 35/49 (71%), 24/43 (56%), 30/46 (65%) *Liver neoplastic nodules [hepatocellular adenoma]* M: 13/46 (28%), 30/42 (71%), 22/42 (52%), 10/48 (21%); F: 2/45 (4%), 13/49 (26%), 21/43 (49%), 16/46 (35%) *Forestomach squamous cell carcinoma* M: 0/46, 0/42, 0/42, 7/48 (15%) F: 0/45, 0/49, 1/43 (2%), 2/46 (4%) *Forestomach papilloma* M: 0/46, 0/42, 1/42 (2%), 4/48 (8%) F: 0/45, 5/49 (10%), 7/43 (16%), 4/46 (9%)	$P < 0.001$ (highest dose) $P < 0.001$ (all treated) $P < 0.001$ (lowest dose) $P < 0.001$ (intermediate & highest dose) $P < 0.01$ (highest dose) NS $P < 0.05$ (highest dose) $P < 0.01$ (intermediate dose)	
Intraperitoneal administration				
Mouse, CD-1 (newborn) (M, F) 52 wk Weyand *et al.* (1993)	Injection of 5, 10 and 20 µL of either DMSO or a 50 mM solution of carbazole in DMSO on PND 1, 8 and 15, respectively. The total dose of carbazole was 1.75 µmol/mouse DMSO control, 38 M, 46 F; carbazole-treated, 34 M, 42 F.	No increase in incidence of tumours	-	Limited exposure to carbazole

d, day; DMSO, dimethyl sulfoxide, F, female; M, male; NR, not reported; PND, postnatal day; wk, week

on a basal diet for 8 weeks. Neoplastic nodules [hepatocellular adenoma] and hepatocellular carcinoma were observed in the liver; the incidence of both types of liver neoplasm in groups treated with carbazole was statistically significantly greater than that in the control group. Additionally, forestomach papilloma and forestomach squamous cell carcinoma were observed, mostly at the intermediate and highest doses, with the exception of forestomach papilloma in female mice that were also observed at the lowest dose. No tumours (squamous cell carcinoma or papilloma) were observed in the forestomach of male or female mice in the control groups.

(b) Subcutaneous administration

A group of 10 male A strain mice (age, 3–4 months), received 10 mg of crystallized carbazole moistened with glycerol, by subcutaneous injection, six times, in the left flank. All 10 mice were still alive after 1 year, and 4 were alive after 19 months. No tumours were reported at the injection site (Shear & Leiter, 1941). [The Working Group noted that the study was poorly reported; limitations included the small number of mice used and the lack of concurrent controls.]

(c) Intraperitoneal administration

Pups (CD-1 mice) were given intraperitoneal doses of 0 or 50 mM carbazole (1.75 µmol per mouse) in a volume of 5, 10 or 20 µL of DMSO on postnatal days 1, 8 and 15, respectively. The liver, lungs and any gross lesions in other tissues were examined histologically. No increase in the incidence of neoplasms was found (Weyand *et al.*, 1993).

3.6.2 Rat

See Table 3.10

Oral administration

In a study of tumour promotion, four groups of male F344 rats were given drinking-water containing 0% or 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine [an initiator of carcinogenesis in the urinary bladder] for 2 weeks, and then fed basal diet containing carbazole at a concentration of 0% or 0.6% for 22 weeks. The incidence of urinary bladder hyperplasia was increased in carbazole-treated male F344 rats compared with controls. No neoplasia or hyperplasia was observed in the liver, kidney, or ureter (Miyata *et al.*, 1985).

In a second study of tumour promotion, male F344 rats were given drinking-water containing *N*-bis(2-hydroxypropyl)nitrosamine at a concentration of 0% or 0.2% for 1 week, and 1 week later were then fed diet containing carbazole at a concentration of 0% or 0.6% for 50 weeks. Carbazole showed no promoting effect in the liver, lung, thyroid or urinary bladder. In addition, carbazole alone did not induce tumours in the lung and thyroid. An increased incidence ($P = 0.02$) of kidney (pelvic) papilloma and carcinoma combined was observed compared with initiator only (Shirai *et al.*, 1988). [The Working Group noted that the purity of carbazole was not reported.]

3.6.3 Syrian golden hamster

See Table 3.11

Oral administration

Two groups of 12 or 18 Syrian golden hamsters (sex not reported) were fed diet containing carbazole at a concentration of 0% or 0.2% for 39 weeks (Moore *et al.*, 1987). An increased incidence of liver foci was observed in the group receiving carbazole. [The Working Group noted the small number of hamsters tested and the short duration of exposure.]

Table 3.10 Studies of carcinogenicity in rats given drinking-water containing carbazole

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 24 wk Miyata *et al.* (1985)	Drinking-water containing 0% or 0.05% BBN for 2 wk followed by diet containing carbazole at 0% or 0.6% for 22 wk. On day 22 of the experiment, the left ureter of all rats was ligated. Control, 44; BBN + carbazole, 14; carbazole, 15	*Tumours of urinary bladder (papilloma)* BBN control: 0/44 Carbazole: 0/15 BBN + carbazole: 2/14 (14%) *Papillary/nodular hyperplasia* BBN control: 3/44 Carbazole: 0/15 BBN + carbazole: 5/14 (36%)*	$^{**}P < 0.05$	
Rat, F344 (M) 52 wk Shirai *et al.* (1988)	Drinking-water containing DHPN at 0% or 0.2% for 1 wk, followed 1 wk later by diet containing carbazole at 0% or 0.6% for 50 wk 19–20 rats/group	For DHPN+carbazole, DHPN, carbazole: *Lung carcinoma*: 11/19 (58%), 16/20 (80%), 0/20 *Lung adenoma*: 17/19 (89%), 18/20 (90%), 0/20 *Thyroid carcinoma*: 15/19 (79%), 14/20 (70%), 0/20 *Thyroid adenoma*: 8/19 (42%), 7/20 (35%), 0/20 *Kidney (pelvic) papilloma and carcinoma*: 11/19 (58%), 4/20 (20%), NR *Bladder papilloma and carcinoma*: 7/19 (37%), 3/20 (15%), NR	DHPN+carbazole *vs* DHPN: NS NS NS NS $P = 0.02$ NS	No untreated controls. Purity of carbazole, NR.

BNN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; d, day; DHPN, *N*-bis(2-hydroxypropyl) nitrosamine; M, male; NR, not reported; NS, not significant; wk, week.

Table 3.11 Study of carcinogenicity in hamsters given diet containing carbazole

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian golden (sex, NR) 40 wk Moore *et al.* (1987)	Diet containing carbazole (technical-grade, purity, 96%) at 0% or 0.2% for 39 wk 12 treated, 18 controls	*Liver foci* 0%, 0.2%: 0/18, 11/12 (92%)* *Forestomach papilloma* 0%, 0.2%: 0/18, 1/12 (8%)	*[$P < 0.0001$]	Small number of animals, short duration of exposure.

NR, not reported; wk, week

3.7 *7H*-Dibenzo[*c,g*]carbazole

Four early studies in mice given 7*H*-dibenzo[*c*,*g*]carbazole by skin application (Boyland & Brues, 1937; Strong *et al.*, 1938; Kirby & Peacock, 1946; Kirby, 1948), six early studies in mice given 7*H*-dibenzo[*c*,*g*]carbazole by subcutaneous administration (Boyland & Brues, 1937; Strong *et al.*, 1938; Andervont & Shimkin, 1940; Andervont & Edwards, 1941; Kirby, 1948; Lacassagne *et al.*, 1955a, b) and one early study in rats given 7*H*-dibenzo[*c*,*g*]carbazole by pulmonary implantation (Boyland & Brues, 1937) showed strong limitations and are not presented in the text or in the tables.

In addition, studies in mice given 7*H*-dibenzo[*c*,*g*]carbazole by oral administration (Armstrong & Bonser, 1950), by skin application and by subcutaneous administration (Taras-Valéro *et al.*, 2000), by intraperitoneal administration (Boyland & Mawson, 1938), by intravenous administration (Andervont & Shimkin, 1940), and by bladder implantation (Bonser *et al.*, 1952), were considered by the Working Group as inadequate for evaluation, and are not presented in the tables. A study in hamsters (Sellakumar & Shubik, 1972) and in a dog (Bonser *et al.*, 1954), although presented in the table and text, were also considered inadequate for evaluation.

Limitations of these studies included the small number of mice tested, the lack of a concurrent vehicle-control group, the lack of information on strain, age and sex, the lack of information on the purity and total amount of 7*H*-dibenzo[*c*,*g*]carbazole administered, and absence of any description of the histological procedures employed.

3.7.1 Mouse

See Table 3.12

(a) Oral administration

In groups of male and female CBA and strong A mice (age not reported) given DBC orally at doses of 0.25–4.0 mg per week in arachis oil, for up to 59 weeks, the induction of forestomach papilloma and carcinoma, liver hepatoma and pulmonary adenoma (more efficiently in males) was reported (Armstrong & Bonser, 1950). [The Working Group noted that the study was limited by the small number of mice tested, the lack of concurrent control group, the lack of information on the purity of the DBC administered, and lack of information on the histopathological procedures employed.]

(b) Skin application

A study of carcinogenicity in skin was performed using highly purified DBC (purity, > 99%). Groups of 50 male C3H mice (age, 6–8 weeks) were treated twice per week with

12.5 µg (46.8 nmol) of DBC in 50 µL of acetone, applied to the interscapular region of the back. Topical applications were continued for 99 weeks, or until a mouse developed a tumour. Control groups included a group receiving no treatment and a group treated with solvent only. Lesions persisting for at least 1 week and with a minimum size of 1 mm^3 were diagnosed as skin papilloma. Histopathological examination was performed. The incidence of skin carcinoma was highly increased ($P < 0.0001$) in mice treated with DBC compared with either control (Warshawsky & Barkley, 1987).

In another study of complete carcinogenicity, 50 female Hsd:(ICR)BR mice (age, 5–6 weeks) were given 50 nmol of DBC in 50 µL of acetone, applied to the shaved back, twice per week, for 99 weeks or until the appearance of a tumour. Groups of untreated mice (n = 11), and mice treated with acetone only (n = 11) were used as negative controls. DBC produced skin tumours in 42 out of 50 (84%) mice, and liver neoplasms in 37 out of 50 (74%) mice (Warshawsky *et al.*, 1994). [The Working Group noted the limited number of controls evaluated by histopathology.]

In a complementary study of tumour initiation, groups of 30 female Hsd:(ICR)BR mice were given a single dose of DBC at 0 or 200 nmol, dissolved in acetone, or 200 nmol of benzo[*a*]pyrene (the positive control for initiation), applied to the shaved back. After 2 weeks, the mice were treated with 2 µg of TPA in 50 µL of acetone, applied twice per week for up 24 weeks. Skin tumours developed in 26 mice in the group receiving DBC plus TPA, and 27 mice in the group receiving benzo[*a*]pyrene plus TPA (Warshawsky *et al.*, 1992).

Female mice of the XVIInc./Z homozygous strain (age, 3 months) were given 50 µg of DBC in acetone, by skin painting, in 34 applications [interval between applications not given]. In groups of mice treated with DBC, the incidence of sarcoma was 70% (22 out of 31) at 6 months and the incidence of hepatoma was 100% (31 out of 31) at 12–14 months (Taras-Valéro *et al.*, 2000). [The Working Group noted the poor description of experimental details.]

(c) Subcutaneous administration

Female mice of the XVIInc./Z homozygous strain (age, 3 months) were given 300 µg of DBC in 0.2 mL of olive oil by subcutaneous injection, three times, at 2-week intervals (Taras-Valéro *et al.*, 2000). In mice treated with DBC, the incidence of sarcoma was 70% (91 out of 130) at 6 months and the incidence of hepatoma was 100% (39 out of 39) at 12–14 months (162 animals in total in the experimental group). [The Working Group noted the poor description of experimental details in this study.]

(d) Intraperitoneal administration

Sixty-five mice were given DBC at a dose of 12.5 mg/kg bw in olive oil as a single intraperitoneal injection. Twenty-eight mice survived 39 days and one mouse developed a sarcoma over 200 days of observation; liver cholangiomas were also seen (Boyland & Mawson, 1938). [Study limitations included the lack of control group, lack of information on sex, age and strain used in the study, and lack of information on the histopathological methods used. The Working Group noted the poor survival of the mice.]

Groups of 20 male A/J mice (age, 6–8 weeks) were given DBC at a dose of 0, 5, 10, 20 or 40 mg/kg bw in 0.2 mL of tricaprylin, as a single intraperitoneal injection. Eight months after the injection, the mice were killed and tumours of the lung counted. Treatment with DBC resulted in a dose-related increase in the incidence (83–100%) and multiplicity (4.7–48.1 tumours per tumour-bearing mouse) of tumours of the lung compared with the controls (55% and 0.6 tumours per tumour-bearing mouse, respectively) (Warshawsky *et al.*, 1996b).

Table 3.12 Studies of carcinogenicity in mice given 7*H*-dibenzo[*c,g*]carbazole

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and multiplicity of tumours	Significance	Comments
Skin application				
Mouse, C3H/Hej (M) 99 wk Warshawsky & Barkley (1987)	Treated topically with 46.8 nmol (12.5 µg) of DBC (purity, 99%) in 50 µl of acetone, or with 50 µl of acetone only, or untreated, 2×/wk, on the shaved interscapular region 50 mice/group	*Skin papillomas* 1/50 (2%), 0/50, 0/50 *Skin carcinomas* 47/50 (94%), 0/50, 0/50.	$P < 0.0001$, for skin carcinomas [one-tailed Fisher exact test]	
Mouse, Hsd:(ICR) BR (F) 99 wk Warshawsky *et al.* (1994, 1996b)	Treated topically with 50 nmol (13.4 µg) of DBC (purity, 99%) in 50 µL acetone, or 50 µL acetone only, or untreated, twice/wk on shaved interscapular region 50 mice/group	*Skin tumours* Untreated, acetone only, DBC 2/11 (18%), 3/11 (27%), 42/50 (84%) Squamous cell carcinoma: 0/11, 1/11 (9%), 27/50 (54%) Papilloma: 0/11, 2/11 (18%), 8/50 (16%) Basal cell carcinoma: 0/11, 0/11, 4/50 (16%) Keratoacanthoma: 0/11, 0/11, 2/50 (4%) Tumours in treated group: Squamous cell carcinoma: 27/50 (54%); papilloma: 8/50 (16%); basal cell carcinoma: 4/50 (8%); keratoacanthoma: 2/50 (4%) *Liver tumours* Hepatocellular carcinoma: 22/50 (44%), 0/5, 0/6 Hepatocellular adenoma: 17/50 (34%), 2/5 (40%), 1/6 (17%)	$P < 0.02$ $P < 0.001$ - - - [$P < 0.001$, squamous cell carcinoma] [$P = 0.041$, hepatocellular carcinoma] [NS]	Only 11 controls evaluated by histopathology

Table 3.12 (continued)

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence and multiplicity of tumours	Significance	Comments
Mouse, Hsd:(ICR) BR (F) 25 wk Warshawsky *et al.* (1992, 1996b)	Initiation–promotion Treated topically with 200 nmol of DBC (53.8 µg; purity, 99%) or BaP in 50 µL of acetone. After 2 wk, treated with 2 µg of TPA in 50 µL acetone. Control groups treated with TPA or DBC; twice/wk on shaved interscapular region 30 mice/group	*Skin tumours* DBC+TPA, BaP+TPA, TPA, DBC: Papilloma: 26/30 (87%), 27/30 (90%), 0/30, 0/30	$P < 0.0001$; one-tailed Fisher exact test (DBC + TPA *vs* TPA)	
Intraperitoneal administration				
Mouse, A/J (M) 32 wk Warshawsky *et al.* (1996b)	Single injection at 0, 5, 10, 20 and 40 mg/kg bw of DBC in 0.2 mL of tricaprylin 55 mice/group	*Lung tumours* Incidence: 11/20 (55%), 15/18 (83%), 18/18 (100%), 12/12 (100%), 14/14 (100%) Multiplicity: 0.6, 4.7*, 13.6*, 14.2*, 48.1*	*$P < 0.05$ using Kurskal-Wallis one-way analysis (vs control group).	Study poorly reported. Limitations included lack of information on DBC purity, and lack of histopathology on organs other than lung.

BaP, benzo[*a*]pyrene; DBC, 7*H*-dibenzo[*c,g*]carbazole; F, female; M, male; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; vs, versus; wk, week

Table 3.13 Studies of carcinogenicity in hamsters given 7*H*-dibenzo[*c,g*]carbazole by intratracheal administration

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (M) 30 wk Sellakumar & Shubik (1972)	Instillations of 0.5 or 3 mg of DBC suspended with an equal amount of haematite dust in 0.2 mL of saline, once/wk for 30 wk and 15 wk, respectively; control group was untreated Hamsters/group: 48 at 0.5 mg; 36 at 3 mg; 90 for controls	*Tumours of the respiratory tract* 40/45 (89%), 30/35 (86%), 0/82 (predominantly squamous cell carcinoma of the trachea, bronchi and larynx)	[$P < 0.0001$]	The study was limited by the lack of appropriate control group. Purity of DBC, NR

DBC, 7*H*-dibenzo[*c,g*]carbazole; M, male; NR, not reported; wk, week

(e) Intravenous administration

Groups of 10–12 strain A mice were given 0.25 mL of a 0.1% aqueous dispersion of DBC as a single injection; exposure duration was 8, 14, or 20 weeks. Lung tumours developed in all treated groups (Andervont & Shimkin, 1940). [A limitation of this study was the lack of concurrent control group.]

(f) Bladder implantation

Eight mice were given 1–2 mg of DBC contained in 10–20 mg paraffin-wax pellets implanted in the bladder. The treated mice showed an increase in the incidence of papilloma and metaplasia of the bladder; carcinoma was also observed (Bonser *et al.*, 1952). Twelve mice that were implanted with paraffin-wax pellets not containing DBC did not develop neoplasms of the bladder. [A limited number of animals was used.]

3.7.2 Syrian hamster

See Table 3.13

Intratracheal administration

Groups of male Syrian hamsters were given 0.5 mg (48 hamsters) or 3 mg (35 hamsters) of DBC (suspended with an equal amount of haematite dust in saline) by weekly instillation

Table 3.14 Study of carcinogenicity in a dog given 7*H*-dibenzo[*c,g*]carbazole by intravesical injection

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Dog mongrel, (F) (age NR) 168 wk Bonser *et al.* (1954)	5 mL of a 0.25% solution of DBC in arachis oil, once/wk for 12 months 1 dog	Multiple papillomas (approximately 40) and one urinary cystic transitional cell carcinoma	-	Study poorly reported, limitations included only one dog studied, no controls, purity of DBC used, NR.

DBC, 7*H*-dibenzo[*c,g*]carbazole; F, female; NR, not reported; wk, week

Table 3.15 Study of carcinogenicity in rats given benzo[*b*]naphthol[2,1-*d*]thiophene by pulmonary implantation

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance
Rat, Osborne-Mendel (F) 140 wk Wenzel-Hartung *et al.* (1990)	Single implantation of 0, 1, 3, or 6 mg (purity, 99.6%) in a 1 : 1 mixture of beeswax and trioctanoin. Positive-control groups given 0.03, 0.1, or 0.3 mg of benzo[*a*]pyrene 35 rats/group	*Squamous cell carcinoma of the lung* 0, 1, 3, or 6 mg of benzo[*b*]naphthol-[2,1-*d*]thiophene: 0/35, 1/35 (3%), 11/35 (31%), 11/35 (31%) 0.03, 0.1, or 0.3 mg of benzo[*a*]pyrene: 3/35 (9%), 11/35 (31%), 27/35 (77%)	[$P < 0.001$] (intermediate and highest dose)

F, female; wk, week

for 30 or 15 weeks, respectively. A group of 90 hamsters served as untreated controls. A total of 69 tumours of the respiratory tract developed in 40 out of 45 (89%) hamsters treated with 0.5 mg of DBC (multiplicity, 1.75), and 42 tumours of the respiratory tract developed in 30 out of 35 (86%) hamsters treated with 3 mg (multiplicity, 1.4). The tumours observed were predominantly squamous cell carcinomas of the trachea, bronchi and larynx. No respiratory tumours (0 out of 82) were observed in the control group (Sellakumar & Shubik, 1972). [There was no haematite control group.]

3.7.3 Dog

See Table 3.14

Intravesical injection

A dog given DBC by intravesical injection developed multiple papillomas and one urinary cystic transitional cell carcinoma (Bonser *et al.*, 1954). [The study was limited by the use of a single animal, and the absence of controls.]

3.8 Dibenzothiophene

No data on the carcinogenicity of dibenzothiophene in experimental animals were available to the Working Group.

3.9 Benzo[*b*]naphtho[2,1-*d*]thiophene

3.9.1 Rat

See Table 3.15

Pulmonary implantation

Groups of 35 inbred female Osborne-Mendel rats (age, 3 months) were given 1, 3, or 6 mg of benzo[*b*]naphtho[2,1-*d*]thiophene (purity, 99.6%) in a 1 : 1 mixture of beeswax and trioctanoin, as a single pulmonary implantation (Wenzel-Hartung *et al.*, 1990). An untreated group and a group that received the vehicle only (a mixture of beeswax and trioctanoin) served as controls. In the positive-control group, 35 rats were given 0.1, 0.3, or 1.0 mg of benzo[*a*]pyrene as a pulmonary implantation. For rats treated with benzo[*b*]naphtho[2,1-*d*]thiophene, increases in the incidence of squamous cell carcinoma were reported at doses of 1, 3 and 6 mg (2.9%, 31.4% and 31.4%, respectively). No tumours of the lung were found in the negative controls.

4. Mechanistic and Other Relevant Data

4.1 Benz[*a*]acridine

4.1.1 Metabolism and distribution

The bioconcentration and metabolism of benz[*a*]acridine in fathead minnows (*Pimephales promelas*) was investigated using ^{14}C-labelled benz[*a*]acridine. The bioconcentration factor was estimated at 106 ± 17, approximately one tenth of that predicted by octanol : water partitioning models. It was estimated that metabolism of benz[*a*]acridine reduced the extent of bioconcentration by 50–90% compared with that expected in the absence of metabolism. The rate constant for the metabolism of benz[*a*]acridine was 0.49 ± 0.07 per hour. Metabolites (not specified) accounted for the bulk of the radiolabel in fish after less than 1 day of exposure (Southworth *et al.*, 1981).

The study by Jacob *et al.* (1982) appeared to be the only comprehensive study on the metabolism of benz[*a*]acridine. Incubations were conducted with liver and lung microsomes from male Wistar rats that were previously untreated, or treated with phenobarbital or benzo[*k*]fluoranthene. The metabolite profile was analysed by GC-MS, following derivatization by silylation. A K-region 5,6-dihydrodiol and a non-K-region dihydrodiol were formed by liver and lung microsomes. Additional metabolites (number not specified) were detected, but not characterized. The K-region dihydrodiol was identified on the basis of the relative intensities of the MS fragment ions. The structure of the non-K-region dihydrodiol could not be assigned unequivocally, although the *trans*-3,4-dihydrodiol isomer was excluded by comparison with an authentic synthetic standard. Pre-treatment with phenobarbital induced K-region oxidation, while pretreatment with benzo[*k*]fluoranthene induced non-K-region oxidation. The ratios of the K-region to non-K-region metabolites were similar in liver and lung (1.8, ~6, and 0.33 for untreated, phenobarbital-treated and benzo[*k*] fluoranthene-treated rats, respectively). No evidence could be obtained for the formation of the putative ultimate carcinogen, *anti*-benz[*a*] acridine-3,4-dihydrodiol-1,2-epoxide, a bay-region diol-epoxide. The metabolic rate was low compared with that observed in concurrent incubations with benz[*c*]acridine (Jacob *et al.*, 1982).

[The bay-region diol-epoxides have yet to be unequivocally identified (either directly or indirectly) *in vivo* or in test systems *in vitro*.]

4.1.2 Genotoxicity and other relevant effects

When benz[*a*]acridine was tested for mutagenicity at concentrations of up to 0.5 mg/plate in *Salmonella typhimurium* TA98 (his^-/his^+) in the presence of an exogenous metabolic system, the results were inconclusive (Ho *et al.*, 1981). Contrasting with these earlier mutagenesis data, benz[*a*]acridine gave positive results a concentration of ~0.01 µM in the Mutatox test, an luminescence assay for reverse bacterial mutation in *Vibrio fischeri* (Bleeker *et al.*, 1999). The mutagenic activities of 1,2,3,4-tetrahydrobenz[*a*] acridine-1,2-epoxide and benz[*a*]acridine-3,4-dihydrodiol-1,2-epoxides were examined in bacteria and mammalian cells, to assess the potential significance of bay-region activation. The *syn*- and *anti*-benz[*a*]acridine-3,4-dihydrodiol-1,2-epoxides (racemic mixture) induced 6 and 60 his^+ revertants/nmol, respectively, in *S. typhimurium* TA98; higher numbers of histidine autotrophs (60/nmol and 240/nmol, respectively) were induced in strain TA100. In comparison, 1,2,3,4-tetrahydrobenz[*a*]acridine-1,2-epoxide (racemic mixture) was considerably more mutagenic (800 and 3000 revertants/nmol in strains TA98 and TA100, respectively). The same trends were observed in Chinese hamster V79–6 cell lines. Benz[*a*]acridine-3,4-dihydrodiol (presumed to have a *trans* configuration) had no

intrinsic mutagenicity [in the absence of metabolic activation] and no significant increase in mutation frequency was observed in *S. typhimurium* TA100 in the presence of liver microsomes from immature male Long Evans rats treated with Aroclor 1254. However, low but statistically significant activation of the compound was observed in the same strain when the incubations were conducted in the presence of a highly purified and reconstituted mono-oxygenase system obtained from the same type of liver microsome (Wood *et al.*, 1983).

Benz[*a*]acridine and its derivatives, the *trans*-benz[*a*]acridine-3,4-dihydrodiol and the *syn*- and *anti*-benz[*a*]acridine-3,4-dihydrodiol-1,2-epoxides (as the racemic mixture), were tested for genotoxicity in two rat hepatoma cell lines, at a single concentration (250 µM) and exposure time (2 hours). The genotoxic effect was measured by alkaline elution (i.e. the appearance of alkali-labile DNA sites). The selected hepatoma cell lines were: H_5, a dedifferentiated cell line that strongly expresses PAH-inducible CYP448-dependent mono-oxygenases (CYP1A and CYP1B), but not CYP450-dependent enzymes (CYP2B); and H_{1-4}, a differentiated hybrid cell line that contains CYP448- and CYP450-dependent mono-oxygenases. The parent benz[*a*]acridine had no effect on any of the cell lines. Likewise, benz[*a*]acridine-3,4-dihydrodiol did not induce DNA-strand breaks in any of the cell lines, in contrast to the analogous benz[*c*]acridine-3,4-dihydrodiol. Each of the benz[*a*]acridine-derived diol-epoxides induced DNA damage in both cell lines. The *anti*-diol-epoxide was more potent than the *syn* isomer and was three times more potent in H_5 cells than in H_{1-4}. The genotoxicity observed with *anti*-benz[*a*]acridine-3,4-dihydrodiol-1,2-epoxide contrasted with the weak mutagenicity of the same compound in the Ames test and in Chinese hamster V79 cells (see above). [Although the authors suggested that the discrepancy between the Ames assay and this assay for genotoxicity might be due to the antibacterial activity of benz[*a*]acridine, the Working Group noted that this would probably not explain the weak mutagenicity in V79 cells]. Overall, benz[*a*]acridine and its derivatives are not extensively metabolized to active mutagens (Loquet *et al.*, 1985).

A recombinant plasmid containing the thymidine kinase (*Tk*) gene (pAGO; 6.36 kb) was reacted *in vitro* with *syn*- and *anti*-benz[*a*]acridine-3,4-dihydrodiol-1,2-epoxide (racemic mixture). The covalent DNA binding and limited restriction by different endonucleases observed *in vitro* were correlated with biological activity by transfer of the plasmid (*Tk* gene) to TK-deficient cells. Upon transfection of mouse Ltk-cells with modified and non-modified plasmid, the benz[*a*]acridine diol-epoxides reduced the number of TK^+ clones formed to a similar, although weaker, degree than that obtained with *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (0.8 and 0.3 ng/10 ng DNA for the benz[*a*]acridine and benzo[*a*]pyrene derivatives, respectively). The inhibition of transformation efficiency was consistent with inactivation of the gene by chemical modification (Schaefer-Ridder *et al.*, 1984).

4.1.3 Mechanistic considerations

Several studies have addressed the induction of specific mono-oxygenases by benz[*a*]acridine. Pre-treatment of male Wistar rats with benz[*a*]acridine resulted in weak induction of liver mono-oxygenase activity, accompanied by a significant change in the microsomal metabolite profile of benz[*a*]anthracene, which favoured K-region 5,6-oxidation (Jacob *et al.*, 1983). Benz[*a*]acridine was also a weak inducer of chrysene metabolism (Jacob *et al.*, 1987). In addition, benz[*a*]acridine was found to markedly increase the rates of ethoxyresorufin and ethoxycoumarin *O*-deethylation by rat liver microsomes and to induce proteins recognized by antibodies to CYP1A1, but not CYP2B1 (Ayrton *et al.*, 1988). More recently, CYP1A1 induction by benz[*a*]

acridine was demonstrated in fish hepatoma PLHC-1 cells (Jung *et al.*, 2001).

The ability of benz[*a*]acridine to induce the aryl hydrocarbon receptor (AhR) was assessed *in vitro* in the CALUX® assay, using a rat hepatoma cell line stably transfected with a luciferase reporter gene under the control of dioxin-responsive elements. In a similar luciferase-reporter test, using the breast carcinoma MVLN cell line, benz[*a*]acridine was a weak inducer of estrogenic activity (Machala *et al.*, 2001). Quantitative structure–activity relationships for potency to activate AhR indicated ellipsoidal volume, molar refractivity, and molecular size as the best descriptors (Sovadinová *et al.*, 2006).

4.2 Benz[*c*]acridine

4.2.1 Metabolism

The study by Jacob *et al.* (1982) appears to be the only comprehensive study on the metabolism of benz[*c*]acridine. Incubations were conducted with liver microsomes from male Wistar rats that were untreated, or treated with phenobarbital, benzo[*k*]fluoranthene, or 5,6-benzoflavone. The metabolite profile was analysed by GC-MS, following derivatization by silylation. Incubation with microsomes from untreated rats yielded five different phenols (unidentified), one diphenol (unidentified) and two dihydrodiols. The major metabolite was identified as the [K-region] 5,6-dihydrodiol, on the basis of the relative intensities of the MS fragment ions. Pretreatment with phenobarbital doubled the total metabolite rate and significantly altered the metabolite profile: only one of the five phenols was detected and its amount had decreased by approximately seven times. This was accompanied by a seven-times increase in the amount of the 5,6-dihydrodiol, which was again the major metabolite. The previously detected other dihydrodiol and two additional non-K-region dihydrodiols (unidentified) were also present. Two K-region triols (i.e. monophenolic derivatives of the K-region dihydrodiol) were also detected, but the position of the phenolic hydroxyl group was not established. Pre-treatment with benzo[*k*]fluoranthene or 5,6-benzoflavone increased the rates of total metabolism approximately 2.8 and 3.9 times, respectively. Both pre-treatments stimulated K-region oxidation and also the formation of phenols and diphenols; the 5,6-dihydrodiol was again the major metabolite. On the basis of MS fragmentation patterns, a small extent of *N*-oxidation also occurred, albeit in very small amounts, compared with a synthetic standard, *trans*-benz[*c*]acridine-3,4-dihydrodiol. Upon incubation of uninduced and benzo[*k*]fluoranthene-induced liver microsomes with the 3,4-dihydrodiol, a diphenol, assumed to be 3,4-dihydroxybenz[*c*]acridine, and a tetrol (tentatively identified as 3,4,5,6-tetrahydroxy-3,4,5,6-tetrahydrobenz[*c*]acridine) were detected. Unequivocal evidence for the formation of the putative ultimate carcinogen, *anti*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxide (a bay-region diol-epoxide), could not be obtained (Jacob *et al.*, 1982).

4.2.2 Genotoxicity and other relevant effects

Two studies reported positive results in tests for mutagenicity with benz[*c*]acridine at a concentration of 25 µg/plate in *S. typhimurium* TA100 (his^-/his^+) in the presence of an exogenous metabolic system (Okano *et al.*, 1979; Baker *et al.*, 1980).

When tested in Chinese hamster Don (lung) cells, benz[*c*]acridine at 1–100 µM induced sister-chromatid exchange without the addition of metabolic activation from S9 (Baker *et al.*, 1983).

The mutagenic activities of 1,2,3,4-tetrahydrobenz[*c*]acridine-1,2-epoxide and of the diol-epoxide metabolites, *syn*- and *anti*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxides, were examined in bacteria and mammalian cells, to assess the potential significance of bay-region

activation. The *syn*- and *anti*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxides (racemic mixture) had comparable mutagenic potencies in *S. typhimurium* TA98 (250 and 300 his^+ revertants/nmol, respectively). In strain TA100, the *syn*-diol-epoxide induced 5100 his^+ revertants/nmol and was approximately twice more active than the *anti* isomer. The order of relative mutagenicities was reversed in Chinese hamster V79–6 cells, in which the anti-diol-1,2-epoxide, which induced 4.5 8-azaguanine-resistant colonies/10^5 surviving cells per nmol, was approximately twofold more active than the *syn* isomer. In both test systems (i.e. *S. typhimurium* TA98 and TA100, and V79 cells), the bay-region diol-epoxides were one to four orders of magnitude more mutagenic than their non-bay-region counterparts (i.e. racemic *anti*-1,2-dihydrodiol-3,4-epoxide, *syn*- and *anti*-8,9-dihydrodiol-10,11-epoxide, and *syn*- and *anti*-10,11-dihydrodiol-8,9-epoxide). In comparison with the analogous benz[*a*]acridine derivatives, the bay-region diol-epoxides from benz[*c*]acridine were more mutagenic by at least one order of magnitude. The bay-region 1,2,3,4-tetrahydrobenz[*c*]acridine-1,2-epoxide (racemic mixture) had high mutagenic activity, about four to eleven times greater than the corresponding benz[*a*]acridine metabolite. Neither the bay-region benz[*c*]acridine diol-epoxides nor 1,2,3,4-tetrahydrobenz[*c*]acridine-1,2-epoxide were metabolized to non-mutagenic derivatives by highly purified epoxide hydrolase. Metabolic-activation experiments were conducted in *S. typhimurium* TA100 in the presence of either liver microsomes from immature male Long Evans rats treated with Aroclor 1254, or a highly purified and reconstituted mono-oxygenase system obtained from the same type of liver microsomes. The results indicated that *trans*-benz[*c*]acridine-3,4-dihydrodiol, the putative immediate precursor of the bay-region diol-epoxides, was at least five times more active than the parent compound, and that none of the other possible *trans*-dihydrodiols (i.e. the 1,2-, 5,6-, 8,9-, and 10,11-dihydrodiols) underwent significant activation to mutagenic derivatives (Wood *et al.*, 1983).

Benz[*c*]acridine and its derivatives, the *trans*-benz[*c*]acridine-3,4-dihydrodiol and the *syn*- and *anti*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxides (as racemic mixture), were tested for genotoxicity in two rat hepatoma cell lines, at a single concentration (250 μM) and exposure duration (2 hours). The genotoxic effect was measured by alkaline elution (i.e. the appearance of alkali-labile DNA sites). The selected cell lines were: H_5, a dedifferentiated cell line that strongly expresses PAH-inducible CYP448-dependent mono-oxygenases (CYP1A and CYP1B), but not CYP450-dependent enzymes (CYP2B); and H_{1-4}, a differentiated hybrid cell line that contains both CYP448- and CYP450-dependent mono-oxygenases. While the parent heterocycle (benz[*c*]acridine) had no effect on any of the cell lines, the 3,4-dihydrodiol induced DNA single-strand breaks at the same order of magnitude in both cell lines, with approximately 60% of the initial DNA remaining in the filter after elution. Each of the benz[*c*]acridine-derived diol-epoxides induced DNA damage in both cell lines. The *anti*-diol-epoxide was more potent than the *syn* isomer and was three times more potent in H_{1-4} cells than in H_5. (Loquet *et al.*, 1985).

A recombinant plasmid containing the mouse thymidine kinase (*Tk*) gene (pAGO; 6.36 kb) was tested *in vitro* with *syn*- and *anti*-benz[*a*]acridine-3,4-dihydrodiol-1,2-epoxide (racemic mixture). The covalent DNA binding and limited restriction by different endonucleases observed *in vitro* were correlated with biological activity by transfer of the plasmid (*Tk* gene) to TK-deficient cells. Upon transfection of mouse LTK^- cells with modified and non-modified plasmid, the benz[*a*]acridine diol-epoxides reduced the formation of TK^+ clones which was similar, although weaker, than that obtained with *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (0.8 and 0.3 ng per 10 ng DNA for the benz[*c*]acridine and benzo[*a*]

pyrene derivatives, respectively). The inhibition of transformation efficiency was consistent with inactivation of the gene by chemical modification (Schaefer-Ridder *et al.*, 1984).

4.2.3 Mechanistic considerations

Several studies have addressed the induction of specific mono-oxygenases by benz[*c*]acridine. Pretreatment of male Wistar rats with benz[*c*]acridine resulted in weak induction of liver mono-oxygenase activity, accompanied by a significant change in the microsomal metabolite profile of benz[*a*]anthracene, which favoured 5,6-oxidation. Benz[*c*]acridine was also a weak inducer of chrysene metabolism (Jacob *et al.*, 1987). The induction of CYP1A1 by benz[*c*]acridine was demonstrated in fish hepatoma PLHC-1 cells (Jung *et al.*, 2001).

The tumorigenicities of benz[*c*]acridine bay-region diol-epoxides and their putative metabolic precursors have been demonstrated (Levin *et al.*, 1983; Chang *et al.*, 1984). The substantially higher activities of the bay-region diol-epoxides from benz[*c*]acridine in bacteria and mammalian cells, compared with their benz[*a*]acridine analogues, are consistent with qualitative arguments of resonance stabilization of the carbocations stemming from epoxide ring opening (Jerina *et al.*, 1976).

The ability of benz[*c*]acridine to induce AhR was assessed in the CALUX® assay *in vitro*, using a rat hepatoma cell line stably transfected with a luciferase reporter gene under the control of dioxin-responsive elements. After exposure for 6 hours, benz[*c*]acridine was six to seven times less potent than benzo[*a*]pyrene. In a similar luciferase-reporter test, using the breast carcinoma MVLN cell line, benz[*c*]acridine did not induce estrogenic activity (Machala *et al.*, 2001). Quantitative structure–activity relationships for potency to activate AhR indicated ellipsoidal volume, molar refractivity, and molecular size as the best descriptors (Sovadinová *et al.*, 2006).

4.3 Dibenz[*a,h*]acridine

4.3.1 Metabolism

The first comprehensive study of the metabolism of dibenz[*a*,*h*]acridine compared the extent of conversion and metabolite patterns after incubation with liver microsomes from male Sprague-Dawley rats pre-treated with dibenz[*a*,*h*]acridine, 3-methylcholanthrene, phenobarbital or corn oil. After an incubation of 6 minutes, the extent of total metabolism of dibenz[*a*,*h*]acridine corresponded to 21, 14, 0.7, or 0.2 nmol/mg protein with microsomes from rats pre-treated with dibenz[*a*,*h*]acridine, 3-methylcholanthrene, phenobarbital, or corn oil, respectively.

Regardless of the type of induction, the product profiles were very similar and the major metabolites were the dihydrodiols that contained bay-region double bonds, specifically, dibenz[*a*,*h*]acridine-3,4-dihydrodiol and dibenz[*a*,*h*]acridine-10,11-dihydrodiol, each accounting for 21–23% of the total when using microsomes from rats induced with 3-methylcholanthrene. Additional metabolites included dibenz[*a*,*h*]acridine-1,2-dihydrodiol (about 5%), two K-region epoxides (dibenz[*a*,*h*]acridine-12,13-epoxide and 5,6-epoxide, at approximately 5% and 2% of the total metabolites, respectively), several unidentified polar metabolites (10–15%), and several unidentified metabolites co-eluting with 3-hydroxy-dibenz[*a*,*h*]acridine (20%). The 8,9-dihydrodiol was not formed (< 2%). In combination, the 3,4-dihydrodiols and 10,11-dihydrodiols accounted for 40–50% of the total metabolism, with no apparent effect of the position of the nitrogen on relative extents of formation. K-region metabolism was a minor pathway, similarly to that reported for dibenz[*a*,*h*]anthracene, an isosteric analogue of dibenz[*a*,*h*]acridine (Steward *et al.*, 1987).

A subsequent study investigated the stereoselectivity of rat-liver enzymes in the conversion of dibenz[*a*,*h*]acridine to its 3,4-dihydrodiol and

10,11-dihydrodiol metabolites and in the conversion of dibenz[*a*,*h*]acridine-10,11-dihydrodiol enantiomers to their bay-region diol-epoxides. Using liver microsomes from immature male Long-Evans rats treated with 3-methylcholanthrene, or controls, the 3,4- and the 10,11-dihydrodiols were formed predominantly as the *R*,*R*-enantiomers, in 38–54% enantiomeric excess. Metabolism of each of the 10,11-dihydrodiol enantiomers by liver microsomes from control rats produced predominantly bay-region diol-epoxides (characterized upon hydrolysis to the tetrols), which accounted for 46–59% of the total metabolites. In contrast, bay-region diol-epoxides accounted for only 14–17% of the total metabolites produced by liver microsomes from rats treated with 3-methylcholanthrene. In all instances, the bay-region diol-epoxides produced were predominantly of the *anti* configuration. (–)-(10*R*,11*R*)-Dibenz[*a*,*h*]acridine-10,11-dihydrodiol was metabolized by liver microsomes from rats treated with 3-methylcholanthrene to the highly mutagenic *anti*-(+)-(8*R*,9*S*,10*S*,11*R*) diol-epoxide in an amount that was 6.5 times more than that of the corresponding *syn*-diol-epoxide. The *anti*/*syn* diol-epoxide ratio was 1.7 when (–)-(10*R*,11*R*)-dibenz[*a*,*h*]acridine-10,11-dihydrodiol was metabolized by liver microsomes from control rats. Metabolism of (10*S*,11*S*)-dibenz[*a*,*h*]acridine-10,11-dihydrodiol by liver microsomes from control rats or rats treated with 3-methylcholanthrene yielded *anti*/*syn* diol-epoxide ratios of 1.5 and 2.3, respectively (Kumar *et al.*, 1995).

A more recent study investigated the biotransformation of dibenz[*a*,*h*]acridine by recombinant human CYP1A1, 1B1, and 3A4, and rat CYP1A1, in the presence of human or rat epoxide hydrolase. Among the human isoforms, CYP1A1 was the most effective (5.38 ± 0.56 pmol/min per pmol CYP), CYP1B1 had moderate activity (0.67 ± 0.07 pmol/min per pmol CYP) and CYP3A4 was the least active (0.20 ± 0.03 pmol/min per pmol CYP). The rate of total dibenz[*a*,*h*]acridine metabolism by human CYP1A1 was less than half that by rat CYP1A1. The major dibenz[*a*,*h*]acridine metabolites produced by human CYP1A1 and CYP1B1 were the *trans*-3,4- and *trans*-10,11-dihydrodiols. CYP1A1 gave a higher proportion of the 10,11-dihydrodiol than of the 3,4-diol (about 45% versus about 24%). In contrast, human CYP1B1 yielded a much greater proportion of 3,4-dihydrodiol than of 10,11-dihydrodiol (about 55% versus about 6%), and rat CYP1A1 did not show regioselectivity, giving nearly equal proportions of the two diols. Despite the differences in regioselectivity, human CYP1A1 and CYP1B1 and rat CYP1A1 had similar stereoselectivities for the formation of the 3,4- dihydrodiols and 10,11-dihydrodiols: in all instances, the *R*,*R* enantiomers were formed almost exclusively (> 91.5%) (Yuan *et al.*, 2004).

4.3.2 Genotoxicity and other relevant effects

Dibenz[*a*,*h*]acridine was reported to enhance viral cell transformation in immortalized rat embryo cells *in vitro* (Freeman *et al.*, 1973).

Dibenz[*a*,*h*]acridine was tested for clastogenicity in a Chinese hamster fibroblast cell line (CHL). Results were negative, both in the absence and in the presence of a S9 metabolic activation system, while dibenz[*a*,*j*]acridine and dibenz[*c*,*h*]acridine gave positive results in the presence of metabolic activation from S9 (see Section 4.4.2; Section 4.5.2; Matsuoka *et al.*, 1982).

Kitahara *et al.* (1978) tested the mutagenicity of dibenz[*a*,*h*]acridine and of the K-region dibenz[*a*,*h*]acridine-12,13-epoxide (racemic mixture) in *S. typhymurium* TA98 and TA100, with or without S9 from rats induced with polychlorinated biphenyls. Dibenz[*a*,*h*]acridine was inactive without metabolic activation, but showed mutagenicity with metabolic activation, particularly in TA100 (2.3 and 39 revertants/μg per plate, in TA98 and TA100, respectively). The K-region 12,13-epoxide was weakly active in TA100 in the absence of metabolic activation (1.8

revertants/μg per plate). However, in the presence of metabolic activation (1.4 and 13 revertants/μg per plate in TA98 and TA100, respectively), it was less mutagenic than dibenz[*a*,*h*]acridine. These data indicated that dibenz[*a*,*h*]acridine-12,13-epoxide is a reactive metabolite, but not an intermediate in the pathway of activation of the parent compound to a mutagen (Kitahara *et al.*, 1978).

Another mutagenicity study in *S. typhimurium* gave negative results at up to 1000 μg/plate in strains TA1535, TA1537, TA1538, TA98, and TA100 in the presence of microsomal S9 from rats induced with Aroclor (Salamone *et al.*, 1979).

Dibenz[*a*,*h*]acridine was mutagenic in *S. typhimurium* TA100 in the presence of liver microsomes from rats co-treated with phenobarbital and 5,6-benzoflavone at 0–100 μg/plate (Karcher *et al.*, 1985).

The mutagenicities of dibenz[*a*,*h*]acridine and dibenz[*a*,*h*]acridine-1,2-, -3,4-, -8,9-, and -10,11-dihydrodiols were assessed in *S. typhimurium* TA100, in the presence of a metabolic activation system from immature Long-Evans male rats pretreated with Aroclor 1254. Dibenz[*a*,*h*]acridine-10,11-dihydrodiol, the precursor of the bay-region dibenz[*a*,*h*]acridine-10,11-dihydrodiol-8,9-epoxides, was about three times more active than dibenz[*a*,*h*]acridine at 125 μM, and approximately twelve times more active than dibenz[*a*,*h*]acridine-3,4-dihydrodiol, the metabolic precursor of the dibenz[*a*,*h*]acridine-3,4-dihydrodiol-1,2-epoxides. Activation of dibenz[*a*,*h*]acridine-1,2-dihydrodiols and dibenz[*a*,*h*]acridine-8,9-dihydrodiols to mutagenic products in TA100 was almost negligible. The mutagenic activities of the four bay-region diol-epoxides from dibenz[*a*,*h*]acridine (racemic *syn*- and *anti*-3,4-dihydrodiol-1,2-epoxide; racemic *syn*- and *anti*-10,11-dihydrodiol-8,9-epoxide) were assessed in bacteria and mammalian cells. The diastereomeric 10,11-dihydrodiol-8,9-epoxides were 20–40 times more mutagenic than the corresponding 3,4-dihydrodiol-1,2-epoxides in *S. typhimurium* TA98 and TA100, with the *anti*-10,11-dihydrodiol-8,9-epoxide being approximately 2.5 times more active in either strain than its *syn* diastereomer. In the Chinese hamster V79–6 cell line, which lacks the capacity for oxidative metabolism of PAHs to mutagens, the 10,11-dihydrodiol-8,9-epoxide diastereomers were 20–80 times more mutagenic than their 3,4-dihydrodiol-1,2-epoxide analogues. The *anti*-10,11-dihydrodiol-8,9-epoxide was twice as cytotoxic and five times more mutagenic than the *syn*-10,11-dihydrodiol-8,9-epoxide. Likewise, the *syn*-10,11-dihydrodiol-8,9-epoxide was twice as cytotoxic, and at least 20 times more mutagenic than the *syn*-3,4-dihydrodiol-1,2-epoxide. The *anti*-3,4-dihydrodiol-1,2-epoxide was the least cytotoxic of the four diol-epoxides tested (Wood *et al.*, 1989).

In a subsequent study, the four enantiomerically pure dibenz[*a*,*h*]acridine-10,11-dihydrodiol-8,9-epoxides (Kumar *et al.*, 1992) were also evaluated for mutagenicity in *S. typhimurium* TA98 and TA100 and in the Chinese hamster V79–4 cell line. The *anti*-(–)-(8*S*,9*R*,10*R*,11*S*) diol-epoxide was the most mutagenic of the four compounds in *S. typhimurium*, inducing 1200 and 6900 his^+ revertants/nmol in strains TA98 and TA100, respectively. The mutagenic activities of the remaining three stereoisomers were 14–72% that of the (*S*,*R*,*R*,*S*) isomer, with the dose–response relationships for the induction of histidine revertants being qualitatively similar in both strains; the two *anti* diol-epoxides were three to seven times more mutagenic than the *syn* isomers. In contrast, in Chinese hamster V79 cells, the *anti*-(+)-(8*R*,9*S*,10*S*,11*R*) diol-epoxide, which induced 68 8-azaguanine-resistant variants/nmol per 10^5 cells, was two to eleven times more mutagenic than the other three diol-epoxides. This is similar to what has been observed with bay-region diol-epoxides from numerous PAHs, where the (*R*,*S*,*S*,*R*) isomer may not be the most active in bacterial assays, but tends to be

the most mutagenic in mammalian cells (Chang *et al.*, 1993).

Groups of six male Sprague-Dawley rats (age, 4–6 weeks) were given dibenz[*a*,*h*]acridine as three equal doses of 25, 50, or 100 mg/kg bw by intratracheal instillation over 24 hours, and killed 6 hours after the third dose. ^{32}P-Postlabelling, using either butanol extraction or nuclease P_1 digestion enrichment procedures, detected one DNA adduct. There was a dose–response effect; at the highest dose, the number of adducts was estimated to be 1.9/10^8 nucleotides when using butanol, and 0.7/10^8 nucleotides when using nuclease P_1. Two cytogenetic end-points, sister-chromatid exchange and micronucleus formation, were also investigated. Although both assays were less sensitive than the ^{32}P-postlabelling assay, the induction of sister-chromatid exchange occurred in lung cells at the two highest doses (number of sister-chromatid exchanges, 10.6 ± 3.5 per cell and 11.2 ± 3.8 per cell with dibenz[*a*,*h*]acridine at a dose of 50 or 100 mg/kg bw, respectively) and micronuclei were induced at the highest dose (Whong *et al.*, 1994).

4.3.3 Mechanistic considerations and additional observations

Ionization potentials were used to predict the mechanism of metabolic activation of carcinogenic PAHs. In the case of dibenz[*a*,*h*]acridine, a high ionization potential (> 8.10 eV) is consistent with a mono-oxygenation pathway, rather than one-electron oxidation (Xue *et al.*, 1999).

Dibenz[*a*,*h*]acridine combines the structural features of benz[*a*]acridine and benz[*c*]acridine. The lack of symmetry, due to the presence of the nitrogen atom in position 7, results in two distinct bay-regions. Thus, metabolism of dibenz[*a*,*h*]acridine yields two pairs of bay-region diol-epoxides that are not structurally equivalent. The differences in structure result in different biological activities that differ between the diol-epoxides and their dihydrodiol precursors. The available data in bacterial and mammalian cells indicated that the bay-region dibenz[*a*,*h*]acridine-10,11-dihydrodiol-8,9-epoxides and their putative metabolic 10,11-dihydrodiol precursor are considerably more mutagenic than the analogous bay-region 3,4-dihydrodiol-1,2-epoxides and their 3,4-dihydrodiol precursor. Of note, the 1,2- and 8,9-dihydrodiols, which cannot be converted to bay-region diol-epoxides, are not activated by metabolic systems to mutagenic products in *S. typhimurium* TA100 (Wood *et al.*, 1989).

A recent model computational study, using the density functional theory, yielded results generally consistent with earlier quantum mechanical calculations of the predicted ease of benzylic carbocation formation at C-1 and C-8 of dibenz[*a*,*h*]acridine diol-epoxides. The computational data suggested that carbocation formation at C-8 is energetically favoured over C-1, which may predict lower reactivity for the 3,4-dihydrodiol-1,2-epoxides compared with the 10,11-dihydrodiol-8,9-epoxides (Borosky & Laali, 2005). A decreased propensity for epoxide ring opening of the 3,4-dihydrodiol-1,2-epoxides may explain their lower mutagenic activity.

The data on mutagenicity in mammalian cells and on tumour initiation on mouse skin implicated *trans*-(–)-(10*R*,11*R*)-dibenz[*a*,*h*]acridine-10,11-dihydrodiol as the proximate carcinogen and the bay-region *anti*-(–)-(8*R*,9*S*,10*S*,11*R*) diol-epoxide as the ultimate carcinogen. The high tumorigenicity of the *R*,*S*,*S*,*R* diol-epoxide reveals a stereoselectivity identical to those exhibited by other homocyclic and *N*-heterocyclic PAHs, including benzo[*a*]pyrene, benz[*a*]anthracene, chrysene, benzo[*c*]phenanthrene, and dibenz[*c*,*h*]acridine (Chang *et al.*, 1993).

Human CYP1A1 is substantially more active in dibenz[*a*,*h*]acridine metabolism than human CYP1B1 and, contrary to rat CYP1A1, is regioselective for formation of the 10,11-dihydrodiol, when compared with the 3,4-dihydrodiol. In addition, the observed stereoselectivity for

production of the (–)-10*R*,11*R* isomer (the proximate carcinogen) suggests that a high expression of CYP1A1 activity may confer increased susceptibility to carcinogenesis induced by dibenz[*a*,*h*]acridine. In contrast, human CYP1B1 appears to play a minor role in the metabolic activation of dibenz[*a*,*h*]acridine (Yuan *et al.*, 2004).

Intratracheal instillation of dibenz[*a*,*h*]acridine in rats resulted in DNA adducts, sister-chromatid exchange, and the induction of micronucleus formation in lung cells. Although similar studies had not been reported with dibenz[*a*,*h*]acridine metabolites, the combined data were consistent with bioactivation of the parent compound to a genotoxicant by metabolism to the (–)-(10*R*,11*R*)-dihydrodiol and subsequent formation of the corresponding *anti* diol-epoxide (Whong *et al.*, 1994).

Dibenz[*a*,*h*]acridine was a potent inducer of mono-oxygenase activities in rat liver. The induction affected the metabolite profile of benz[*a*]anthracene in the presence of metabolic activation, suppressing 10,11-oxidation and favouring 5,6-, and 8,9- (but not bay-region) oxidation (Jacob *et al.*, 1985). Also, liver microsomes from rats pretreated with dibenz[*a*,*h*]acridine stimulate chrysene metabolism to the proximate carcinogen, *trans*-chrysene-1,2-dihydrodiol (Jacob *et al.*, 1987).

The ability of dibenz[*a*,*h*]acridine to induce AhR was quantified *in vitro* in the CALUX® assay, using a rat hepatoma cell line stably transfected with a luciferase reporter gene under the control of dioxin-responsive elements. After an exposure of 6 hours, dibenz[*a*,*h*]acridine was 2.45 times more potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and 217 times more potent than benzo[*a*]pyrene, suggesting that it may contribute significantly to overall AhR-mediated activity in the environment (e.g. river sediments). The same study did not detect statistically significant estrogenic activity with dibenzo[*a*,*h*]acridine (Machala *et al.*, 2001). Quantitative structure–activity relationships for potency in activation of AhR indicated ellipsoidal volume, molar refractivity, and molecular size as the best descriptors (Sovadinová *et al.*, 2006).

4.4 Dibenz[*a,j*]acridine

4.4.1 Distribution and metabolism

(a) Distribution

When male Wistar rats were given [^{3}H]dibenz[*a*,*j*]acridine at a dose of 0.5 mg/kg bw in DMSO by intraperitoneal administration, faecal excretion accounted for the bulk of the radiolabel and occurred essentially within 48 hours. When [^{3}H]dibenz[*a*,*j*]acridine was given at the same dose by intravenous administration to cannulated rats, there was rapid (within 6 hours) biliary excretion. After treatment with β-glucuronidase and arylsulfatase, about 25% of the excreted radiolabel was soluble in ethyl acetate. This fraction contained 3-hydroxydibenz[*a*,*j*]acridine and 4-hydroxydibenz[*a*,*j*]acridine, polar products of secondary oxidation, and only a small amount (1–2%) of the 3,4-dihydrodiol. In the absence of enzymatic hydrolysis, the total amount of radiolabel extracted into ethyl acetate did not exceed 3% (Robinson *et al.*, 1990).

In mice, topical application of [^{3}H]dibenz[*a*,*j*]acridine resulted in radiolabel peaks in the kidney at 6 hours and in the liver at 12 hours. The total concentration of radiolabel in the skin decreased by approximately 50% over 96 hours. After 48 hours, the parent compound accounted for 25–30% of the total radiolabel in the liver, and two metabolites, presumed to be the 1,2-diols and 3,4-diols, accounted for 2–6% of the total radiolabel. Two additional, less polar, metabolites were present at 2.5% and 5–16% in the skin and liver, respectively, but remained unidentified (Warshawsky *et al.*, 1993).

(b) Metabolism

There were numerous reports on the metabolism of dibenz[*a*,*j*]acridine, both *in vitro* and *in vivo*; these had been partially reviewed (Warshawsky *et al.*, 1996a).

An initial study used isolated preparations of perfused rabbit lung. The total rate of appearance of dibenz[*a*,*j*]acridine metabolites in the blood was lower than that for the structurally similar *N*-heterocyclic compound, 7*H*-dibenzo[*c*,*g*]carbazole (DBC), both in preparations from untreated rabbits (numbers not given) and from rabbits pre-treated with corn-oil (204 ± 34 ng/g lung per hour at a dose of 175 ± 12.5 µg of dibenz[*a*,*j*]acridine, *versus* 936 ± 144 ng/g lung per hour at a dose of 300 µg of DBC). When the rabbits were pre-treated with benzo[*a*]pyrene at a dose of 20 mg/kg bw in 3 mL of corn oil, administered intraperitoneally, 24 hours before being killed, a statistically significant increase in the metabolism of dibenz[*a*,*j*]acridine (to 1089 ± 235 ng/g lung per hour, $P = 0.05$) was observed. This increase was associated with a statistically significant increase ($P = 0.01$) in the production of non-extractable (i.e. conjugated) metabolites. Based upon a combination of ultraviolet (UV) and fluorescence spectroscopy and mass spectrometry, one of the major metabolites was identified as the 3,4-dihydrodiol of dibenz[*a*,*j*]acridine. A second major metabolite had a mass spectrum consistent with a monohydroxylated derivative of dibenz[*a*,*j*]acridine (Warshawsky *et al.*, 1985).

Subsequent studies provided structural proof for a variety of dibenz[*a*,*j*]acridine metabolites, using a combination of HPLC-UV with diode-array detection or fluorescence and mass spectrometry, and authentic standards. The metabolites identified in liver and lung microsomal preparations from Wistar rats induced with 3-methylcholanthrene were two dihydrodiols (trans-dibenz[*a*,*j*]acridine-3,4-dihydrodiol and trans-dibenz[*a*,*j*]acridine-5,6-dihydrodiol), dibenz[*a*,]acridine-5,6-epoxide, and two phenols, 3-hydroxy- and 4-hydroxydibenz[*a*,*j*]acridine; the 1,2-dihydrodiol was not detected. Additional secondary metabolites, for which unequivocal characterization or strong structural evidence could be provided, included the 3,4,10,11-tetrols, 3,4,8,9-tetrols, and 1,2,3,4-tetrols, the 3,4-dihydrodiol-8,9-epoxide and the 5,6-dihydrodiol-8,9-epoxide (no stereochemical information provided), the *syn*- and *anti*-3,4-dihydrodiol-1,2-epoxide (bay-region diol-epoxide), dibenz[*a*,*j*]acridine-5,6,8,9-diepoxide, and incompletely characterized phenolic 3,4- and 5,6-dihydrodiols. The metabolite profiles in liver and lung microsomal preparations were very similar: *trans*-dibenz[*a*,*j*]acridine-3,4-dihydrodiol was the major metabolite (30–40%), dibenz[*a*,*j*]acridine-5,6-epoxide was the second most abundant metabolite, and the two phenols, particularly the 4-hydroxy isomer, were also present in significant proportions (Gill *et al.*, 1986, 1987). In addition to the previously mentioned metabolites, dibenz[*a*,*j*]acridine-*N*-oxide was detected as a minor product (about 1%) in similar incubations conducted with liver microsomes from rats that were not induced, or rats that had been induced with phenobarbital. Incubation of rat liver microsomes from uninduced or phenobarbital-induced rats in the presence of 3,3,3-trichloropropene-1,2-oxide (1.5 mM), an inhibitor of epoxide hydrolase, led to an approximately 20% decrease in the extent of total metabolism and the amount of *trans*-dibenz[*a*,*j*]acridine-3,4-dihydrodiol was reduced 30–40 times. Induction of epoxide hydrolase by pretreatment with *trans*-stilbene oxide failed to produce a clear decrease in the proportion of dibenz[*a*,*j*]acridine-5,6-epoxide compared with the control experiments (Gill *et al.*, 1987). Further metabolism of *trans*-dibenz[*a*,*j*]acridine-3,4-dihydrodiol in 3-methylcholanthrene-induced liver microsomes led predominantly to the 3,4-dihydrodiol-8,9-epoxide and a phenolic 3,4-dihydrodiol (44.4%, combined); the bay-region diol-epoxides

accounted for approximately 6% of the total metabolites (Gill *et al.*, 1987).

Similar studies conducted with liver microsomes from male Sprague-Dawley rats and female Hsd:(ICR)BR mice induced with 3-methylcholanthrene yielded essentially the same type of metabolic profile, although the specific metabolite distributions were not as extensively differentiated as indicated above; the only noteworthy difference was the identification of small amounts of the previously undetected 1,2-dihydrodiol (stereochemistry not specified). Treatment of rats and mice with 3-methylcholanthrene led to statistically significant increases ($P \leq 0.05$) in liver microsomal metabolism to dihydrodiols and phenols compared with animals treated with corn oil, similar to observations in parallel incubations conducted with benzo[*a*]pyrene as positive control (Wan *et al.*, 1992). The use of synchronous fluorescence spectroscopy yielded quantitative data on metabolite compositions in good agreement with those obtained from radioactivity measurements (Schneider *et al.*, 1994).

The absolute configurations of the two major dibenz[*a*,*j*]acridine metabolites formed with liver microsomes from uninduced, phenobarbital-induced and 3-methylcholanthrene-induced male Wistar rats or male SW mice were established on the basis of comparison with synthetic standards. About 63–70% of 3,4-dihydrodiol was in the (–)-*3R*,*4R* configuration regardless of species or treatment. In contrast, the 5,6-epoxide was predominantly present as the *5R*,*6S* isomer in uninduced and phenobarbital-induced preparations (60% and 75%, respectively, with the mouse liver microsomes; 81% and 79%, respectively, with rat liver microsomes). A reversed stereochemical preference was found in preparations from both species induced with 3-methylcholanthrene, with highly stereoselective formation of the *5S*,*6R* isomer dihydrodiol (91% with mouse liver microsomes and 95% with rat liver microsomes) (Duke & Holder, 1988; Duke *et al.*, 1988).

Trans-dibenz[*a*,*j*]acridine-3,4-dihydrodiol was also the major metabolite (57.8 ± 2.6%) produced in incubations of dibenz[*a*,*j*]acridine with human liver microsomes (Sugiyanto *et al.*, 1992). Human CYP1A1, CYP1A2, CYP3A4, and CYP3A5 catalysed the formation of *trans*-dibenz[*a*,*j*]acridine-3,4-dihydrodiol; the CYP3A4 isoform was the most selective for this metabolite, whereas CYP1A2 was selective for K-region 5,6-oxidation. Regardless of the specific CYP, the 3,4-dihydrodiol had a *3R*,*4R*-configuration, with an optical purity of close to 100%. Likewise, the K-region 5,6-dihydrodiol of dibenz[*a*,*j*]acridine was formed by CYP1A1 and CYP1A2 as the *R*,*R* diastereomer with an optical purity of almost 100%, while dibenz[*a*,*j*]acridine-5,6-epoxide was formed by CYP1A1 predominantly as the *5S*,*6R* isomer (80%), as observed with liver microsomes from rodents induced with 3-methylcholanthrene (Roberts-Thomson *et al.*, 1995).

Dibenz[*a*,*j*]acridine metabolism occurred readily *in vitro* in hepatocytes from male Wistar rats pre-treated with phenobarbital, or 3-methylcholanthrene, or untreated, with the formation of water-soluble conjugates and non-conjugated metabolites. The water-soluble metabolites accounted for > 50% of the total when 80% of the substrate had been metabolized by hepatocytes from rats induced with 3-methylcholanthrene. Hydrolysis of the cell homogenates with β-glucuronidase/aryl sulfatase before extraction with ethyl acetate resulted in a decrease of only 10% in water-soluble radiolabel, indicating that this fraction was mostly composed of thioether conjugates. This was further confirmed by preincubation of diethyl maleate with hepatocytes from rats induced with 3-methylcholanthrene, which decreased the glutathione concentrations by 56%, with a concomitant increase in the total organic solvent-soluble radioactivity (to 75% in the absence of enzymatic hydrolysis and 80% with β-glucuronidase/aryl sulfatase treatment). The major metabolites present in the organic solvent-soluble fraction, with or without

β-glucuronidase and arylsulfatase hydrolysis, were 3- and 4-hydroxy-dibenz[*a*,*j*]acridine and *trans*-3,4-dihydro-3,4-dihydroxydibenz[*a*,*j*]acridine; the 3,4-dihydrodiol accounted for 34–66% of the total organic solvent-soluble metabolites. Contrary to observations with rat liver microsomes, the K-region 5,6-epoxide and the 5,6-dihydrodiol were minor metabolites in the hepatocyte incubations. Increased hepatocyte densities (10^7 cells per mL) and prolonged incubation times led to a higher extent of metabolism, which was associated with increased DNA binding and protein binding of the radiolabel. At the end of the incubation period, the 3,4-dihydrodiol had undergone substantial metabolism, but the specific structures of the secondary metabolites were not elucidated (Robinson *et al.*, 1990).

Quantitative comparisons of total dibenz[*a*,*j*]acridine metabolism by preparations of liver microsomes or S9 from male Sprague-Dawley rats or female Hsd:(ICR)BR mice pretreated with different inducers (3-methylcholanthrene, Aroclor 1254, dibenz[*a*,*j*]acridine itself, DBC, or phenobarbital) were conducted to assess whether metabolism occurred by PAH- or aromatic amine-type biotransformation. The results indicated that with liver preparations from both species, dibenz[*a*,*j*]acridine was metabolized by a set of enzymes in microsomes similar to those that metabolize other PAHs (Warshawsky *et al.*, 1996a). [The Working Group noted that this reference was a review; it was not clear whether the original data were reported elsewhere].

4.4.2 Genotoxicity and other relevant effects

Dibenz[*a*,*j*]acridine was mutagenic in *S. typhimurium* TA98 and TA100 at concentrations as low as 5 μg/plate in the presence of an exogenous metabolic system (McCann *et al.*, 1975; Kitahara *et al.*, 1978; Baker *et al.*, 1980; Ho *et al.*, 1981); while a negative result was obtained for induction of unscheduled DNA synthesis in primary rat hepatocytes *in vitro* (Probst *et al.*, 1981).

Dibenz[*a*,*j*]acridine was tested for clastogenicity in a Chinese hamster fibroblast cell line. Chromosomal aberrations were produced in the presence, but not in the absence, of an S9 metabolic-activation system (Matsuoka *et al.*, 1982).

Dibenz[*a*,*j*]acridine and several of its metabolites were tested for mutagenicity in *S. typhimurium* TA98 and TA100, using S9 fractions from the livers of male Sprague-Dawley rats induced with Aroclor 1254 or of guinea-pigs induced with 3-methylcholanthrene. The latter was also used as the activation system for V79 Chinese hamster lung cells. Dibenz[*a*,*j*]acridine was mutagenic in TA100 over a dose range of 2–16 nmol/plate. Within the same dose range, 4-hydroxydibenz[*a*,*j*]acridine and 6-hydroxydibenz[*a*,*j*]acridine, the 5,6-epoxide, and the *N*-oxide were not mutagenic. Among the test compounds requiring the guinea-pig metabolic-activation system, which included 1,2-dihydrodiol, 3,4-dihydrodiol, and 5,6-dihydrodiol, the 3,4-dihydrodiol was the most mutagenic, both in TA100 and in V79 cells. No differences in mutagenicity were observed in TA100 between the 3,4-dihydrodiol enantiomers or the racemic mixture. In V79 cells, only the *3R*,*4R*-dihydrodiol was active, the activity being approximately three times that of the racemic mixture. The 1,2-dihydrodiol was the most mutagenic in TA98. Much weaker responses were obtained in TA100 when the guinea-pig metabolic-activation system was replaced by that from rats pre-treated with Aroclor 1254; under these conditions, no activity was detected in TA98 with any of the compounds. The most mutagenic compounds in mammalian cells and bacteria were the bay-region diol-epoxides, which did not require metabolic activation. *anti*-Dibenz[*a*,*j*]acridine-3,4-dihydrodiol-1,2-epoxide was more mutagenic than its *syn* isomer in all the cell systems tested. These results indicated a mutagenicity pattern comparable to those observed in PAHs (Bonin *et al.*, 1989).

Dibenz[*a*,*j*]acridine was analysed for cytotoxic and genotoxic effects on human lymphocytes. An

effect on the frequency of micronucleus formation above that in controls was observed only at the higher concentrations (5 and 10 μg/mL). Cytotoxicity was moderate, as indicated by a 28% decrease in the mitotic index at the highest concentrations (Warshawsky *et al.*, 1995a).

Epithelial cells from the buccal mucosa of Wistar rats were demonstrated to metabolize dibenz[*a*,*j*]acridine to DNA-binding species. Upon incubation with [^{14}C]dibenz[*a*,*j*]acridine (1.6 μM) for 18 hours, covalent binding was determined to be 4.5 ± 0.3 pmol per 10 mg DNA by liquid-scintillation counting (Autrup & Autrup, 1986).

Using ^{32}P-postlabelling, the DNA-adduct patterns and organ distributions were investigated in rodents given dibenz[*a*,*j*]acridine by topical application. Comparison of DNA binding in weanling female Sprague-Dawley rats, ICR mice and Syrian hamsters showed qualitatively similar profiles for the three species, with two main adducts being observed. Although liver and kidney were investigated, DNA binding occurred almost exclusively in the skin. Based upon relative adduct labelling, mice displayed the highest levels of DNA adducts (Li *et al.*, 1990).

Subsequent studies in female Hsd:(ICR)BR mice confirmed the almost exclusive formation of DNA adducts from dibenz[*a*,*j*]acridine in the skin, in agreement with its pattern of carcinogenicity. After topical application of the parent compound and of the *trans*-1,2-, 3,4-, and 5,6-dihydrodiols and subsequent DNA isolation, ^{32}P-postlabelling was conducted under conditions of limiting [^{32}P]ATP. The highest level of binding to skin DNA was shown by the 3,4-dihydrodiol. Dibenz[*a*,*j*]acridine formed two adducts in the skin, which were identical to those obtained from the 3,4-dihydrodiol. Two chromatographically different adducts, which were not produced by the parent compound, were detected upon application of the 5,6-dihydrodiol. No adducts from the 1,2-dihydrodiol were detected. When the nuclease P1 digestion enrichment procedure was used, 3,4-dihydrodiol gave rise to the formation of all four adducts. These results were consistent with formation of the 3,4-dihydrodiol as the major route of activation of dibenz[*a*,*j*]acridine leading to DNA binding in the skin, with subsequent metabolism to a bay-region diol-epoxide. An additional pathway to DNA-binding species may involve the 3,4,5,6-bis-dihydrodiol-1,2-oxide (Roh *et al.*, 1993; Talaska *et al.*, 1995). [The Working Group noted that routes of administration other than topical application have not been investigated for DNA adducts.]

In the presence of liver microsomes from rats treated with 3-methylcholanthrene, dibenz[*a*,*j*]acridine was shown to bind to calf thymus DNA, yeast RNA, and the polynucleotides polyG, polyA, polyU, and polyC. Among the polynucleotides, the greatest extent of binding was observed with polyG. The relative extents of binding of dibenz[*a*,*j*]acridine to the four polynucleotides were similar to those obtained with benzo[*a*]pyrene. Analysis of the effect of different modifiers (α-naphthoflavone, 3,3,3-trichloropropene-1,2-oxide, cyclohexene oxide, and styrene oxide) upon the binding levels revealed that the binding of dibenz[*a*,*j*]acridine to polyG was dependent upon a microsomal hydroxylating-enzyme system (Warshawsky *et al.*, 1996a).

In a more recent study, female Hsd:(ICR) BR mice were given dibenz[*a*,*j*]acridine (300 μg) and synthetic (+/–)-*anti*-dibenz[*a*,*j*]acridine-3,4-dihydrodiol-1,2-epoxide (50 μg), applied to the back. The mice were killed 48 hours later and the skin DNA was analysed by ^{32}P-postlabelling. Four adducts were formed *in vivo*. For comparison, the synthetic diol-epoxide was reacted *in vitro* with purine nucleotides (3′- and 5′-deoxyadenosine monophosphate [dAMP], 3′- and 5′-deoxyguanosine monophosphate [dGMP]) and calf thymus DNA. The synthetic 3′-dAMP adduct and 94% of the calf thymus DNA adducts formed from the diol-epoxide were chromatographically identical to the major (89%) adduct

from the same diol-epoxide *in vivo*. On the other hand, 86% of the synthetic dGMP adducts formed from the diol-epoxide were chromatographically consistent with the major (> 50%) adduct obtained *in vivo* upon application of dibenz[*a*,*j*]acridine (Xue *et al.*, 2001).

4.4.3 Mechanistic considerations

Data on ionization potentials were used to predict the metabolic activation of carcinogenic PAHs. In the case of dibenz[*a*,*j*]acridine, a high ionization potential (about 8.0 eV) is consistent with a mono-oxygenation pathway, rather than one-electron oxidation (Xue *et al.*, 1999).

Dibenz[*a*,*j*]acridine was found to be a moderate inducer of hepatic 7-ethoxyresorufin *O*-deethylase (EROD) activity in Ah-responsive C57BL/6J mice. EROD activity was closely related to the levels of expression of liver CYP1A1 and CYP1B1 when data from a series of 23 test PAHs were combined (Shimada *et al.*, 2003). The data on EROD activities in mice contrasted with those for recombinant human enzymes, where dibenz[*a*,*j*]acridine was a potent inhibitor of CYP1A1, CYP1A2, and particularly CYP1B1, with IC_{50} [concentration at which activity is inhibited by 50%] values of 56 ± 7, 41 ± 8, and 15 ± 2 nM, respectively (Shimada & Guengerich, 2006).

When dibenz[*a*,*j*]acridine was applied following procedures known to induce skin papilloma and carcinoma on the back of mice, A to T and G to T transversions were found in codons 12, 13, and 61 of the Ha-*Ras* gene in papillomas and carcinomas. The mutational spectra in the Ha-*Ras* gene were consistent with the observed binding of dibenz[*a*,*j*]acridine to dG and dA in DNA *in vivo* (Xue *et al.*, 2001).

When tested in the freshwater green alga, *Selenastrum capricornutum*, under different light sources, dibenz[*a*,*j*]acridine was not phototoxic. In comparison, benzo[*a*]pyrene was phototoxic, as a result of photochemical production of quinones (Warshawsky *et al.*, 1995b).

4.5 Dibenz[*c*,*h*]acridine

4.5.1 Metabolism and distribution

Reports on studies of the metabolism of dibenz[*c*,*h*]acridine are limited. The compound has two identical bay regions expected to undergo bioactivation.

Two enantiomerically pure *trans*-3,4-dihydrodiols and the racemic mixture were assessed for metabolism by rat liver enzymes. The racemic dihydrodiol was metabolized at a rate of 2.4 nmol/nmol CYP1A1 per minute with liver microsomes from immature male Long-Evans rats treated with 3-methylcholanthrene. This rate was more than 10 times that observed with liver microsomes from uninduced rats or rats treated with phenobarbital. The major metabolites (68–83%) were a diastereomeric pair of *bis*-dihydrodiols having the new dihydrodiol group at the 8,9-position. The tetrols derived from the bay-region 3,4-dihydrodiol-1,2-epoxides accounted for 15–23% of the total metabolites. A small amount of a phenolic dihydrodiol, formed from the 3,4-dihydrodiol-8,9-epoxide, was also detected. The assignment of a phenolic structure was based on the pH-dependence of the UV spectrum and on the mass spectral information. Although the specific position of the new hydroxyl group was not assigned unequivocally, formation of the 9-hydroxy isomer was assumed, since hydroxylation at the 8-position would involve an unstable intermediate with a resonance contributor bearing a positive charge on the nitrogen. The rate of metabolite formation by a highly purified mono-oxygenase system reconstituted with CYP1A1 and epoxide hydrolase (17 nmol of metabolites/nmol of CYP1A1 per minute) was considerably higher, although the metabolite profile was very similar to that observed with liver microsomes from rats treated

wih 3-methylcholanthrene. Stereoselective formation of the 3,4-dihydrodiol-1,2-epoxide was inferred from the absolute configurations of the tetrols. The (+)-(3*S*,4*S*)-dihydrodiol yielded predominantly the *syn*-diol-epoxide, whereas the (–)-(3*R*,4*R*)-dihydrodiol gave mainly the *anti*-diol-epoxide. The major *bis*-dihydrodiol metabolites (dibenz[*c*,*h*]acridine-3,4,8,9-bis-dihydrodiol) had the same absolute configuration at the 8,9-position, assumed to be 8*R*,9*R* from analysis of the circular dichroism spectra; this implies the (8*R*,9*S*)-epoxide as their precursor (Adams *et al.*, 1999).

Microspectrofluorimetry on single living cells (mouse embryo 3T3 fibroblasts) was used to compare the metabolic profiles of dibenz[*c*,*h*]acridine, benzo[*a*]pyrene, and 6-aminochrysene. The results indicated similarities between the profiles of dibenz[*c*,*h*]acridine and benzo[*a*]pyrene, and important differences between those of dibenz[*c*,*h*]acridine and 6-aminochrysene, consistent with a PAH-type, rather than aromatic amine-type metabolism, for dibenz[*c*,*h*]acridine. Inhibition of the metabolism of dibenz[*c*,*h*]acridine occurred in the presence of benzo[*a*]pyrene, while dibenz[*c*,*h*]acridine did not inhibit the metabolism of benzo[*a*]pyrene. This indicated that benzo[*a*]pyrene is a better substrate for the metabolizing enzymes under the conditions of the assay (Lahmy *et al.*, 1987).

4.5.2 Genotoxicity and other relevant effects

The mutagenicities of dibenz[*c*,*h*]acridine and the K-region dibenz[*c*,*h*]acridine-5,6-epoxide (racemic mixture) were tested in *S. typhimurium* TA98 and TA100, both in the absence and in the presence of liver microsomal S9 from rats induced with polychlorinated biphenyls. The parent compound was inactive in the absence of metabolic activation, but showed mutagenicity in the presence of metabolic activation (11 and 95 revertants/μg per plate, in TA98 and TA100, respectively). In comparison, benzo[*a*]pyrene induced 80 revertants/μg per plate in TA100, but was more active in TA98. The 5,6-epoxide was inactive without activation, and much less mutagenic than dibenz[*c*,*h*]acridine in the presence of activation (0.7 and 8.5 revertants/μg per plate in TA98 and TA100, respectively). These data indicated that the major pathway of dibenz[*c*,*h*]acridine activation to a mutagen is not through K-region oxidation (Kitahara *et al.*, 1978).

In another study of mutagenicity, dibenz[*c*,*h*]acridine gave positive results in four strains of *S. typhimurium* (TA1535, TA1538, TA98 and TA100) in the presence of liver microsomal S9 from male Sprague-Dawley rats induced with Aroclor 1254. In TA1538, the maximum effect was a 75-times increase in the number of revertants compared with the value for the negative controls, obtained at 5000 μg/plate; in TA100, the number of revertants increased six times above background at a concentration of 4 μg/plate (Anderson & Styles, 1978).

Dibenz[*c*,*h*]acridine was mutagenic in *S. typhimurium* TA100 in the presence of liver microsomes from rats cotreated with phenobarbital and 5,6-benzoflavone at 0–100 μg/plate (Karcher *et al.*, 1985).

Dibenz[*c*,*h*]acridine was tested for clastogenicity in a Chinese hamster fibroblast cell line. Chromosomal aberrations were induced in the presence, but not in the absence, of metabolic activation from S9 (Matsuoka *et al.*, 1982).

The mutagenic activities of the enantiomers of the diastereomeric pair of bay-region dibenz[*c*,*h*]acridine-3,4-dihydrodiol-1,2-epoxides have been evaluated in *S. typhimurium* TA98 and TA100 and in the 8-azaguanine-sensitive Chinese hamster V79–6 cell line, which lacks the capacity for metabolic oxidation of PAHs to mutagens. In both strains of bacteria, the *anti*-diol-epoxide enantiomers [(+)-1*R*,2*S*,3*S*,4*R* and (–)-1*S*,2*R*,3*R*,4*S*] were two to four times more mutagenic than the *syn* [(+)-1*S*,2*R*,3*S*,4*R* and (–)-1*R*,2*S*,3*R*,4*S*] enantiomers. There was not a significant difference in mutagenicity between

the enantiomers of each pair or between each enantiomer and the corresponding racemic mixture. Contrasting with the results in bacteria, the *anti*-(+)-(1*R*,2*S*,3*S*,4*R*)-3,4-dihydrodiol-1,2-epoxide isomer was five to seven times more mutagenic in the mammalian cell line than any of the other dibenz[*c*,*h*]acridine-3,4-dihydrodiol-1,2-epoxides. Purified rat liver epoxide hydrolase did not catalyse the conversion of any of the 3,4-dihydrodiol-1,2-epoxide isomers to inactive products. Additional experiments on bacterial mutagenesis with dibenz[*c*,*h*]acridine and its derivatives requiring metabolic activation were conducted in the presence of hepatic microsomes from immature male Long-Evans rats treated with Aroclor 1254. Among the test compounds, 3,4-dihydrodibenz[*c*,*h*]acridine, the putative precursor of a bay-region tetrahydroepoxide, was activated to the most powerful mutagen. The second most active compound was the (–)-3*R*,4*R*-dihydrodiol. Of the three metabolically possible *trans*-dihydrodiols (at the 1,2- 3,4- and 5,6- positions; all tested as racemic mixtures), dibenz[*c*,*h*]acridine-3,4-dihydrodiol was the most mutagenic; it was also considerably more mutagenic than the parent compound. The 1,2,3,4-tetrahydro-3,4-diol, which lacks the bay-region 1,2 double bond, was not activated to a mutagen. These observations are consistent with metabolic activation of dibenz[*c*,*h*]acridine to a bay-region diol-epoxide (Wood *et al.*, 1986).

4.5.3 Mechanistic considerations

Activation of dibenz[*c*,*h*]acridine to a bay-region diol-epoxide is consistent with the mutagenicity data and with the relative tumorigenicities of the parent compound and several of its metabolites (see Section 3). In agreement with predictions of the bay-region theory, the 3,4-dihydrodiol is a proximate carcinogen and the bay-region 3,4-dihydrodiol-1,2-epoxides are ultimate carcinogens. The data on metabolism by rat liver microsomes suggested that a high level of CYP1A1 activity may confer increased susceptibility to dibenz[*c*,*h*]acridine-induced carcinogenesis.

The rates of solvolysis of the dibenz[*c*,*h*]acridine-3,4-dihydrodiol-1,2-epoxides were found to be comparable to those of the corresponding diol-epoxides from dibenz[*a*,*j*]anthracene. This contrasted with much slower rates of solvolysis for the dibenz[*a*,*j*]acridine-3,4-dihydrodiol-1,2-epoxides. These observations were consistent with the fact that the benzylic cation stemming from opening of the dibenz[*a*,*j*]acridine-derived epoxide has a resonance contributor bearing a positive charge on the nitrogen, while this is not the case for the dibenz[*c*,*h*]acridine-derived epoxide (Sayer *et al.*, 1990).

The bay-region (+)-(1*R*,2*S*,3*S*,4*R*)-3,4-dihydrodiol 1,2-epoxide was the most tumorigenic of the four possible isomeric bay-region diol-epoxides from dibenz[*c*,*h*]acridine, both in an initiation–promotion model in mouse skin and in newborn mice. Similar observations have been reported with various carbocyclic PAHs for which the *R*,*S*,*S*,*R* bay-region diol-epoxides typically display high tumorigenic activities (Chang *et al.*, 2000).

The mutational activation of the Ha-*Ras* protooncogene in skin tumours of female CD-1 mice was investigated in an initiation–promotion model using a single application of dibenz[*c*,*h*]acridine (200 nmol), followed 10 days later by long-term treatment with TPA (16 nmol given twice per week for 20–25 weeks). The DNA isolated from carcinoma induced by dibenz[*c*,*h*]acridine efficiently transformed NIH 3T3 cells, and a high percentage of the transformed foci had an amplified Ha-*Ras* gene containing an A to T transversion in the second base of codon 61. The same mutation was detected in DNA from primary tumours in a high percentage of the carcinomas induced by dibenz[*c*,*h*]acridine, and also in NIH 3T3 cells transformed with DNA from benign skin papillomas induced by dibenz[*c*,*h*]acridine. The latter observation suggested that the mutation

is an early event in mouse skin carcinogenesis induced by dibenz[*c*,*h*]acridine. In a concurrent study of complete carcinogenesis in cells treated repeatedly with 7,12-dimethylbenz[*a*]anthracene, an A to T transversion in the second base of codon 61 of the Ha-*ras* gene was also identified. By analogy with 7,12-dimethylbenz[*a*] anthracene, for which metabolic activation to a bay-region diol-epoxide leads to the formation of a deoxyadenosine adduct, the bay-region diol-epoxide from dibenz[*c*,*h*]acridine may bind preferentially to adenine residues in DNA (Bizub *et al.*, 1986).

In a later study, Chinese hamster V79 cells were exposed to high or low concentrations of the highly carcinogenic *anti*-(+)-(*R*,*S*,*S*,*R*) or the less active *anti*-(–)-(*S*,*R*,*R*,*S*) bay-region diol-epoxides of dibenz[*c*,*h*]acridine. Independent 8-azaguanine-resistant clones were isolated, and base substitutions at the hypoxanthine (guanine) phosphoribosyltransferase *(Hprt*) locus were determined. While the proportion of mutations at AT base pairs increased as the concentration of the *anti*-(+)-(*R*,*S*,*S*,*R*) diol-epoxide decreased, concentration-dependent differences in the mutation profile were not observed for the *anti*-(–)-(*S*,*R*,*R*,*S*) diol-epoxide. Similar results were obtained with bay-region diol-epoxides of benzo[*a*]pyrene and benzo[*c*]phenanthrene. In a DNA repair-deficient variant of V79 cells, no concentration-dependent differences were found in the mutation profile induced by the (*R*,*S*,*S*,*R*) diol-epoxide of benzo[*a*]pyrene, suggesting that the occurrence of concentration-dependent differences requires an intact DNA-repair system (Conney *et al.*, 2001).

4.6 Carbazole

4.6.1 Metabolism

3-Hydroxycarbazole has been reported to be a major urinary metabolite of carbazole in rats (Johns & Wright, 1964). In a more recent study, carbazole was characterized as a noncompetitive inhibitor of CYP1A (Wassenberg *et al.*, 2005).

4.6.2 Genotoxicity and other relevant effects

In early studies, carbazole was reported to be inactive when tested for mutagenicity in *S. typhimurium* strains TA1535, TA1538, TA98 and TA100 in the presence of metabolic activation (Anderson & Styles, 1978). Similarly, other studies reported that carbazole was not mutagenic with metabolic activation from S9 in *S. typhimurium* TA98 (Ho *et al.*, 1981) and TM677 (Kaden *et al.*, 1979).

Carbazole was reported to be moderately clastogenic in the bone marrow of Swiss albino mice. At intraperitoneal doses of 25, 50, 100, 150 or 200 mg/kg bw, carbazole caused significant reductions in the mitotic index and increases in chromosomal aberrations at the two higher doses; these effects were observed after treatment for 14 hours, but not after 42 hours (Jha *et al.*, 2002).

Carbazole induced dominant lethality and sperm-head abnormalities in male Swiss albino mice (Jha & Bharti, 2002). In the former test, statistically significantly positive results were reported for mice given carbazole as five daily intraperitoneal doses at 30 or 60 mg/kg bw; in the latter test, there was a significant dose–response relationship in the range of 50–300 mg/kg bw when carbazole was given as a single dose.

Carbazole (50–500 µg/L) caused a twofold induction of EROD activity in embryos of *Fundulus heteroclitus* (killifish; saltwater minnow). The strong stimulation of EROD activity by the AhR agonist β-naphthoflavone (1 µg/L) was considerably diminished upon coincubation of the fish embryos with carbazole (Wassenberg *et al.*, 2005). Although not embryotoxic itself, carbazole enhanced the embryotoxicity of β-naphthoflavone.

4.7 *7H*-Dibenzo[*c,g*]carbazole

4.7.1 Distribution and metabolism

The toxicokinetics of DBC have been reviewed (Xue & Warshawsky, 2005).

In hamsters given a dose of 3 mg per animal by intratracheal instillation, once per week for 5 weeks, DBC passed from the lungs to the intestinal tract and was excreted mainly in the faeces (Nagel *et al.*, 1976). After inhalation as an aerosol at a concentration of 1.1–13 µg/L air for 60 minutes, [^{14}C]-labelled DBC was widely distributed in rat tissues. Within 1 hour after exposure, the highest amounts of radiolabel were observed in the respiratory tract, upper gastrointestinal tract, liver and adrenal glands. Tissue clearance was rapid, with half-lives ranging from 1 to 16 hours. DBC was extensively metabolized, and excreted primarily in the faeces (Bond *et al.*, 1986).

When incubated with liver microsomal fractions from rats or mice pre-treated with 3-methylcholanthrene, DBC was metabolized to twelve different compounds, of which five were identified as mono-hydroxylated derivatives, namely 5-OH-DBC and 3-OH-DBC as major metabolites, and 2-OH-DBC, 4-OH-DBC and 6-OH-DBC as minor products. A dihydrodiol was tentatively identified as 3,4-dihydroxy-3,4-dihydro-DBC (Périn *et al.*, 1981). A subsequent study with rat liver microsomes identified 2-OH-DBC and 3-OH-DBC, but not 4-OH-DBC, as metabolites, while cultured rat hepatocytes also metabolized DBC predominantly to phenols (Stong *et al.*, 1989). In another study by the same research group, mouse and rat liver microsomes were reported to metabolize DBC to 5-OH-DBC, 3-OH-DBC, and 2-OH-DBC, with 1-OH-DBC being formed in trace amounts; dihydrodiols were not detected as metabolites (Wan *et al.*, 1992). On the basis of mass spectral analysis, *N*-OH-DBC was reported to be a major rat-liver microsomal metabolite; it was also formed in an isolated preparation of perfused rabbit lung (Warshawsky & Myers, 1981). However, the formation of this metabolite was not confirmed in subsequent studies (Xue *et al.*, 1993). The same group then used conventional and synchronous fluorescence spectroscopy to identify 1-OH-DBC, 3-OH-DBC, and 5-OH-DBC as metabolites of DBC formed by liver microsomes from rats induced by 3-methylcholanthrene (Schneider *et al.*, 1994).

Converting the phenolic metabolites of DBC, which are relatively unstable, to more stable acetoxy-DBC derivatives by use of acetic anhydride/pyridine facilitates their analysis (Xue *et al.*, 1993). By means of this procedure, the major DBC metabolites formed in microsomes from livers of rats induced with 3-methylcholanthrene were quantified and close agreement was found with the outcome of a radiometric analysis; the order of abundance was 5-OH-DBC > 1-OH-DBC > 3-OH-DBC.

Experiments with knockout mice in which *Cyp1a1, Cyp1a2* or *Cyp1b1* was deleted showed that DBC is metabolized mainly by Cyp1a1 in the liver and by Cyp1a1 and Cyp1b1 in the lung of mice induced with β-naphthoflavone, and by hepatic Cyp1a2 in non-induced mice. Comparison of metabolic profiles generated by different enzymes indicated that Cyp1a1 produces 1-OH-DBC, 2-OH-DBC, and (5+6)-OH-DBC, Cyp1a2 generates mainly (5+6)-OH-DBC, and Cyp1b1 produces 4-OH-DBC. Similar results were obtained *in vitro* with Supersomes™ [microsomes derived from baculovirus-infected insect cells] expressing human CYP1 enzymes (Shertzer *et al.*, 2007).

4.7.2 Genotoxicity and other relevant effects

(a) Mutagenicity in bacterial systems

DBC was reported to induce revertants in *S. typhimurium* TA98 in the presence of metabolic activation from S9 at 2.3 times the spontaneous reversion rate, but the results were not consistent (Salamone *et al.*, 1979). In another study in TA98,

DBC have negative results at concentrations of up to 100 µg/plate and was toxic to TA98 at 250 µg/plate, in the presence of metabolic activation (Ho *et al.*, 1981). In a subsequent study, DBC was reported to be mutagenic in TA100 with metabolic activation from mouse liver S9 (Périn *et al.*, 1988). In another test it was reported that DBC was not mutagenic in TA98 and TA100 either in the absence or the presence of metabolic activation from S9 from livers of rats, mice or hamsters pre-treated with various inducers (Schoeny & Warshawsky, 1987). However, a weakly positive response was observed with *S. typhimurium* TM677 in an assay for forward mutation. In the presence of S9 from rats pre-treated with Aroclor 1254, three phenolic metabolites were also mutagenic in TM677, in the order 3-OH-DBC > 4-OH-DBC > 2-OH-DBC.

(b) Mutagenicity in cell lines

When tested in Chinese hamster V79 cell lines expressing human CYP1A1 or CYP1A2, DBC caused an increase in mutation frequency (6-thioguanine resistance) in both cell lines (Gábelová *et al.*, 2002). In V79 cells expressing CYP1A1, DBC caused a dose-dependent increase in the frequency of micronucleus formation (Farkasová *et al.*, 2001). The effect of DBC in decreasing colony-forming ability in the same cell line, and also in HepG2 cells, correlated with its DNA-damaging activities, as measured with the alkaline DNA-unwinding assay and the modified single-cell gel electrophoresis (SCGE) assay (comet assay) (Gábelová *et al.*, 2000).

DBC and several phenolic metabolites were investigated for mutagenic activity in DPI-3 cells, an epithelial line derived from hamster embryos, co-cultured with rat liver cells. At a concentration of 40 µM, 3-OH-DBC gave 4.4 ± 0.8 mutants per 10^5 survivors, 13c-OH-DBC gave 8.0 ± 3.1 mutants, and DBC itself 8.0 ± 2.8 mutants. In the DMSO control, the number of mutants was 1.0 ± 0.2 per 10^5 survivors. Under these conditions the metabolites 2-OH- and 4-OH-DBC were not mutagenic (Stong *et al.*, 1989).

DBC was mutagenic (inducing resistance to 6-thioguanine) in human DNA repair-deficient *Xeroderma pigmentosum* cells that were co-cultured with human Hs703T cells (an epithelial cell line derived from a human liver carcinoma) to provide a source of metabolizing activity (Parks *et al.*, 1986).

DBC induced a dose-dependent increase in the frequency of micronucleus formation in cultured lymphocytes from two donors (Warshawsky *et al.*, 1995a).

In tests for mutagenic activity *in vivo* in the Muta™Mouse, DBC induced a 30 times increase in the frequency of *LacZ* mutants in the liver, 28 days after a single subcutaneous injection, and a 3.4 times increase in the frequency of mutation in the skin, 28 days after a single topical application (Renault *et al.*, 1998).

(c) Formation of DNA adducts

Many studies have investigated the formation of DNA adducts by DBC and its metabolites, both *in vitro* and *in vivo*. Most of these studies have used sensitive ^{32}P-postlabelling analysis for detection and characterization of DNA adducts.

In one of the earliest studies of this type, female mice received DBC as a single subcutaneous injection at 44 µmol/kg bw, which resulted in very high adduct levels in the liver, relative to levels in other tissues (Schurdak & Randerath, 1985). The order of binding was liver >> kidney > lung > spleen > skin > brain, the level in liver being approximately 25 times higher than that in kidney.

In a time-course and dose–response study on the formation of DBC–DNA adducts in the lung of mice given DBC as a single intraperitoneal injection at 0, 5, 10, 20 or 40 mg/kg bw, the highest adduct levels were found at 40 mg/kg bw after 5–7 days. At lower doses, the maximum levels shifted to earlier time-points (1–3 days). Up to

seven adducts were detected by ^{32}P-postlabelling analysis (Warshawsky *et al.*, 1996b).

The patterns of DNA adducts in the skin and liver of mice treated topically or intraperitoneally with DBC, 2-OH-, 3-OH- or 4-OH-DBC were compared. In liver, the patterns from DBC and 3-OH-DBC were similar to each other, and distinct from those formed by 2-OH-DBC and 4-OH-DBC. On the other hand, in skin none of the phenolic metabolites produced an adduct pattern that resembled that of DBC; and the pattern produced by DBC in skin was different from that produced by DBC in liver. Thus DBC is activated by a pathway that involves metabolism to 3-OH-DBC in the liver, but a different pathway appears to be involved in the skin (Schurdak *et al.*, 1987a).

Levels of DNA adducts in mouse liver after topical or intraperitoneal administration of *N*-methyl-DBC were ~300 times lower than after treatment with DBC, but the difference was only about twofold in skin (Schurdak *et al.*, 1987b). The adduct patterns formed by the two compounds in liver were qualitatively similar; in skin the adduct pattern elicited by either compound was different from that seen in the liver, and the two patterns were substantially different from each other. *N*-Methyl-DBC bound preferentially to skin DNA, DBC bound preferentially to liver DNA. These results were in accordance with the target-organ specificity for carcinogenicity of the two compounds, and also indicated that a non-substituted nitrogen is required for genotoxicity in mouse liver, but not in mouse skin (see also Talaska *et al.*, 1994). Regardless of the route of administration (topical, oral or subcutaneous), DNA-adduct formation by DBC in mouse liver was always substantially higher (~10–140 times) than in other tissues (kidney, lung and skin) (Schurdak & Randerath, 1989). Microsomal activation of DBC *in vitro* in the presence of polynucleotides indicated that guanine moieties in DNA were the principal sites of modification and that binding of DBC can occur both via the nitrogen atom and through the 1,2,3,4-ring of the molecule (Lindquist & Warshawsky, 1989).

Among seven different phenolic derivatives of DBC, 3-OH-DBC gave rise to adducts that were similar to those formed by DBC itself; 4-OH-DBC also induced substantial adduct formation, albeit with a different pattern. In addition, 2-OH-DBC induced a low level of adducts, while 1-OH-DBC, 5-OH-DBC, 6-OH-DBC, and 13c-OH-DBC did not give rise to any detectable formation of adducts (Talaska *et al.*, 1994).

In a comparison with benzo[*a*]pyrene (a skin carcinogen), uptake of DBC from skin was found to be 70% higher than for benzo[*a*]pyrene over the first 24 hours after topical application. As a result, binding to skin protein and DNA was higher for benzo[*a*]pyrene, while binding to liver protein and DNA was higher for DBC. The amounts of protein adducts in blood were similar for the two compounds (Meier & Warshawsky, 1994).

DBC formed DNA adducts in primary mouse embryo cells (Gábelová *et al.*, 1997).

The formation of adducts by DBC was investigated in liver DNA from female mice, with separate examination of mitochondrial and nuclear DNA. At 24 hours after an intraperitoneal dose of DBC at 5 mmol/kg bw, the levels of adducts in nuclear DNA were twofold those in mitochondrial DNA; at 48 hours, the amount of adducts in nuclear DNA had decreased and that in mitochondrial DNA had increased, such that the two values were similar. At a higher dose of 15 mmol/kg bw, similar levels of adducts were observed in nuclear and mitochondrial DNA at 24 hours, although this dose and a higher dose (30 mmol/kg bw) were cytotoxic to liver cells (Périn-Roussel *et al.*, 1995). Subsequently, levels of DNA adducts were compared in parenchymal and non-parenchymal cells of the liver of mice given DBC at an intraperitoneal dose of 5 mmol/kg bw for 48 hours. Both cell types showed formation of DBC–DNA adducts, although the

amount was higher by nearly 15-fold in parenchymal cells (Périn-Roussel *et al.*, 1997).

DBC was tested for DNA-adduct formation and other DNA-damaging effects in WB-F344 progenitor cells from the rat liver (Valovicová *et al.*, 2009). Exposure to DBC at 10 µM for 24 hours led to formation of DBC–DNA adducts at a frequency of 56.3/10^8 nucleotides, as measured by ^{32}P-postlabelling. In the dose range 0.1–20 µM, DBC induced a dose-dependent increase in DNA breakage detected in the comet assay, and caused a statistically significant increase in the frequency of micronucleus formation at concentrations of 0.5–2.5 µM. DBC did not give rise to additional DNA damage in the comet assay in the presence of formamidopyrimidine-DNA glycosylase/AP endonuclease (Fpg endonuclease), which suggests that the lesions observed were not the result of oxidative damage.

Repeated topical administration of DBC at low doses to the dorsal skin of mice caused a steady increase in the formation of liver DNA adducts, which eventually reached a plateau. The early increase in levels of DNA adducts was not accompanied by stimulation of DNA synthesis or histological signs of cell proliferation, these effects becoming evident only after several treatments had been given and a certain level of DNA adducts had accumulated (Dorchies *et al.*, 2001).

Topical application of DBC produced significantly higher amounts of adducts in liver DNA of the Car-R mice (a mouse strain resistant to skin carcinogenesis) than in Car-S mice (a strain susceptible to skin carcinogenesis) (Périn *et al.*, 1998).

Topical application of DBC induced DNA adducts in mouse skin and liver, levels being considerably higher in the liver. DBC weakly induced CYP1A2, but had no effect on the expression of CYP1A1 in these tissues (Taras-Valéro *et al.*, 2000).

Incubation of DBC with horseradish peroxidase or rat-liver microsomes gave rise to radical cation formation and yielded, in the presence of DNA, several instable "depurinating" DNA adducts. These were identified as being guanine derivatives modified at the N7 position bound to the 5- or 6-position of DBC, or the N3 or N7 position of adenine bound to the 5-position of DBC (Chen *et al.*, 1997). The DBC–5-N7-Gua adduct was detected *in vivo* in the liver of mice treated with DBC, but it accounted for only ~0.4% of the total, the remainder being stable, covalently-modified nucleotide adducts (Dowty *et al.*, 2000).

As a step towards adduct characterization, HPLC was used to separate five of the seven DBC–DNA adducts in mouse liver that were detected by ^{32}P-postlabelling analysis and partially resolved by multidirectional thin-layer chromatography (O'Connor *et al.*, 1997). One of the DBC–DNA adducts formed in mouse liver was subsequently identified as being chromatographically identical to one of the synthetic adducts formed upon reaction between the reactive DBC-3,4-dione and nucleic acid bases and nucleotides, products that were characterized by mass spectrometry and nuclear magnetic resonance. These analyses suggested that the 4-NH2 position of cytosine was the site of adduction, and the adduct was identified as *N*4-[3,4-dione-DBC-1-yl]-Cyt (Xue *et al.*, 2002).

In Chinese hamster V79 cell lines stably expressing human metabolic enzymes, DBC induced higher levels of DNA adducts in cells that expressed CYP1A1 than in those expressing CYP1A2 (24.5 ± 7.2 *versus* 0.7 ± 0.2 adducts/10^8 nucleotides). In the parental cell lines, which are devoid of CYP activity, no DNA adducts were formed. DBC induced micronucleus formation in the CYP1A2-expressing cells in a dose-dependent manner, and gave also a positive response in the comet assay with endonucleases Fpg and EndoIII, suggesting that oxidative damage, rather than DNA-adduct formation, may be responsible for the genotoxic activity observed (Gábelová *et al.*, 2004).

In Chinese hamster V79 cells stably expressing human CYP3A4, DBC formed DNA adducts at a low level (0.25 ± 0.18 adducts/10^8 nucleotides at 10 µM). It also induced micronucleus formation and *Hprt* mutation (at the highest dose only) in these cells, which suggested that CYP3A4 plays a role in the metabolic activation of DBC (Mesárošová *et al.*, 2011).

DBC induced DNA adducts, DNA damage (as detected by the comet assay), and micronucleus formation in human hepatoma HepG2 cells. These effects were accompanied by induction of *CYP1A1/2* and *CYP1B1* mRNA (Gábelová *et al.*, 2011).

In studies *in vivo* comparing mice lacking Cyp1a2 activity (*Cyp1a2*$^{-/-}$) and mice lacking AhR activity (*Ahr*$^{-/-}$) with wildtype mice, no significant difference was found in the extent of DNA-adduct formation in lung, skin and liver after topical application of DBC. In contrast, the formation of DNA adducts was significantly reduced in both types of knockout mouse after topical application of benzo[*a*]pyrene (Talaska *et al.*, 2006). When the compound was given by intraperitoneal administration, the level of DBC–DNA adducts in liver and lung was significantly higher in *Ahr*$^{(-/-)}$ mice than in wildtype mice given the compound by intraperitoneal administration (Shertzer *et al.*, 2007).

DBC has been shown to form covalent DNA adducts in the liver of English sole (*Pleuronectes vetulus*) (Stein *et al.*, 1993) and the liver, intestinal mucosa, gills and brain of northern pike (*Esox lucius*) (Ericson *et al.*, 1999; Ericson & Balk, 2000).

(d) Cell death and cell proliferation

Dependent on concentration, DBC caused both necrosis (at ~80 µM) and apoptosis (at < 1 µM) in HepG2 human hepatoma cells (O'Brien *et al.*, 2000). Subsequent studies by these authors demonstrated that human liver-cell lines differ in their ability to metabolize the compound to toxic species and that apoptosis is only observed when detectable metabolites and DNA adducts are formed (O'Brien *et al.*, 2002).

Induction of apoptosis by DBC in mouse liver *in vivo* was accompanied by an increase in expression of *Bax* mRNA and Bax protein, as well as upregulation of TGFβ1 in parenchymal cells; another change related to cell proliferation included overexpression of *Bcl2*, an anti-apoptotic gene (Martín-Burriel *et al.*, 2004).

DBC weakly induced AhR in WB-F344 rat-liver epithelial cells (a model of liver-progenitor cells *in vitro*) and it inhibited gap-junctional intercellular communication (Vondrácek *et al.*, 2006).

(e) Mutational spectrum in tumours

Tumours induced in the lungs of A/J mice given DBC at a dose of 5–40 mg/kg bw by intraperitoneal injection carried mutations in the K-*Ras* gene in 46 out of 49 cases. Of these, 35 (76%) had an AT to TA transversion in the third base of codon 61. The mutation spectrum was the same for tumours induced by DBC at a dose of 5, 20 or 40 mg/kg bw (Warshawsky *et al.*, 1996b). In a subsequent study, lung tumours in A/J mice given DBC at a dose of 10 mg/kg bw by intraperitoneal injection also had a high frequency (83%) of K-*Ras* mutations. Ten of the twelve tumours analysed had a detectable mutation; seven mutations were found at codon 61: all were AT to TA transversions (six were CAA to CAT; one was CAA to CTA) (Gray *et al.*, 2001).

When DBC was administered topically at a dose of 50 nmol or 100 nmol to the dorsal skin of Hsd:(ICR)BR mice twice per week for up to 70 weeks, the tumours induced in the skin and liver had a high frequency of Ha-*Ras* mutations (67% of skin tumours at both doses; 45% of liver tumours at the higher dose, but none [0 out of 10] at the lower dose). In all cases, the mutations were AT to TA transversions in the second base of codon 61 (CAA to CTA) (Mitchell & Warshawsky, 1999).

In Hsd:(ICR)BR mice given a single topical treatment of DBC at 200 nmol applied to the dorsal skin, followed by multiple applications of TPA at 2 mg twice per week for 28 weeks, the skin papillomas induced were frequently mutated at Ha-*Ras* (Mitchell & Warshawsky, 2001). Of the papillomas tested, 71% had mutations at codon 61, 4% had mutations at codon 12, 4% had mutations at codon 13 and 21% did not carry Ha-*Ras* mutations. CAA to CTA transversions accounted for all the mutations at codon 61. The mutation at codon 12 was a GGA to GAA transition, and the mutation at codon 13 was a GGC to GTC transversion.

Ionization potentials have been used to predict the metabolic activation of carcinogenic PAHs. Those with a high ionization potential are likely to proceed via mono-oxygenation. Carbazoles, which may be activated via one-electron transfer, mono-oxygenation or a combination of both, have lower ionization potentials than, for example, acridine derivatives, which are activated through mono-oxygenation. Thus ionization potentials predict to a certain extent the pathways of activation of carcinogenic *N*-heterocyclic PAHs (Xue *et al.*, 1999).

4.7.3 Structure–activity considerations for carbazole derivatives

Table 4.1 shows the relative carcinogenic potencies of carbazole and DBC derivatives (Warshawsky, 1992; see also references therein).

When administered in the diet, carbazole is a carcinogen in the liver and forestomach. None of the benzo[*a*]carbazoles are strong carcinogens: there is weak activity observed in skin for 7*H*-benzo[*a*]carbazole and slightly higher activity for the 10-methyl derivative, when injected subcutaneously. DBC is a potent skin carcinogen when administered topically or subcutaneously, and a potent liver carcinogen when given orally or subcutaneously, also inducing pulmonary and forestomach tumours.

Addition of a methyl group at the 7-position of DBC decreases the carcinogenic activity when applied topically, but not when injected subcutaneously. 5-Methyl-DBC, 6-methyl-DBC, 6,8-dimethyl-DBC and *N*-acetyl-DBC are potent carcinogens when given subcutaneously, but of these only *N*-acetyl-DBC is a liver carcinogen when injected intraperitoneally. 3-Methyl-DBC, 5,9-dimethyl-DBC and 5,9-diethyl-*N*-acetyl-DBC are active in liver, but not in subcutaneous tissue, as are 3-methoxy-DBC, 3-acetoxy-DBC and 3-hydroxy-DBC. 4-Methoxy-DBC and 4-acetoxy-DBC are active in both tissues, while 3,11-dimethyl-DBC and 5,9,*N*-trimethyl-DBC are inactive in both. These data suggested that the 5-, 6-, and 7-positions of DBC are involved in its sarcomagenic activity, while the 3- and/or the 5- and the 9-positions are involved in the hepatocarcinogenic activity of the compound.

4.8 Dibenzothiophene

4.8.1 Metabolism

The metabolism of dibenzothiophene was studied with liver microsomes from rats pretreated with 3-methylcholanthrene, phenobarbital, dibenzothiophene or Aroclor 1254 (Vignier *et al.*, 1985). Two metabolites were identified: dibenzothiophene-5-oxide (dibenzothiophene sulfoxide), the major metabolite, and dibenzothiophene-5,5-dioxide (dibenzothiophene sulfone). No metabolites involving oxidation of carbon–carbon bonds were identified. Induction with 3-methylcholanthrene, phenobarbital, and Aroclor 1254 strongly enhanced the formation of dibenzothiophene sulfoxide, while dibenzothiophene had no effect as an inducer. In subsequent studies, the same authors showed that dibenzothiophene sulfoxide was converted to dibenzothiophene sulfone, indicating two sequential oxidation steps at the sulfur atom. The role of CYPs in dibenzothiophene oxidation reactions was also studied. Carbon monoxide, an inhibitor

Table 4.1 Relative carcinogenic activity of carbazoles in mice

Compound	Skin/subcutaneous tissue	Liver
Carbazole		++
11*H*-Benzo[*a*]carbazole	–	
8-Methyl-2-nitrobenzo[*a*]carbazole	–	
7,10-Dimethylbenzo[*a*]carbazole	–	
8-Bromobenzo[*a*]carbazole	–	
8-Chlorobenzo[*a*]carbazole	–	
2-Chlorobenzo[*a*]carbazole	–	
2-Chloro-6-methylbenzo[*a*]carbazole	–	
7*H*-Benzo[*a*]carbazole	+/–	
10-Methylbenzo[*a*]carbazole	+	
7*H*-Dibenzo[*c*,*g*]carbazole	+++	+++
1-Aza-7*H*-dibenzo[*c*,*g*]carbazole	+++	NR
4-Aza-7*H*-dibenzo[*c*,*g*]carbazole	++	NR
N-Methyl-7*H*-dibenzo[*c*,*g*]carbazole	++/+++	–
3-Methyl-7*H*-dibenzo[*c*,*g*]carbazole	–	+++
5-Methyl-7*H*-dibenzo[*c*,*g*]carbazole	+++	–
6-Methyl-7*H*-dibenzo[*c*,*g*]carbazole	+/++	–
3,11-Dimethyl-7*H*-dibenzo[*c*,*g*]carbazole	–	–
5,9-Dimethyl-7*H*-dibenzo[*c*,*g*]carbazole	–	+++
6,8-Dimethyl-7*H*-dibenzo[*c*,*g*]carbazole	+++	–
5,9,*N*-Trimethyl-7*H*-dibenzo[*c*,*g*]carbazole	–	–
N-Acetyl-7*H*-dibenzo[*c*,*g*]carbazole	+++	+++
5,9-Dimethyl-*N*-acetyl-7*H*-dibenzo[*c*,*g*]carbazole	–	+++
5-Acetylamino-7*H*-dibenzo[*c*,*g*]carbazole	+	–
5-Nitro-7*H*-dibenzo[*c*,*g*]carbazole	+/++	–
5,6-Dihydro-7*H*-dibenzo[*c*,*g*]carbazole	+/++	–
2-Methoxy-7*H*-dibenzo[*c*,*g*]carbazole	+/++	NR
3-Methoxy-7*H*-dibenzo[*c*,*g*]carbazole	–	+++
4-Methoxy-7*H*-dibenzo[*c*,*g*]carbazole	+++	++
6-Methoxy-7H-dibenzo[c,g]carbazole	++	–
3,11-Dimethoxy-7*H*-dibenzo[*c*,*g*]carbazole	–	–
3-Acetoxy-7*H*-dibenzo[*c*,*g*]carbazole	–	+++
4-Acetoxy-7*H*-dibenzo[*c*,*g*]carbazole	+	+++
6-Acetoxy-7*H*-dibenzo[*c*,*g*]carbazole	–	–
3-Hydroxy-7*H*-dibenzo[*c*,*g*]carbazole	–	++
5-Hydroxy-7*H*-dibenzo[*c*,*g*]carbazole	–	–
N-Ethyl-7*H*-dibenzo[*c*,*g*]carbazole	+	NR

–, not active; +, weakly active; ++, moderately active; +++, highly active; NR, not reported
Adapted from Warshawsky (1992)

of CYP activity, reduced sulfoxide formation by about 55% and sulfone formation by about 92% (Vignier *et al.*, 1985).

In a more recent study, the metabolism of dibenzothiophene was studied using of liver microsomes from rats pre-treated with phenobarbital, 5,6-benzoflavone or Aroclor 1254. The same two metabolites described above were identified: dibenzothiophene sulfoxide, the major metabolite, and dibenzothiophene sulfone (Jacob *et al.*, 1991). No metabolites involving oxidation of carbon–carbon bonds were found. The yield of the sulfone metabolite was increased by each inducer, but the amount of sulfoxide formed was only marginally affected.

Dibenzothiophene was characterized as a noncompetitive inhibitor of CYP1A (Wassenberg *et al.*, 2005).

4.8.2 Genotoxicity, structure–activity relationships and other relevant data

Dibenzothiophene has been evaluated in two studies on bacterial mutagenicity in two strains of *S. typhimurium* (Table 4.2). No increase in mutagenic activity was observed for dibenzothiophene at concentrations of 0–100 μg/plate in *S. typhimurium* strain TA98 with exogenous metabolic activation (McFall *et al.*, 1984). Another study was conducted with both *S. typhimurium* strains TA98 and TA100 in the presence or absence of exogenous metabolic activation from S9 from rats induced with Aroclor 1254, with strain TA98 with metabolic activation in the pre-incubation protocol, and with strain TA100 without metabolic activation in the pre-incubation protocol. Except in the latter case, dibenzothiophene did not significantly induce mutation in these studies (Pelroy *et al.*, 1983).

Dibenzothiophene formed several unidentified DNA adducts after incubation for 24–28 hours in human HepG2 cells in culture, as determined by ^{32}P-postlabelling (Amat *et al.*, 2004).

While no formal structure–activity studies have been reported on dibenzothiophene and related three-ring thiophene-based polycyclic aromatic compounds, some information can be gleaned from the data on bacterial mutagenesis. Dibenzothiophene is a symmetrical three-ringed thiophene. Three other, asymmetric, three-ringed thiophenes were evaluated for mutagenic activity (Pelroy *et al.*, 1983). Naphtho[1,2-*b*]thiophene induced mutations in both strains, while the other isosteres, naphtho[2,3-*b*]thiophene and naphtho[2,1-*b*]thiophene, were inactive. These results suggest that a phenanthrenoid arrangement of the thio-PAH with the sulfur atom in the bay region was required for biological activity of three-ringed thiophenes.

Dibenzothiophene (10–500 μg/L) reduced EROD activity in embryos of *Fundulus heteroclitus* (killifish; saltwater minnow) by about 60%. The strong stimulation of EROD activity by the AhR agonist β-naphthoflavone (1 μg/L) was considerably diminished upon coincubation of the fish embryos with dibenzothiophene (Wassenberg *et al.*, 2005). Although not embryotoxic itself, dibenzothiophene enhanced the embryotoxicity of β-naphthoflavone.

In a study of oral toxicity, male CD-1 mice were given dibenzothiophene at a single dose of 0–1609 mg/kg bw. From the results, an LD_{50} of 470 mg/kg bw was calculated. In a companion study, male CD-1 mice were pre-treated with 3-methylcholanthrene at a single dose of 80 mg/kg bw by intraperitoneal injection, and simultaneously with phenobarbital as three consecutive intraperitoneal injections at 50 mg/kg bw per day. After 24 hours, these mice were given dibenzothiophene at doses of 0–744 mg/kg bw. The LD_{50} of the induced mice treated with dibenzothiophene was 335 mg/kg bw, suggesting that increased levels of CYP increased the toxicity of this compound (Leighton, 1989).

Table 4.2 Studies of mutagenicity with dibenzothiophene in bacteria

S. typhimurium strain	Concentration range (µg/plate)	Metabolic activation	Result	Reference
TA98	0–328	± S9	–	Pelroy *et al.* (1983)
TA100	0–500	± S9	–	Pelroy *et al.* (1983)
TA98	0–500	+ S9 (pre-incubation)	–	Pelroy *et al.* (1983)
TA100	0–250	– S9 (pre-incubation)	+	Pelroy *et al.* (1983)
TA98	0–100	+ S9	–	McFall *et al.* (1984)

S9, 9000 × *g* rat liver supernatant

4.9 Benzo[*b*]naphtho[2,1-*d*]thiophene

4.9.1 Metabolism

The metabolism of benzo[*b*]naphtho[2,1-*d*]thiophene upon incubation with microsomes from rat liver takes places on the sulfur atom – producing benzo[*b*]naphtho[2,1-*d*]thiophene sulfoxide and benzo[*b*]naphtho[2,1-*d*]thiophene sulfone – and on the aromatic carbons of both the benzo- and naphtha-rings – producing *trans*-benzo[*b*]naphtho[2,1-*d*]thiophene-1,2-dihydrodiol, *trans*-benzo[*b*]naphtho[2,1-*d*]thiophene-3,4-dihydrodiol, and 7-, 8-, and 9-hydroxybenzo[*b*]naphtho[2,1-*d*]thiophene (Jacob *et al.*, 1986, 1991; Misra & Amin, 1990; Murphy *et al.*, 1992). Formation of several other metabolites, including several triols, has been reported, but these were not fully characterized (Jacob *et al.*, 1986, 1991). The appearance of these metabolites by incubation with liver homogenates was dependent on the rat strain and on pre-treatment with specific inducers. In male Wistar rats, induction with Aroclor 1254 generally increased the level of the sulfone metabolite to a greater extent than that of the sulfoxide metabolite. The same effect was seen after induction with phenobarbital, while induction with 5,6-benzoflavone increased the levels of the two metabolites to a similar extent (Jacob *et al.*, 1991). Induction of Wistar rats with 1,1-bis-(*p*-chlorophenyl)-2,2,2-trichloroethane; DDT) increased the formation of a sulfone-phenol (Jacob *et al.*, 1986, 1988). When the yield of benzo[*b*]naphtho[2,1-*d*]thiophene metabolites was compared in non-induced liver homogenates from Wistar and F344 rats, microsomes from Wistar rats produced higher levels of the sulfoxide, sulfone and benzo[*b*]naphtho[2,1-*d*]thiophene-1,2-diol metabolites compared with those from F344 rats (Murphy *et al.*, 1992). Pre-treatment of F344 rats with Aroclor 1254 increased the liver microsome-mediated metabolism of benzo[*b*]naphtho[2,1-*d*]thiophene, and produced all of the known metabolites (Murphy *et al.*, 1992).

4.9.2 Genotoxicity, structure–activity relationships and other relevant data

The potential genotoxic activity of benzo[*b*]naphtho[2,1-*d*]thiophene has been evaluated in a series of studies of mutation in bacteria and one assay in mammalian cells (Table 4.3). Benzo[*b*]naphtho[2,1-*d*]thiophene did not induce mutations in *S. typhimurium* strain TA98 with or without a source of exogenous metabolic activation in a standard plate-incorporation test or in a liquid pre-incubation assay (Pelroy *et al.*, 1983; McFall *et al.*, 1984). Three studies were conducted with *S. typhimurium* strain TA100, in which benzo[*b*]naphtho[2,1-*d*]thiophene did not induce mutations in the presence or absence of exogenous metabolic activation. However, this substance was mutagenic in *S. typhimurium* TA100 in the presence of exogenous metabolic activation in the pre-incubation protocol (Pelroy *et al.*, 1983). In another study benzo[*b*]

Table 4.3 Studies of mutagenicity with benzo[*b*]naphtho[2,1-*d*]thiophene in bacteria

S. typhimurium strain or human cell line	Concentration range (μg/plate)[a]	Metabolic activation	Result	Reference
TA98	0–100	+ S9	–	McFall *et al.* (1984)
TA98	0–500	± S9	–	Pelroy *et al.* (1983)
TA100	0–500	± S9	–	Pelroy *et al.* (1983)
TA98	0–250	+ S9 (pre-incubation)	–	Pelroy *et al.* (1983)
TA100	0–250	– S9 (pre-incubation)	+	Pelroy *et al.* (1983)
TA100	0–320	+ S9	+	Misra & Amin (1990)
haA1v2 (*TK* locus)	0–10 μg/mL	NA	–	Durant *et al.* (1996)

[a] Unless otherwise specified
NA, not applicable; S9, 9000 × *g* rat liver supernatant

naphtho[2,1-*d*]thiophene was also mutagenic in *S. typhimurium* TA100 after exogenous metabolic activation (Misra & Amin, 1990). Benzo[*b*]naphtho[2,1-*d*]thiophene was not mutagenic in a human lymphoblastoid cell line (h1A1v2) known to express the metabolic enzyme CYP1A1 constitutively (Durant *et al.*, 1996).

Benzo[*b*]naphtho[2,1-*d*]thiophene formed one unidentified DNA adduct after incubation for 24–28 hours in human HepG2 cells in culture, as determined by ^{32}P-postlabelling (Amat *et al.*, 2004).

No formal structure–activity studies of four-ringed thiophenes, including benzo-naphthothiophenes, anthrathiophenes and phenanthrothiophenes, have been reported. However, results from tests of mutagenicity in bacteria provide some information on potential structure–activity relationships. Pelroy *et al.* (1983) studied the mutagenic activities of 13 four-ringed thiophenes in *S. typhimurium* TA98 and TA100 in the presence of exogenous metabolic activation. Phenanthro[3,4-*b*]thiophene was the most active compound, with a mutagenic activity in TA100 (≈195 revertants/μg) equal to that of benzo[*a*]pyrene. Anthra[2,1-*b*]thiophene induced nine TA100 revertants/μg and anthra[1,2-*b*]thiophene and anthra[2,3-*b*]thiophene each induced about four TA100 revertants/μg. In another study, phenanthro[3,4-*b*]thiophene and its isostere, phenanthro[4,3-*b*]thiophene, were compared with respect to their mutagenic activity in *S. typhimurium* TA98, TA100 and TA104 in the presence of exogenous metabolic activation from S9 from rats induced with Aroclor 1254 with the plate-incorporation protocol. Phenanthro[3,4-*b*]thiophene was mutagenic in *S. typhimurium* TA100 only (550 revertants/μg), while phenanthro[4,3-*b*]thiophene was mutagenic in TA98 (≈14 revertants/μg) and TA100 (≈13 revertants/μg) (Swartz *et al.*, 2009). All five thiophenes have phenanthrenoid structures with the thiophene ring at the distal end of the molecule in a bay or fjord configuration, a region known to enhance the mutagenic and carcinogenic activities of PAHs (Xue & Warshawsky, 2005).

4.9.3 Mechanistic considerations

The structure of benzo[*b*]naphtho[2,1-*d*]thiophene is similar to that of the carbocyclic hydrocarbon chrysene. Chrysene is metabolized to two major dihydrodiols upon incubation with liver microsomes from rats induced with 3-methylcholanthrene. Both chrysene-1,2-diol and chrysene-3,4-diol have a functionalized terminal benzo-ring. The K-region 5,6-dihydrodiol was also detected, at much lower levels (Nordqvist *et al.*, 1981). Chrysene-1,2-diol was found to be metabolized to a reactive diol epoxide, *r*-1,*t*-2-dihydroxy-*t*-3,4-oxy-1,2,3,4-tetrahydrochrysene, which forms DNA adducts in rodent and human

skin (Weston *et al.*, 1985). Benzo[*b*]naphtho[2,1-*d*]thiophene is metabolized to diols that are structurally analogous to those of chrysene: *trans*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]naphtho[2,1-*d*]thiophene and *trans*-3,4-dihydroxy-3,4-dihydrobenzo[*b*]naphtho[2,1-*d*]thiophene. Both dihydrodiols were mutagenic in *S. typhimurium* TA100, with pre-incubation in the presence of a liver homogenate from rats induced with Aroclor 1254. The mutagenicity of the 3,4-dihydrodiol was comparable to that of the parent compound benzo[*b*]naphtho[2,1-*d*]thiophene, while the 1,2-dihydrodiol was a weaker mutagen (Misra & Amin, 1990). These results suggest the potential for further metabolism of benzo[*b*]naphtho[2,1-*d*]thiophene diols to diol epoxides, which could form DNA adducts and mutations, although there are no studies on benzo[*b*]naphtho[2,1-*d*]thiophene diol epoxide or DNA-adduct formation to confirm this.

5. Summary of Data Reported

5.1 Exposure data

Seven nitrogen-heterocyclic polycyclic aromatic hydrocarbons (azaarenes: benz[*a*]acridine, benz[*c*]acridine, dibenz[*a*,*h*]acridine, dibenz[*a*,*j*]acridine, dibenz[*c*,*h*]acridine, carbazole, 7*H*-dibenzo[*c*,*g*]carbazole) and two sulfur-heterocyclic polycyclic aromatic hydrocarbons (thiaarenes: dibenzothiophene and benzo[*b*]naphtho[2,1-*d*]thiophene) were reviewed. These compounds are formed during the incomplete combustion of nitrogen- and sulfur-containing organic material from natural sources (volcanic activities, wildfires, fossil fuels) and from anthropogenic sources (automobile exhausts, some industrial activities, tobacco smoke, cooking emissions). These compounds have been detected at low concentrations in the environment, in ambient air (total of four-ring azaarenes, including benz[*a*]acridine and benz[*c*]acridine, at concentrations below the nanogram-per-cubic-metre level), water (at the microgram-per-litre level in groundwater and tar-contaminated sites) and soil (at the microgram-per-kg level). For comparison, the mainstream smoke of cigarettes contains 0.1 ng per cigarette dibenz[*a*,*h*]acridine, up to 10 ng per cigarette dibenz[*a*,*j*]acridine and 700 ng per cigarette 7*H*-dibenzo[*c*,*g*]carbazole.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

5.3.1 Benz[a]acridine

Benz[*a*]acridine has been evaluated for carcinogenicity in one study using dermal application in mice and one study of pulmonary implantation in rats. The study in mice was inadequate to evaluate the carcinogenicity of benz[*a*]acridine. Pulmonary implantation of benz[*a*]acridine did not increase the incidence of tumours of the lung in rats.

5.3.2 Benz[c]acridine

Benz[*c*]acridine has been evaluated for carcinogenicity in two studies of dermal application and four studies of dermal initiation–promotion in mice, a study of pulmonary implantation and a study of bladder implantation in rats, and a bioassay in neonatal mice.

The two studies of dermal application in mice were considered to be inadequate for evaluation of the carcinogenicity of benz[*c*]acridine. In two of the studies of initiation–promotion in mice, benz[*c*]acridine gave a positive response as an initiator; the other two initiation–promotion studies were considered to be inadequate. Benz[*c*]acridine did not increase the incidence of tumours of the lung when implanted into the lungs of rats; the study of bladder implantation in rats was

considered to be inadequate. When administered intraperitoneally to newborn mice, benz[*c*]acridine caused an increase in the incidence of tumours of the lung (primarily adenomas) in both sexes. The metabolites, benz[*c*]acridine-3,4-dihydrodiol and benz[*c*]acridine-*anti*-3,4-dihydrodiol-1,2-epoxide, were potent skin-tumour initiators in mice and induced tumours of the lung and liver when given to newborn mice.

*5.3.3 Dibenz[*a,h*]acridine*

Dibenz[*a,h*]acridine has been evaluated for carcinogenicity in one study of oral administration, five studies of dermal application, two studies using dermal initiation–promotion, four studies of subcutaneous injection, and a study of intravenous injection in mice. It was also tested in a study of subcutaneous injection and in a study of pulmonary implantation in rats. The studies of oral administration, dermal application, and subcutaneous injection in mice were considered to be inadequate for the evaluation of the carcinogenicity of dibenz[*a,h*]acridine. In the two initiation–promotion studies in mice, dibenz[*a,h*]acridine gave a positive response as an initiator. Dibenz[*a,h*]acridine also increased the incidence of adenoma of the lung when given to mice by intravenous injection, and of carcinoma of the lung when implanted into the lungs of rats. The metabolites dibenz[*a,h*]acridine-10,11-dihydrodiol, dibenz[*a,h*]acridine-*anti*-10,11-dihydrodiol-8,9-epoxide, and (+)-dibenz[*a,h*]acridine-*anti*-10*S*,11*R*-dihydrodiol-8*R*,9*S*-epoxide were potent skin-tumour initiators in mice.

*5.3.4 Dibenz[*a,j*]acridine*

Dibenz[*a,j*]acridine has been evaluated for carcinogenicity in one study of oral administration, seven studies of dermal application, one initiation–promotion study, and four studies of subcutaneous injection in mice, and in one study of pulmonary implantation in rats. The studies of oral administration and subcutaneous injection in mice were considered to be inadequate for the evaluation of the carcinogenicity of dibenz[*a,j*]acridine. Dibenz[*a,j*]acridine caused an increase in the incidence of skin cancer in two of the studies of dermal application and, as an initiator, in the initiation–promotion study in mice; the other studies of dermal application were considered to be inadequate. Dibenz[*a,j*]acridine did not increase the incidence of tumours of the lung when implanted into the lungs of rats.

*5.3.5 Dibenz[*c,h*]acridine*

Dibenz[*c,h*]acridine has been evaluated for carcinogenicity in one initiation–promotion study in mice and in one bioassay in neonatal mice. In the initiation–promotion study, dibenz[*c,h*]acridine, as an initiator, caused an increase in the incidence and multiplicity of skin papilloma. In the bioassay in neonatal mice, dibenz[*c,h*]acridine given by intraperitoneal injection caused an increase in the incidence of tumours of the lung (primarily adenomas) in both sexes, and in the incidence and multiplicity of liver adenoma in males. The metabolites (–)-dibenz[*c,h*]acridine-3*R*,4*R*-dihydrodiol and (+)-dibenz[*c,h*]acridine-*anti*-3*S*,4*R*-dihydrodiol-1*R*,2*S*-epoxide, and two isosteric analogues, were potent skin-tumour initiators in mice, and induced tumours of the lung and liver when given to neonatal mice.

5.3.6 Carbazole

Carbazole has been evaluated for carcinogenicity in one feeding study, three studies of dermal application and one study of subcutaneous injection in mice, in one study of intraperitoneal injection in neonatal mice, and in two studies of tumour promotion in rats. It was also tested in a feeding study in hamsters. The studies of dermal application and subcutaneous injection in mice and the feeding study in hamsters

were inadequate to evaluate the carcinogenicity of carbazole. In mice given diet containing carbazole, a dose-dependent increase in the incidence of liver neoplastic nodules (adenomas) and hepatocellular carcinoma was observed. In the forestomach of these animals, papillomas (in males and females) and carcinomas (in males only) were also detected. No increase in tumour incidence was seen in the study of intraperitoneal injection in neonatal mice. In male rats, carbazole administered in the diet did not show a promoting effect in one study, but promoted the development of kidney papilloma and carcinoma in another study.

5.3.7 7H-*Dibenzo[*c,g*]carbazole*

7*H*-Dibenzo[*c,g*]carbazole has been evaluated for carcinogenicity in mice in one study of oral administration, seven studies of dermal application, seven studies of subcutaneous injection, two studies of intraperitoneal injection, one study of intravenous injection, one study of bladder implantation, and one tumour-initiation study. This substance was also evaluated for carcinogenicity after intratracheal implantation in hamsters, and after intravesical injection in a dog. The oral study, five of the studies of dermal application, all studies of subcutaneous injection, one of the studies of intraperitoneal injection, the study of intravenous injection and the study of bladder implantation in mice, as well as the study of intratracheal implantation in hamsters and the study of intravesical injection in the dog were inadequate to evaluate the carcinogenicity of 7*H*-dibenzo[*c,g*]carbazole. In mice given 7*H*-dibenzo[*c,g*]carbazole by dermal application, a statistically significant increase in the incidence of skin carcinoma was observed in one study, and a statistically significantly increased incidence of skin tumours and liver neoplasms was observed in one other study. Intraperitoneal administration of 7*H*-dibenzo[*c,g*]carbazole to mice resulted in a dose-related increase in the incidence and multiplicity of lung adenomas. A skin-painting initiation–promotion study in mice indicated that 7*H*-dibenzo[*c,g*]carbazole had tumour-initiating ability.

5.3.8 Dibenzothiophene

No data were available to the Working Group

*5.3.9 Benzo[*b*]naphto[2,1-*d*]thiophene*

In one study of pulmonary implantation in female rats, benzo[*b*]naphto[2,1-*d*]thiophene increased the incidence of squamous cell carcinoma of the lung.

5.4 Mechanistic and other relevant data

*5.4.1 Benz[*a*]acridine*

The metabolism of benz[*a*]acridine by rat liver and lung microsomes yielded benz[*a*]acridine-5,6-dihydrodiol (a K-region dihydrodiol) and an uncharacterized non-K-region dihydrodiol, which was not benz[*a*]acridine-3,4-dihydrodiol. Evidence for the formation of the bay-region diol-epoxide, *trans*-benz[*a*]acridine-3,4-dihydrodiol-1,2-oxide, has not been obtained. Benz[*a*]acridine was a weak inducer of mono-oxygenase activity in rat liver, and was shown to induce proteins recognized by antibodies to cytochrome 1A1, but not cytochrome 2B1. Mutagenicity tests conducted with benz[*a*]acridine in *Salmonella typhimurium* TA98 (his^-/his^+), in the presence of an exogenous metabolic system, were inconclusive. However, benz[*a*]acridine was positive in the Mutatox™ test. This assay is based on the use of a dark variant of the luminescent bacterium *Vibrio fischeri*, which can be used to detect genotoxic activity in aqueous samples; the presence of genotoxic compounds results in mutations and consequently in restoration of photoluminescence. The *cis*- and *trans*-benz[*a*]

acridine-3,4-dihydrodiol-1,2-oxides were mutagenic in *S. typhimurium* TA98 and TA100 and in Chinese hamster V79–6 cell lines. Benz[*a*]acridine-3,4-dihydrodiol was mutagenic in *S. typhimurium* TA100 in the presence of a highly purified and reconstituted mono-oxygenase system obtained from rat liver microsomes. The *cis*- and *trans*-benz[*a*]acridine-3,4-dihydrodiol-1,2-oxides induced DNA damage in two rat hepatoma cell lines. Benz[*a*]acridine itself and its metabolite *trans*-benz[*a*]acridine-3,4-dihydrodiol were inactive in the same test systems.

There is inadequate evidence for a mutagenic mechanism underlying the carcinogenicity of benz[*a*]acridine on the basis of experimental data.

5.4.2 Benz[c]acridine

The metabolism of benz[*c*]acridine by rat liver microsomes yielded several mono- and diphenols and dihydrodiols. The major metabolite was the K-region dihydrodiol, while *trans*-benz[*c*]acridine-3,4-dihydrodiol was formed in very small amounts. A small amount of *N*-oxidation products was also formed. Unequivocal evidence of the formation of *trans*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxide was not obtained. Benz[*c*]acridine was a weak inducer of mono-oxygenase activity in rat liver.

Benz[*c*]acridine was mutagenic in *S. typhimurium* TA100 in the presence of exogenous metabolic activation. The bay-region *cis*- and *trans*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxides were mutagenic in *S. typhimurium* TA98 and TA100 and in Chinese hamster V79–6 cells. In the same test systems, non-bay-region diol-epoxides were one to four orders of magnitude less mutagenic. *trans*-Benz[*c*]acridine-3,4-dihydrodiol, the precursor of the bay-region diol-epoxides, was at least five times more active than benz[*c*]acridine in *S. typhimurium* TA100 in the presence of exogenous metabolic activation. None of the other possible *trans*-dihydrodiols was significantly activated under these conditions. Benz[*c*]acridine induced sister-chromatid exchange in Chinese hamster Don (lung) cells without the addition of an exogenous metabolic system. *trans*-Benz[*c*]acridine-3,4-dihydrodiol and the *cis*- and *trans*-benz[*c*]acridine-3,4-dihydrodiol-1,2-epoxides induced DNA damage in two rat hepatoma cell lines. The bay-region diol-epoxides of benz[*c*]acridine had substantially higher activities in bacterial and mammalian cells than their benz[*a*]acridine analogues. These differences are consistent with qualitative arguments regarding resonance stabilization of the carbocations resulting from opening of the epoxide ring.

There is weak evidence for a mutagenic mechanism underlying the carcinogenicity of benz[*c*]acridine on the basis of experimental data.

5.4.3 Dibenz[a,h]acridine

Dibenz[*a*,*h*]acridine metabolism yields two types of bay-region diol-epoxide, i.e. *cis*- and *trans*-dibenz[*a*,*h*]acridine-3,4-dihydrodiol-1,2-epoxide and *cis*- and *trans*-dibenz[*a*,*h*]acridine-10,11-dihydrodiol-8,9-epoxide. The differences in structure result in different biological activities between the diol-epoxides and between their dihydrodiol precursors. The dibenz[*a*,*h*]acridine-10,11-dihydrodiol-8,9-epoxides and their metabolic precursor, dibenz[*a*,*h*]acridine-10,11-dihydrodiol, are considerably more mutagenic in bacterial and mammalian test systems than the analogous bay-region 3,4-dihydrodiol-1,2-epoxides and their 3,4-dihydrodiol precursor. The *trans*-dibenz[*a*,*h*]acridine-1,2- and -8,9-dihydrodiols, which cannot be converted to bay-region diol-epoxides, are not activated to mutagenic products in *S. typhimurium* TA100. Computational data suggest that carbocation formation at C-8 is energetically favoured over that at C-1, which may determine the lower reactivity of the 3,4-dihydrodiol-1,2-epoxides in comparison

with that of the 10,11-dihydrodiol-8,9-epoxides. A decreased propensity for epoxide ring opening of the 3,4-dihydrodiol-1,2-epoxides may explain their lower mutagenic activity. Intratracheal instillation of rats with dibenz[*a*,*h*]acridine resulted in formation of DNA adducts, sister-chromatid exchange, and micronucleus formation in lung cells. The data on mutagenicity in mammalian cells and tumour initiation on mouse skin implicate *trans*(–)-(10*R*,11*R*)-dibenz[*a*,*h*]acridine-10,11-dihydrodiol as the proximate carcinogen and the bay-region *trans*(+)-(8*R*,9*S*,10*S*,11*R*) diol-epoxide as the ultimate carcinogen. The observed stereoselectivity is identical to that exhibited by other carbocyclic and aza-polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene, benz[*a*]anthracene, chrysene, benzo[*c*]phenanthrene and dibenz[*c*,*h*]acridine. Human cytochrome 1A1 is substantially more active in metabolizing dibenz[*a*,*h*]acridine than human cytochrome 1B1 and, in contrast to rat cytochrome 1A1, is regioselective for the formation of dibenz[*a*,*h*]acridine-10,11-dihydrodiol compared with dibenz[*a*,*h*]acridine-3,4-dihydrodiol. In addition, stereoselectivity for the production of the proximate carcinogen, 10*R*,11*R*-dibenz[*a*,*h*]acridine-10,11-dihydrodiol, by cytochrome 1A1 suggests that a high expression of this enzyme activity may confer increased susceptibility to dibenz[*a*,*h*]acridine-induced carcinogenesis. Dibenzo[*a,h*]acridine was about 2.5 times more potent than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and more than 200 times more potent than benzo[a]pyrene in activating the aryl hydrocarbon receptor in a rat hepatoma cell line *in vitro*.

There is moderate evidence for a mutagenic mechanism underlying the carcinogenicity of dibenz[*a*,*h*]acridine on the basis of experimental data.

*5.4.4 Dibenz[*a,j*]acridine*

Dibenz[*a,j*]acridine is converted by rat, mouse and human liver microsomes and by rat lung microsomes to a series of hydroxylated metabolites, including dihydrodiols, tetrahydrotetrols, phenols and diol-epoxides. *trans*-Dibenz[*a,j*]acridine-3,4-dihydrodiol is typically the major metabolite, predominantly as the *3R,4R* isomer. Human cytochrome 1A1, 1A2, 3A4 and 3A5 catalysed the formation of *trans*-dibenz[*a,j*]acridine-3,4-dihydrodiol *in vitro*; the 3A4 isoform was the most selective for this metabolite, while cytochrome 1A2 was selective for K-region 5,6-oxidation. Regardless of the specific cytochrome, the 3,4-dihydrodiol had a *3R,4R*-configuration, with almost 100% optical purity. Extensive phase-II metabolism, including glutathione conjugation, was demonstrated to occur with rat hepatocytes *in vitro*.

Dibenz[*a,j*]acridine was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an exogenous metabolic system. The compound induced chromosomal aberrations in Chinese hamster fibroblasts in the presence of exogenous metabolic activation. The most mutagenic dibenz[*a,j*]acridine metabolites in both bacterial and mammalian cells were the bay-region diol-epoxides, *cis*- and *trans*-dibenz[*a,j*]acridine-3,4-dihydrodiol-1,2-oxide, which did not require metabolic activation. The *trans* diol-epoxide was consistently more mutagenic than its *cis* isomer. Dibenz[*a,j*]acridine increased the frequency of micronucleus formation in human lymphocytes *in vitro*. In the presence of liver microsomes from rats treated with 3-methylcholanthrene, dibenz[*a,j*]acridine was shown to bind to calf thymus DNA, yeast RNA, polyG, polyA, polyU and polyC. The greatest extent of binding was observed with polyG. Epithelial cells from rat buccal mucosa metabolized dibenz[*a,j*]acridine to DNA-binding species. Upon topical application to rats, mice and hamsters, similar profiles of DNA adducts were detected by

^{32}P-postlabelling, almost exclusively in the skin, with higher levels of adduct being seen in mice. Topical application of the *trans*-dibenz[*a,j*]acridine-1,2-, -3,4-, and -5,6-dihydrodiols to mice, followed by ^{32}P-postlabelling analysis of skin DNA, demonstrated that the 3,4-dihydrodiol is an intermediate in the major route of dibenz[*a,j*]acridine activation leading to DNA binding in the skin. Topical application of dibenz[*a,j*]acridine and (+/–)-*trans*-benz[*a,j*]acridine-3,4-dihydrodiol-1,2-epoxide yielded four skin DNA adducts, detected by ^{32}P-postlabelling. The major adduct from dibenz[*a,j*]acridine co-eluted with a synthetic deoxyguanosine adduct and the major adduct formed by the diol-epoxide *in vivo* was a deoxyadenosine adduct. Skin papillomas and carcinomas formed after topical application of dibenz[*a,j*]acridine to mice harboured A to T and G to T transversions in codons 12, 13 and 61 of the *Hras* gene. The mutation spectra in the *Hras* gene were consistent with the observed binding of dibenz[*a,j*]acridine to deoxyguanosine or deoxyadenosine *in vivo*.

There is strong evidence for a mutagenic mechanism underlying the carcinogenicity of dibenz[*a,j*]acridine on the basis of experimental data.

5.4.5 Dibenz[c,h]acridine

Dibenz[*c,h*]acridine is mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an exogenous metabolic activation system. Activation of dibenz[*c,h*]acridine to the bay-region diol-epoxide, dibenz[*c,h*]acridine-3,4-dihydrodiol-1,2-oxide, is consistent with its mutagenicity in bacterial and mammalian systems. In Chinese hamster V79–6 cells, the (+)-*anti*-(1*R*,2*S*,3*S*,4*R*)-dibenz[*c,h*]acridine-3,4-dihydrodiol-1,2-epoxide was more mutagenic than any of the other 3,4-dihydrodiol-1,2-epoxides. It was also the most tumorigenic of the four possible isomeric bay-region diol-epoxides of dibenz[*c,h*]acridine, both in an initiation–promotion model on mouse skin and in newborn mice (see above). Data on metabolism in rat liver microsomes suggest that a high expression of cytochrome 1A1 activity may confer increased susceptibility to dibenz[*c,h*]acridine-induced carcinogenesis. Exposure of Chinese hamster V79 cells to the (*R,S,S,R*) and (*S,R,R,S*) bay-region diol epoxides from dibenz[*c,h*]acridine resulted in AT base-pair mutations at the hypoxanthine (guanine) phosphoribosyltransferase locus. Dibenz[*c,h*]acridine produced chromosomal aberrations in Chinese hamster fibroblasts in the presence of exogenous metabolic activation. Following an initiation–promotion protocol, the DNA isolated from dibenz[*c,h*]acridine-induced carcinomas in female CD-1 mice efficiently transformed NIH 3T3 cells. A high percentage of the transformed foci had an amplified *Hras* gene containing an A to T transversion in the second base of codon 61. The same mutation was detected in primary tumour DNA in a high percentage of the dibenz[*c,h*]acridine-induced carcinomas and also in NIH 3T3 cells transformed with DNA from dibenz[*c,h*]acridine-induced benign skin papillomas.

There is strong evidence for a mutagenic mechanism underlying the carcinogenicity of dibenz[*c,h*]acridine, despite the absence of studies demonstrating the formation of DNA adducts induced by dibenz[*c,h*]acridine.

5.4.6 Carbazole

The major metabolite of carbazole in rats is 3-hydroxycarbazole. It is characterized as a non-competitive inhibitor of cytochrome 1A enzymes. Carbazole is not mutagenic to bacteria. It is moderately clastogenic in mice when administered intraperitoneally. It induced dominant lethality and sperm-head abnormalities in male mice. Carbazole has been reported to be a major active component of coal tar; it displays anti-angiogenic and anti-inflammatory properties *in vitro*.

There is inadequate evidence for a mutagenic mechanism underlying the carcinogenicity of carbazole on the basis of experimental data.

5.4.7 7H-*Dibenzo[*c,g*]carbazole*

When administered to rodents, *7H*-dibenzo[*c*,*g*]carbazole is widely distributed in tissues, extensively metabolized, and excreted mainly in the faeces. In mice, *7H*-dibenzo[*c*,*g*]carbazole is mainly metabolized by cytochromes 1A1 and 1A2 in the liver and by cytochromes 1A1 and 1B1 in the lung. Similar results were obtained *in vitro* in test systems expressing human cytochrome 1 enzymes. The major metabolites formed by liver cells and microsomal fractions are monohydroxylated derivatives; a dihydrodiol is also formed.

7H-Dibenzo[*c*,*g*]carbazole gave positive results in some, but not all, tests for mutagenicity in bacteria. *7H*-Dibenzo[*c*,*g*]carbazole induced mutations and micronucleus formation in Chinese hamster V79 cells expressing human cytochrome 1A1 and/or 1A2. It was mutagenic in DNA repair-deficient human xeroderma pigmentosum cells, and induced micronucleus formation in cultured human lymphocytes cells *in vitro*. *7H*-Dibenzo[*c*,*g*]carbazole was mutagenic in transgenic mice, inducing mutations in the liver and skin. The substance formed DNA adducts in rodent cells *in vitro* and in mice *in vivo*. The order of binding in mouse tissues after subcutaneous injection of *7H*-dibenzo[*c*,*g*]carbazole was liver >> kidney > lung > spleen > skin > brain. The pattern of DNA adducts observed in the liver was different from that in skin. The pattern in the liver resembled that formed by 3-hydroxydibenzo[*a*]acridine. *7H*-Dibenzo[*c*,*g*]carbazole induced DNA damage in rodent cells *in vitro*, measured as alkali-labile lesions (converted to strand breaks). There was conflicting evidence regarding its contribution to formation of oxidative damage in DNA. *7H*-Dibenzo[c,g]carbazole caused necrosis and apoptosis in the HepG2 human hepatoma cell line, and apoptosis in mouse liver *in vivo*. It weakly induced the aryl hydrocarbon receptor and inhibited gap-junction intercellular communication in WB-F344 rat-liver epithelial cells, a property of tumour promoters. A high percentage of tumours induced in mouse lung by *7H*-dibenzo[*c*,*g*]carbazole contained mutations in *Kras*, the majority of which were AT to TA transversions in the third base of codon 61. Similarly, skin tumours induced by *7H*-dibenzo[*c*,*g*]carbazole frequently contained mutations in *Hras1*, mostly AT to TA transversions.

There is moderate evidence for a mutagenic mechanism underlying the carcinogenicity of *7H*-dibenzo[*c*,*g*]carbazole; this compound is mutagenic by a genotoxic mechanism.

5.4.8 Dibenzothiophene

Dibenzothiophene was not mutagenic in several strains of *S. typhimurium*. Metabolism studies conducted with preparations of rat liver identified only sulfur-oxidation metabolites and a study in human liver-tumour cells indicated the formation of unidentified DNA adducts.

There is inadequate evidence for a mutagenic mechanism underlying the carcinogenicity of dibenzothiophene on the basis of experimental data.

*5.4.9 Benzo[*b*]naphtho[2,1-*d*]thiophene*

Benzo[*b*]naphtho[2,1-*d*]thiophene was mutagenic in two strains of S. *typhimurium*. The metabolism of this compound has been studied with preparations of rat liver microsomes, which revealed formation of two dihydrodiol metabolites, both of which were mutagenic in one strain of *S. typhimurium*. A study in HepG2 human liver-tumour cells indicated the formation of unidentified DNA adducts by benzo[*b*]naphtho[2,1-*d*]thiophene.

There is weak evidence for a mutagenic mechanism underlying the carcinogenicity of benzo[*b*]naphtho[2,1-*d*]thiophene on the basis of experimental data.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *inadequate evidence* in experimental animals for the carcinogenicity of benz[*a*]acridine.

There is *limited evidence* in experimental animals for the carcinogenicity of benz[*c*]acridine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of dibenz[*a,h*]acridine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of dibenz[*a,j*]acridine.

There is *limited evidence* in experimental animals for the carcinogenicity of dibenz[*c,h*]acridine.

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of 7*H*-dibenzo[*c,g*]carbazole.

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

There is *limited evidence* in experimental animals for the carcinogenicity of benzo[*b*]naphto[2,1-*d*]thiophene.

6.3 Overall evaluation

Benz[*a*]acridine is *not classifiable as to its carcinogenicity (Group 3).*

Benz[*c*]acridine is *not classifiable as to its carcinogenicity (Group 3).*

Dibenz[*a,h*]acridine is *possibly carcinogenic to humans (Group 2B).*

Dibenz[*a,j*]acridine is *probably carcinogenic to humans (Group 2A).* In making the overall evaluation for dibenz[*a,j*]acridine, the Working Group considered mechanistic and other relevant data.

Dibenz[*c,h*]acridine is *possibly carcinogenic to humans (Group 2B).* In making the overall evaluation for dibenz[*c,h*]acridine, the Working Group considered mechanistic and other relevant data.

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

7*H*-Dibenzo[*c,g*]carbazole is *possibly carcinogenic to humans (Group 2B).*

Dibenzothiophene is *not classifiable as to its carcinogenicity to humans (Group 3).*

Benzo[*b*]naphtho[2,1-*d*]thiophene is *not classifiable as to its carcinogenicity to humans (Group 3).*

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GLOSSARY

Air blowing: Process by which compressed air is blown into a bitumen feedstock typically at 230–260 °C. This process results in complex reactions that raise the softening-point and viscosity of the bitumen. See **oxidized bitumens**.

Air-blown asphalts: See **oxidized bitumens.**

Air-rectified bitumen or **Air-refined bitumen** (synomym for **semi-blown bitumen**): A bitumen that has been subjected to mild oxidation with the goal of producing a bitumen that meets paving-grade bitumen specifications, typically having a penetration index of ≤ 2.0

Asphalt: A mixture of bitumen and mineral materials used as a paving material that is typically produced at temperatures in the range of 140–160 °C.

Asphalt binder: A term used in the USA and some other countries for **bitumen**.

Asphalt cement: A term used in the USA and some other countries for **bitumen**.

Asphalt cold mixes: Asphalt mixtures made using cutback bitumens or bitumen emulsions that can be applied at ambient temperature.

Asphalt paving mixtures: Mixtures of graded mineral aggregates (sized stone fractions, sands and fillers) with a controlled amount of **straight-run** or **paving bitumen**.

Bitumen, petroleum-derived: A dark brown to black cement-like residuum obtained from the distillation of suitable crude oils. The distillation processes may involve one or more of the following atmospheric distillation, vacuum distillation, steam distillation. Further processing of distillation residuum may be blended to yield a material, the physical properties of which are suitable for commercial applications. These additional processes can involve air oxidation, solvent stripping or blending of residua of different stiffness characteristics.

Bitumen emissions: The complex mixture of aerosols, vapours and gases from heated bitumen and products containing bitumen; although the term "bitumen fume" is often used in reference to total emissions, technically bitumen fume refers only to the aerosolized fraction of total emissions (i.e. solid particulate matter, condensed vapour and liquid bitumen droplets).

Bitumen-emission condensate: The condensate of emissions from heated bitumen; the chemical composition may vary with the temperature and the type of bitumen.

Bitumen emulsion [Class 4]: Mixtures of two normally immiscible components (bitumen and water) and an emulsifying agent (usually a surfactant); bitumen emulsions are used in paving, roofing and waterproofing operations. These materials are also called **asphalt emulsion** (North America).

Bitumen extract: The fraction of bitumen that is soluble in organic solvents such as benzene, toluene, carbon disulfide or dimethyl sulfoxide.

Bitumen fume: Refers to the aerosolized fraction of total emissions (i.e. solid particulate matter, condensed vapour and liquid bitumen droplets); term wrongly used to define **bitumen emissions**.

Bitumen vapour: Refers to vapours and gases from heated bitumen.

Built-up roofing asphalt (BURA): In North America, **oxidized bitumen** used in the construction of low slope built-up roofing systems; specification defined by ASTM D312. The **oxidized bitumen** typically has a penetration index of ≥ 2.0.

Coal tar: A dark brown to black, highly aromatic material manufactured during the high-temperature carbonization of bituminuous coals, which differs from bitumen substantially in composition and physical characteristics. It was previously used in the roofing and paving industries as an alternative to bitumen.

Coal-tar pitch: A black or dark brown cementitious solid that is obtained as a residue in the partial evaporation or fractional distillation of **coal tar**. Coal-tar pitch has been used in the past in roofing as an alternative to bitumen.

Cutback bitumens [Class 3]: Bitumens, the viscosity of which has been reduced by the addition of a **cutback solvent** derived from petroleum.

Cracking-residue bitumen: See **thermally cracked bitumens.**

Hard bitumens: Bitumens produced using extended vacuum distillation with some air rectification from propane-precipitated bitumen. Hard bitumens have low penetration values and high softening-points.

Macadam: A type of **asphalt** mix with a high stone content and containing 3–5% bitumen by weight.

Mastic asphalt: A type of **asphalt** made using a very fine mineral aggregate with a **hard bitumen**. These materials can be poured and levelled by hand. The application temperatures are typically between 200 and 250 °C.

Modified bitumens [Class 5]: Products or specialized applications made by incorporating polymers, elastomers or other products into **straight-run** or **oxidized bitumens**.

Natural asphalt: Naturally occurring mixture of bitumens and mineral matter formed by oil seepages in the Earth's crust.

Oxidized bitumens [Class 2]: Bitumens produced by reaction with air under temperature-controlled conditions, typically 260 °C. Also referred to as **air-blown asphalts** or **roofing asphalts** in the USA.

Penetration index or **grade**: A measure of change in penetration with temperature.

Propane-precipitated asphalt. See **solvent precipitation**.

Road oils: A term sometimes used to describe very soft vacuum residue or other low-viscosity bitumen products that are generally used to produce paving products for use on very low-volume roads in moderate to cold climates.

Roofing asphalts: See **oxidized bitumens**.

Roofing felt: A sheet material saturated and coated with bitumen, generally supplied in rolls and used for waterproofing roofs.

Solvent precipitation: Process by which **propane-precipitated asphalt** [bitumen] is separated from a vacuum residue by solvent precipitation, usually with propane. In the USA, the term used is "solvent deasphalting".

Solvent-refined asphalt: Term used in the USA to define **propane-precipitated asphalt**.

Stone-mastic asphalt: A high stone-content paving mixture used in some countries.

Steam-refined bitumens: Vaccum residues that have been subjected to injection of steam to aid vacuum distillation. See **straight-run bitumens.**

Straight-run bitumens or **paving bitumens** [**Class 1**]: These are usually produced from the residue from atmospheric distillation of petroleum crude oil by applying further distillation under vacuum, solvent precipitation or a combination of these processes. Also called "steam-refined bitumens" or "straight-reduced bitumens".

Thermally cracked bitumens or **thermal bitumens** [**Class 6**]: Bitumens produced by thermal cracking at high temperatures, typically 440–500 °C.

Warm-mix asphalt: A type of hot-mixed asphalt, which is produced at lower than normal temperatures. Typically warm-mixed asphalts are produced at temperatures between 100 and 130 °C.

LIST OF ABBREVIATIONS

1-OHP	1-hydroxypyrene
AFC	asphalt-fume condensate
AhR	aryl hydrocarbon receptor
AM	arithmetic mean
BaP	benzo[*a*]pyrene
BDL	below the detection limit
BPDE	7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene
BSF/BSM	benzene-soluble fraction/benzene-soluble matter
CAS	Chemical Abstracts Service
CI	confidence interval
CSM	cyclohexane-soluble matter
DBC	7*H*-dibenzo[*c*,*g*]carbazole
DMBA	7,12-dimethylbenz[*a*]anthracene
dmm	decimillimetre
DMSO	dimethyl sulfoxide
EINECS	European Inventory of Existing Commercial Chemical Substances
ELISA	enzyme-linked immunosorbent assay
EROD	ethoxyresorufin-*O*-deethylase
FD	fluorescence detection
FID	flame ionization detection
GC	gas chromatography
GM	geometric mean
GSD	geometric standard deviation
HPLC	high-performance liquid chromatography
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry
NIOSH	National Institute for Occupational Safety and Health
OH-PAH	hydroxylated polycyclic aromatic hydrocarbon
OR	odds ratio
PAC	polycyclic aromatic compound
PAH	polycyclic aromatic hydrocarbon
PG	penetration grade
PI	penetration index
PEN	penetration

PMR	proportional mortality rate
ppm	parts per million
RAP	reclaimed asphalt pavement
RPM	respirable particulate matter
RR	relative risk
RT-PCR	reverse-transcription polymerase chain reaction
SIR	standardized incidence ratio
SMR	standardized mortality ratio
STV	standard tar viscometer
TPA	12-*O*-tetradecanoylphorbol 13-acetate
TOM	total organic matter
TPM	total particulate matter
TWA	time-weighted average
USERIA	ultrasensitive enzymatic radioimmunoassay
VOC	volatile organic compounds
WPT	waste plastic and tall-oil pitch
w/v	weight per volume
w/w	weight per weight

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IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of bitumens and their emissions, the *N*-heterocyclic polycyclic aromatic hydrocarbons benz[*a*]acridine, benz[*c*]acridine, dibenz[*a,h*]acridine, dibenz[*a,j*]acridine, dibenz[*c,h*]acridine, carbazole and *7H*-dibenzo[*c,g*]carbazole, as well as the *S*-heterocyclic polycyclic aromatic hydrocarbons benzo[*b*]naphtho[2,1-*d*]thiophene and dibenzothiophene. Bitumens are produced by distillation of crude oil during petroleum refining, and also occur naturally. Bitumens can be divided into six broad classes, according to their physical properties and specifications required for different applications. The major use (about 80%) of bitumens is for road paving; other uses include roofing, waterproofing, sealing and painting. The term "bitumen" should not be confused with "asphalt", which refers to the mixture of bitumen (4-10% by weight), small stones, sand and filler used for road paving. Bitumens are complex mixtures that contain a large number of organic chemical compounds. Application of bitumens may generate emissions (fumes and vapours) that may contain, among volatile and non-volatile compounds, a number of known or probable carcinogens. An *IARC Monographs* Working Group reviewed epidemiological evidence, animal bioassays, and mechanistic and other relevant data to reach conclusions as to the carcinogenic hazard to humans of various occupations that entail exposure to bitumens and bitumen emissions, including road paving, roofing, and application of mastic asphalt, and to various heterocyclic polycyclic aromatic compounds.