WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

VOLUME 92

Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures

LYON, FRANCE
2010

WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

VOLUME 92

Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures

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 2005

2010

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, life-style 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 Internet: 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 831057 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

Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures/IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2005: Lyon, France)

(IARC monographs on the evaluation of carcinogenic risks to humans; v. 92)

1. Carcinogens 2. DNA Adducts 3. Environmental Pollutants – toxicity
4. Neoplasms – etiology 5. Polycyclic Hydrocarbons, Aromatic – toxicity
I. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans II. Series

ISBN 978 92 832 1292 8 (NLM Classification: W1)
ISSN 1017-1606

PRINTED IN FRANCE

Photograph: Copyright © IARC by Georges Mollon
Historical image of young chimney sweep: Copyright © Mary Evans Picture Library London, United Kingdom; used with permission
Chemical structures (from lower left to upper right): Benzo[*a*]pyrene, dibenzo[*a,l*]pyrene, naphtho[2,1-*a*]fluoranthene

Cover design: Roland Dray and Georges Mollon, IARC

CONTENTS

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 under some circumstances. 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.

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.

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

VOLUME 92
SOME NON-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS AND SOME RELATED EXPOSURES

Lyon, 11–18 October 2005

LIST OF PARTICIPANTS

Working Group Members[1,2]

Janet Arey, Air Pollution Research Center, University of California–Riverside, Riverside, CA 92521, USA (*Subgroup Chair, Exposure Data*)

Frederick A. Beland, Division of Biochemical Toxicology (HTF-110), National Center for Toxicological Research, 3900 NCTR Road, Jefferson, Arkansas 72079-9502, USA (*Subgroup Chair, Cancer in Experimental Animals*)

Jürgen Borlak, Center of Pharmacology and Toxicology, Medical School of Hannover, Fraunhofer Institute of Toxicology and Experimental Medicine, Nikolai-Fuchs-Strasse 1, D-30625 Hannover, Germany

Scott W. Burchiel, 1 University of New Mexico, Mail Code MSC09 5360, New Mexico NIEHS Center, Albuquerque, NM 87131-0001, USA

Ercole Cavalieri[3], Eppley Cancer Institute, University of Nebraska Medical Center, Nebraska Medical Center, Omaha, NE 68198-6805, USA

Victor J. Feron, TNO Quality of Life (retired), Utrechtseweg 48, NL-3704 HE Zeist, The Netherlands

---

[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only.

[2] NOTE: Minor pertinent interests are not listed. Examples of minor interests include stock valued at no more than US$10 000 overall, research grants that provide no more than 5% of the unit's research budget, and consulting and speaking engagements, on matters not related to courts or government agencies, that do not exceed 2% of time or compensation. All consulting or speaking on matters before a court or government agency is listed as a significant pertinent interest.

[3] Dr Cavalieri has dissociated himself from this volume, having concluded that radical cations play the major role in the initiation of carcinogenesis by benzo[*a*]pyrene, dibenzo[*a,l*]pyrene, and 7,12-dimethylbenz[*a*]-anthracene and that depurinating adducts are the major actors in the initiation of carcinogenesis by these three compounds.

Tony Fletcher, Public and Environmental Health Research Unit, London School of Hygiene & Tropical Medicine, Keppel Street, GB-London WC1E 7HT, United Kingdom

Lynn Flowers, National Center for Environmental Assessment, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW (8601D), Washington, DC 20460, USA

Per Gerde, Institute of Environmental Medicine, Division of Physiology, Karolinska Institute, Scheeles Väg 1, Box 287, SE-171 77 Stockholm, Sweden

Per Gustavsson, Institute of Environmental Medicine, Karolinska Institute, SE-171 76 Stockholm, Sweden (*Subgroup Chair, Cancer in Humans*)

Ron A. Herbert[4], Pathology Support Group, Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709, USA

Daniel Krewski, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Room 320, One Stewart Street, Ottawa, Ontario K1N 6N5, Canada (*Meeting Chair*)

M. Matilde Marques, Centro de Química Estrutural, Departamento de Engenharia Química e Biológica, Instituto Superior Técnico, Av. Rovisco Pais, P-1049-001 Lisboa, Portugal

Hasan Mukhtar, Department of Dermatology, University of Wisconsin, Medical Sciences Center, Room B-25, 1300 University Avenue, Madison, WI 53706, USA

Stephen Nesnow, Integrated Systems Toxicology Division/NHEERL, U.S. Environmental Protection Agency, 109 T.W. Alexander Drive, B143-06, Research Triangle Park, NC 27711, USA (*Subgroup Chair, Other Relevant Data*)

Timo Partanen, Instituto Regional de Estudios en Sustancias Toxicas (IRET), Facultad de Ciencias de la Tierra y el Mar, National University, Apartado Postal 86, 3000 Heredia, Costa Rica

Trevor M. Penning, Department of Pharmacology, University of Pennsylvania, School of Medicine, 130C John Morgan Bldg, 3620 Hamilton Walk, Philadelphia, PA 19104-6084, USA

Tsutomu Shimada, Department of Biochemistry and Center in Molecular Toxicology, School of Medicine, Vanderbilt University, 638 Robinson Research Building, Nashville, Tennessee 37232-0146, USA (*unable to attend*)

Rashmi Sinha, Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, EPS, Rm 3046, Rockville, MD 20892-7273, USA (*not present for evaluations*)

Isabelle Stücker, INSERM U170, IFR69, 16 Avenue Paul Vaillant Couturier, F-94807 Villejuif Cedex, France

[4] Partner had been employed by R.J. Reynolds Tobacco Company. There has been no continuing professional relationship for more than one year.

Kirsi Vähäkangas, Department of Pharmacology and Toxicology, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland

Invited Specialists

Robert Herrick[5], Department of Environmental Health, Harvard School of Public Health, Harvard University, 401 Park Drive, Landmark Center, Room 404E, Boston MA 02215, USA

Ted Junghans[6], Technical Resources International Inc., 6500 Rock Spring Drive, Suite 650, Bethesda, MD 20817-1197, USA

Hans Kromhout[7], Environmental Epidemiology Division, Institute for Risk Assessment Sciences (IRAS), Utrecht University, PO Box 80.178, Jenalaan 18d, NL-3508 TD Utrecht, The Netherlands

Steve Olin[6], ILSI Research Foundation, Risk Science Institute, One Thomas Circle, NW, 9th Floor, Washington, DC 20005-5802, USA

Representatives of National or International Health Agencies

Christopher De Rosa, Division of Toxicology and Environmental Medicine, Centers for Disease Control and Prevention (CDC), Agency for Toxic Substances and Disease Registry (ATSDR), 1600 Clifton Road NE, Mailstop F-32, Atlanta GA 30333, USA

Jeffrey A. Ross, Cancer Biology Branch, Mail Drop B143-06, Environmental Carcinogenesis Division, NHEERL, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA

Observers

Observer for CONCAWE[8]

Geoff Granville, Toxicology & Product Stewardship, Shell Canada Limited, PO Box 100, Station M, Calgary, Alberta T2P 2H5, Canada

Observer for Michelin

David Lee Williams, Occupational Medicine/Toxicology, Manufacture Michelin, SGEP/M Bât. T15, Infirmerie, 23 Place des Carmes, F-63040 Clermont-Ferrand cedex 9, France

IARC Secretariat

Robert Baan (*Rapporteur, Other Relevant Data*)
Vincent James Cogliano (*Head of Programme*)
Fatiha El Ghissassi (*Co-rapporteur, Other Relevant Data*)

---

[5] Received research support from the National Asphalt Paving Association.

[6] Participation funded by the U.S. National Cancer Institute under a contract to Technical Resources International, Inc., and a subcontract to the International Life Sciences Institute.

[7] Received research support from the Dutch Pavers Association. Future support is under consideration.

[8] European association of oil companies

Yann Grosse (*Rapporteur, Cancer in Experimental Animals*)
Maria León
Verena Loch, WHO Programme on Cancer Control, Geneva
Jane Mitchell (*Editor*)
Nikolai Napalkov
Magali Olivier
Ann Olsson
Ghislaine Scélo
Béatrice Secretan (*Rapporteur, Exposure Data*)
Kurt Straif (*Responsible Officer; Rapporteur, Cancer in Humans*)
Andreas Ullrich, WHO Programme on Cancer Control, Geneva

Administrative assistance
Sandrine Egraz
Michel Javin
Brigitte Kajo
Martine Lézère
Helene Lorenzen-Augros

Post-meeting assistance
Lamia Benbrahim-Tallaa
Laurent Galichet
Neela Guha
Anne-Sophie Hameau
Sylvia Moutinho
Karine Racinoux
Dorothy Russell

IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

PREAMBLE

1. BACKGROUND

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme to evaluate the carcinogenic risk of chemicals to humans and to produce monographs on individual chemicals. The *Monographs* programme has since been expanded to include consideration of exposures to complex mixtures of chemicals (which occur, for example, in some occupations and as a result of human habits) and of exposures to other agents, such as radiation and viruses. With Supplement 6 (IARC, 1987a), the title of the series was modified from *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* to *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, in order to reflect the widened scope of the programme.

The criteria established in 1971 to evaluate carcinogenic risk to humans were adopted by the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monographs series*. Those criteria were subsequently updated by further ad-hoc working groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987b, 1988, 1991a; Vainio *et al.*, 1992).

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* may also indicate where additional research efforts are needed.

The *Monographs* represent the first step in carcinogenic risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that certain exposures could alter the incidence of cancer in humans. The second step is quantitative risk estimation. Detailed, quantitative evaluations of epidemiological data may be made in the *Monographs*, but without extrapolation beyond the range of the data available. Quantitative extrapolation from experimental data to the human situation is not undertaken.

The term ‘carcinogen’ is used in these monographs to denote an exposure that is capable of increasing the incidence of malignant neoplasms; the induction of benign neo-

plasms may in some circumstances (see p. 19) contribute to the judgement that the exposure is carcinogenic. The terms 'neoplasm' and 'tumour' are used interchangeably.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several 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, 1991a; Vainio *et al.*, 1992; see also pp. 25–27).

The *Monographs* may assist national and international authorities in making risk assessments and in formulating decisions concerning any necessary preventive measures. The evaluations of IARC working groups are scientific, qualitative judgements about the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which regulatory measures may be based. Other components of regulatory decisions vary from one situation to another and from country to country, responding to different socioeconomic and national priorities. **Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments and/or other international organizations.**

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of a wide range of human exposures. A survey of users in 1988 indicated that the *Monographs* are consulted by various agencies in 57 countries. About 2500 copies of each volume are printed, for distribution to governments, regulatory bodies and interested scientists. The Monographs are also available from IARC*Press* in Lyon and via the Marketing and Dissemination (MDI) of the World Health Organization in Geneva.

3. SELECTION OF TOPICS FOR MONOGRAPHS

Topics are selected on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some evidence or suspicion of carcinogenicity. The term 'agent' is used to include individual chemical compounds, groups of related chemical compounds, physical agents (such as radiation) and biological factors (such as viruses). Exposures to mixtures of agents may occur in occupational exposures and as a result of personal and cultural habits (like 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. The IARC information bulletins on agents being tested for carcinogenicity (IARC, 1973–1996) and directories of on-going research in cancer epidemiology (IARC, 1976–1996) often indicate exposures that may be scheduled for future meetings. Ad-hoc working groups convened by IARC in 1984, 1989, 1991, 1993 and 1998 gave

recommendations as to which agents should be evaluated in the IARC Monographs series (IARC, 1984, 1989, 1991b, 1993, 1998a,b).

As significant new data on subjects on which monographs have already been prepared become available, re-evaluations are made at subsequent meetings, and revised monographs are published.

4. DATA FOR MONOGRAPHS

The *Monographs* do not necessarily cite all the literature concerning the subject of an evaluation. Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to biological and epidemiological data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the working groups. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see pp. 25–27). In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, unpublished sources of information may be used.

5. THE WORKING GROUP

Reviews and evaluations are formulated by a working group of experts. The tasks of the group 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 mechanism of action; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans.

Working Group participants who contributed to the considerations and evaluations within a particular volume are listed, with their addresses, at the beginning of each publication. 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. In addition, nominees of national and international agencies and industrial associations may be invited as observers.

6. WORKING PROCEDURES

Approximately one year in advance of a meeting of a working group, the topics of the monographs are announced and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are

collected by the Carcinogen Identification and Evaluation Unit of IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as MEDLINE and TOXLINE.

For chemicals and some complex mixtures, the major collection of data and the preparation of first drafts of the sections on chemical and physical properties, on analysis, on production and use and on occurrence are carried out under a separate contract funded by the United States National Cancer Institute. Representatives from industrial associations may assist in the preparation of sections on production and use. Information on production and trade is obtained from governmental and trade publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available because their publication could disclose confidential information. 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, or is used by IARC staff, to prepare sections for the first drafts of monographs. The first drafts are compiled by IARC staff and sent before the meeting to all participants of the Working Group for review.

The Working Group meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, edited and prepared for publication. The aim is to publish monographs within six months of the Working Group meeting.

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, directly impinging on its interpretation, should be brought to the attention of the reader, a comment is given in square brackets.

7. EXPOSURE DATA

Sections 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 included at the beginning of each monograph.

Most monographs on individual chemicals, groups of chemicals or complex mixtures include sections on chemical and physical data, on analysis, on production and use and on occurrence. In monographs on, for example, physical agents, occupational exposures and cultural habits, other sections may be included, such as: historical perspectives, description of an industry or habit, chemistry of the complex mixture or taxonomy. Mono-

graphs on biological agents have sections on structure and biology, methods of detection, epidemiology of infection and clinical disease other than cancer.

For chemical exposures, the Chemical Abstracts Services Registry Number, the latest Chemical Abstracts primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. For biological agents, taxonomy and structure are described, and the degree of variability is given, when applicable.

Information on chemical and physical properties and, in particular, data relevant to identification, occurrence and biological activity are included. For biological agents, mode of replication, life cycle, target cells, persistence and latency and host response are given. 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.

The purpose of the section on analysis or detection is to give the reader an overview of current methods, with emphasis on those widely used for regulatory purposes. Methods for monitoring human exposure are also given, when available. No critical evaluation or recommendation of any of the methods is meant or implied. The IARC published a series of volumes, *Environmental Carcinogens: Methods of Analysis and Exposure Measurement* (IARC, 1978–93), that describe validated methods for analysing a wide variety of chemicals and mixtures. For biological agents, methods of detection and exposure assessment are described, including their sensitivity, specificity and reproducibility.

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents which 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. 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.

Data on production, international trade and uses are obtained for representative regions, which usually include Europe, Japan and the United States of America. 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.

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. In the case of mixtures, industries, occupations or processes, information is given about all agents

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 time and place. For biological agents, the epidemiology of infection is described.

Statements concerning regulations and guidelines (e.g., pesticide registrations, maximal levels permitted in foods, occupational exposure limits) are included for some countries as indications of potential exposures, 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 vaccines and therapy, are described.

8. STUDIES OF CANCER IN HUMANS

(a) Types of studies considered

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

Cohort and case–control studies relate the exposures under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence or mortality in those exposed to incidence or mortality in those not exposed) as the main measure of association.

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, mixture or exposure circumstance under study. Because individual exposure is not documented, however, a causal relationship is less easy to infer from correlation studies than from cohort and case–control studies. Case reports 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 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 surrounding interpretation of case reports 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, relevant case reports or correlation studies may add materially to the judgement that a causal relationship is present.

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

(b) Quality of studies considered

The Monographs are not intended to summarize all published studies. Those that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when they provide the only data available. Their inclusion 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 the study description.

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. By 'bias' is meant the operation of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between disease and an agent, mixture or exposure circumstance. By 'confounding' is meant a situation in which 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. In evaluating the extent to which these factors have been minimized in an individual study, working groups consider a number of aspects of design and analysis as described in the report of the study. 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.

Firstly, 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.

Secondly, the authors should have taken account in the study design and analysis of 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 be more appropriate than those with national rates. Internal comparisons of disease frequency among individuals at different levels of exposure should also have been made in the study.

Thirdly, 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. The methods used should preferably have been the generally accepted techniques that have been refined since the mid-1970s. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c) Inferences about mechanism of action

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 and time since exposure ceased, are reviewed and summarized when available. The analysis of temporal relationships can be useful in formulating models of carcinogenesis. In particular, 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 the mechanism of action. Special attention is given to measurements of biological markers of carcinogen exposure or action, such as DNA or protein adducts, as well as markers of early steps in the carcinogenic process, such as proto-oncogene mutation, when these are incorporated into epidemiological studies focused on cancer incidence or mortality. Such measurements may allow inferences to be made about putative mechanisms of action (IARC, 1991a; Vainio *et al.*, 1992).

(d) Criteria for causality

After the individual epidemiological studies of cancer have been summarized and the quality assessed, a judgement is made concerning the strength of evidence that the agent, mixture or exposure circumstance in question is carcinogenic for humans. In making its judgement, the Working Group considers several criteria for causality. A strong association (a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that relative risks of small magnitude do not imply lack of causality and may be important if the disease is common. Associations that are replicated in several studies of the same design or using 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 amount of exposure), and results of studies judged to be of high quality are given more weight than those of studies judged to be methodologically less sound. When suspicion of carcinogenicity arises largely from a single study, these data are not combined with those from later studies in any subsequent reassessment of the strength of the evidence.

If the risk of the disease in question increases with the amount of exposure, this is considered to be a strong indication of causality, although absence of a graded response is not necessarily evidence against a causal relationship. 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.

Although a carcinogen may act upon more than one target, the specificity of an association (an increased occurrence of cancer at one anatomical site or of one morphological type) adds plausibility to a causal relationship, particularly when excess cancer occurrence is limited to one morphological type within the same organ.

Although rarely available, results from randomized trials showing different rates 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, the judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first of all that the studies giving rise to it 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 be consistent with a relative risk of unity for any observed level of exposure and, when considered together, should provide a pooled estimate of relative risk which is at or near unity and has 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 for the relative risk of cancer to increase with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained in this way from several epidemiological studies can apply only to the type(s) of cancer studied and to dose levels and intervals between first exposure and observation of disease that are the same as or less than those observed in all the studies. Experience with human cancer indicates that, in some cases, the period from first exposure to the development of clinical cancer is seldom less than 20 years; studies with latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

9. STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

All known human carcinogens that have been studied adequately in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (aflatoxins, 4-aminobiphenyl, azathioprine, betel quid with tobacco, bischloromethyl ether and chloromethyl methyl ether (technical grade), chlorambucil, chlornaphazine, ciclosporin, coal-tar pitches, coal-tars, combined oral contraceptives, cyclophosphamide, diethylstilboestrol, melphalan, 8-methoxypsoralen plus ultraviolet A radiation, mustard gas, myleran, 2-naphthylamine, nonsteroidal estrogens, estrogen replacement therapy/steroidal estrogens, solar radiation, thiotepa and 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

and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, **in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is *sufficient evidence* (see p. 24) of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans**. The possibility that a given agent may cause cancer through a species-specific mechanism which does not operate in humans (see p. 27) should also be taken into consideration.

The nature and extent of impurities or contaminants present in the chemical or mixture being evaluated are given when available. Animal strain, sex, numbers per group, age at start of treatment and survival are reported.

Other types of studies summarized include: experiments in which the agent or mixture was administered in conjunction with known carcinogens or factors that modify carcinogenic effects; studies in which the end-point was not cancer but a defined precancerous lesion; and experiments on the carcinogenicity of known metabolites and derivatives.

For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

An assessment is made as to the relevance to human exposure of samples tested in experimental animals, which may involve consideration of: (i) physical and chemical characteristics, (ii) constituent substances that indicate the presence of a class of substances, (iii) the results of tests for genetic and related effects, including studies on DNA adduct formation, proto-oncogene mutation and expression and suppressor gene inactivation. The relevance of results obtained, for example, with animal viruses analogous to the virus being evaluated in the monograph must also be considered. They may provide biological and mechanistic information relevant to the understanding of the process of carcinogenesis in humans and may strengthen the plausibility of a conclusion that the biological agent under evaluation is carcinogenic in humans.

(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 and schedule of exposure, species, strain, sex, age, 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.

As mentioned earlier (p. 11), the *Monographs* are not intended to summarize all published studies. Those studies in experimental animals that are inadequate (e.g., too short a duration, too few animals, poor survival; see below) or are judged irrelevant to the evaluation are generally omitted. Guidelines for conducting adequate long-term carcinogenicity experiments have been outlined (e.g. Montesano *et al.*, 1986).

Considerations of importance to the Working Group 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 adequately monitored, particularly in inhalation experiments; (iii) whether the doses and duration of treatment were appropriate and whether the survival of treated animals was similar to that of controls; (iv) whether there were adequate numbers of animals per group; (v) whether animals of each sex were used; (vi) whether animals were allocated randomly to groups; (vii) whether the duration of observation was adequate; and (viii) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, should be taken into account in the evaluation of tumour response.

When benign tumours occur together with and originate from the same cell type in an organ or tissue as malignant tumours in a particular study and appear to represent a stage in the progression to malignancy, it may be valid to combine them in assessing tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be pre-neoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent or mixture induces only benign neoplasms that appear to be end-points that do not readily progress to malignancy, it should nevertheless be suspected of being a carcinogen and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain and age of the animal, the dose of the carcinogen and the route and length of exposure. Evidence of an increased incidence of neoplasms with increased level 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. Both DNA damage and increased cell division are important aspects of carcinogenesis, and cell proliferation is a strong determinant of dose–response relationships for some carcinogens (Cohen & Ellwein, 1990). Since many chemicals require metabolic activation before being converted into their reactive intermediates, both metabolic and pharmacokinetic 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, as could saturation of processes such as DNA repair (Hoel *et al.*, 1983; Gart *et al.*, 1986).

(c) Statistical analysis of long-term experiments in animals

Factors considered by the Working Group include the adequacy of the information given for each treatment group: (i) the number of animals studied and the number examined histologically, (ii) the 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). When there is no difference in survival between control and treatment groups, the Working Group usually compares the proportions of animals developing each tumour type in each of the groups. Otherwise, consideration is given as to whether or not appropriate adjustments have been made for differences in survival. These adjustments can include: comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour is discovered), in the case where most differences in survival occur before tumours appear; life-table methods, when tumours are visible or when they may be considered 'fatal' because mortality rapidly follows tumour development; and the Mantel-Haenszel test or logistic regression, when occult tumours do not affect the animals' risk of dying but are 'incidental' findings at autopsy.

In practice, classifying tumours as fatal or incidental may be difficult. Several survival-adjusted methods have been developed that do not require this distinction (Gart *et al.*, 1986), although they have not been fully evaluated.

10. OTHER DATA RELEVANT TO AN EVALUATION OF CARCINOGENICITY AND ITS MECHANISMS

In coming to an overall evaluation of carcinogenicity in humans (see pp. 25–27), the Working Group also considers related data. The nature of the information selected for the summary depends on the agent being considered.

For chemicals and complex mixtures of chemicals such as those in some occupational situations or involving cultural habits (e.g. tobacco smoking), the other data considered to be relevant are divided into those on absorption, distribution, metabolism and excretion; toxic effects; reproductive and developmental effects; and genetic and related effects.

Concise information is given on absorption, distribution (including placental transfer) and excretion in both humans and experimental animals. Kinetic factors that may affect the dose–response relationship, such as saturation of uptake, protein binding, metabolic activation, detoxification and DNA repair processes, are mentioned. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data on humans and on animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be of particular importance for extrapolation between species. Data are given on acute and chronic toxic effects (other than cancer), such as organ toxicity, increased cell proliferation, immunotoxicity and endocrine effects. The presence and toxicological significance of cellular receptors is described. Effects on reproduction, teratogenicity, fetotoxicity and embryotoxicity are also summarized briefly.

Tests of genetic and related effects are described in view of the relevance of gene mutation and chromosomal damage to carcinogenesis (Vainio *et al.*, 1992; McGregor *et al.*, 1999). The adequacy of the reporting of sample characterization is considered and, where necessary, commented upon; with regard to complex mixtures, such comments are

similar to those described for animal carcinogenicity tests on p. 18. The available data are interpreted critically by phylogenetic group according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and cell transformation. The concentrations employed are given, and mention is made of whether use of an exogenous metabolic system *in vitro* affected the test result. These data are given as listings of test systems, data and references.

Positive results in tests using prokaryotes, lower eukaryotes, plants, insects and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information about the types of genetic effect produced and about the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g., gene mutations and chromosomal aberrations), while others are to a greater or lesser degree associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour-promoting activity and for cell transformation 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. A critical appraisal of these tests has been published (Montesano *et al.*, 1986).

Genetic or other activity detected in experimental mammals and humans is regarded as being of greater relevance than that in other organisms. The demonstration that an agent or mixture can induce gene and chromosomal mutations in whole mammals indicates that it may have carcinogenic activity, although this activity may not be detectably expressed in any or all species. Relative potency in tests for mutagenicity and related effects is not a reliable indicator of carcinogenic potency. 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 to rule out carcinogenicity of agents or mixtures that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative proliferation, peroxisome proliferation) (Vainio *et al.*, 1992). Factors that may lead to misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al.*, 1986).

When available, data relevant to mechanisms of carcinogenesis that do not involve structural changes at the level of the gene are also described.

The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is evaluated by the same criteria as are applied to epidemiological studies of cancer.

Structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent are also described.

For biological agents — viruses, bacteria and parasites — other data relevant to carcinogenicity include descriptions of the pathology of infection, molecular biology (integration and expression of viruses, and any genetic alterations seen in human tumours)

and other observations, which might include cellular and tissue responses to infection, immune response and the presence of tumour markers.

11. SUMMARY OF DATA REPORTED

In this section, the relevant epidemiological and experimental data are summarized. Only reports, other than in abstract form, that meet the criteria outlined on p. 11 are considered for evaluating carcinogenicity. Inadequate studies are generally not summarized: such studies are usually identified by a square-bracketed comment in the preceding text.

(a) Exposure

Human exposure to chemicals and complex mixtures is summarized on the basis of elements such as production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are given when available. Exposure to biological agents is described in terms of transmission and prevalence of infection.

(b) Carcinogenicity in humans

Results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized.

(c) Carcinogenicity in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species and route of administration, it is stated whether an increased incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. If the agent or mixture produced tumours after prenatal exposure or in single-dose experiments, this is also indicated. Negative findings are also summarized. Dose– response and other quantitative data may be given when available.

(d) Other data relevant to an evaluation of carcinogenicity and its mechanisms

Data on biological effects in humans that are of particular relevance are summarized. These may include toxicological, kinetic and metabolic considerations and evidence of DNA binding, persistence of DNA lesions or genetic damage in exposed humans. Toxicological information, such as that on cytotoxicity and regeneration, receptor binding and hormonal and immunological effects, and data on kinetics and metabolism in experimental animals are given when considered relevant to the possible mechanism of the carcinogenic action of the agent. The results of tests for genetic and related effects are summarized for whole mammals, cultured mammalian cells and nonmammalian systems.

When available, comparisons of such data for humans and for animals, and particularly animals that have developed cancer, are described.

Structure–activity relationships are mentioned when relevant.

For the agent, mixture or exposure circumstance being evaluated, the available data on end-points or other phenomena relevant to mechanisms of carcinogenesis from studies in humans, experimental animals and tissue and cell test systems are summarized within one or more of the following descriptive dimensions:

(i) Evidence of genotoxicity (structural changes at the level of the gene): for example, structure–activity considerations, adduct formation, mutagenicity (effect on specific genes), chromosomal mutation/aneuploidy

(ii) Evidence of effects on the expression of relevant genes (functional changes at the intracellular level): for example, alterations to the structure or quantity of the product of a proto-oncogene or tumour-suppressor gene, alterations to metabolic activation/inactivation/DNA repair

(iii) Evidence of relevant effects on cell behaviour (morphological or behavioural changes at the cellular or tissue level): for example, induction of mitogenesis, compensatory cell proliferation, preneoplasia and hyperplasia, survival of premalignant or malignant cells (immortalization, immunosuppression), effects on metastatic potential

(iv) Evidence from dose and time relationships of carcinogenic effects and interactions between agents: for example, early/late stage, as inferred from epidemiological studies; initiation/promotion/progression/malignant conversion, as defined in animal carcinogenicity experiments; toxicokinetics

These dimensions are not mutually exclusive, and an agent may fall within more than one of them. Thus, for example, the action of an agent on the expression of relevant genes could be summarized under both the first and second dimensions, even if it were known with reasonable certainty that those effects resulted from genotoxicity.

12. EVALUATION

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

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, mixture or exposure circumstance to a higher or lower category than a strict interpretation of these criteria would indicate.

(a) Degrees of evidence for carcinogenicity in humans and in experimental animals and supporting evidence

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) nor to the mechanisms involved. A classification may change as new information becomes available.

An evaluation of degree of evidence, whether for a single agent or a mixture, 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 degree of evidence.

(i) *Carcinogenicity in humans*

The applicability of an evaluation of the carcinogenicity of a mixture, process, occupation or industry on the basis of evidence from epidemiological studies depends on the variability over time and place of the mixtures, processes, occupations and industries. The Working Group seeks to identify the specific exposure, process or activity which is 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.

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, mixture or exposure circumstance 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.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent, mixture or exposure circumstance 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 human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent, mixture or exposure circumstance and any studied cancer at any observed level of exposure. 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.

(ii) *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 or mixture 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) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Exceptionally, a single study in one species 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.

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; or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.

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 or mixture is not carcinogenic. A conclusion of evidence suggesting lack of carcinogenicity is inevitably limited to the species, tumour sites and levels of exposure studied.

(b) Other data relevant to the evaluation of carcinogenicity and its mechanisms

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

Data relevant to mechanisms of the carcinogenic action are also evaluated. The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is assessed, using terms such as weak, moderate or strong. Then, the Working Group assesses if that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans come 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.

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.

(c) Overall evaluation

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation of the carcinogenicity to humans of an agent, mixture or circumstance of exposure.

An evaluation may be made for a group of chemical compounds that have been evaluated by the Working Group. In addition, when supporting data indicate that other, related compounds for which there is no direct evidence of 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 compounds if the strength of the evidence warrants it.

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

Group 1 — The agent (mixture) is carcinogenic to humans.
The exposure circumstance entails exposures that are carcinogenic to humans.

This category is used when there is *sufficient evidence* of carcinogenicity in humans. Exceptionally, an agent (mixture) 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 (mixture) acts through a relevant mechanism of carcinogenicity.

Group 2

This category includes agents, mixtures and exposure circumstances 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, mixtures and exposure

circumstances 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 other relevant data.

Group 2A — The agent (mixture) is probably carcinogenic to humans.
The exposure circumstance entails exposures that are 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 (mixture) may be classified in this category when there is *inadequate evidence* of carcinogenicity in humans, *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, mixture or exposure circumstance may be classified in this category solely on the basis of *limited evidence* of carcinogenicity in humans.

Group 2B — The agent (mixture) is possibly carcinogenic to humans.
The exposure circumstance entails exposures that are possibly carcinogenic to humans.

This category is used for agents, mixtures and exposure circumstances 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, mixture or exposure circumstance for which there is *inadequate evidence* of carcinogenicity in humans but *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group.

Group 3 — The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans.

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

Exceptionally, agents (mixtures) 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, mixtures and exposure circumstances that do not fall into any other group are also placed in this category.

Group 4 — The agent (mixture) is probably not carcinogenic to humans.

This category is used for agents or mixtures for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents or mixtures 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 other relevant data, may be classified in this group.

13. REFERENCES

Breslow, N.E. & Day, N.E. (1980) *Statistical Methods in Cancer Research*, Vol. 1, *The Analysis of Case–Control Studies* (IARC Scientific Publications No. 32), Lyon, IARC*Press*

Breslow, N.E. & Day, N.E. (1987) *Statistical Methods in Cancer Research*, Vol. 2, *The Design and Analysis of Cohort Studies* (IARC Scientific Publications No. 82), Lyon, IARC*Press*

Cohen, S.M. & Ellwein, L.B. (1990) Cell proliferation in carcinogenesis. *Science*, **249**, 1007–1011

Gart, J.J., Krewski, D., Lee, P.N., Tarone, R.E. & Wahrendorf, J. (1986) *Statistical Methods in Cancer Research*, Vol. 3, *The Design and Analysis of Long-term Animal Experiments* (IARC Scientific Publications No. 79), Lyon, IARC*Press*

Hoel, D.G., Kaplan, N.L. & Anderson, M.W. (1983) Implication of nonlinear kinetics on risk estimation in carcinogenesis. *Science*, **219**, 1032–1037

Huff, J.E., Eustis, S.L. & Haseman, J.K. (1989) Occurrence and relevance of chemically induced benign neoplasms in long-term carcinogenicity studies. *Cancer Metastasis Rev.*, **8**, 1–21

IARC (1973–1996) *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity/Directory of Agents Being Tested for Carcinogenicity*, Numbers 1–17, Lyon, IARC*Press*

IARC (1976–1996), Lyon, IARC*Press*

Directory of On-going Research in Cancer Epidemiology 1976. Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1977 (IARC Scientific Publications No. 17). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1978 (IARC Scientific Publications No. 26). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1979 (IARC Scientific Publications No. 28). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1980 (IARC Scientific Publications No. 35). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1981 (IARC Scientific Publications No. 38). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1982 (IARC Scientific Publications No. 46). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1983 (IARC Scientific Publications No. 50). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1984 (IARC Scientific Publications No. 62). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1985 (IARC Scientific Publications No. 69). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1986 (IARC Scientific Publications No. 80). Edited by C.S. Muir & G. Wagner

Directory of On-going Research in Cancer Epidemiology 1987 (IARC Scientific Publications No. 86). Edited by D.M. Parkin & J. Wahrendorf

Directory of On-going Research in Cancer Epidemiology 1988 (IARC Scientific Publications No. 93). Edited by M. Coleman & J. Wahrendorf

Directory of On-going Research in Cancer Epidemiology 1989/90 (IARC Scientific Publications No. 101). Edited by M. Coleman & J. Wahrendorf

Directory of On-going Research in Cancer Epidemiology 1991 (IARC Scientific Publications No.110). Edited by M. Coleman & J. Wahrendorf

Directory of On-going Research in Cancer Epidemiology 1992 (IARC Scientific Publications No. 117). Edited by M. Coleman, J. Wahrendorf & E. Démaret

Directory of On-going Research in Cancer Epidemiology 1994 (IARC Scientific Publications No. 130). Edited by R. Sankaranarayanan, J. Wahrendorf & E. Démaret

Directory of On-going Research in Cancer Epidemiology 1996 (IARC Scientific Publications No. 137). Edited by R. Sankaranarayanan, J. Wahrendorf & E. Démaret

IARC (1977) *IARC Monographs Programme on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. Preamble (IARC intern. tech. Rep. No. 77/002)

IARC (1978) *Chemicals with* Sufficient Evidence *of Carcinogenicity in Experimental Animals* — IARC Monographs *Volumes 1–17* (IARC intern. tech. Rep. No. 78/003)

IARC (1978–1993) *Environmental Carcinogens. Methods of Analysis and Exposure Measurement*, Lyon, IARC*Press*

Vol. 1. Analysis of Volatile Nitrosamines in Food (IARC Scientific Publications No. 18). Edited by R. Preussmann, M. Castegnaro, E.A. Walker & A.E. Wasserman (1978)

Vol. 2. Methods for the Measurement of Vinyl Chloride in Poly(vinyl chloride), Air, Water and Foodstuffs (IARC Scientific Publications No. 22). Edited by D.C.M. Squirrell & W. Thain (1978)

Vol. 3. Analysis of Polycyclic Aromatic Hydrocarbons in Environmental Samples (IARC Scientific Publications No. 29). Edited by M. Castegnaro, P. Bogovski, H. Kunte & E.A. Walker (1979)

Vol. 4. Some Aromatic Amines and Azo Dyes in the General and Industrial Environment (IARC Scientific Publications No. 40). Edited by L. Fishbein, M. Castegnaro, I.K. O'Neill & H. Bartsch (1981)

Vol. 5. Some Mycotoxins (IARC Scientific Publications No. 44). Edited by L. Stoloff, M. Castegnaro, P. Scott, I.K. O'Neill & H. Bartsch (1983)

Vol. 6. N-Nitroso Compounds (IARC Scientific Publications No. 45). Edited by R. Preussmann, I.K. O'Neill, G. Eisenbrand, B. Spiegelhalder & H. Bartsch (1983)

Vol. 7. Some Volatile Halogenated Hydrocarbons (IARC Scientific Publications No. 68). Edited by L. Fishbein & I.K. O'Neill (1985)

Vol. 8. Some Metals: As, Be, Cd, Cr, Ni, Pb, Se, Zn (IARC Scientific Publications No. 71). Edited by I.K. O'Neill, P. Schuller & L. Fishbein (1986)

Vol. 9. Passive Smoking (IARC Scientific Publications No. 81). Edited by I.K. O'Neill, K.D. Brunnemann, B. Dodet & D. Hoffmann (1987)

*Vol. 10. Benzene and Alkylated Benzenes (*IARC Scientific Publications No. 85). Edited by L. Fishbein & I.K. O'Neill (1988)

Vol. 11. Polychlorinated Dioxins and Dibenzofurans (IARC Scientific Publications No. 108). Edited by C. Rappe, H.R. Buser, B. Dodet & I.K. O'Neill (1991)

Vol. 12. Indoor Air (IARC Scientific Publications No. 109). Edited by B. Seifert, H. van de Wiel, B. Dodet & I.K. O'Neill (1993)

IARC (1979) *Criteria to Select Chemicals for* IARC Monographs (IARC intern. tech. Rep. No. 79/003)

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 4, *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans* (IARC Monographs, Volumes 1 to 29), Lyon, IARC*Press*

IARC (1983) *Approaches to Classifying Chemical Carcinogens According to Mechanism of Action* (IARC intern. tech. Rep. No. 83/001)

IARC (1984) *Chemicals and Exposures to Complex Mixtures Recommended for Evaluation in IARC Monographs and Chemicals and Complex Mixtures Recommended for Long-term Carcinogenicity Testing* (IARC intern. tech. Rep. No. 84/002)

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 6, *Genetic and Related Effects: An Updating of Selected* IARC Monographs *from Volumes 1 to 42*, Lyon, IARC*Press*

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, IARC*Press*

IARC (1988) *Report of an IARC Working Group to Review the Approaches and Processes Used to Evaluate the Carcinogenicity of Mixtures and Groups of Chemicals* (IARC intern. tech. Rep. No. 88/002)

IARC (1989) *Chemicals, Groups of Chemicals, Mixtures and Exposure Circumstances to be Evaluated in Future IARC Monographs, Report of an ad hoc Working Group* (IARC intern. tech. Rep. No. 89/004)

IARC (1991a) *A Consensus Report of an IARC Monographs Working Group on the Use of Mechanisms of Carcinogenesis in Risk Identification* (IARC intern. tech. Rep. No. 91/002)

IARC (1991b) *Report of an ad-hoc* IARC Monographs *Advisory Group on Viruses and Other Biological Agents Such as Parasites* (IARC intern. tech. Rep. No. 91/001)

IARC (1993) *Chemicals, Groups of Chemicals, Complex Mixtures, Physical and Biological Agents and Exposure Circumstances to be Evaluated in Future* IARC Monographs, *Report of an ad-hoc Working Group* (IARC intern. Rep. No. 93/005)

IARC (1998a) *Report of an ad-hoc* IARC Monographs *Advisory Group on Physical Agents* (IARC Internal Report No. 98/002)

IARC (1998b) *Report of an ad-hoc* IARC Monographs *Advisory Group on Priorities for Future Evaluations* (IARC Internal Report No. 98/004)

McGregor, D.B., Rice, J.M. & Venitt, S., eds (1999) *The Use of Short and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evaluation* (IARC Scientific Publications No. 146), Lyon, IARC*Press*

Montesano, R., Bartsch, H., Vainio, H., Wilbourn, J. & Yamasaki, H., eds (1986) *Long-term and Short-term Assays for Carcinogenesis — A Critical Appraisal* (IARC Scientific Publications No. 83), Lyon, IARC*Press*

Peto, R., Pike, M.C., Day, N.E., Gray, R.G., Lee, P.N., Parish, S., Peto, J., Richards, S. & Wahrendorf, J. (1980) Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In: *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 2, *Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal*, Lyon, IARC*Press*, pp. 311–426

Tomatis, L., Aitio, A., Wilbourn, J. & Shuker, L. (1989) Human carcinogens so far identified. *Jpn. J. Cancer Res.*, **80**, 795–807

Vainio, H., Magee, P.N., McGregor, D.B. & McMichael, A.J., eds (1992) *Mechanisms of Carcinogenesis in Risk Identification* (IARC Scientific Publications No. 116), Lyon, IARC*Press*

Vainio, H., Wilbourn, J.D., Sasco, A.J., Partensky, C., Gaudin, N., Heseltine, E. & Eragne, I. (1995) Identification of human carcinogenic risk in IARC Monographs. *Bull. Cancer,* **82**, 339–348 (in French)

Waters, M.D., Stack, H.F., Brady, A.L., Lohman, P.H.M., Haroun, L. & Vainio, H. (1987) Appendix 1. Activity profiles for genetic and related tests. In: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 6, *Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1 to 42*, Lyon, IARC*Press*, pp. 687–696

Wilbourn, J., Haroun, L., Heseltine, E., Kaldor, J., Partensky, C. & Vainio, H. (1986) Response of experimental animals to human carcinogens: an analysis based upon the IARC Monographs Programme. *Carcinogenesis*, **7**, 1853–1863

GENERAL REMARKS

This ninety-second volume of *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of 60 polycyclic aromatic hydrocarbon (PAH) compounds and several occupations involving exposures to coal-derived PAHs. This is the first of several volumes related to agents that contribute to air pollution; subsequent volumes will cover certain particulate or chemical agents, indoor emissions from household combustion of solid fuels and high-temperature frying, asphalt/bitumen, motor vehicle emissions, and ultimately outdoor air pollution. Many of the PAHs and occupations evaluated in this volume were last reviewed more than 20 years ago in Volumes 32–35, a four-part series on polynuclear aromatic compounds. Newer experimental and epidemiological information have been published since that time and are reviewed in this volume.

There is a long history to the identification of PAHs as human cancer hazards. In 1775 Sir Percivall Pott identified soot as the cause of scrotal cancer in chimney sweeps (Brown and Thornton, 1957), the first attribution of an occupational cancer to a specific cause. In the early 1900s laboratory scientists began the search that led to the isolation of benz[*a*]anthracene, dibenz[*a,h*]anthracene, benzo[*a*]pyrene, and other PAH compounds and the demonstration that they can induce cancer in experimental animals (Phillips, 1983).

The evaluations of carcinogenic hazard in this volume are qualitative assessments of the evidence that a PAH congener or mixture can increase the incidence of cancer. The same evidence also demonstrates that PAH congeners and mixtures vary widely in the level of carcinogenic response induced by a given dose. Although benzo[*a*]pyrene is the marker of PAH exposure that is most often used, there is evidence that a few PAH congeners, for example, dibenzo[*a,l*]pyrene, are more potent in their ability to induce lung cancer or skin cancer in experimental systems. These potent congeners should be measured in environmental and biological samples, as they may contribute substantially to the risk of human cancer attributable to PAH mixtures.

Some occupations evaluated in this volume may entail exposures to carcinogens other than coal-derived PAHs, and it is possible that the observed cancer risks could be attributable in part to these other exposures. In aluminium production, for example, there is an excess of bladder cancer, and potential confounding by other occupational exposures could not be ruled out with reasonable confidence.

The *IARC Monographs* use the term 'carcinogen' to denote an exposure that is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. In reviewing bioassay data for this volume, the Working Group considered mouse skin initiation-promotion studies (single or multiple applications of a PAH followed by repeated applications of a tumour promoter such as 12-*O*-tetradecanoylphorbol-13-acetate) to be insufficient for assessing the carcinogenicity of PAHs *per se*. The Working Group also reviewed a number of newborn mouse assays in which the PAHs were administered to infant mice (e.g. days 1, 8, and 15 of life). Some of these newborn treatments led to neoplasms, which were in some instances malignant. The Working Group considered positive newborn mouse assays to be indicative of the genotoxic potential of the compound under investigation rather than as unambiguous proof of carcinogenic potential. The Working Group further felt that results of newborn mouse assays had to be viewed in light of other available data before making an assessment of the overall carcinogenic potential of an exposure.

A significant source of PAH exposure in the general population is the consumption of particular foods, notably toasted cereals and grilled meats. These foods contain measurable levels of benzo[*a*]pyrene and other PAHs that are *carcinogenic, probably carcinogenic,* or *possibly carcinogenic to humans*, and there is strong evidence that some of these compounds, including benzo[*a*]pyrene, induce digestive-tract tumours in experimental animals when administered by ingestion. There are, however, few epidemiological studies investigating directly the possible association between dietary PAH intake and cancer. The studies conducted to date used questionnaires about meat-cooking in conjunction with benzo[*a*]pyrene as a marker for total PAH intake. Three case-control studies observed a small-to-moderate increase in the risk of colorectal adenoma, a precursor of colon cancer, with higher estimated intake of benzo[*a*]pyrene. There was, however, no association with benzo[*a*]pyrene intake in a case-control study of colon cancer. One case-control study of pancreatic cancer also observed a moderate increase in risk with benzo[*a*]pyrene intake. There was no association with benzo[*a*]pyrene in one study of prostate cancer and non-Hodgkin lymphoma. This volume reviews the epidemiological studies that suggest a possible association between consumption of PAHs in foods and increased risks of colorectal adenoma and pancreatic cancer. These epidemiological studies, however, are limited to one geographical area and are too small to be considered conclusive. Large-scale independent cohort studies are needed to more definitively investigate these associations.

A summary of the findings of this volume appears in *The Lancet Oncology*.

References

Brown JR, Thornton JL. (1957) Percivall Pott (1714–1788) and chimney sweeper's cancer of the scrotum. *Occup Environ Med* 14: 68–70.

Phillips, DH. (1983) Fifty years of benzo(*a*)pyrene. *Nature* 303: 468–472.

MONOGRAPH ON SOME NON-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS AND SOME RELATED EXPOSURES

SOME NON-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS AND SOME RELATED EXPOSURES

The compounds covered in this monograph are listed in Table 1.1

1. Exposure Data

Polycyclic aromatic hydrocarbons (PAHs) are very widespread environmental contaminants, due to their formation during the incomplete combustion or pyrolysis of organic material. They are found in air, water, soils and sediments, generally at trace levels except near their sources. Tobacco smoke contains high concentrations of PAHs. They are present in some foods and in a few pharmaceutical products that are applied to the skin.

Occupational exposure to PAHs in several work environments can lead to body burdens among exposed workers that are considerably higher than those in the general population. In particular, industrial processes that involve the pyrolysis or combustion of coal and the production and use of coal-derived products are major sources of PAHs and are the focus of this monograph.

1.1 Chemical and physical data

1.1.1 *Nomenclature, structure and properties*

The term polycyclic aromatic hydrocarbons (PAHs) commonly refers to a large class of organic compounds that contain only carbon and hydrogen and are comprised of two or more fused aromatic rings.

The PAHs that have been chosen for inclusion in this monograph are presented in the Appendix; their nomenclature is listed in Table 1 and their structures are given in Figure 1 therein. The International Union of Pure and Applied Chemistry (IUPAC) Systematic Name (IUPAC, 1979; Sander & Wise, 1997), the Chemical Abstracts Services (CAS) Registry Number, the molecular formula and the relative molecular mass for each compound are given in Table 1 of the Appendix. The chemical and physical properties, the latest Chemical Abstracts Primary Name (9th Collective Index), common synonyms and sources for spectroscopic data are given in Table 2 and the main text of this Appendix. The nomenclature of PAHs has been inconsistent and the more commonly

used names that appear in this monograph may not be those used in the primary CAS Index or by IUPAC.

Table 1.1. *IARC Monographs* volume[a] and evaluation of the polycyclic aromatic hydrocarbons covered in this monograph

Common name	Volume(s)	Group
Acenaphthene	–	–
Acepyrene	–	–
Anthanthrene	32	3
Anthracene	32	3
11*H*-Benz[*b*,*c*]aceanthrylene	–	–
Benz[*j*]aceanthrylene	–	–
Benz[*l*]aceanthrylene	–	–
Benz[*a*]anthracene	3, 32	2A
Benzo[*b*]chrysene	–	–
Benzo[*g*]chrysene	–	–
Benzo[*a*]fluoranthene	–	–
Benzo[*b*]fluoranthene	3, 32	2B
Benzo[*ghi*]fluoranthene	32	3
Benzo[*j*]fluoranthene	3, 32	2B
Benzo[*k*]fluoranthene	32	2B
Benzo[*a*]fluorene	32	3
Benzo[*b*]fluorene	32	3
Benzo[*c*]fluorene	32	3
Benzo[*ghi*]perylene	32	3
Benzo[*c*]phenanthrene	32	3
Benzo[*a*]pyrene	3, 32	2A
Benzo[*e*]pyrene	3, 32	3
Chrysene	3, 32	3
Coronene	32	3
4*H*-Cyclopenta[*def*]chrysene	–	–
Cyclopenta[*cd*]pyrene	32	3
5,6-Cyclopenteno-1,2-benzanthracene	–	–
Dibenz[*a*,*c*]anthracene	32	3
Dibenz[*a*,*h*]anthracene	3, 32	2A
Dibenz[*a*,*j*]anthracene	32	3
Dibenzo[*a*,*e*]fluoranthene	32	3
13*H*-Dibenzo[*a*,*g*]fluorene	–	–
Dibenzo[*h*,*rst*]pentaphene	3	3
Dibenzo[*a*,*e*]pyrene	3, 32	2B
Dibenzo[*a*,*h*]pyrene	3, 32	2B
Dibenzo[*a*,*i*]pyrene	3, 32	2B
Dibenzo[*a*,*l*]pyrene	3, 32	2B
Dibenzo[*e*,*l*]pyrene	–	–
1,2-Dihydroaceanthrylene	–	–
1,4-Dimethylphenanthrene	32	3
Fluoranthene	32	3
Fluorene	32	3
Indeno[1,2,3-*cd*]pyrene	3, 32	2B

Table 1.1 (contd)

Common name	Volume(s)	Group
1-Methylchrysene	32	3
2-Methylchrysene	32	3
3-Methylchrysene	32	3
4-Methylchrysene	32	3
5-Methylchrysene	32	2B
6-Methylchrysene	32	3
2-Methylfluoranthene	32	3
3-Methylfluoranthene	32	3
1-Methylphenanthrene	32	3
Naphtho[1,2-*b*]fluoranthene	–	–
Naphtho[2,1-*a*]fluoranthene	–	–
Naphtho[2,3-*e*]pyrene	–	–
Perylene	32	3
Phenanthrene	32	3
Picene	–	–
Pyrene	32	3
Triphenylene	32	3

[a] Vol. 3 published in 1973, Vol. 32 in 1983 and Suppl. 7 in 1987 (IARC, 1973, 1983, 1987).

The chemical structures and ring numbering shown in the Appendix follow the IUPAC rules for fused-ring systems. Structures are typically oriented such that (i) the greatest number of rings in a row are aligned horizontally, (ii) the maximum number of rings is positioned in the upper right quadrant and (iii) the least number of rings is positioned in the lower left quadrant. Numbering begins with the uppermost ring the furthest to the right; the most counterclockwise carbon atom is not involved with ring fusion. The numbering proceeds clockwise around the structure with hydrogenated carbon atoms. The numbering of anthracene and phenanthrene are 'retained exceptions' to this rule. Numbering of atoms engaged in ring fusion (numbers not shown in this monograph) are given letters, such as *a*, *b* and *c*, after the number of the preceding atom.

The 35 IUPAC 'parent compounds' are used in the nomenclature, and structures are built from these by adding prefixes (e.g. benzo-, cyclopenta- or a group of rings such as indeno-), followed by an italic letter or letters denoting the bond or bonds of the base (which has as many rings as possible) at which fusion occurs. The letter *a* refers to the 1,2-bond, and all bonds are then lettered sequentially whether or not they carry hydrogen atoms (Lee *et al.*, 1981). The IUPAC parent compounds are given an order of increasing priority with increasing ring number. The parent with the highest priority is used to name the structure. An exception to this rule is the choice of benzo[*a*]pyrene over benzo[*def*]chrysene.

The important chemical and physical properties of each pure PAH are summarized in Table 2 of the Appendix and include, where available: melting-point, vapour pressure,

partition coefficient for *n*-octanol:water (log K_{ow}), water solubility, and Henry's law constant. These physicochemical properties of PAHs — namely, very low water solubility, low vapour pressure and high log K_{ow} — control the transport and distribution of PAHs in the environment. A more complete set of data that includes the available descriptions of crystals, and data on boiling-point, density and rate constant for atmospheric gas-phase reactions (low molecular weights only) are given in the Appendix. Only experimental data are reported here and, for consistency, log K_{ow} values generally include evaluated values only (Sangster Research Laboratories, 2005).

1.1.2 *Analysis*

(a) Analysis of ambient exposure to PAHs

Chemical analysis of PAHs often requires extensive separation schemes because of their lack of distinct functional groups, the existence of numerous structural isomers and the need to analyse PAHs in diverse environmental matrices. Methods for the analysis of PAHs were described in detail in the 1980s (for example, Lee *et al*., 1981; Bjorseth, 1983; IARC, 1983; Bjorseth & Ramdahl, 1985) and have recently been updated (IPCS, 1998; Neilson, 1998). Unfortunately, the PAHs that have been quantified in ambient and occupational samples are often very limited in number; for example, the 16 'US Environmental Protection Agency priority pollutant PAHs' are often measured, and the larger PAHs (molecular weight >300), which have been suggested to have an important carcinogenic impact (Grimmer *et al.*, 1984), have been addressed only recently (Schubert *et al*., 2003).

(i) *Collection and sampling*

Two- to four-ring PAHs are present, at least partially, in the gas phase in ambient and industrial atmospheres (Coutant *et al.*, 1988), and sampling of total PAHs requires that an adsorbent be placed downstream from the filter that samples the particle-associated PAHs. In addition, air stripping, that is caused by the passage of large volumes of air, can cause volatile components to be lost from the filter, and, again, it is very important that sampling techniques include adsorbents downstream of the filter (NIOSH, 2000). Common adsorbents used include Amberlite XAD resins, polyurethane foam and Tenax-GC (Chuang *et al.*, 1987; Reisen & Arey, 2005). Size-fractionated sampling of particles is now often used to apportion the sources of ambient particles and to investigate the health impact of ambient particles. The US Environmental Protection Agency classifies particle diameters as 'coarse' (2.5–10 μm), 'fine' (≤2.5 μm) and 'ultrafine' (<0.1 μm).

During ambient sampling, reaction of the PAHs on the filter with ambient gaseous species such as ozone can result in an underestimation of the actual concentrations (Schauer *et al.*, 2003). Such 'artefacts' are probably less important in workplace atmospheres where higher PAH concentrations allow shorter sampling times.

(ii) *Extraction*

Extraction techniques used include solvent, Soxhlet, ultrasonic, microwave-assisted, supercritical fluid, accelerated solvent and solid-phase extraction, and these have been evaluated for use with different sample matrices (Colmsjö, 1998). The addition of deuterated internal standards of specific PAHs and quantification by 'isotope-dilution' mass spectrometry (MS) is one technique that is often employed to correct for losses of analyte during sample preparation (Boden & Reiner, 2004).

(iii) *Quantification and identification of PAHs in isolated mixtures of polycyclic aromatic compounds*

Due to the existence of numerous structural isomers of the PAHs, chromatographic separation either by gas chromatography (GC) or high-performance liquid chromatography (HPLC) is generally employed for isomer-specific identification and quantification. In addition, HPLC provides a useful fractionation technique for isolating PAHs from complex sample mixtures and allows quantification with universal or selective detectors after further separation, for example, by GC with MS (GC–MS) (Reisen & Arey, 2005). The development of standard reference materials (SRMs) with certified values for PAHs in complex environmental matrices allows evaluation of new analytical techniques (Wise *et al.*, 1993; Schubert *et al*., 2003).

(iv) *Liquid chromatography (LC)*

The development of reverse-phase (RP) HPLC columns coupled with ultraviolet (UV) absorbance and fluorescence detection has improved the analysis of a range of PAHs including high-molecular-weight species (Fetzer & Biggs, 1993; Wise *et al.*, 1993). The length:breadth ratio is a shape-descriptive parameter that has been used in numerous studies of PAH retention in both LC and GC (see Poster *et al*., 1998 and references therein), and a useful listing of length:breadth ratio for many of the PAHs has been compiled (Sander & Wise, 1997). For a comprehensive review of the selectivity of monomeric and polymeric C_{18} RP HPLC columns for PAH analysis, the reader is referred to Poster *et al.* (1998).

(v) *Gas chromatography*

High-efficiency capillary GC columns with thermally stable stationary phases are used routinely for the analysis of PAHs. Using GC–MS and three different GC stationary phases, 23 isomers of molecular weight 302 and four isomers of molecular weight 300 were recently quantified in four different environmental–matrix SRMs: coal tar (SRM 1597), sediment (SRM 1941) and air particulate matter (SRMs 1648 and 1649a) (Schubert *et al.*, 2003).

(vi) *Other methods of quantification*

Laser-excited time-resolved Shpol'skii spectroscopy has recently been reported as a method for the unambiguous determination of dibenzo[*a*,*e*]pyrene, dibenzo[*a*,*h*]pyrene, dibenzo[*a*,*i*]pyrene, dibenzo[*a*,*l*]pyrene and dibenzo[*e*,*l*]pyrene in HPLC fractions (Yu &

Campiglia, 2004). The use of multidimensional GC, LC, coupled LC–GC and supercritical fluid chromatography have been reported (Sonnefeld *et al.*, 1982; Benner, 1998; Poster *et al.*, 1998; Marriott *et al.*, 2003). The use of single-particle or particle-beam MS offers the possibility of real-time analysis of PAHs on size-resolved particles but, without chromatographic separation, complete information on structural isomers cannot be achieved (Noble & Prather, 2000).

(b) Analysis of occupational exposure

Since the 1940s, the exposure of workers to PAHs has been assessed by measurements of workroom air. In the 1970s, personal air sampling of inhalable dust replaced static air sampling (Kenny *et al.*, 1997). In many studies, a surrogate — namely coal-tar pitch volatiles as benzene-soluble or cyclohexane-soluble matter — has been used as an indicator of airborne PAH. Only in the last decade has the direct determination of the 16 'priority pollutant' PAHs or that of a single marker — namely benzo[*a*]pyrene — in workroom air been chosen to measure industrial exposure to PAHs. The sampling method used to evaluate PAH exposures has been changed so that not only the particulates are sampled, but also the gaseous fraction of the PAH (Notø *et al*., 1996). Methods for the extraction and analysis of ambient air samples are also applied to occupational air samples.

There are currently no standardized methods to measure dermal exposures to PAH. Using polypropylene pads as adsorbing materials, Jongeneelen *et al.* (1988a) evaluated dermal exposures among workers exposed to coal-tar pitch. Wolff *et al.* (1989) measured dermal exposures among roofers by collecting pre- and post-shift skin wipes from measured areas of each worker's forehead. These samples are extracted and analysed by methods similer to those used for air samples.

(c) Analysis of PAH metabolites in urine

A specific metabolite of pyrene, 1-hydroxypyrene, in urine has been suggested as a biomarker of human exposure to PAHs (Jongeneelen *et al*., 1985; Jongeneelen, 2001). Recently, the glucuronide of 1-hydroxypyrene has also been used as an indicator of exposure, since the majority of 1-hydroxypyrene is conjugated and the fluorescence intensity of the conjugate is higher, but its additional value has not yet been assessed (Strickland *et al*., 1996). The measurement of various hydroxylated phenanthrenes has also been reported as a biomarker of exposure; analysis by GC–MS (Grimmer *et al*., 1991, 1993) and HPLC has been used to measure hydroxylated phenanthrenes and 3-hydroxybenzo[*a*]pyrene (Gundel *et al*., 1996; Popp *et al*., 1997; Gendre *et al.*, 2002). A recent attempt at immunoaffinity separation of PAH metabolites from the urine of exposed workers showed the presence of both 1-hydroxypyrene and several hydroxyphenanthrenes (Bentsen-Farmen *et al*., 1999). Urinary 1-hydroxypyrene remains, at the present time, the most reliable and pratical marker for monitoring individual exposures or exposures of the population to PAHs (Dor *et al*., 1999).

1.2 Occurrence and exposure

1.2.1 *Sources of exposure to PAHs for the general population*

Sources of PAH exposure for the general population have been reviewed previously (IARC, 1983) and also more recently (IPCS, 1998). Exposures to PAHs can occur through tobacco smoke, ambient air, water, soils, food and pharmaceutical products. PAHs are ubiquitous in the environment, and result in measurable background levels in the general population (IPCS, 1998). Biological monitoring of 1-hydroxypyrene in the urine of occupationally non-exposed individuals or representative samples of the general population has shown detectable levels in nearly all individuals at median concentrations that are typically less than 0.1 μmol/mol creatinine (reported in Huang *et al.*, 2004). In the USA, the National Health and Nutrition Examination Survey (NHANES) analysed 2312 urine samples collected from the general population in 1999–2000 and showed a geometric mean concentration of 1-hydroxypyrene of 0.039 μmol/mol creatinine (95% confidence interval (CI), 0.034–0.046 μmol/mol). Adult smokers had a three-fold higher level than nonsmokers (geometric mean, 0.080 versus 0.025 μmol/mol). These data are comparable with other recent data on occupationally non-exposed populations in Europe and Canada (Huang *et al.*, 2004). Occupational exposures in some industries can result in urinary levels of 1-hydroxypyrene that are orders of magnitude higher (see Section 1.2.2). The NHANES survey data for 2001–2002 (CDC, 2005) also include urinary analyses of 22 PAH metabolites in over 2700 individuals.

Mainstream tobacco smoke is a major source of exposure to PAHs for smokers (IARC, 2004). A recent study (Ding *et al.*, 2005) reported PAH levels in mainstream smoke from 30 US domestic brands of cigarette. The 14 PAHs measured (of the 16 priority PAHs of the Environmental Protection Agency) had either *sufficient* or *limited evidence* of carcinogenicity in experimental animals. Levels of total PAHs in mainstream smoke ranged from 1 to 1.6 μg/cigarette. Sidestream smoke is a source of PAHs in indoor air; levels of benzo[*a*]pyrene in sidestream smoke have been reported to range from 52 to 95 ng/cigarette — more than three times that in mainstream smoke (IARC, 2004).

PAHs are widely detected as ambient air pollutants, primarily bound to particulate matter but also in the gas phase (especially the lower-molecular-weight PAHs). Average concentrations of individual PAHs in the ambient air of urban areas typically range from 1 to 30 ng/m^3 (excluding naphthalenes), and the more volatile PAHs are generally more abundant; however, concentrations up to several tens of nanograms per cubic metre have been reported in road tunnels or in large cities that use coal or other biomasses as residential heating fuels extensively (IPCS, 1998). Estimates of annual emissions of PAHs from anthropogenic sources in the 1990s were 8600 tonnes/year in Europe (Boström *et al.*, 2002) and 2000 tonnes/year in Canada (Government of Canada, 1994). Major sources of PAHs in ambient air (both outdoors and indoors) include residential and commercial heating with wood, coal or other biomasses (oil and gas heating produce much lower quantities of PAH), other indoor sources such as cooking and tobacco smoke, motor vehicle exhaust (especially from diesel engines), industrial emissions and forest

fires (IARC, 1983; IPCS, 1998). PAHs present in ambient air in the gas phase generally have durations of less than a day, whereas particle-associated PAHs may persist for weeks and undergo long-range atmospheric transport (Arey & Atkinson, 2003).

Most PAHs in water originate from surface run-off, particularly in urban areas; smaller particles derive from atmospheric fall-out and larger particles from the abrasion of asphalt pavement. Industrial effluents can also contribute to PAH loads in surface waters, and sediment levels may range up to several thousand micrograms per kilogram. Although concentrations of PAHs in water are usually very low because of the low solubility of these compounds, surface water concentrations are typically 1–50 ng/L, with higher concentrations in some contaminated areas (IPCS, 1998). Comparison of PAH levels in rainwater with those in surface waters showed higher levels in rainwater (10–200 ng/L, with levels up to 1000 ng/L in snow and fog) (IPCS, 1998). Recently, it has been reported that urban run-off from asphalt-paved car parks treated with coats of coal-tar emulsion seal could account for the majority of PAHs in many watersheds in the USA (Mahler *et al.*, 2005). PAH levels in drinking-water are typically much lower (IPCS, 1998).

Food is a major source of intake of PAHs for the general population (see Section 1.2.3). Estimates of PAH intake from food vary widely, ranging from a few nanograms to a few micrograms per person per day. Sources of PAHs in the diet include barbecued/grilled/broiled and smoke-cured meats; roasted, baked and fried foods (high-temperature heat processing); breads, cereals and grains (at least in part from gas/flame drying of grains); and vegetables grown in contaminated soils or with surface contamination from atmospheric fall-out of PAHs (IARC, 1983; IPCS, 1998; JECFA, 2005).

Skin contact with PAH-contaminated soils and the use of dermal pharmaceutical products based on coal tar have also been identified as sources of exposure to and uptake of PAHs for the general population (Jongeneelen *et al.*, 1985; Wright *et al.*, 1985; Viau & Vyskocil, 1995; IPCS, 1998).

1.2.2 *PAHs in occupational settings: production processes and exposure*

(a) Processing and use of coal and coal-derived products

The processing and use of coal and coal-derived products is fundamental to many of the industries described below. A brief introduction to coal pyrolysis and liquefaction is informative.

Pyrolysis (also called thermolysis) is the thermal decomposition of organic substances such as coal during heating to more than 300 °C in an oxygen-free atmosphere. It is the generic term for carbonization, coking and devolatilization. It is also the primary reaction in gasification, combustion and direct liquefaction. The decomposition products of pyrolysis are pyrolysis gas (mainly hydrogen, carbon monoxide, carbon dioxide, methane and C_2–C_5 hydrocarbons), liquid products (tar, oil, crude benzene and water) and coke as a solid residue and the main product. Depending on the properties of the coal, different

sulfur and nitrogen compounds are formed during the pyrolysis process. The distribution and composition of pyrolysis products are mainly determined by the type of coal but can be influenced by parameters in the process such as heating rate, temperature, atmosphere and pressure (Crelling *et al.*, 2005).

Low-temperature carbonization and coking involve the heating of coal with the exclusion of air. This process removes condensable hydrocarbons (pitch, tar and oil), gas and gas liquour, which leaves a solid residue of coke. Low-temperature carbonization (up to 800 °C) and coking (> 900 °C) are differentiated by the final temperature. The two processes also differ considerably in the rate of heating of the coal and the residence time in the reactor. These parameters have a direct effect on the product yields. Low-temperature carbonization produces fine coke and fairly large quantities of liquid and gaseous products, whereas high-temperature coking is used primarily for the production of a high-temperature lump coke for blast furnaces and cupola ovens (Crelling *et al.*, 2005).

High-temperature coking of coal is carried out entirely in batch-operated coke ovens, the majority of which are of the horizontal chamber type. The feedstock is a coking coal of given size and composition. The coking properties depend chiefly on softening and resolidification temperatures and on swelling behaviour. Coking takes place at 1000–1300 °C for 15–30 h. The coking time depends on the operating conditions and width of the oven. The main product is metallurgical coke that is required for the production of pig iron. Metallurgical coke is characterized by its suitable size and high resistance to abrasion even under the conditions of a blast furnace. Coke-oven gas and liquid by-products are also produced. In western Europe, these by-products influence the economy of coking and, therefore, are reprocessed (Crelling *et al.*, 2005). High-temperature coking is associated with higher levels of exposure to PAHs than low-temperature processes (Price *et al.*, 2000).

Considerable technical improvements in coke production have led to greater cost effectiveness. These include the mechanization and automation of oven operations, the reduction of coking time and an increase in specific throughput by the use of thinner bricks of higher thermal conductivity and larger oven sizes (Crelling *et al.*, 2005).

Tables 1.2–1.13 summarize the information available on exposures from 1983 to 2005 for the 10 industrial sectors addressed in this monograph. Each table was constructed to identify the country in which the sampling was carried out, the year in which measurements were made, the identity of the job or task sampled, the number subjects for whom measurements were made, the number of measurements taken, tobacco-smoking status of the subjects (when reported), levels of total PAHs, pyrene and benzo[*a*]pyrene in the air and dermal levels of pyrene and benzo[*a*]pyrene, as well as composite measures such as benzene-soluble fractions and cyclohexane-soluble material. The air samples reported are personal exposure measurements. In most cases, the study did not take into account concomitant exposures in the workplace; however, when this information was reported, it has been indicated in the text on the relevant industrial sector. Approximately one-third of the studies reported measurements of urinary metabolites, usually 1-pyrenol

(1-hydroxypyrene). These have also been indicated below, together with results of the dermal sampling that was usually conducted to measure levels of pyrene and benzo[*a*]pyrene on the skin surface.

(b) General considerations

Based on the CAREX database, it has been estimated that in 15 countries in Europe in 1990–93 almost 1 000 000 people were exposed to PAHs above background levels through their occupations (Kauppinen *et al.*, 2000). A study in Costa Rica showed that 17 700 men and women were occupationally exposed to PAHs, excluding environmental tobacco smoke and diesel exhaust (Partanen *et al.*, 2003).

The production and use of coal tar and coal tar-derived products are major sources of occupational exposure to PAHs. Crude coal tar is a by-product of coke production and was formerly also a by-product of gas works. Crude coal tar is usually distilled, and blends of distillation fractions are used for various purposes, such as wood conservation, paints, road tars and roofing materials. PAH concentrations in coal-tar products may range from less than 1% up to 70% or more (Jongeneelen, 2001; ATSDR, 2002).

Most PAHs are relatively non-volatile compounds. Airborne PAHs with fewer than four aromatic rings (molecular weight range, 128–178) are sufficiently volatile to be present as gaseous compounds in the working environment. PAHs with four rings (molecular weight, 202) may be present both in the gas phase and as adsorbed particulates. PAHs with higher molecular weights (>228) are typically bound to airborne particulates (Jongeneelen, 2001).

Occupational exposure to PAHs occurs primarily through inhalation and skin contact. Monitoring of workplace air and personal air sampling for individual PAHs, sets of PAHs or surrogates (e.g. coal-tar pitch volatiles) have been used to characterize inhalation exposures; more recently, biological monitoring methods have been applied to characterize the uptake of certain PAHs (e.g. pyrene, benzo[*a*]pyrene) as biomarkers of total exposure (see Sections 1.1.2 and 1.3).

There is growing awareness that occupational uptake of PAHs through the skin is substantial (Jongeneelen, 2001). For example, uptake of pyrene by the dermal route was estimated to account for as much as 75% of total body dose for coke-oven workers (VanRooij *et al*, 1993a); for creosote-impregnating workers, dermal pyrene uptake was on average 15-fold higher than the estimated respiratory uptake (VanRooij *et al.*, 1993b).

Geographical distribution of the industries described in the following sections varies considerably from industry to industry and over time within an industry. Coke production increased more than fivefold in the People's Republic of China between 1970 and 1995, with concomitant decreases in Europe and North America. In 1995 and 1999, the People's Republic of China provided over one-third of the world's production of coke and more than half of global coke exports (Terjung, 2000).

(c) Coal liquefaction

Coal liquefaction is a conversion process in which liquid fuels and liquid chemicals are obtained from solid coal. Coal liquefaction can be accomplished in two ways. In the first, which is called direct liquefaction or coal hydrogenation, the coal is suspended in suitable oils and treated with either hydrogen in the presence of a catalyst or hydrogenating solvents to yield oil products and some unreactive residue. In the second, which is called indirect liquefaction, coal is gasified to yield a mixture of hydrogen and carbon monoxide (synthesis gas) from which liquid products can be synthesized in one or more steps. Both methods were developed into industrial-scale processes during the 1930s and were used extensively during the Second World War in Germany. Currently, (indirect) coal liquefaction is employed on an industrial scale only in South Africa. Further improvements were made to develop large pilot plant operations, mainly in Germany, Japan and the USA. These activities reached their peak between 1975 and 1985, and have continued at lower levels since that time (Quinlan *et al.*, 1995a,b,c; Crelling *et al.*, 2005).

Concentrations of PAHs in the air and the skin and urine of workers in coal liquefaction are summarized in Table 1.2.

Quinlan *et al.* (1995a) studied a pilot coal liquefaction plant. Inhalation exposures to cyclohexane-soluble material were measured and spot urine samples were collected. There were no statistically significant relationships between the levels of cyclohexane-soluble material and those of 1-hydroxypyrene, and the authors attributed elevated levels of 1-hydroxypyrene primarily to dermal absorption of PAHs among engineers.

Quinlan *et al.* (1995b) also conducted an in-depth study to investigate the relationships between work activities, exposures to PAHs and excretion of 1-hydroxypyrene among coal liquefaction workers. The study demonstrated that there was an increase in the daily (pre- versus post-shift) levels of 1-hydroxypyrene excretion, as well as an increase in the day-to-day levels (shift 1 to shift 4). The levels of exposure to cyclohexane-soluble material ranged from < 5 to 49 μg/m^3. Pyrene was reported to comprise 7% of the extract; its concentration in the particulate phase ranged from 0.8 to 2.8 μg/m^3, while benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene and dibenzo[*a*,*h*]pyrene totalled 0.5% of the cyclohexane-soluble extract.

(d) Coal gasification

Coal gasification is the process of reacting coal with oxygen, steam and carbon dioxide to form a gas that contains hydrogen and carbon monoxide. Gasification is essentially incomplete combustion. The chemical and physical processes in gasification and combustion are quite similar, the main difference being the nature of the final products. With regard to processing, the main difference in operations is that gasification consumes the heat evolved during combustion. Under the reducing environment of gasification, sulfur in the coal is released as hydrogen sulfide rather than sulfur dioxide and nitrogen in the coal is converted mostly to ammonia rather than nitrogen oxides. These

Table 1.2. Concentrations of PAHs in the air, skin and urine of workers in coal liquefaction in the United Kingdom [year of study not reported]

Reference	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Dermal levels (ng/cm^2)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range	Mean	Range
Quinlan *et al*. (1995a)	Engineer	5	6	2		0.07	NR				
	Technician	5	9	3							
	Engineer	5	6	2	1-Hydroxypyrene					8.53	< 1*–72.8
	Technician	5	9	3						3.74	0.5*–7*
Quinlan *et al*. (1995b)	Operators[a]	5	NR	NR	16 individual PAHs, vapour-phase only		ND–3340[b]				
			10	NR	CSM		ND–49				
			[b]	NR	Pyrene[c]	1323	630–2870				
										Geometric mean	
	Operators[d]	5	38	NR	1-Hydroxypyrene					NR	0.59–20.02
	Maintenance[d]	5	35	NR						NR	0.24–13.72
	Laboratory[d]	2	16	NR						NR	0.29–2.22
	Operators[e]	7	7	NR						2.9	0.87–6.58
	Maintenance[e]	9	9	NR						3.35	0.56–14.18
	Laboratory[e]	9	9	0						0.53	0.22–2.28
	Office[e]	10	10	1						0.26	0.15–2.06

Table 1.2 (Contd)

Reference	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Dermal levels (ng/cm^2)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range	Mean	Range
Quinlan *et al*. (1995c)	Engineer	5	10	0	Pyrene			21.5	ND–47.7		
	Technician	5	10	3				17.8	ND–78.3		
	Engineer	5	20	0	Benzo[*a*]pyrene			ND	ND		
	Technician	5	20	3				ND	ND		
	Engineer	5	20	0	1-Hydroxypyrene						0.73–48.47
	Technician	5	20	3							2.19–15.43

CSM, cyclohexane-soluble material; ND, not detected; NR, not reported; PAH, polycyclic aromatic hydrocarbon

* Read from graph

[a] Values measured over 1 week

[b] Value measured for phenanthrene

[c] Calculated from mean CSM value, assuming 7% pyrene content in CSM extract

[d] Values measured over 4 weeks

[e] Spot measurements at the end of working period

reduced forms of sulfur and nitrogen are easily isolated, captured and used, and thus gasification is a clean-coal technology with better environmental performance than coal combustion (Shadle *et al*., 2002).

Depending on the type of gasifier and the operating conditions, gasification can be used to produce a fuel gas that is suitable for a number of applications. A low heating-value fuel gas is produced from an air-blown gasifier for use as an industrial fuel and for power production. A medium heating-value fuel gas is produced from enriched oxygen-blown gasification for use as a synthesis gas in the production of chemicals such as ammonia, methanol and transportation fuels. A high heating-value gas can be produced by passing the medium heating-value gas product over catalysts to produce a substitute or synthetic natural gas (Shadle *et al*., 2002).

The earliest gasification processes were developed using a countercurrent, fixed-bed gasifier. In a fixed-bed gasifier, coal is fed onto the top of the bed and travels downwards against the current to the flow of gases. Atmospheric fixed-bed gasifiers of various design are still occasionally found in small-scale industrial use. On a large scale, some Lurgi fixed-bed pressurized gasification plants are currently operating commercially, e.g. in the Republic of South Africa and in the USA (Shadle *et al*., 2002; Crelling *et al.*, 2005).

Fluidized-bed gasification, invented in 1922 by Winkler at BASF, has the advantage of a fairly simple reactor design. In this process, the reactor vessel is designed so that the air and steam flow required for gasification is sufficient to fluidize the bed of coal, char and ash. Fluidization occurs when the velocity of the gas flow lifts the particles and causes the gas–solid mixture to flow like a fluid (Shadle *et al*., 2002; Crelling *et al.*, 2005).

Entrained-flow gasification takes place in a flame-like reaction zone, usually at a very high temperature, to produce a liquid slag. For economical operations, a high-standard heat recovery system is mandatory, but the gas product typically has a very low methane content and is free of tars, oils and phenols, which thereby simplifies gas and water treatment considerably. Entrained-flow gasifiers of the Koppers-Totzek design that are operated at atmospheric pressure are used industrially in many countries to produce hydrogen or synthesis gas (Shadle *et al*., 2002; Crelling *et al.*, 2005).

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

Concentrations of PAHs in the air of workers in the coal gasification industry were reported by Gustavsson and Reuterwall (1990) to be similar to those described by Lindstedt and Sollenberg (1982) in American plants [data not presented in Tables or Figures]. In addition to PAHs, workers in coal gasification may be exposed to many

compounds, including asbestos, silica, amines, arsenic, cadmium, lead, nickel, vanadium, hydrocarbons, sulfur dioxide, sulfuric acid and aldehydes (IARC, 1984).

(e) Coke production and coke ovens

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

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

The vast majority of coke is produced from slot-type by-product coke ovens. Individual coke ovens are built of interlocking silica bricks that are produced in numerous shapes for special purposes. It is not uncommon for batteries of modern coke ovens to contain 2000 different shapes and sizes of brick. Typical coke ovens are 12–14 m in length, 4–6 m in internal height and be less than 0.5 m in internal width. On each side of the oven are heating flues that are also built of silica brick. Batteries of adjacent ovens, where ovens share heating flues, contain as many as 85 ovens. At each end of each oven, refractory-lined steel doors are removed and re-seated for each oven charge and push. Coke batteries are generally heated with part of the coke-oven gas that is generated in the process of coke production; however, they can also be heated with blast-furnace gas and natural gas. Once heated, the battery generally remains hot for its entire life because cooling causes a mineralogical change in the silica that lowers the strength of the silica brick (Kaegi *et al.*, 1993).

Above the ovens is a roof system that is capable of supporting the moving Larry car from which coal is discharged into each oven through three to five charging holes in the top of each oven. The Larry car is filled for each oven charge from a large blended coal silo that is constructed above the rail of the Larry car, usually at one end of the coke

battery. Modern Larry car technology includes telescopic charging chutes to minimize dust emissions during charging. Many facilities also include automatic removal and replacement of the charging-hole lid. After completion of charging and replacement of the charging-hole lids, a small flap at the top of one of the oven doors is opened and a steel levelling bar is inserted along the length of the oven above the coal charge. The levelling bar is moved back and forth over the coal to produce a level charge that has sufficient free space above it. This free space is important to ensure balanced heating of the coal and is needed to convey the volatile carbonization products out of the oven. Most coke batteries charge wet coal into the ovens; however, a few facilities are equipped with pre-heaters that not only remove all moisture from the coal, but pre-heat it to 150–200 °C in order to expedite the carbonization process. The pre-heated charge facilities function very similarly to wet charge facilities except that more attention is paid to potentially higher levels of charging emissions caused by the dryness of the coal (Kaegi *et al*., 1993).

On top of the battery, at either one or both ends of each oven, refractory-lined standpipes are mounted on additional roof openings into each oven. The volatile gases generated from the coal during carbonization flow to the top of the oven, into the free space and out through the standpipes. The standpipes are all connected to large collecting mains that run along the length of the battery. These mains transport the gases to the by-product plant in which they are processed into various materials. Water is sprayed into the mains in order to cool the gases and to condense out some of the tar (Kaegi *et al*., 1993).

At the end of the coking cycle, which ranges from about 15 to 30 h depending on production needs and on the condition of the battery, the doors are removed from each oven. A pusher machine equipped with a large water-cooled ram then pushes the coke from the oven into a hot or quench car. After the coke is pushed from the oven, the doors are replaced to maintain oven heat and oven carbon content. The hot car may or may not have a moveable or partial roof to minimize gaseous and particulate emissions. The car moves on rails and positions the hot coke beneath a large water tank that is equipped with nozzles on its underside. The water flow is regulated to quench the coke with a minimal amount of excess water remaining on the cooled coke. After quenching, the hot car moves again to dump the coke onto a refractory, covered coke wharf that is sloped away from the hot car. The coke flows to the bottom of the wharf, at which point it drops onto a conveyor system for transportation to a blast furnace, storage pile or out of the plant (Kaegi *et al*., 1993; Crelling *et al.*, 2005).

In 1990, total worldwide coke production was about 378 million tones and was essentially unchanged since that in 1970. In 1990, the former USSR was the largest coke producer (80 million tonnes), followed closely by the People's Republic of China (73 million tonnes). Japan produced 53 million tonnes and the USA produced about 27 million tonnes. Since 1970, production in the former USSR has remained in the range of 75–85 million tonnes, but massive shifts in production have occurred in the USA, Japan and the People's Republic of China. Between 1970 and 1990, production in the USA decreased by more than 50% while Japanese production increased by 50%. During the same period, the People's Republic of China increased coke production by over 300%

(Kaegi *et al*., 1993). By 1999, worldwide coke production had declined to about 326 million tonnes, of which 121 million tonnes were produced in the People's Republic of China (Terjung, 2000).

Concentrations of PAHs in the air and urine of workers in coke ovens are summarized in Table 1.3 and Figure 1.1.

More than 30 studies of exposure among coke-oven workers have been reported since 1983, six of which included profiles of three or more PAHs; seven others reported levels of pyrene, benzo[*a*]pyrene or both; and the remainder reported composite measurements (benzene-soluble fraction, cyclohexane-soluble material) or urinary measurements only. A variety of sites in the coke plants were sampled, and the overall pattern (regardless of the exposure that was measured) was that topside workers (including lidmen, tar chasers and Larry car operators) had the highest exposures, followed by workers by the side of the ovens (such as coke-side machine operators, benchmen, door repairers, wharfmen, quenchers, pushers and temperature controllers). Workers in other areas of the plant such as maintenance, office and control workers had the lowest exposures (see Table 1.3). It has been reported that modernization of coke plants, including improved control measures, can substantially reduce exposures (Quinlan *et al.*, 1995c).

In addition to PAHs, coke-oven workers may be exposed to a large number of compounds, including asbestos, silica, amines, arsenic, cadmium, lead, nickel, vanadium, hydrocarbons, sulfur dioxide, sulfuric acid and aldehydes (IARC, 1984).

(f) Coal-tar distillation

Coal tar is the condensation product obtained by cooling the gas that evolves from the destructive distillation of coal to approximately ambient temperature. It is a black, viscous liquid that is denser than water and is composed primarily of a complex mixture of condensed-ring aromatic hydrocarbons. It may contain phenolic compounds, aromatic nitrogen bases and their alkyl derivatives, and paraffinic and olefinic hydrocarbons. Coal-tar pitch is the residue from the distillation of coal tar. It is a black solid that has a softening-point of 30–180 °C (Betts, 1997). Figure 1.2 portrays the process of coal-tar production and its conversion to coal-tar distillates and residual coal-tar pitch, and also illustrates the uses of creosote (see this section) and of coal-tar pitch (see Sections 1.2.2(*e*),(*g*),(*h*)).

The largest source of tar and pitch is the pyrolysis or carbonization of coal. The importance of coal tar as an industrial raw material dates back to the first half of the eighteenth century, when the carbonization of coal and the production of tar as a by-product were expanding rapidly in the United Kingdom. Initially, the crude tar was subjected to a simple flash distillation in pot stills to yield a solvent (naphtha), creosote for timber preservation and a residue of pitch that was used as a binder for coal briquettes. Later, coal tar was the main source of aromatic hydrocarbons, phenols and pyridine bases that were needed by the rapidly expanding dyestuffs, pharmaceuticals and explosives industries. The development of by-product coke ovens and recovery of crude benzene at both coke ovens and gas works greatly increased the supplies of crude tar and tar distillates

Table 1.3. Concentrations of PAHs in the air, skin and urine of workers in coke ovens

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Andersson *et al.* (1983), Sweden, NR	Top side	1	1	NR	11 PAHs Benzo[*a*]pyrene	1513 (total) 38			
Haugen *et al.* (1986), Norway, NR	Top side	4	4	22	38 PAHs	Outside RPE, 266 Inside RPE, 110	212–315 51–162		
Hemminki *et al.* (1990), Poland, NR	Top side	NR	NR	NR	Benzo[*a*]pyrene	39.1	9.4–90		
	Side	NR	NR	NR		4.09	0.54–13.6		
	Pusher side	NR	NR	NR		6.42	2.5–11.2		
	Sorting	NR	NR	NR		0.82	0.25–1.4		
	Office	NR	NR	NR		0.19	0.03–0.45		
	Distillation	NR	NR	NR		0.06	0.06		
Jongeneelen *et al.* (1990), Netherlands, NR	Side	5		NR	13 PAHs; pyrene	6.9[a]; < 0.6[a]	< 1–46; < 0.6–4.8		
	Top side oven 1	20		55%		17.0[a]; 2.0[a]	7.3–39; < 0.6–4.4		
	Push side	7		29%		13.9[a]; 1.6[a]	3.6–77; < 0.6–9.8		
	Maintenance	10		NR		13.6[a]; 1.8[a]	< 1–43; < 0.6–6.1		
	Top side oven 2	9		56%		12.9[a]; 1.7[a]	1.8–37; 1.8–7.3		
	Side	7		57%	1-Hydroxy-pyrene: end of shift; increase over shift			2.0[a]; 0.13[a]	0.7–2.6; –1.2–1.5
	Top side oven 1	19		NR				3.3[a]; 2.0[a]	0.8–7.5; 0–4.9
	Push side	7		29%				1.9[a]; 0.67[a]	0.6–3.5; –0.4–2.0
	Maintenance	11		64%				1.9[a]; 1.2[a]	1.31–4.1; 0.33–3.0
	Top side oven 2	9		56%				2.7[a]; 1.3[a]	1.3–6.5; –1.3–4.6
Reuterwall *et al.* (1991), Sweden, NR	Oven in steel mill	12	NR	0	14 PAHs; benzo[*a*]pyrene	NR; [3.5 estimated]	6–570		

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Buchet *et al.* (1992), Belgium, NR	Oven bench side	10	10	6	13 PAHs	14.2[b]; 25.1[c]	0.7–74.2		
	Oven top side	6	6	3		198.7[b]; 241.2[c]	26.6–959		
	Oven bench side	10	10	6	Pyrene	0.05 NS[b]; 0.09 S[b]			
	Oven top side	6	6	3		15.9 NS[b];5.62 S[b]			
	Oven bench side	10	10	6	1-Hydroxypyrene			*Nonsmoker*[b] 2.27 pre; 2.36 post 4.67 pre; 10.91 post	*Smoker*[b] 0.46 pre; 1.45 post 3.22 pre; 11.72 post
	Oven top side	6	6	3					
Assennato *et al.* (1993), Italy, 1992		69	NR	45.6%					
	Supervisor	1	1	NR	Benz[*a*]anthracene; chrysene; benzo[*a*]pyrene; total PAHs		0.41; 0.29; 0.32; 6.98		
	Door maintenance	NR	NR	36.4%			4.26–14.79; 2.31–6.37; 2.34–6.53; 30.37–96.96		
	Machine operator	NR	NR	NR			0.11–33.19; 0.08–13.17; 0.03–12.63; 2.94–218.9		
	Gas regulator	NR	NR	NR			0.21–2.1; 0.12–1.61; 0.13–1.6; 7.24–26.48		
	Temperature operator	NR	NR	NR		NR	1.77–10.07; 1.37–5.03; 0.98–4.78; 20.98–64.48		
	Top side	NR	NR	NR		NR	0.45–3.4; 0.47–4.73; 0.23–2.42; 8.91–47.93		

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Grimmer *et al.* (1993), Germany, NR		4	16	3/4	19 PAHs	Summary data not reported			
	Battery top	1	4	0	Pyrene			60.5	43.7–80.3
	Battery top	1	4	1	metabolites			33.4	29.2–39.2
	Driver of containers	1	4	1				16.9	6.1–26.3
	Machinist	1	4	1				4.3	3.1–5.2
Van Hummelen *et al.* (1993), Belgium, NR		33	33	26/33	13 PAHs	23.7	SE, 10.8		
					1-Hydroxypyrene			0.51 pre; 0.75 post	SE, 0.08 SE, 0.17
VanRooij *et al.* (1993a), Netherlands, 1990		12	60	8/12	Pyrene	1.53	0.09–5.37		
						Total dermal levels (μg)			
					Pyrene (8-h)	74.4	21.2–165.9		
					1-Hydroxy-pyrene (7-day)			111.4 nmol	36–239 nmol
Ferreira *et al.* (1994), Belgium, NR		56	56	31/56	Total 13 PAHs	15.9	0.5–1106.4		
					1-Hydroxypyrene			0.8 pre 1.5 post	0.04–29.3 0.02–93.5

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Clonfero *et al.* (1995), Italy NR		95	95	54	1-Hydroxypyrene			1.28	0.04–5.59
Levin *et al.* (1995), Sweden, 1988	Various	10	10	6/10	Benzo[*a*]pyrene	4[c]	0.9–37		
					Sum of 7 PAHs		20–480		
					1-Hydroxypyrene			14 ng/mL[c]	4–90 ng/mL
1990					Benzo[*a*]pyrene	0.7[c]			
					Sum of 7 PAHs		< 10–70		
					1-Hydroxypyrene			3.8 ng/mL[c]	1–17 ng/mL
Øvrebø *et al.* (1995), Norway, NR		*Jan; June*			1-Hydroxypyrene			*Jan; June*	
	Top side	18; 13		50%				4.26; 5.53	
	Bench side	26; 18		61%				1.80; 2.93	
	Maintenance	23; 17		56%				1.11; 1.32	
Popp *et al.* (1995), Germany, NR		29	29	16/29	Benzo[*a*]pyrene	1.7	0.5–3.6		
					Total 19 PAHs	49.2	14.0–127.4		
	Top side				Benzo[*a*]pyrene	2.3			
					Total 19 PAHs	67.1			
	Coke side				Benzo[*a*]pyrene	1.4			
					Total 19 PAHs	38.7			
Pyy *et al.* (1995), Finland, 1987–90		160	Dust, 510; gas, 90	NR	Fluorene	0.58–24.64			
	10 working areas				Phenanthrene	0.16–18.76			
					Benzo[*a*]pyrene	0.05–10.30			

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Malkin *et al.* (1996),		10	10	NR	CTPV		ND–350		
					Pyrene		ND–1		
USA, 1994	Coal-tar sludge handling area	18	18	9	1-Hydroxy-pyrene (pre; post)			1.0; 1.7	0.16–3.0; 0.24–4.85
								1.6; 3.7	
	Labourer	2	2	NR				0.4; 0.6	
	Coal handler operator	6	6	NR				1.4; 2.4	
	Coal handler maintenance	5	5	NR					
	Other	5	5	NR				1; 1.6	
Winker *et al.* (1996), Austria, NR		24	1	14/24	Sum of 16 PAHs	*Old facility*	101		
						New facility	32		
Mielzyńska *et al.* (1997), Poland, NR	Total	24		> 50%	Benzo[*a*]pyrene (air) and 1-Hydroxy-pyrene (pre; post urine)	2.1[c]	0.1–15.1		
	Top side oven	7	25	NR		3.97	0.6–14.1	2.0; 3.57	0.1–7.76; 0.14–10.74
	Side of oven	8	28	NR		2.57	0.2–15.1	1.54; 2.37	0.09–4.94; 0.08–18.92
	Gas fitting operators	3	10	NR		1.27	0.3–4.5	1.24; 2.96	0.15–2.66; 0.92–5.3
	Dry quenching	6	11	NR		0.27	0.1–1.5	0.46; 0.87	0.07–1.76; 0.06–2.2
Pan *et al.* (1998), China, NR		75	95	51/75					
	Topside	25		15	Total PAHs; pyrene; benzo[*a*]-pyrene; 1-hydroxypyrene (pre urine)	264.9[c]; 4.27[c]; 4.30[c]		12.0	
	Push side	10		8		139.3[c]; 1.6[c]; 2.0[c]		9.1	
	Coke side	15		10		82.4[c]; 0.46[c]; 0.58[c]		5.7	
	Bottom	25		18		134.0; 0.86; 4.0		4.0	

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Romundstad *et al.* (1998), Norway, 1976–87		NR	594	NR	Total particulate PAH		*95% CI*		
	Top side								
	1976		12			300	139–461		
	1978–87		221			125	113–138		
	Inside helmet, 1977–87		212			37	33–41		
	Side oven								
	1976–87		75			44	25–63		
	Inside helmet		60			10	7–12		
	Ram car								
	1976		5			30	5–55		
	1978		5			6	0–17		
	Quench, 1976		4			2	0–6		
Wu *et al.* (1998), Taiwan, China, 1995–96	Top side	18	54	12	BSF (air) and 1-hydroxy-pyrene (pre; post urine)	528[b]	144–6309	29[d]; 199[d]	1–101[d]; 8–3261[d]
	Side oven	41	123	21		74[b]	11–1130	5[d]; 13[d]	0.7-23[d]; 0.2–520[d]
	Side/control	21	63	11		49[b]	16–111	3[d]; 11[d]	0.3-24[d]; 3–31[d]
Brescia *et al.* (1999), Italy, NR		76			PAH; benzo[*a*]-pyrene, 1-hydroxypyrene	18.98; 1.72	12.58–42.66; 0.87–2.88	1.44	0.04–3.75
	Top side	27	27	55.6%					
	Bench	32	32	62.5%		20.03; 1.56	12.58–63.66; 0.48–6.33	1.30	0.051–5.59
	Bottom	17	17	45.0%		15.37; 0.78	6.9–16.86; 0.32–0.86	1.35	0.068–4.18

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Chen *et al.* (1999), Taiwan, China, 1995–96		88	264	NR	BSF				
	Lidman		33			515[b]	72–18181		
	Tar chaser		21			432[b]	51–4334		
	Larry car operator		15			185[b]	55–649		
	Cokeside machine operator		21			121[b]	32–2965		
	Benchman		18			97	33–488		
	Door repair		30			82	11–352		
	Wharfman		15			42	10–117		
	Quencher		24			29	ND–395		
	Pusher		24			25	ND–98		
	Temperature controller		12			55	30–156		
	Body repairman		21			55	10–136		
	Heater		12			38	21–85		
	Supervisor		18			26	ND–91		
Pavanello *et al.* (2000), Italy, NR	Top side	30	30	0	1-Hydroxypyrene			0.82	0.12–5.15
	Other workers	30	30	0				0.39	0.03–1.23

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Price *et al.* (2000), United Kingdom, 1998	Low-temperature	13	13	6	Sum of 19 PAHs	50.03	5.87–131.6		
					HSE11[e]	7.03	0.01–19.4		
					Benzo[*a*]pyrene	1.15	0.01–3.2		
					Pyrene	2.03	0.05–7.44		
					1-Hydroxy-pyrene			2.64	0.41–6.91
	High-temperature	11	11	5	Sum of 19 PAHs	79.26	8.8–184.7		
					HSE11[e]	16.45	1.27–44.8		
					Benzo[*a*]pyrene	2.26	0.18–6.26		
					Pyrene	2.12	0.43–9.90		
					1-Hydroxy-pyrene			1.72	0.25–5.42
	High-temperature	13	13	5	Sum of 19 PAHs	70.73	9.94–294.7		
					HSE11[e]	5.77	0.226–29.25		
					Benzo[*a*]pyrene	0.81	0.02–4.13		
					Pyrene	0.63	0.05–2.49		
					1-Hydroxy-pyrene			2.07	0.25–7.1
van Delft *et al.* (2001), Netherlands, 1997									*SD*
	Oven (high exposure)	35	35	15	1-Hydroxypyrene			51.04 (NS); 1.52 (S)	0.67 (NS); 1.40 (S)
	Distilleries and maintenance (low exposure)	37	37	18				0.27 (NS); 0.7 (S)	0.22 (NS); 0.39 (S)
Zhang *et al.* (2001), China, NR		162	NR	108	1-Hydroxypyrene			9.86[b]	0.9–89.8

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Lu *et al.* (2002), Taiwan, China, NR					BSF				
	Top side	24	72	15	1-Hydroxypyrene	483.0[b]		S pre: 6.6	2.3–16.7
								S post: 17.0	6.0–32.5
								NS pre: 3.8	0.4–18.6
								NS post: 7.3	1.0–35.0
	Coke side	50	150	23		70.8[c]		S pre: 0.9	0.3–2.7
								S post: 1.6	0.3–5.0
								NS pre: 0.8	0.2–2.4
								NS post: 1.4	0.3–11.5
	Office	14	42	6		43.4[c]		S pre: 1.0	0.4–2.2
								S post: 1.3	0.7–3.1
								NS pre: 1.2	0.5–3.0
								NS post: 1.5	0.7–3.6
Marczynski *et al.* (2002), Germany, NR		20	20	15	Sum of 16 PAHs	54.26	4.51–316.4		
					Benzo[*a*]pyrene	2.77	0.12–16.26		
Strunk *et al.* (2002), Germany, NR		24	24	16	Sum of 16 PAHs				
	Top side	5	5		(air); 1-hydroxy-	491.2	82.81–1679	19.7; 39.18	6.84–34.82; 19.06–79.36
	Bench side	8	8		pyrene; sum of	26.61	1.65–88.53	7.01; 12.95	1.22–15.03; 5.87–23.66
	Complete area	11	11		hydroxyphenan-	76.18	1.04–237.8	3.57; 8.70	0.51–10.2; 3.31–21.26
					threne (urine)				
Waidyanatha *et al.* (2003), China, NR	Side and bottom	13	13	8	Phenanthrene[d];			1.70; 0.003	0.037–8.66; 0.001–0.007
	Top side	15	15	9	pyrene[d]			3.42; 0.005	0.013–19.3; 0.0005–0.017

Table 1.3 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Pavanello *et al.* (2004), Poland, 2002		95	95	57	1-Hydroxypyrene			6.93	0.25–31.4

BSF, benzene-soluble fraction; CI, confidence interval; CTPV, coal-tar pitch volatiles; ND, not detected; NR, not reported; NS, nonsmoker; PAH, polycyclic aromatic hydrocarbon; pre, pre shift; post, post shift; RPE, respiratory protective equipment; S, smoker; SD, standard deviation; SE, standard error

[a] Geometric mean of the mean of three or fewer observations per worker

[b] Geometric mean

[c] Median

[d] Reported in μg/L

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

Figure 1.1. Range in concentrations of post-shift urinary 1-hydroxypyrene (in μmol/mol creatinine) and benzo[*a*]pyrene (in μg/m^3) in occupational settings with exposure to PAHs[a]

Coke ovens

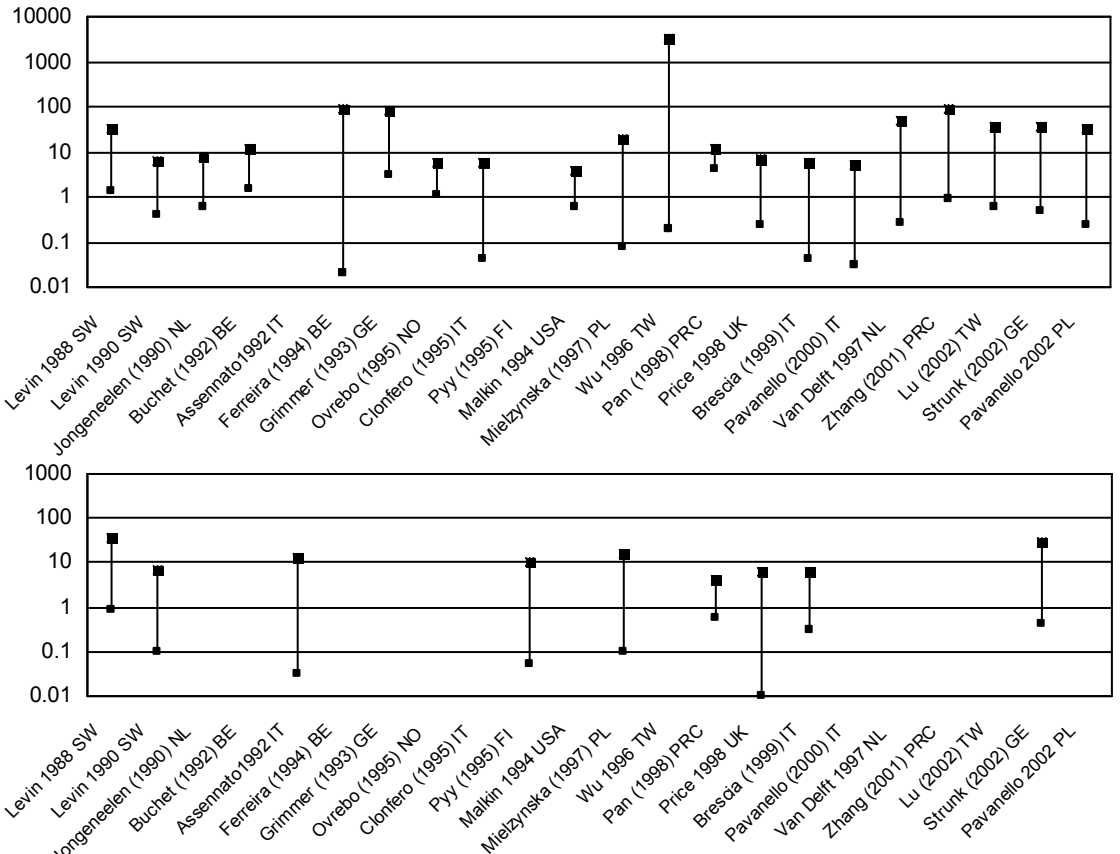


Figure 1.1 (contd)

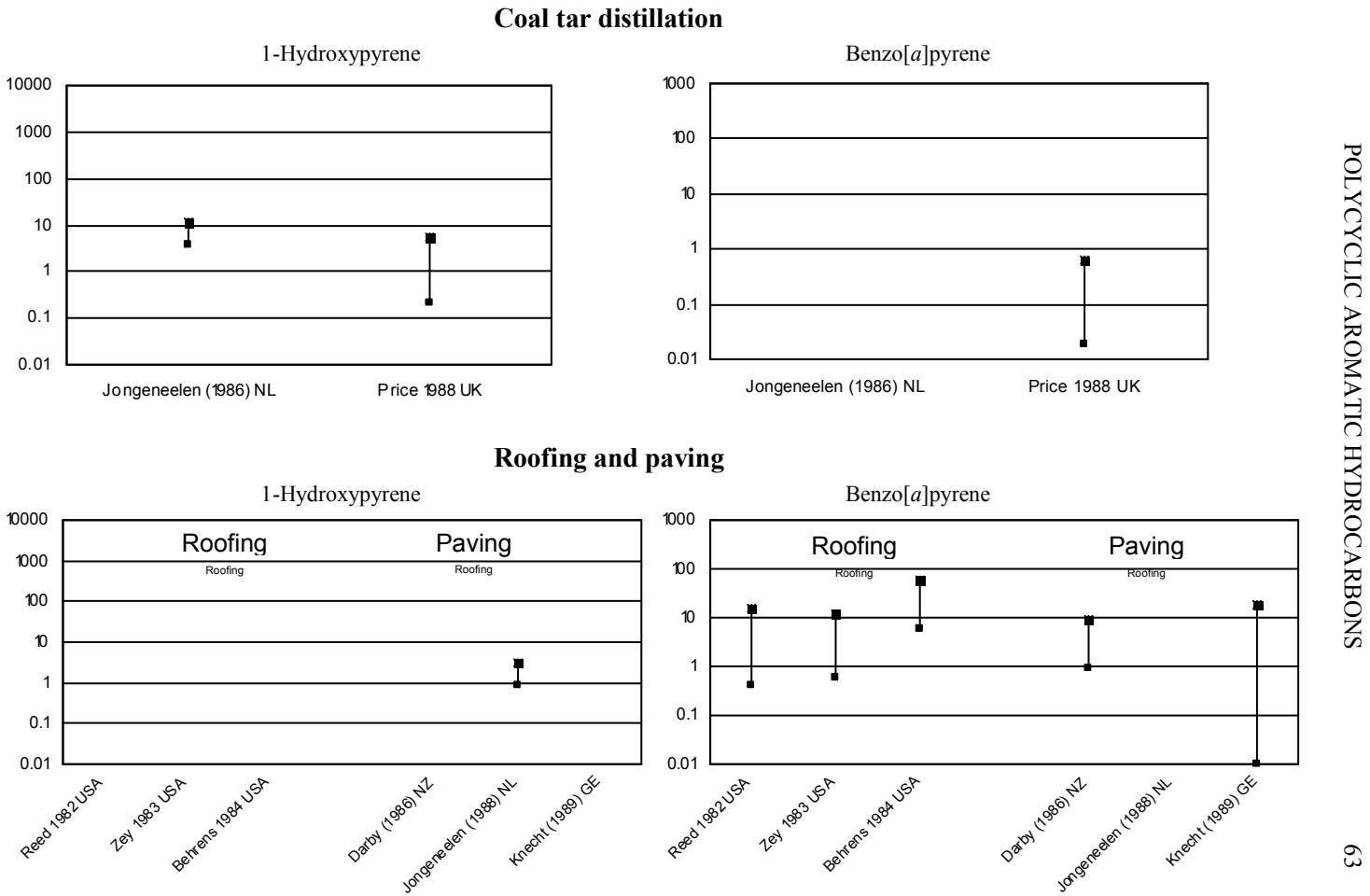


Figure 1.1 (contd)

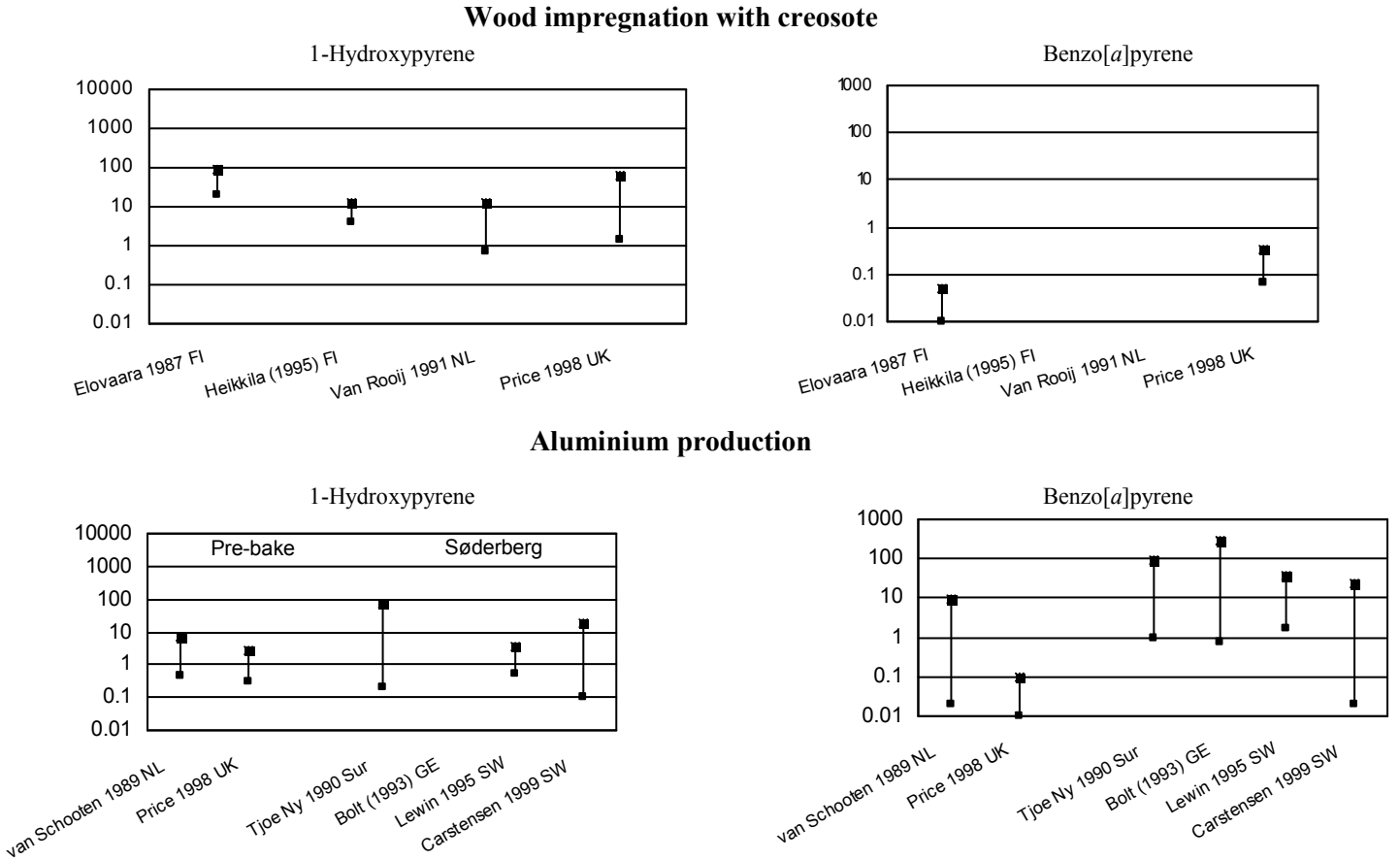


Figure 1.1 (contd)

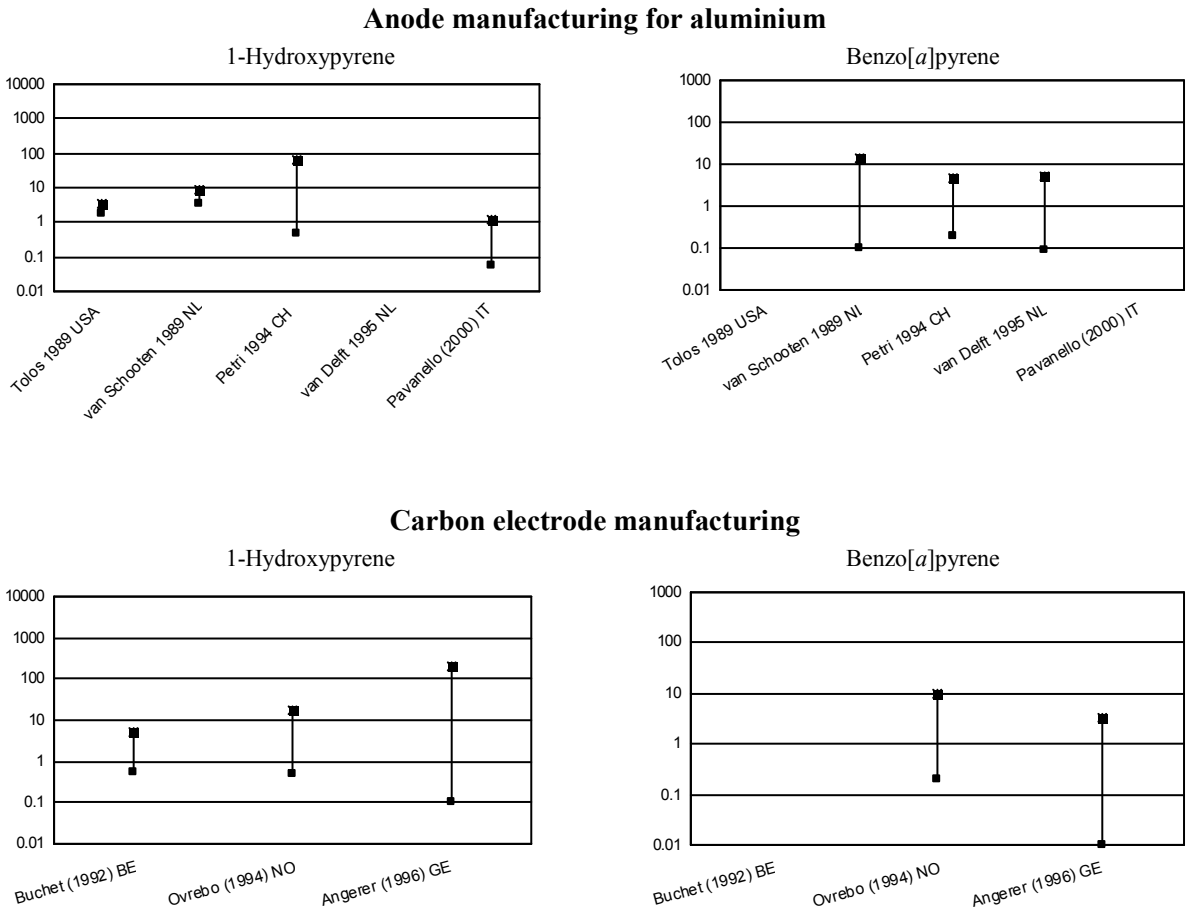
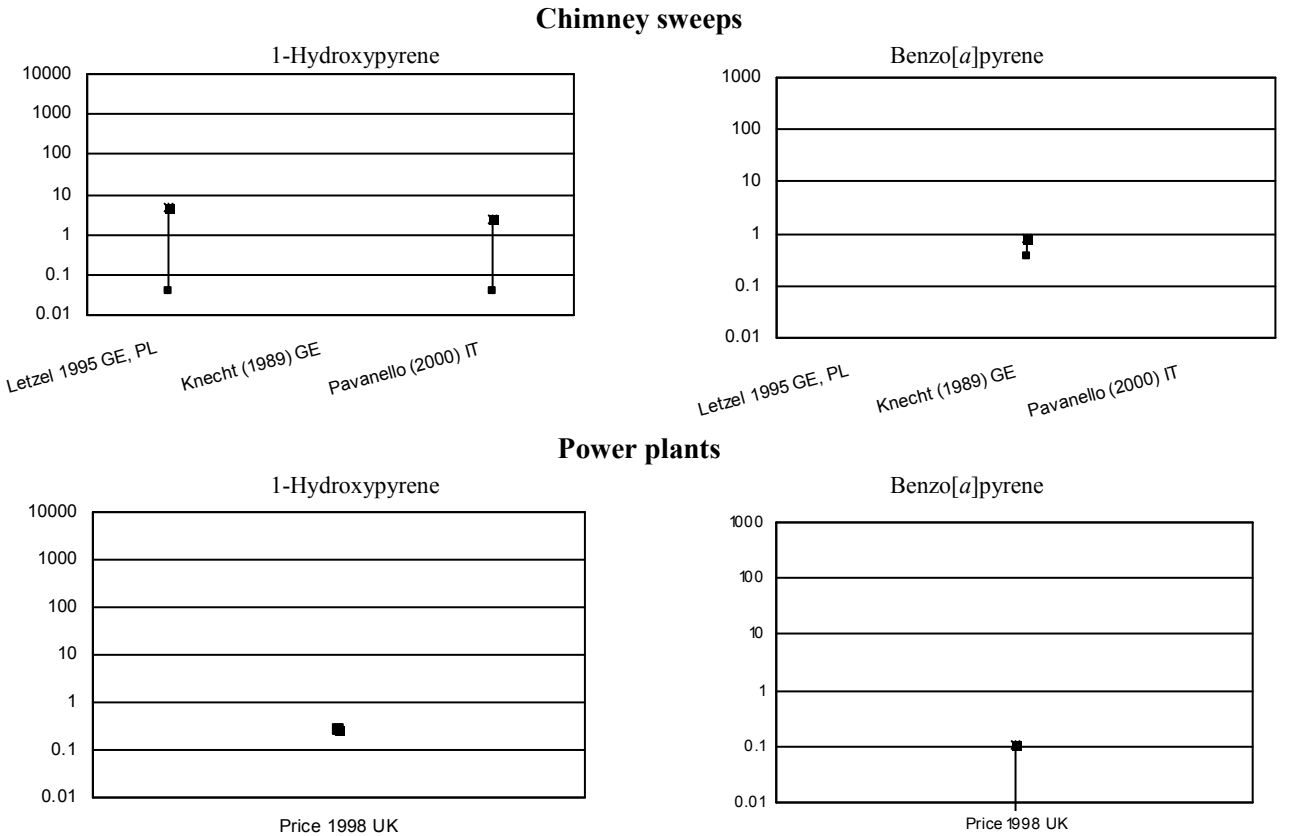
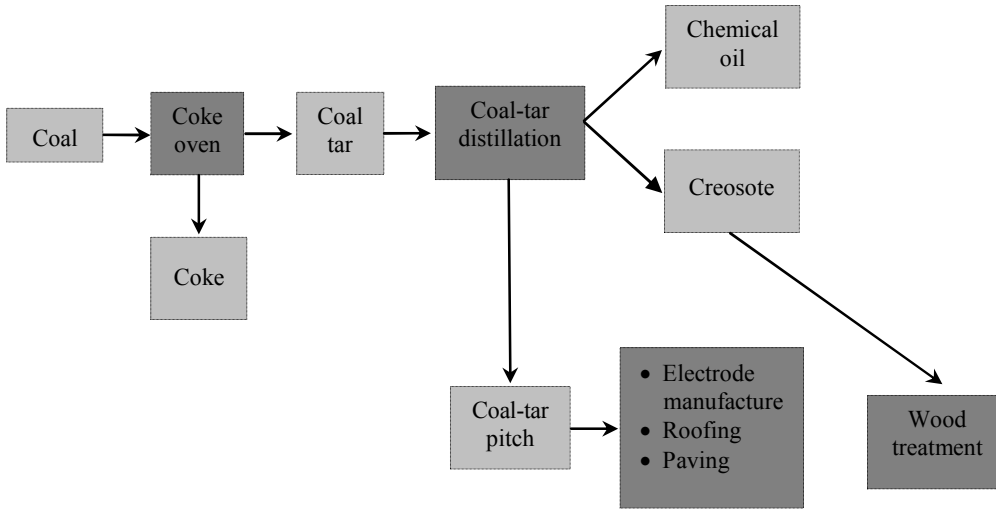


Figure 1.1 (contd)



[a] Data in brackets are publication date; data without brackets are study date, not every study had data for both 1-Hydroxypyrene and Benzo[*a*]pyrene
BE, Belgium; CH, Switzerland; FI, Finland; GE, Germany; IT, Italy; NL, Netherlands; NO, Norway; NZ, New Zealand; PL, Poland; PRC, People's Republic of China; SUR, Surinam; SW, Sweden; TW, Taiwan, China; UK, United Kingdom; USA, USA

Figure 1.2. Simple schema for generation of coal tar and coal-tar products



for the recovery of tar chemicals, i.e. benzene, toluene, xylenes, phenol, cresols and cresylic acids, pyridine and methylpyridines, naphthalene and anthracene, in addition to the so-called bulk products, e.g. creosote, tar paints, road tars and pitch binders (Betts, 1997).

Until the end of the Second World War, coal tar was the main source of these aromatic chemicals. However, the large increase in demand from the rapidly expanding plastics and synthetic fibre industries has greatly surpassed the potential supply from coal carbonization, which has led to the development of petroleum-based processes. This situation was exacerbated in the early 1970s by the cessation of the manufacture in Europe of town gas from coal, a process that was carried out preponderantly in continuous vertical retorts. By the 1990s, over 90% of the world production of aromatic chemicals was derived from the petrochemical industry, and coal tar became chiefly a source of anti-corrosion coatings, wood preservatives, feedstocks for the manufacture of carbon black and binders for electrodes (Betts, 1997).

Apart from the presence of a few per cent (usually below 5%) of aqueous liquor that contains inorganic salts and 1 or 2% of coal–char–coke dust that arises from the carry-over of particles in the carbonization process, coal-tar distillation products comprise essentially two components: (i) the distillate, which distills at up to ~400 °C at atmospheric pressure, is primarily a complex mixture of mono- and polycyclic aromatic hydrocarbons, a proportion of which are substituted with alkyl, hydroxyl and amine and/or hydro sulfide groups and, to a lesser extent, their sulfur-, nitrogen- and oxygen-containing analogues. For those tars produced from coal carbonization at lower temperatures, the distillate also contains hydroxy aromatic compounds, alkanes and alkenes. The distillate is typically removed by way of several fractions, which include

'chemical oils' and creosote; (ii) the second product is the residue from the distillation (pitch), which represents at least 50% of the coal-tar products formed by high-temperature carbonization and consists of a continuation of the sequence of mono- and polycyclic aromatic and heterocyclic compounds, but also extends to molecules containing 20–30 rings (Betts, 1997).

Crude coal tar is of value only as a fuel. Although large amounts were formerly burned, this practice has largely been abandoned. In the 1990s, 99% of the tar produced in the United Kingdom and Germany and 75% of that produced in the USA were distilled. In the USA, most of the crude tar is first topped in simple continuous stills to recover a chemical oil, i.e. a fraction that distills at 235 °C and contains most of the naphthalene (Betts, 1997).

Although smaller mild-steel or wrought-iron pot stills, that are equipped with fractionating columns, may still be used, continuous stills that have daily capacities of 100–700 tonnes are the primary means of coal-tar distillation worldwide (Betts, 1997).

The various designs of continuous tar stills are basically similar. The crude tar is filtered to remove large-sized solid particles, dehydrated by heat exchange and passage through a waste-heat coil, then heated under pressure to ~360 °C and flashed to separate volatile oils from the non-volatile pitch. The volatile oils are separated into a series of fractions of increasing boiling range by fractional condensation in a sidestream column or a series of columns. The diverse designs differ in the extent to which heat exchange is used, in the plan of the pipe-still furnace, in the distillation pressure (i.e. atmospheric pressure or reduced pressure) and the recycling or not of pitch or base tar (Betts, 1997).

The tars recovered from commercial carbonization plants are not primary products of the thermal decomposition of coal, since the initial products undergo a complex series of secondary reactions. Even tars produced at the lowest commercial carbonization temperatures are very different from primary tars. Low-temperature tar, continuous vertical-retort tar and coke-oven tar form a series in which the yield of tar decreases, the aromaticity of the tar increases, the content of paraffins and phenols decreases and the ratio of substituted aromatic and heterocyclic compounds to their unsubstituted parent molecules decreases. These differences are reflected in the densities and carbon:hydrogen ratios of the tars. Higher aromaticity correlates with higher density and carbon:hydrogen ratio. The reactions that account for these changes (i.e. cracking and cyclization of paraffins, dehydration of phenols and dealkylation of aromatic and heterocyclic ring compounds) are those that would be expected, on thermodynamic grounds, to occur at the temperatures that prevail in carbonization retorts (Betts, 1997).

The part of coke-oven tar that is normally distillable at atmospheric pressure boils at up to ~400 °C and amounts to up to 50% of the whole. It contains principally aromatic hydrocarbons. In particular, benzene, toluene and the xylene isomers, tri- and tetra-methylbenzenes, indene, hydrindene (indane) and coumarone occur in the first fraction that is normally removed; this represents about 3.5% of the tar and boils at up to ~200 °C. This fraction also contains polar compounds including tar acids (phenol and cresols) and tar bases (pyridine, picolines (methylpyridines) and lutidines (dimethylpyridines)). The

most abundant component of this type of tar is naphthalene, which is taken in the second fraction and represents about 10% of the tar. It is contaminated with small but significant amounts of thionaphthene, indene and other compounds. The next fraction contains the two methylnaphthalene isomers and is equivalent to 2% of the tar. Subsequent fractions contain biphenyl, acenaphthene and fluorene (each in the range of 0.7–1% of the tar) and then diphenylene oxide (about 1.5% of the tar). Anthracene and phenanthrene are usually present at about 1 and 6%, respectively. The series continues with components that boil at up to 400 °C, which represents approximately the limit of the usual commercial distillation range, i.e. pyrene and fluoranthene (Betts, 1997).

Continuous vertical-retort tars differ from coke-oven tars in that, whereas the latter contain relatively small amounts of non-aromatic hydrocarbons, continuous vertical-retort tars contain a relatively high proportion of normal straight-chain or slightly branched-chain paraffins, alkylated aromatics and phenols (Betts, 1997).

Of the total tar bases in coke-oven and continuous vertical-retort tars in the United Kingdom, pyridine makes up about 2%, 2-methylpyridine, 1.5%, 3- and 4-methylpyridines, about 2%, and ethylpyridine and dimethylpyridines, 6%. Primary bases, anilines and methylanilines account for about 2% of the bases in coke-oven and continuous vertical-retort tars and 3.5% of the bases in low-temperature tars. The main basic components in coke-oven tars are quinoline (16–20% of the total), isoquinoline (4–5%) and methylquinolines. These dicyclic bases are less prominent in continuous vertical-retort and low-temperature tars, in which only a minority of the basic constituents have been identified (Betts, 1997).

Much less is known about the composition of pitch, the residue from coal-tar distillation. Studies of coke-oven pitch indicate that it contains the following high-molecular-weight constituents: aromatic hydrocarbons with four rings, e.g. chrysene, fluoranthene, pyrene, triphenylene, naphthacene and benzanthracene; five-membered ring systems are represented by picene, benzopyrenes (benzo[*a*]pyrene and benzo[*e*]pyrene), benzofluoranthenes and perylene; the main components of the next highest fraction are six-membered ring systems such as dibenzopyrenes, dibenzofluoranthenes and benzoperylenes; seven-ring systems, e.g. coronene, have also been identified. These basic hydrocarbon structures are accompanied by methyl and polymethyl derivatives and, in the case of the pitches from continuous vertical-retort and low-temperature tars, by mono- and polyhydroxy derivatives. As in the case of the distillate oil range, heterocyclic compounds are also present (Betts, 1997).

Above this relatively low-molecular-weight range, which constitutes approximately 40–50% of a medium-soft coke-oven pitch, the information concerning the chemical structure of pitch is only qualitative and is derived mainly from statistical structural analysis and mass spectra. As molecular weight increases, more heterocyclic atoms appear in the molecule, whereas the number and length of alkyl chains decreases and the hydrocarbon structures are not fully condensed. In the lower-temperature pitches, some ring structures appear to be partly hydrogenated (Betts, 1997).

Concentrations of PAHs in the air and urine of workers in coal-tar distillation are summarised in Table 1.4 and Figure 1.1. The levels of exposure to PAHs overall were similar among the high-temperature processes and much lower in the low-temperature distillation facility.

(g) Paving and roofing involving coal-tar pitch

The exposures associated with roofing are the result of two operations. First, the old roof is removed by cutting, prying and scraping the existing roofing material from the roof, and discarding it. A new roof is then installed by melting solid blocks of coal-tar pitch, then pumping or carrying buckets of the molten material to the roof, where layers of roofing felt and liquid coal-tar pitch are spread upon the surface to produce a build-up. In recent years, coal-tar pitch has been removed from paving and roofing asphalts and has been replaced by bitumen (NIOSH, 2000; IPCS, 2004).

Concentrations of PAHs in the air of workers who used coal-tar pitch in roofing are summarized in Table 1.5 and Figure 1.1.

NIOSH conducted three health hazard evaluations (Reed, 1982; Zey, 1983; Behrens & Liss, 1984) between 1982 and 1984 of the tear-off and installation of coal-tar roofs. In the three investigations, air samples were collected to measure exposures to total PAHs, the benzene-soluble fraction and six to 12 individual PAHs. Exposures varied widely between sites. In one study, the majority of exposures were below the limits of detection; in another study, exposures ranged up to 64.5 μg/m^3 for benzo[*a*]pyrene.

Wolff *et al.* (1989) evaluated dermal and inhalation exposure among roofers by collecting pre-shift and post-shift skin wipes from a measured area of each worker's forehead and air samples from the workers' breathing zone. Samples were collected during the portion of each job when old coal-tar roofs were cut into pieces, ripped up and cleared away. Substantial increases were measured in the levels of skin contamination (pre- to post-shift) for seven PAHs and total PAHs (mean pre-shift, 83.9 ng/9 cm^2 skin area versus post-shift, 1521 ng/9 cm^2 skin area). Mean inhalation exposure levels during the tear-off of coal-tar roofs ranged from 9.6 to 23.0 μg/m^3 for the sum of eight PAHs.

Other exposures of roofers include silica, diesel exhaust, asbestos and organic solvents.

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

Detailed information on cessation of the use of coal tar in the European paving industry has been collected in the course of an IARC study on cancer mortality among asphalt workers. In Table 1.6, the last reported year of use of coal tar in paving by any company that participated in the cohort study is presented. The data originated from a company questionnaire and its ensuing evaluation by country-specific experts (Burstyn *et*

Table 1.4. Concentrations of PAHs in the air and urine of workers in coal-tar distillation

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air (μg/m^3)		Urinary 1-hydroxypyrene (μmol/mol creatinine)	
						Mean	Range	Mean	Range
van de Ven & Nossent (1984), Netherlands, NR	Operators, cleaners, maintenance	NR	49	NR	Sum of 11 PAHs	31	<1–277		
Jongeneelen *et al*. (1986), Netherlands, NR			*Air; urine*		Sum of 11 PAHs; pyrene				
	Operator pitch unit	1	8; 2	0		26; 8.5 }		3.7	NR
	Operator batch distillery	1	6; 2	1		14; 5.1 }	<2–280;	11.8	NR
	Operator pump station	1	5; 4	0		4.7; 1.4 }	<2–96	4.0	NR
	Cleaner	1	4; 4	1		16; 5.2 }		4.6	NR
Price *et al*. (2000), United Kingdom, 1998	Low-temperature	8	8	1	Total 19 PAHs	12.17	3.99–38.59	0.36	0.21–1.05
					HSE 11[a]	0.008	<0.004–0.008		
					Benzo[*a*]pyrene	ND	ND		
					Pyrene	0.037	0.013–0.068		
	High-temperature	12	12	4	Total 19 PAHs	279.04	51.9–1130.5	2.60	0.78–5.69
					HSE 11[a]	0.95	0.15–4.87		
					Benzo[*a*]pyrene	0.283	0.019–0.642		
					Pyrene	1.24	0.14–6.73		

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

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

Table 1.5. Concentrations of PAHs in the air of workers in roofing involving coal-tar pitch

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)	
						Mean	Range
Reed (1982), USA, 1982	Coal-tar roof tear-off	7	11	NR	BSF	720	300–1100
					Phenanthrene	8.3	0.8–22.0
					Anthracene	2.4	0.2–6.7
					Fluoranthene	13.1	1.5–39.5
					Pyrene	11.6	1.0–34.7
					Benzo[*a*]anthracene	5.7	0.5–14.4
					Chrysene	6.4	0.6–15.6
					Benzo[*a*]pyrene	5.9	0.4–15.7
Zey (1983)[a], USA, 1983	Coal-tar roof tear-off and application	13	24	NR	BSF	ND	140–2970
					Acenaphthene	24	ND–91.6
					Fluorene	25	ND–26
					Phenanthrene	12	ND–21
					Anthracene	22	ND–16.8
					Fluoranthene	9	ND–47.3
					Pyrene	9	ND–31.3
					Benzo[*c*]phenanthrene	29	ND–3
					Benzo[*a*]anthracene	14	ND–8.4
					Chrysene	15	ND–17.6
					Benzo[*b*]- + benzo[*k*]-fluoranthene	17	ND–11.5
					Benzo[*e*]pyrene	30	ND–4.5
					Benzo[*a*]pyrene	23	ND–11.9
					Total PAHs	42.7	ND–388

Table 1.5 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)	
						Mean	Range
Behrens & Liss (1984), USA, 1984	Coal-tar roof tear-off	6	6	NR	BSF	2.2	600–5300
					Fluoranthene	69.0	13.3–186.6
					Pyrene	52.8	10.6–140.6
					Benzo[*a*]anthracene	32.7	6.8–82.9
					Chrysene	27.9	6.0–71.4
					Benzo[*a*]pyrene	23.7	6.0–59.9
					Benzo[*e*]pyrene	21.8	4.3–64.5
					Phenanthrene	59.2	10.6–161.3
					Benzo[*ghi*]pyrene	17.9	3.3–43.8
Wolff *et al.* (1989)[b], USA, 1987	Tear-off of coal-tar roofs						*SD*
	Morning job	NR	8	NR	Sum of 8 PAHs	23.0	9.5
	Afternoon job	NR	2			9.6	0.9
	Single job	NR	1			15.3	–
	Morning job	NR	7			14.5	5.2
	Single job	NR	2			13.4	7.1
						Dermal levels (ng)	
	Skin wipe	3	6		pre	83.9	37.7
					post	1521	1373

BSF, benzene soluble fraction; ND, not detected; NR, not reported; PAH, polycyclic aromatic hydrocarbons; pre, pre-shift; post, post-shift; SD, standard deviation

[a] The Working Group noted some inconsistency and lack of information in the article, which made determination of number of workers, number of samples and PAH air levels difficult.

[b] The paper presents some inconsistencies in the number of air and skin wipe samples that were taken.

Table 1.6. Cessation of use of coal tar in asphalt paving (surface dressing)

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

From Burstyn *et al.* (2003)

al., 2003). A gradient in cessation of use can be seen, with Scandinavian countries ending use earlier than central and southern European countries, such as the Netherlands, France and Germany. However, even within countries, large differences in the use of coal tar have occurred between companies, depending on the supplier of the asphalt mixes and the presence of coke ovens in the neighbourhood. Even after the cessation of use of coal tar, workers in paving have been exposed to coal tar due to the use of recycled asphalt that contained coal tar in some countries.

Concentrations of PAHs in the air and urine of workers in paving that involves coal tar are summarized in Table 1.7 and Figure 1.1.

Darby *et al*. (1986) investigated the exposure of workers who performed coal-tar chip sealing as part of road paving operations in New Zealand. Personal exposures to six PAHs were measured in two samples taken in the area of workers' breathing zones and were reported to contain up to 9 μg/m^3 benzo[*a*]pyrene.

Jongeneelen *et al.* (1988a) measured exposures in highway chip sealing with coal-tar and reported inhalation levels of cyclohexane-soluble material, dermal levels of pyrene and pre- and post-shift levels of urinary excretion of 1-hydroxypyrene. The geometric mean for inhalation exposures to cyclohexane-soluble material was 0.6 mg/m^3 and that for dermal exposure to pyrene was < 10 ng; mean pre- to post-shift urinary 1-hydroxypyrene levels increased from 0.7 to 0.9 μmol/mol creatinine. In another study of road surfacing with blends of bitumen with refined coal-tar, the mean increase between pre- and post-shift levels of urinary 1-hydroxypyrene was 0.54 μmol/mol creatinine (Jongeneelen *et al.*, 1988b).

Knecht and Woitowitz (1989) measured air samples located in the breathing zone of workers who applied a coal tar–bitumen blend in road paving. Median air concentrations of benzo[*a*]pyrene were reported to be 0.7 μg/m^3.

Table 1.7. Concentrations of PAHs in the air and urine of workers in paving involving coal-tar pitch

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels ($\mu g/m^3$)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Darby *et al.* (1986), New Zealand, NR	Coal-tar chip sealing	2	2	NR	Benzo[*a*]anthracene + chrysene		1.2–17.8		
					Benzo[*b,j,k*]fluorene		1.1–11.4		
					Benzo[*e*]pyrene		0.7–5.4		
					Benzo[*a*]pyrene		0.9–9.0		
					Dibenz[*a,j*]anthracene		0.2–1.6		
					Indeno[1,2,3-*cd*]pyrene		0.7–6.3		
Jongeneelen *et al*. (1988a), Netherlands, NR	Chip sealing with refined coal-tar and blended bitumens					*Geometric mean*		*Geometric mean*	
		3	2	2	CSM	0.25 mg/m^3			
			3		Pyrene (wrist)	<10 ng			
			9		1-Hydroxypyrene			pre 1.8	
			8					post 2.8	
		6	18	2	CSM	0.20 mg/m^3			
			23		Pyrene (wrist)	24 ng			
			23		Pyrene rinse post	97.5 μg			
			23		1-Hydroxypyrene			pre 1.2	
			23					post 1.5	
		3	6	1	CSM	0.2 mg/m^3			
			9		Pyrene (wrist)	24 ng			
			7		Pyrene rinse post	216 μg			
			8		1-Hydroxypyrene			pre 1.5	
			8					post 1.9	

Table 1.7 (contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels ($\mu g/m^3$)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Jongeneelen *et al*. (1988a) (contd)	Chip sealing with refined coal-tar	4	1 4 5 3 4	0	CSM Pyrene (wrist) Pyrene rinse post 1-Hydroxypyrene	0.6 mg/m^3 < 10 ng 37.4 ng		 pre 0.7 post 0.9	
Jongeneelen *et al*. (1988b), Netherlands, NR	Road surfacing with blended bitumen and refined coal-tar	28	NR		1-Hydroxypyrene				pre 0.8–3.1 post 0.9–3.2
Knecht & Woitowitz (1989), Germany, NR	Road surfacing with blended 30% refined coal-tar + 70% bitumen	NR Stationary samples taken near work stations	250	NR	 Benzo[*a*]pyrene Indeno[1,2,3-*cd*]pyrene Benzo[*bjk*]fluoranthene Dibenzo[*a*,*h*]anthracene Chrysene	*Median* 0.7 0.2 9.3 0.03 2.8			

CSM, cyclohexane-soluble matter; NR, not reported; PAH, polycyclic aromatic hydrocarbon; pre, pre-shift; post, post-shift

(h) Creosote as a wood preservative

Coal-tar creosote is a distillate of coal tar that generally distills in the 200–400 °C range and is composed primarily of about 85% PAHs and 2–17% phenolic compounds. PAHs in coal-tar creosote are mainly two- and three-ring structures (Price *et al.*, 2000; ATSDR, 2002).

Coal-tar creosote has been used as a wood preservative since the nineteenth century, and wood preservation accounts for over 97% of its current use. Coal-tar creosote is applied to wood by commercial pressure treatment at 50–60 °C or, at least in the past, by individuals dipping or brushing the wood by hand. It is applied to railroad ties, utility poles, marine pilings, fence posts and other wood products for outdoor use (Price *et al.*, 2000; ATSDR, 2002).

Several studies have investigated exposure to PAHs among workers involved in the treatment of timber with creosote (Table 1.8 and Figure 1.1). Total PAH concentrations can be very high, especially due to relatively high concentrations of low-molecular-weight PAHs such as naphthalene, acenapthene and phenanthrene. In this industry, exposure to PAHs may occur both through inhalation and via the skin by contact with treated timber and contaminated surfaces. Based on urinary 1-hydroxypyrene levels, Elovaara *et al.* (1995) showed that the major uptake of PAHs was attributable to exposure to creosote through the skin. An intervention study (VanRooij *et al.*, 1993b) also provided evidence that skin is the main route of uptake of PAHs in this industry. Workers who wore a coverall under their working clothes had a 35% reduction in dermal exposure to pyrene and their urinary concentration of 1-hydroxypyrene was halved from 6.6 µg to 3.2 µg over a period of 22 h.

Workers involved in the creosote industry are also exposed to diesel exhaust, asbestos, silica, sulfur-substituted hydrocarbons, solvents, aliphatic amines and aldehydes. Other potential exposures may include other wood preservatives such as inorganic arsenicals or pesticides based on pentachlorophenol.

(i) Aluminium production, including the manufacture of anodes

Aluminium, the third most abundant element in the earth's crust, is usually combined in nature with silicon and oxygen as aluminium silicate. When aluminium silicate is subjected to tropical weathering, aluminium hydroxide may be formed. Rock that contains high concentrations of aluminium hydroxide is called bauxite. Although bauxite is normally the starting material for the production of aluminium, the industry generally refers to metallurgical grade alumina (Al_2O_3) that is extracted from bauxite by the Bayer process as the ore, from which aluminium is obtained by electrolysis (Sanders, 2002).

Since the discovery of the process by Hall and Héroult in 1886, nearly all aluminium has been produced by electrolysis of alumina dissolved in a molten cryolite (Na_3AlF_6)-based bath. The aluminium is deposited in molten form on a carbon cathode, which also serves as the melt container. Simultaneously, oxygen is deposited on and consumes the carbon–carbon anode(s) of the cell. Pure cryolite melts at 1012 °C, but alumina and additives,

Table 1.8. Concentrations of PAHs in the air, skin and urine of workers in the timber impregnation industry (creosote)

Reference country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Dermal levels (μg)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range	Mean	Range
Heikkilä *et al*. (1987), Finland, [1985]	Plant 1	9	26		3–6 ring PAHs 4–6 ring PAHs	3–106[a] 0.3–8.1[a]					
	Plant 2	4	8		3–6 ring PAHs 4–6 ring PAHs	0.1–104[a] 0.06–7.5[a]					
VanRooij *et al*. (1993b), Netherlands, 1991	Assembly hall	7	7	5/7	Pyrene 1-Hydroxypyrene		0.3–3.0	440	47–1368	6.4 μg	0.7–12.7[c]
	Cylinder	3	3	0	Pyrene 1-Hydroxypyrene	1.1	0.9–1.3	649	120–1511	7.5 μg	2.6–12.2[c]
Elovaara *et al*. (1995), Finland, 1987	Wood impregnation plant	6	30	3	Total 10 PAHs[b] Benzo[*a*]pyrene Pyrene	5.7 0.01 0.97	1.23–13.74 0.01–0.05 0.23–2.10				
		6	18	3	1-Hydroxypyrene (end of shift)					64	19–85
Heikkilä *et al*. (1995), Finland, NR	Handling of creosote-impregnated timber	3	9	3	1-Hydroxypyrene					6.8	3.8–12.8

Table 1.8 (Contd)

Reference country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Dermal levels (μg)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range	Mean	Range
Price *et al*. (2000), United Kingdom, 1998	Several	11	11	6	Total of 19 PAHs	835	30.2–1913				
					HSE11[d]	0.28	0.07–0.38				
					Benzo[*a*]pyrene	0.22	0.07–0.35				
					Pyrene	0.33	0.06–0.69				
					1-Hydroxypyrene					16.0	1.44–60.0

PAH, polycyclic aromatic hydrocarbon
[a] Range of means
[b] Excluding naphthalene
[c] Sample covering 8 h-work shift and the 14 consecutive hours
[d] Benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,h*]anthracene, anthanthrene, cyclopenta[*c,d*]pyren

namely 4–8% calcium fluoride, 5–13% aluminum fluoride, 0–7% lithium fluoride and 0–5% magnesium fluoride, lower the melting-point, which allows operations to proceed at 920–980 °C (Sanders, 2002). Commercialization of the Hall-Héroult process was rapid, and led to the swift growth of the aluminium industry in Europe and North America in the last decade of the nineteenth century (Frank *et al.*, 2005).

A modern alumina-smelting cell consists of a rectangular steel shell which is lined with refractory insulation that surrounds an inner lining of baked carbon. Few materials other than carbon are able to withstand the combined corrosive action of molten fluorides and molten aluminium. Thermal insulation is adjusted to provide sufficient heat loss to freeze a protective coating of electrolyte on the inner walls but not on the bottom, which must remain uncovered to allow for electrical contact with the molten aluminium cathode. Steel (collector) bars are joined to the carbon cathode at the bottom to conduct the electric current from the cell. The current enters the cell either through pre-baked carbon anodes or through a continuous self-baking Søderberg anode (Sanders, 2002).

The Søderberg anode is formed continuously from a paste of petroleum coke and coal-tar pitch which are typically added to the top of a rectangular steel casing. When it passes through the casing, the paste bakes to form carbon and replaces the anode that is being consumed. The baked portion extends past the casing into the molten electrolyte. The electric current enters the anode through vertical or sloping steel spikes (also called pins), and molten aluminium is generally removed from the cells daily by siphoning into a crucible. Normally, the metal is 99.6–99.9% pure, and the principal impurities (iron, silica, titanium, vanadium and manganese) derive mainly from the anode, but also from the alumina (Sanders, 2002).

Pre-baked anodes are produced by molding petroleum coke and coal-tar pitch binder into blocks which are baked at 1000–1200 °C. Petroleum coke is used because of its low impurity (ash) content. The more noble impurities, such as iron and silicon, deposit in the aluminium whereas less noble impurities, such as calcium and magnesium, accumulate as fluorides in the bath. Coal-based coke could be used, but extensive and expensive pre-purification would be required. Steel stubs seated in the anode that uses cast iron support the anodes (via anode rods) in the electrolyte and conduct electric current into the anodes (Sanders, 2002).

In industrial electrowinning (separation by electrolysis) of aluminium, part of the energy required to reduce the alumina is supplied by electricity and part derives from consumption of the carbon anode. Carbon is also used to line the cathode. For each kilogram of aluminium produced, 0.4–0.5 kg of anode is consumed and this represents the major carbon requirement. High-purity carbon is desirable because any ash from the carbon would contaminate either the aluminium produced or the electrolyte. In addition, certain impurities, such as vanadium, are particularly harmful because they catalyse air burning of the carbon; other impurities, such as phosphorus, accumulate in the electrolyte, undergo cyclic redox reactions (partial reduction followed by re-oxidation) and consume electric current unproductively. The coke residue from petroleum refining is reasonably

pure and has therefore been used as the major source of carbon for anodes (Frank *et al*., 2005).

Anthracite has been the major constituent of cathode blocks in the cells, although graphite and metallurgical coke have also been used to some extent. Anthracite is calcined at 1200 °C or above, crushed and sized, mixed with coal-tar pitch, molded into blocks and baked. These blocks, mortared together with a carbonaceous seam mix, form the pot lining, which is the container for both the aluminium and the electrolyte. High purity is not as important for the cathode blocks because leaching of impurities is very slow. Consumption of cathode carbon amounts to 0.02–0.04 kg per kilogram of aluminium produced (Frank *et al*., 2005).

Workers in the aluminium industry and the related carbon-electrode manufacturing industry have been monitored most intensively for exposure to PAHs and large studies have recently been conducted in Europe and South America (Table 1.9 and Figure 1.1). Exposure studies up to the early 1980s were reviewed previously (IARC, 1984), and only studies published since that time are reviewed below.

Exposure to PAHs, sulfur dioxide and fluorides have decreased over time (Benke *et al*., 1998). At two plants that operated the vertical stud Søderberg potrooms in Norway, exposures decreased on average by a factor of four between the late 1950s and the late 1980s (Romundstad *et al.*, 1999). The decrease in exposure is most probably a result of the implementation of improved technology in combination with increased use of effective personal protective devices. In addition, the increasing predominance of pre-bake potrooms also probably contributed to the decline of exposures to PAHs (Benke *et al.*, 1998), although this might apply to the anode pre-baking plants. Urinary levels of 1-hydroxypyrene in anode manufacturing for the aluminium industry did not decrease considerably between the mid-1980s and mid-1990s (Table 1.10 and Figure 1.1).

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

In addition to exposures to sulfur dioxide and fluorides, several other exposures can occur among aluminium workers, including aluminium fluoride, fibrous sodium aluminium tetrafluoride particles, fluorspar, alumina, carbon monoxide, carbon dioxide and various trace metals (e.g. vanadium, chromium and nickel) (Benke *et al.*, 1998). Exposure to asbestos has also been reported (Dufresne *et al.*, 1996). In addition to these chemical exposures, workers in the aluminium industry are also exposed to extreme heat and high static magnetic fields (Benke *et al*., 1998).

Table 1.9. Concentrations of PAHs in the air and urine of workers in the aluminium industry[a]

Reference, country	Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
							Mean	Range	Mean	Range
Bolt & Golka (1993), Germany	NR	Søderberg plant	16	NR	NR	Benzo[*a*]pyrene	NS	0.8–292		
Tjoe Ny *et al*. (1993), [Surinam]	1990	Technicians, engineers, electricians, laboratory workers	6	6	1	Total 16[d]	12	3–22		
						Benzo[*a*]pyrene	<1.0	<1.0–<1.0		
						Pyrene	3.5	<1.4–8.3		
			5	5	1	1-Hydroxypyrene			1.0	0.2–2.7
		Foremen, tappers	6	6	NR	Total 16[d]	38	12–60		
						Benzo[*a*]pyrene	0.93	<1.0–1.5		
						Pyrene	7.9	2.2–14		
			4	4	2	1-Hydroxypyrene			3.0	1.2–4.5
		Crane operators, all rounders	10	10	NR	Total 16[d]	328	59–715		
						Benzo[*a*]pyrene	14	1.5–43.2		
						Pyrene	39	16–121		
			8	8	4	1-Hydroxypyrene			18	3.7–39.6
		Potmen	10	10	NR	Total 16[d]	170	30–388		
						Benzo[*a*]pyrene	2.7	1.0–5.7		
						Pyrene	25	2.5–49		
			9	9	3	1-Hydroxypyrene			35	16.5–75.9
		Electrode men	6	6	NR	Total 16[d]	1040	256–2430		
						Benzo[*a*]pyrene	48	7.5–94		
						Pyrene	130	52–262		
			4	4	4	1-Hydroxypyrene			43	23.5–66.5

Table 1.9 (Contd)

Reference, country	Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
							Mean	Range	Mean	Range
Levin *et al*. (1995), Sweden	NR	Several	9	9	1	Total 7	91	30–400		
						Benzo[*a*]pyrene	7.07	1.7–36		
			9	9	1	1-Hydroxypyrene			5.9	1.3–10.7 ng/mL
van Schooten *et al*. (1995), Netherlands	1989	Pot-relining	NR	41	NR	Total 12[b]	150[c]	0.9–1709		*SD*
						Benzo[*a*]pyrene[b]	1.05[c]	<0.02–9		
						Pyrene[b]	32.3[c]	0.15–223		
			8	NS	6	1-Hydroxypyrene			S, 6.72	4.25
									NS, 6.20	8.44
		Electrolysis	NR	23	NR	Total 12[b]	1.0[c]	0.3–4.6		
						Benzo[*a*]pyrene[b]	0.03[c]	<0.02–0.2		
						Pyrene[b]	0.12[c]	0.03–0.7		
			22	NR	13	1-Hydroxypyrene			S, 0.88	0.42
									NS, 0.48	0.27
Carstensen *et al*. (1999) Sweden	NR	Potroom workers	97	97	31%	Total 22[b]	13.2[e]	0.01–270		
			96	96		Total 7 (gaseous)	16.3[e]	0.01–132		
			93	93		Benzo[*a*]pyrene[b]	0.97[e]	0.02–23.5		
			94	94		Pyrene[b]	1.11[e]	0.02–34.4		
			95	95		Pyrene (gaseous)	1.56[e]	0.01–9.5		
			96	96		1-Hydroxypyrene			4.3	0.1–17.7

Table 1.9 (Contd)

Reference, country	Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
							Mean	Range	Mean	Range
Romundstad *et al*. (1999), Norway	1978–87	Pot operator	195		NR	PAHs[b]	66			
	1980–86		60		NR		88			
	1987–95		201		NR		10			
	1988–96		98		NR		14			
	1980–86	Stud puller	54		NR		842			
	1986–87		35		NR		89			
	1987–89		30		NR		248			
	1988–96	Stud puller with	130		NR		11			
	1990–95	one-man cabins with filtered air	49		NR		12			
	1978–79	Flex raiser	47		NR		145			
	1980–86		21		NR		120			
	1987–95		46		NR		9			
	1978–87	Tapper	90		NR		37			
	1986		2		NR		65			
	1987–95		60		NR		10			
	1988–96		97		NR		9			
	1978–87	Other jobs in	288		NR		53			
	1980–86	potroom	161		NR		52			
	1987–95		193		NR		10			
	1988–89		15		NR		13			
	1978–87	Daytime	264		NR		86			
	1980–86	maintenance	33		NR		219			
	1987–95		74		NR		24			
	1988–96		271		NR		23			

Table 1.9 (Contd)

Reference, country	Year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels ($\mu g/m^3$)		Urinary levels (μmol/mol creatinine)	
							Mean	Range	Mean	Range
Price *et al.* (2000), United Kingdom	1998	Several	10	10	1/9	Sum of 19 PAHs	60.9	0.01–138		
						HSE 11[f]	0.31	0.001–0.85		
						Benzo[*a*]pyrene	0.03	0.01–0.10		
						Pyrene	0.86	0.02–2.91		
						1-Hydroxypyrene			0.7	0.3–2.6

NR, not reported; NS, non-smoker; PAH, polycyclic aromatic hydrocarbon; S, smoker; SD, standard deviation

[a] All studies were of the Søderberg process, except those in the Netherlands (van Schooten *et al.*, 1995) and the United Kingdom (Price *et al.*, 2000), which were of the pre-baking process

[b] Only particulate PAHs were measured

[c] Geometric mean

[d] PAHs recommended for sampling by the US National Institute of Occupational Safety and Health

[e] Median

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

Table 1.10. Concentrations of PAHs in the air and urine of workers in anode manufacturing for the aluminium industry

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels ($\mu g/m^3$)		Urinary levels (μmol/mol creatinine)	
						Mean	Range or SD	Mean	Range
Tolos *et al*. (1990), USA, NR	Anode bake area								
	Crane operators	9	9	NR	Total 17	25.8	13.8–56.4		
					Pyrene	2.5	1.2–6.5		
					1-Hydroxypyrene			2.9	2.0–3.6
	Packer-puller	7	7	NR	Total 17	24.7	10.1–57.2		
					Pyrene	2.8	1.5–7.4		
					1-Hydroxypyrene			2.4	1.9–3.3
	Equipment operators	2	2	NR	Total 17	52.1	9.5–94.6		
					Pyrene	2.1	1.5–2.8		
					1-Hydroxypyrene			3.0	2.5–3.5
van Schooten *et al*. (1995) Netherlands, 1989	Bake oven	NR	22	NR	Total 12[a]	8.7[b]	3.0–107		*SD*
					Benzo[*a*]pyrene[a]	0.35[b]	0.1–14.4		
					Pyrene[a]	1.5[b]	0.5–22.7		
		6	NR	6	1-Hydroxypyrene			8.43	4.08
		9	NR	0				3.65	2.11
	Anode factory	NR	40	NR	Total 12[a]	23[b]	1.1–854		
					Benzo[*a*]pyrene[a]	1.51[b]	0.1–11.6		
					Pyrene[a]	5.6[b]	0.3–318		
		2	NR	2	1-Hydroxypyrene			4.84	3.64
		5	NR	0				3.25	1.89
Petry *et al*. (1996), Switzerland, 1994	Green anode section; several jobs	6	30	4	Total 26	NR	4.0–121		
					Benzo[*a*]pyrene	NR	0.2–4.9		
					Pyrene	NR	0.4–12.8		
					1-Hydroxypyrene			NR	0.5–61.8

Table 1.10 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range or SD	Mean	Range
van Delft *et al*. (1998), Netherlands, 1995	Anode plant (high exposure	5	18		Total 16	32	2.3–185		
					Benzo[*a*]pyrene	1.2	0.43–3.2		
					Pyrene	2.45	0.28–46		
		17	17	9	1-Hydroxypyrene[c]			13.2 μg/day	7.7–45.1
	Maintenance (medium exposure)	7	12		Total 16	8.4	1.8–80		
					Benzo[*a*]pyrene	0.37	0.09–5.0		
					Pyrene	0.51	0.10–4.4		
		19	19	7	1-Hydroxypyrene[c]			5.66 μg/day	2.2–20.2
	Laboratory and office (low exposure)	19	19	5	1-Hydroxypyrene[c]			1.59 μg/day	0.66–3.54
Pavanello *et al*. (2000), Italy, NR	Anode plant workers	22	22	0	1-Hydroxypyrene			0.32	0.06–1.17

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

[a] Only particulate PAH were measured

[b] Geometric mean

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

(j) Carbon-electrode manufacture other than for aluminium production

With the exception of the use of carbon in the manufacture of aluminium, the largest use of carbon and graphite is as electrodes in electric-arc furnaces. In general, the use of graphite electrodes is restricted to open-arc furnaces of the type used in steel production, whereas carbon electrodes are employed in submerged-arc furnaces used in the manufacture of phosphorus, ferroalloy and calcium carbide (Criscione *et al*., 1992).

Graphite electrodes are produced commercially in many sizes ranging from 32 mm in diameter by 610 mm in length to 700 mm in diameter by 2800 mm in length; each diameter is generally available in two or three lengths. These electrodes are used in open-arc furnaces for the manufacture of steel, iron and steel castings, brass, bronze, copper and its alloys, nickel and its alloys, magnesium, lead, tin, fused cast refractories, fused refractory grain and mineral wool insulation and for the treatment of toxic wastes. The largest use of graphite electrodes by far is in the manufacture of steel and, as a consequence, the growth of graphite production has been closely related to the growth in steel production in electric furnaces. A small but growing number of arc furnaces now use direct current and a single-electrode column positioned in the centre of the furnace. In both cases, steel is produced by filling the cylindrical shell with ferrous scrap, directly reduced iron or occasionally molten pig iron, then melting and refining the metallic charge with the intense heat derived from the electric arc that is generated at the tips of the electrodes (Criscione *et al*., 1992).

Before the mid-1940s, the arc furnace was used almost exclusively for the production of low-tonnage, high-quality steels such as stainless and alloy steels. Since then, its use has been extended to production of the more common high-tonnage steel grades, including sheet steels. The growth of steel production in arc furnaces in the USA has been dramatic; it rose from 6% of total steel production in 1950, to 20% in 1975 and 36% in 1990. Worldwide, over 210 million tonnes of steel were produced in electric-arc furnaces in 1990 (approximately 26% of total world steel production) and these furnaces consumed over 800 000 tonnes of graphite electrodes (Criscione *et al*., 1992).

Graphite electrodes are consumed in the melting process. For iron and steel production, the average consumption is approximately 2–5 kg/tonne, depending on the quality of the charge material, the quality of the electrodes and numerous factors related to the productivity and operation of the arc furnace. A combination of these factors has resulted in a reduction of about 40% in specific electrode consumption over the past 15 years (Criscione *et al*., 1992).

Graphite electrodes are produced in two broad-grade classifications — regular grade and premium grade. The principal differences between the two grades are that the premium grade is made from a super premium needle coke and is impregnated with pitch before graphitization. The premium-grade electrode is used when very high performance is required, such as in ultrahigh-powered arc furnaces. The capacity of an electrode column to carry current depends on many characteristics of the furnace operation as well

as those of the electrode and electrode joint, and, over the years, significant progress has been achieved in improving this capacity (Criscione *et al*., 1992).

Concentrations of PAHs in the air and urine of workers in the carbon-electrode industry are summarized in Table 1.11 and Figure 1.1. Exposure conditions in these carbon manufacturing plants are relatively similar to those in anode manufacturing for aluminium plants. Three recent studies from Belgium (Buchet *et al.*, 1992), Germany (Angerer *et al.*, 1997) and Norway (Øvrebø *et al.*, 1994) reported differences in PAH concentrations within the plants. Much higher exposures were observed at the beginning of the production process when raw materials are mixed and the electrode is dipped in liquid tar in the studies in Belgium and Germany.

(k) Chimney sweeping and other exposures to soot

For hundreds of years, chimneys have been swept with long steel brushes inserted manually into the chimney from the top and from the bottom. By this procedure, chimney sweepers are exposed to soot particulates.

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

Two German studies investigated the exposure of chimney sweeps to PAHs (Table 1.12 and Figure 1.1). Knecht *et al.* (1989) assessed exposures in the breathing zone of chimney sweeps during so-called ‘dirty’ or ‘black work’ over a period of 11 days. Samples were taken per ‘job’ that was classified based on the fuel fired: oil fuel, oil/solid or solid fuels. Twenty PAHs were quantified in a total of 115 samples. Higher concentrations were seen when solid fuels had been fired. A more recent biomonitoring study carried out in Germany and Poland in 1995 reported 1-hydroxypyrene levels in the urine ranging from below the detection limit of 0.1 μg/L to 12.8 μg/L (Letzel *et al.*, 1999). Urinary concentrations in Poland were on average five times higher, most probably due to the fact that coal and wood are more often used there as fuel. The urinary concentrations in workers in Germany were relatively low. The use of personal protective devices among this group of 100 chimney sweeps was not reported.

Chimney sweeps have also been reported to be exposed concurrently to sulfur dioxide and arsenic (Bagchi & Zimmerman, 1980).

Table 1.11. Concentrations of PAHs in the air and urine of workers in carbon electrode manufacturing other than for the aluminium industry

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Buchet *et al*. (1992), Belgium, NR	Conditioning	10	NR	7	Total of 13 Pyrene	2.8 0.10 NS; 0.04 S	1.0–9.3		
					1-Hydroxypyrene			0.55 NS; 0.55 S	
	Second thermal treatment	25	NR	17	Total of 13 Pyrene	4.1 0.05 NS; 0.05 S	0.2–50.9		
					1-Hydroxypyrene			0.57 NS; 0.79 S	
	Heating of raw electrodes	6	NR	4	Total of 13 Pyrene	17.4 0.20 NS ; 0.39 S	100–117 [sic][a]		
					1-Hydroxypyrene			2.56 NS; 3.13 S	
	Maintenance	17	NR	2	Total of 13 Pyrene	12.5 0.21 NS ; 0.13 S	0.6–251		
					1-Hydroxypyrene			1.21 NS; 3.76 S	
	Grinding and mixing raw materials	14	NR	9	Total of 13 Pyrene	96.0 10.75 NS ; 3.52 S	17.7–551		
					1-Hydroxypyrene			2.98 NS; 2.83 S	

Table 1.11 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels ($\mu g/m^3$)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Buchet *et al*. (1992) (contd)	Electrode impregnation	8	NR	5	Total of 13	222.6	20.8–1212		
					Pyrene	27.27 NS ; 3.35 S			
					1-Hydroxypyrene			4.14 NS; 4.96 S	
Angerer *et al*. (1997), Germany, NR	Crushing	2	4	NR	Total of 8	5.19	0.37–10.0		
					Benzo[*a*]pyrene	0.09	0.01–0.17		
					Pyrene	0.72	0.04–1.40		
		2	NR		1-Hydroxypyrene			5.0	0.6–9.4
					Sum of OH-PHE			5.4	0.98–9.7
	Baking	5	10	NR	Total of 8	29.3	4.94–70.7		
					Benzo[*a*]pyrene	1.15	0.14–3.39		
					Pyrene	4.54	0.81–12.4		
		30	NR		1-Hydroxypyrene			12.1	0.9–169
					Sum of OH-PHE			12.0	1.7–56.0
	Impregnation	3	6	NR	Total of 8	23.4	16.9–31.1		
					Benzo[*a*]pyrene	1.09	0.47–1.46		
					Pyrene	3.12	2.19–4.55		
		9	NR		1-Hydroxypyrene			11.4	3.2–42.1
					Sum of OH-PHE			18.7	8.9–75.6
	Graphitization	4	8	NR	Total of 8	1.27	0.45–1.74		
					Benzo[*a*]pyrene	0.01	0.007–0.01		
					Pyrene	0.22	0.01–0.38		
		24	NR		1-Hydroxypyrene			0.93	0.1–3.3
					Sum of OH-PHE			2.4	0.77–8.5

Table 1.11 (Contd)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Angerer *et al*. (1997) (contd)	Conditioning	2	4	NR	Total of 8	0.37	0.11–0.63		
					Benzo[*a*]pyrene	0.01	0.003–0.02		
					Pyrene	0.03	0.01–0.05		
		2	NR		1-Hydroxypyrene			1.2	0.9–1.5
					Sum of OH-PHE			3.9	2.1–5.8

NR, not reported; NS, nonsmoker; OH-PHE, monohydrated metabolites of phenanthrene; PAH, polycyclic aromatic hydrocarbon; S, smoker
[a] [The lower value of 100 is inconsistent with a mean of 17.4.]

Table 1.12. Concentrations of PAHs in the air and urine of chimney sweeps

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH measured	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Knecht *et al*. (1989), Germany, NR		NR		NR					
	Oil fuel		37		Benzo[*a*]pyrene	0.36			
	Oil/solid fuel		34			0.83			
	Solid fuel		44			0.82			
								Median	
Letzel *et al*. (1999), Germany, Poland, 1995		100	100	42	1-Hydroxypyrene[a]			NR	0.04–4.5
	Apprentices, journeymen	79	79	NR				0.28	
	District master	21	21	NR				0.1	
	Workers from Germany	93	93	38				0.14–0.32	
	Workers from Poland	7	7	4				1.2	
	Smokers	42	42	42				0.39	
	Nonsmokers	58	58	0				0.19	
Pavanello *et al*. (2000), Italy, NR		27	27	0	1-Hydroxypyrene			0.56	0.04–2.34

NR, not reported; PAH, polycyclic aromatic hydrocarbon

[a] Originally reported in μg/L. Conversion factor: 1 μmol/mol creatinine = 1.93 μg/g creatinine = 0.013 μmol/L = 2.84 μg/L

(l) Thermoelectric power plants

Thermal power plants use the energy of heat to make electricity. Coal is burned to heat water in a boiler and convert it to high-pressure steam. The high-pressure steam is directed into a steam turbine which turns the turbine shaft. This shaft is connected to an electrical generator which produces electricity. A condenser converts the exhausted steam from the turbine back into water which is re-used in the boiler. The condenser contains tubes that have water circulating through them to cool the steam. The water is supplied by a nearby reservoir or river. This condensing process increases the efficiency of electricity generation (ATCO Power, 2008).

Coal for the generating stations may be extracted from a nearby coal mine or brought in by rail or ship. At the generating station, the coal is crushed and stockpiled. A conveyor belt carries the crushed coal from the stockpile to bunkers within the power station. The coal is fed as required into pulverizers, where it is ground to a fine dust, the consistency of talcum powder. A fan blows the coal into the boiler's furnace. Water flows through tubes that form the walls of the furnace and the intense heat of the burning coal causes the water inside the tubes to boil. The boiling water rises into the steam drum at the top of the boiler where the steam is separated from the water. The steam, at high pressure, is super-heated to still higher temperatures and then used to turn the steam turbine and an electricity generator. After leaving the turbine, the steam — now at a lower temperature and lower pressure — passes through the condenser, which condenses the steam back into water that is pumped back to the boiler to repeat the cycle. The fine, powdery ash produced when coal is burned is called fly-ash. The hot gases and fly-ash move out of the boiler's furnace and into electrostatic precipitators, which are a series of electrically charged metal plates that attract and hold the fly-ash particles. The collected fly-ash is either sold for use as an additive to concrete or transported to the mine site to be used for fill (ATCO Power, 2008).

Few studies have focused on the exposure to PAHs of workers in power plants that transform coal into electricity. A recent study by the Health and Safety Executive (Price *et al*., 2000) evaluated exposures to PAHs of workers employed in a coal-fired power station (Table 1.13 and Figure 1.1). Twelve individuals (including drivers of bulldozers and other equipment, operators and fitters and welders) were monitored. The overall mean exposure was low (1.4 μg/m^3) and none of the end-of-shift urine samples contained levels of 1-hydroxypyrene above the detection limit (0.5 μmol/mol creatinine).

Other potential exposures present in the environment of workers in thermoelectric power plants are to residual coal or oil fly-ash, trace metals such as chromium, vanadium, nickel and mercury, and combustion gases such as sulfur dioxide and nitrogen oxides. Depending on the fuel that is used to drive vehicles such as bulldozers and other heavy equipment, there is also potential exposure to diesel exhaust emissions. Since the majority of workers in a power plant are involved in maintenance work, exposure to solvents, greases and oils, asbestos and welding fumes should also be taken into account (Price *et al.*, 2000).

Table 1.13. Concentrations of PAHs in the air and urine of workers in thermoelectric power plants (utility industry)

Reference, country, year of study	Job/task	No. of subjects	No. of samples	No. of smokers	PAH	Air levels (μg/m^3)		Urinary levels (μmol/mol creatinine)	
						Mean	Range	Mean	Range
Price *et al*. (2000), United Kingdom, 1998	Several	12	12	6	Sum of 19 PAHs	1.42	0.46–2.15		
					HSE11[a]	0.14	0.04–0.49		
					Benzo[*a*]pyrene	0.02	0.007–0.11		
					Pyrene	0.025	0.006–0.055		
					1-Hydroxypyrene			0.25	0.25–0.25

PAH, polycyclic aromatic hydrocarbon

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

(m) Overall ranking of industries by level of PAHs

To rank exposure to PAHs by industry, the Working Group compared the measurements of the analytes most frequently reported in air samples collected throughout the 10 industrial sectors. Relatively few of the studies conducted a detailed analysis to identify six or more of the component PAHs that comprised the overall exposure. Therefore, airbone concentrations of benzo[*a*]pyrene and urinary levels of 1-hydroxypyrene were compared across industries. Urinary levels of 1-hydroxypyrene also reflect dermal uptake and therefore should be interpreted as a measure of uptake via both inhalation and the skin. Ranges of personal measurements of benzo[*a*]pyrene and urinary levels of 1-hydroxypyrene were plotted by year and study (Figure 1.1). The relative ranking of the industries for 1-hydroxypyrene in urine and benzo[*a*]pyrene in air is summarized in Figure 1.3.

In the case of inhalation exposures to benzo[*a*]pyrene, reported levels were highest in the aluminium production industry (operating the Søderberg process), with values of up to 100 μg/m^3. These levels were substantially higher than those measured in the pre-bake aluminium production process. Levels in electrode manufacturing for aluminium reduction or manufacture of graphite electrodes for the metal industry were also considerably lower (up to 10 μg/m^3).

The levels of benzo[*a*]pyrene observed in studies of coke ovens were somewhat lower than those observed in aluminium production, and there was some evidence of a trend towards lower exposures over time within the studies conducted in western Europe and the USA; a study from Sweden documented substantial reductions by comparing exposures before and after a major plant renovation in 1990 (Levin *et al.*, 1995). A distinct reduction in exposures to PAHs was also evident from the analysis of longitudinal data from two Norwegian Søderberg plants that covered three decades (Romundstad *et al.*, 1999).

Studies conducted after 1995 do not support the downward trend in exposure, although this may be because more recent studies were performed in coke manufacture in Asian and eastern European countries. This coincides with a shift in coke production away from Europe and the USA. The extent to which controls of coke-oven emissions and associated exposure are in place in these facilities has not been reported. Given the reported (sometimes extreme) concentrations, it is unlikely that the control measures in place meet modern standards.

Comparison of the urinary levels of 1-hydroxypyrene between aluminium production and coke-oven workers shows a pattern of higher exposures among the coke-oven workers (up to 3261 μmol/mol creatinine), which is probably a result of the higher levels of exposure to pyrene in coke-oven environments than those in aluminium reduction plants.

Concentrations of benzo[*a*]pyrene measured during roofing and paving with coal tar were relatively similar, with ranges generally up to 10–20 μg/m^3. One exception was a study in the USA, in which exposure to benzo[*a*]pyrene ranged up to 64.5 μg/m^3 during

Figure 1.3. Relative ranking of industries by levels of exposure to (by inhalation only) and by urinary levels of 1-hydroxypyrene (inhalation and dermal exposure)

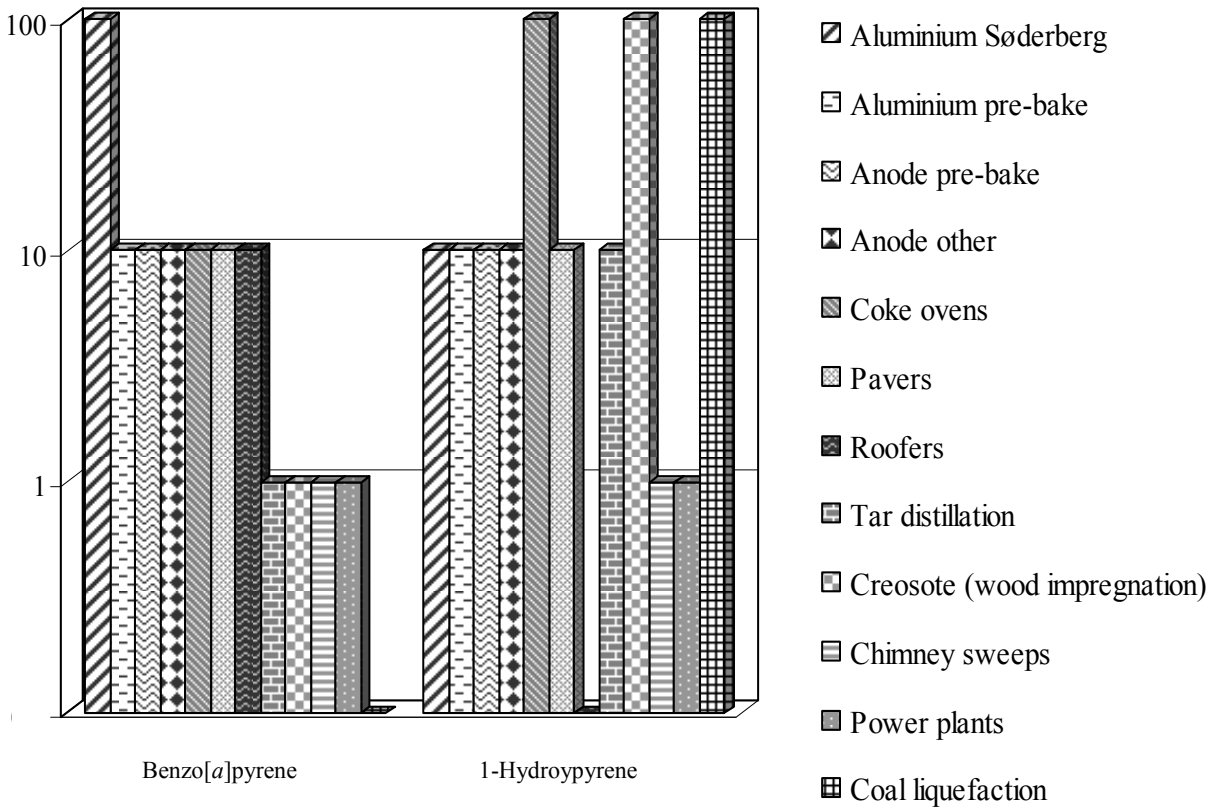


Figure compiled by the Working Group

tear-off of coal-tar roofing; however, no measures of 1-hydroxypyrene were reported among roofers. One study that measured 1-hydroxypyrene among pavers found levels of up to 3.2 μmol/mol creatinine, which are much lower than urinary levels seen in workers in aluminium production plants, electrode manufacturing plants, coke ovens and tar distillation plants. No trends in exposure to PAHs were apparent for roofers and pavers in the studies reviewed for the period 1982–89. The fact that coal tar had stopped being used in these applications would have resulted in greatly reduced exposures to PAHs. However, during tear-off in roofing and recycling in paving, exposure to coal tar can still occur.

The lowest tier of exposure to benzo[*a*]pyrene (at or below 1 $\mu g/m^3$) included workers in coal-tar distillation, coal liquefaction, power plants, chimney sweeping and wood impregnation (Figure 1.3). The pattern of urinary 1-hydroxypyrene among these industrial groups did not parallel airborne levels of benzo[*a*]pyrene; workers in coal liquefaction and wood impregnation with coal-tar creosote had levels of 1-hydroxypyrene of 10–100 μmol/mol. This may reflect the poor correspondence between exposure to PAHs by inhalation and urinary excretion of 1-hydroxypyrene, since dermal absorption may be a very significant route of exposure to PAHs. For wood impregnation, the high urinary levels of 1-hydroxypyrene may reflect a predominance of low-molecular-weight PAHs in this environment.

Substantial differences in exposures have been reported within industries and time periods. In Figure 1.1, more detailed temporal and geographical information is given. Differences between job/task within industries are shown in the tables.

(n) PAH profiles

A comparison of industries by composition of the PAH profiles is to some extent limited by the differences in the sets of PAH compounds that were measured in the individual studies. The set of PAHs measured also changed over time, as sampling and analytical methods evolved, making it possible to identify and quantify more components of these complex mixtures. Since the mid-1980s, benzo[*a*]pyrene and pyrene were measured more frequently. The heavier PAHs (heavier than coronene) have not been measured in these industries to date, largely because of the lack of SRMs in the past and difficulties in analytical techniques.

Studies that reported at least six individual PAHs are presented in Table 1.14. Some patterns are apparent in the profiles by industry, which are strongly influenced by the source material in the particular process and the temperature at which the operations were conducted. For example, coke ovens operate at high temperatures (up to 1300 °C), at which extensive pyrolysis of coal constituents occurs very readily. As a result, the studies showed higher levels of exposure over the entire range of PAH compounds and the greatest prevalence of high-molecular-weight PAH compounds (molecular weight > 252). At the other extreme, wood impregnation with creosote reflects a lower-temperature process that uses a distillate fraction of coal tar as a preservative. The resulting PAH exposure profile for this process is rich in low-molecular-weight PAH compounds (such

as naphthalene, acenaphthylene, acenaphthene and fluorene), while very low to non-detectable levels of PAHs with a molecular weight of > 216 (benzo[*a*]fluorene) are reported. Some industries present intermediate profiles; for example, carbon electrode manufacture uses coal-tar pitch as the process material but does not raise the pitch to temperatures that promote the volatilization of the higher-molecular-weight compounds (molecular weight, 252 and above). The profiles for these operations reflect this, as the highest exposures in these industries are to pyrene (molecular weight, 202) and lower-molecular-weight PAHs. No profiles were available for the aluminum pre-bake process, coal gasification or chimney sweeps.

1.2.3 *Dietary exposure to PAHs*

The general population can be exposed to PAHs via grilled and smoked foods, foods grown in polluted environments, polluted air, drinking-water and tobacco smoking. Due to the ubiquitous nature of PAHs in foods, the estimation of their dietary consumption is challenging; thus, etiological studies of cancer must rely on exposure measures of dietary intake from questionnaires as well as on biological markers, such as urinary metabolites (Roth *et al*., 2001; Strickland *et al*., 2002) and DNA adducts (see Ramesh *et al*., 2004 for a comprehensive review of dietary PAHs).

Two general approaches have been used to measure PAH levels in the diet. The first approach is to measure a large number of PAHs (see Guillén, 1994; Phillips, 1999), while the second method measures benzo[*a*]pyrene as a surrogate marker for all PAHs (Kazerouni *et al*., 2001). The first approach provides a more realistic notion of the total burden from diet but individual PAHs in any particular foods may vary widely. Measurement of many of the PAHs was previously found to be difficult, and many studies have measured only benzo[*a*]pyrene as a marker of total PAHs in foods because of its carcinogenic potency in experimental animals.

To verify that benzo[*a*]pyrene is a representative marker of total PAHs in food, Kazerouni *et al*. (2001) calculated the correlation coefficients (*r*) between values of benzo[*a*]pyrene and the concentrations of individual and total PAHs in a selection of food items. Selected samples from each major food group were analysed for acenaphthene, anthracene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]-perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, fluoranthene, fluorene, indeno-[1,2,3-*cd*]pyrene, naphthalene, phenanthrene and pyrene by HPLC. The correlation coefficient between concentrations of total PAHs (the sum of all those measured in this study) and benzo[*a*]pyrene was 0.87 (*p*-value = 0.0001). The correlation value between PAHs that are known to have carcinogenic potential in experimental animals (i.e. benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene and indeno[1,2,3-*cd*]pyrene) and benzo[*a*]pyrene was 0.98 (*p*-value = 0.0001).

A large body of international data on PAHs in food has recently been reviewed (http://epic-spain.com/libro.html) (Jakszyn *et al*., 2004). The data consist of information

Table 1.14. PAH profiles of the industries reviewed in this monograph[a]

No. of rings	PAH	MW	Coke ovens					Coke production		Coal-tar distillation	
			(top)					High temp.	Low temp.	High temp.	Low temp.
	Reference:		**A**	**B**	**C**	**D**	**E**	**F**	**F**	**F**	**F**
2/3	Naphthalene	128	5				5	4	4	5	3
	Acenaphthylene	152		4		3	5	3	3	2	1
	Acenaphthene	154		3		3	0	3	2	4	3
	Fluorene	166	5	4		3	4	3	3	3	2
	Anthracene	178	5	4	4	2	3	2	3	2	1
	Phenanthrene	178	5	4	5	3	4	3	3	4	2
4	Fluoranthene	202	5	4	4	3	4	3	2	2	1
	Pyrene	202		4	4	3	3	2	2	2	1
	Benzo[*a*]fluorene	216	5	3	3						
	Benzo[*ghi*]fluoranthene	226		2	3						
	Cyclopenta[*c,d*]pyrene	226		3	3						
	Benzo[*a*]anthracene	228	5	3	4	2	3	2	2	1	0
	Benzo[*c*]phenanthrene	228		2	3						
	Chrysene	228	5	3	4	2	3	2	2	1	0
5	Benzo[*a*]pyrene	252	4	3	4	3	3	2	2	2	0
	Benzo[*b*]fluoranthene	252	4	2	3	4	3	2	2	1	0
	Benzo[*e*]pyrene	252		3	4	3					
	Benzo[*j*]fluoranthene	252		2	3						
	Benzo[*k*]fluoranthene	252	4	2	2	3	3	2	2	1	0
	Perylene	252		2	3						
6	Anthanthrene	276		3	3			2	0	0	0
	Benzo[*g,h,i*]perylene	276		3	3	3	3	2	1	1	0
	Indeno[1,2,3-*c,d*]pyrene	276		3	3	4	3	2	0	0	0
	Benzo[*b*]chrysene	278									
	Dibenzo[*a,c*]anthracene	278		2		2					
	Dibenzo[*a,h*]anthracene	278			3		1	2	0	0	0
	Picene	278									
	Coronene	300		3	3						

[a] No profiles were available to the Working Group for coal liquefaction, coal gasification, aluminium production by the prebake process or chimney sweeps.

MW, molecular weight; PAH, polycyclic aromatic hydrocarbon; temp., temperature

A, Andersson *et al.* (1983); **B**, Haughen *et al.* (1986); **C**, Grimmer *et al.* (1993); **D**, Winker *et al.* (1996); **E**, Strunk *et al.* (2002); **F**, Price *et al.* (2000); **G**, Reed (1982); **H**, Zey (1983); **I**, Behrens & Liss (1984); **J**, Wolff *et al.* (1989); **K**, Darby *et al.* (1986); **L**, Knecht & Woitowitz (1989); **M**, Heikkilä *et al.* (1987); **N**, Tjoe Ny *et al.* (1993); **O**, Carstensen *et al.* (1999); **P**, Petry *et al.* (1996)

Categorised levels of exposure to selected PAHs: **0**: < detection limit; **1**: < 0.1 μg/m^3; **2**: 0.1–2 μg/m^3; **3**: 2–10 μg/m^3; **4**: 10–50 μg/m^3; **5**: > 50 μg/m^3

Table 1.14 (contd)

Coal-tar roofing				Paving		Creosote		Aluminium production			Carbon electrode manufacturing	Thermo-electric power plants
		Tear off only		Coal tar	Tar bitumen blend			Søderberg		Anode manufac-turing		
G	**H**	**I**	**J**	**K**	**L**	**M**	**F**	**N**	**O**	**P**	**F**	**F**
						5	5	5		3	4	2
							2	3		2	2	1
	0					5	5	4		4	3	1
	0		3			2	4	4		3	3	1
3	0		2				2	3	1	2	2	1
3	2	5				3	4	4	2	3	3	1
4	3	5			5		2	5	2	3	2	1
4	3	5	3		5	2	2	3	2	3	2	1
					4	1			2			
				3	3					1		
3	2	5	2	3	4		1	3	2	2	1	1
	0				1					1		
3	2	5		3	4	2	1	4	3	2	2	1
3	2	5	2	3	3	1	2	4	2	2	1	1
	3		2		2		0	4	3	2	1	1
	0	5		3	3	1			2	2		
		5			2				2	2		
			2		2	1	0	4	2	2	1	1
					1				2	2		
					1		0			2	0	1
		5	2		1	1	0	3	2	2	1	0
				2	1		0	4	2	2	1	0
										2		
										2		
				2	1	1	0	4		2	0	0
									2			
					1					1		

on food type, methods of cooking and preservation, benzo[*a*]pyrene and total PAH content, analytical technique, sampling method, authors and year of the study and the country where the food was obtained (see Table 1.15 as an example).

PAHs can contaminate foods by two major routes. They are generated through pyrolysis during the grilling/barbecueing of meat products when fat drips from the meat onto hot coals or a heated surface and produces smoke that coats the food with the compounds (Larsson *et al.*, 1983; Lijinsky, 1991). They are also introduced by certain methods of preserving meat and other food products. The other major source of PAH contamination of food is from environmental sources such as petroleum or coal-tar combustion by-products (Lijinsky, 1991).

Grilled foods of any type that are exposed to smoke are likely to have a high content of PAHs. The benzo[*a*]pyrene content of meat items depends on the method, temperature and duration of cooking (Larsson *et al*., 1983; Larsson, 1986; Lodovici *et al*., 1995).

Smoke curing of foods is commonly used for preservation and to add flavour. Although the methods differ, they all expose the food to potentially carcinogenic compounds such as PAHs. The level of contamination with PAHs can vary substantially depending on the procedure used in the smoking (Gomaa *et al*., 1993; García Falcón *et al*., 1999; Anastasio *et al*., 2004). Currently, traditional smoking techniques are being partially replaced by liquid smoke flavour. The advantage is a more uniform flavour, which is easier to reproduce and allows greater control of the levels of toxic compounds. However, the level of benzo[*a*]pyrene in liquid smoke flavour samples can vary substantially, from not detected to 336.6 μg/kg (Gomaa *et al*., 1993; Yabiku *et al*., 1993).

Unprocessed foods such as vegetables, fruits, vegetable oils, dairy products and seafood can be contaminated with PAHs from the atmosphere, and by deposition and uptake from soil, water and sediment. The higher content in green leafy vegetables such as kale and collards compared with other vegetables is probably due to greater surface contact with ambient air and consequently deposition of airborne contaminants (Kazerouni *et al*., 2001; Ramesh *et al*., 2004). Vegetables can be highly contaminated when grown close to highways or on contaminated soils. As a result of stricter air emission regulations and improved environmental conditions in several developed countries, levels of PAHs in vegetables and fruits are now lower than those reported in the 1970s. Oils from different vegetable sources, such as rapeseed, olive oil, sunflower, soya bean and maize, contain considerable levels of PAHs (Ramesh *et al*., 2004), which are probably formed during processing rather than as a result of airborne contamination. Similarly, PAH levels are low in cereals and beans, but drying techniques used for preservation, such as combustion-gas heating and smoking, increases concentrations of PAHs (Roth *et al.*, 1998; Ramesh *et al.*, 2004).

In addition to grilling and smoking processes, the accumulation of PAHs in foods of animal origin, especially livestock, is also due to the consumption of contaminated feed. Consumption of seafoods, in particular bottom-feeding shellfish and finfish, may contribute considerably to the amount of PAHs in the diet (Ramesh *et al.*, 2004). [High trophic-

Table 1.15. Examples of PAH concentrations in selected fruit and vegetables

Food	Cooking method	Preservation method	Mean BaP μ/kg	Total PAH	Analytical method	Reference	Country of study
Fruit							
Apple	Raw	Fresh	0.10		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Apple	Raw	NA	0.53	8.27	HPLC	Lodovici *et al*. (1995)	Italy
Apple (peeled)	Raw	NA	0.06	2.35	HPLC	Lodovici *et al*. (1995)	Italy
Banana	Raw	Fresh	0.16		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Fruits (citrus)	Raw	NA	0.03	1.67	HPLC	Lodovici *et al*. (1995)	Italy
Grapefruit	Raw	Fresh	0.02		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Orange	Raw	Fresh	0.16		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Peach	Raw	Fresh	0.17		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Peanuts	Toasted	NA	0.01		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Strawberry	Raw	Fresh	0.01		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Walnuts	Toasted	NA	0.03		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Vegetables							
Beans (greens)	NA	Canned	0.14		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Beets (greens)	Raw	NA	0.096	14.0	HPLC	Lodovici *et al*. (1995)	Italy
Broccoli	Raw	Fresh/frozen	0.17		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Carrot	Raw	Fresh/frozen	0.15		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Cauliflower	Raw	Fresh/frozen	0.12		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Cauliflower	Raw	NA	0.006	2.79	HPLC	Lodovici *et al*. (1995)	Italy
Cole slaw	Raw	NA	0.02		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Collars greens	Raw	Fresh/frozen	0.48		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Corn	NA	Canned	0.17		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Corn	NA	NA	0.022	0.85	HPLC	Lodovici *et al*. (1995)	Italy
Greens (mixed)	NA	Frozen	0.14		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Kale	Raw	Fresh/frozen	0.47		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Kale	NA	Frozen	0.15		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Lettuce	Raw	NA	0.007	2.61	HPLC	Lodovici *et al*. (1995)	Italy

Table 1.15 (Contd)

Food	Cooking method	Preservation method	Mean BaP μ/kg	Total PAH	Analytical method	Reference	Country of study
Peas	NA	Canned	0.09		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Spinach	NA	Frozen/fresh	0.10		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Spinach	NA	Frozen	0.12		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Squash	Raw	NA	0.45	8.90	HPLC	Lodovici *et al*. (1995)	Italy
Tomato	NA	Fresh/canned	0.19		HPLC + TLC	Kazerouni *et al*. (2001)	USA
Tomato	Raw	NA	0.003	0.64	HPLC	Lodovici *et al*. (1995)	Italy

BaP; benzo[*a*]pyrene; HPLC, high-performance liquid chromatography;NA, not available; PAH, polycyclic aromatic hydrocarbon; TLC, thin-layer chromatography
Adapted from http://epic-Spain.com/libro.html

level consumers, such as humans, do not bioaccumulate due to a higher capacity to metabolize PAHs.]

A comparison of daily intakes of PAHs across studies requires the consideration of various issues, such as selection of food items, methodology used for extraction, identification and quantification. Estimated daily dietary intake of PAHs in selected countries has been summarized (Ramesh *et al*., 2004) (Table 1.16).

Table 1.16. Dietary intakes of PAHs in various countries

Country	Intake (μg/person/day)	Reference
USA	0.16–1.6	Santodonato *et al.* (1981)
	0.04–0.06[a]	Kazerouni *et al.* (2001)
	0.12–2.8[a]	Hattemer-Frey & Travis (1991)
United Kingdom	3.7	Dennis *et al.* (1991)
Germany	0.02–0.04	State Committee for Air Pollution Control (1992)
Austria	3.4 (0.7–15.6)[b]	Pfannhauser (1991)
Italy	3.0	Lodovici *et al.* (1995)
Spain	6.3–8.4	Falćo *et al.* (2003)
Greece	1.6–4.5	Voutsa & Samara (1998)
Netherlands	5–17	de Vos *et al*. (1990)
Sweden	0.08	Beckman Sundh *et al.* (1998)

PAH, polycyclic aromatic hydrocarbon
From Ramesh *et al.* (2004)
[a] Values reported were for benzo[*a*]pyrene concentrations only.
[b] Median and range for the sum of 16 PAHs

The FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) recently selected a representative mean intake of 4 ng/kg bw benzo[*a*]pyrene per day and a high-level intake of 10 ng/kg bw per day for their evaluation of PAHs (JECFA, 2005). It also noted that some population groups who regularly consume food cooked over open fires or barbecues, or people who habitually consume foods from areas of higher contamination, may have higher intakes.

In spite of the differences observed in international studies, it is clear that dietary intake is the major route of exposure to PAHs for a large proportion of the nonsmoking, non-occupationally exposed population (Ramesh *et al*., 2004). In a study in the USA, Phillips (1999) estimated that, for nonsmokers who are not exposed occupationally, about 70% of exposure to PAHs typically comes from dietary sources. For a nonsmoking man aged between 19 and 50 years, total PAH intake was estimated at 3.12 μg per day, of which 96.2% was from food, 1.6% from air, 0.2% from water and 1.9% from soil

(Menzie *et al*., 1992). However, in a study in which the relative contributions of dietary and inhalation exposures were compared directly, weekly intakes of benzo[*a*]pyrene by inhalation (mainly from indoor air) were greater than those from the diet in half of the subjects mostly because the range of dietary exposure to benzo[*a*]pyrene (1–500 ng/day) was wider than that of inhalation exposure (10–50 ng/day) (Lioy *et al*., 1988; Lioy & Greenberg, 1990).

In a study conducted in the USA on the distribution of dietary intake of benzo[*a*]pyrene in 228 subjects, approximatively 31% of the subjects had an intake in the range of 40.1–60 ng/day (Kazerouni *et al*., 2001).

Figure 1.4 presents the percentage contribution of various food groups to the mean daily intake of benzo[*a*]pyrene. The dietary information was collected using a food-frequency questionnaire that included detailed questions on the cooking methods of meat combined with a newly developed benzo[*a*]pyrene database (Sinha *et al*., 2005). Bread and other cereal products (29%) followed by grilled/barbecued meat (21%) contributed most to the total daily intake of benzo[*a*]pyrene. In contrast, fat, sweets and dairy food groups contributed the least. Taking into account the concentration of benzo[*a*]pyrene in individual items in the cereal, fruit and vegetable food groups, the large contribution of each group is due to the size of the portion consumed. In terms of the percentage contribution to total dietary intake of benzo[*a*]pyrene, this result is consistent with the study by Lodovici *et al*. (1995) with regard to cereal products, vegetables and fruit groups, but not to grilled/barbecued meats. The studies by Larsson (1986) and by de Vos *et al.* (1990) reported a much higher contribution from fat/oil and from sugar and sweets, respectively.

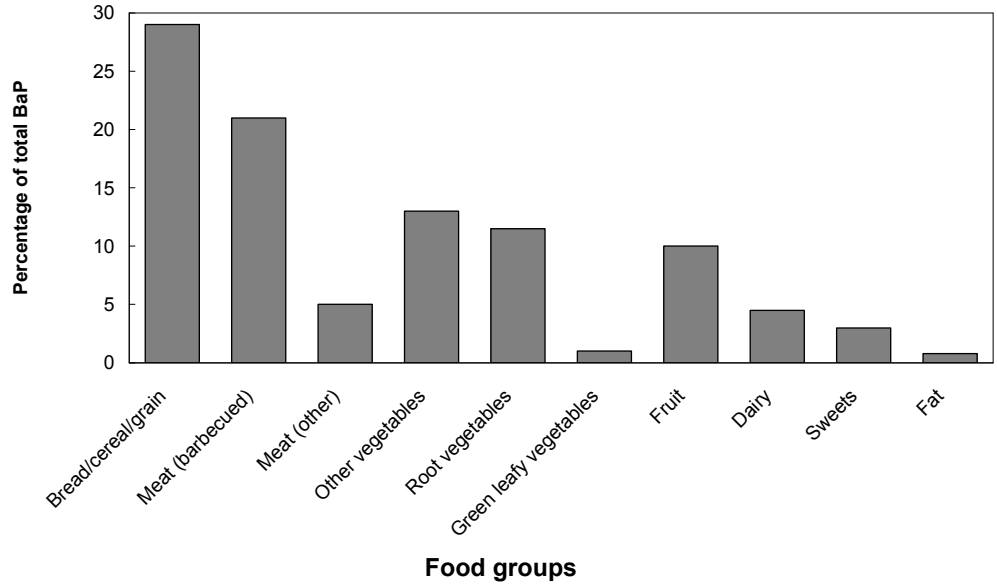
A database has been developed that can be used in conjunction with a food-frequency questionnaire to estimate the intake of PAHs (Kazerouni *et al*., 2001; Sinha *et al*., 2005). Multiple meat samples were cooked by different methods and to varying degrees, and additional foods were sampled and measured for benzo[*a*]pyrene as a marker for PAHs. The software application, CHARRED, which was developed to compute PAH values from meat intake, is now available (www.charred.cancer.gov).

1.3 Regulations and guidelines

Several countries have set occupational exposure limits for coal-tar pitch volatiles (CTPVs) or particulate PAHs. The values are typically based on the benzene- or cyclohexane-soluble matter of the particulates present in air. Some countries have exposure limits for individual PAHs, notably for benzo[*a*]pyrene.

For CTPVs, Belgium, Canada (Alberta, British Columbia, Ontario [short-term exposure limit (STEL)], Quebec), China, Malaysia, New Zealand, Spain and the USA have set the occupational exposure limit at a 0.2-mg/m^3 time-weighted average (TWA) for the benzene-soluble fraction (which includes acridine, anthracene, benzo[*a*]pyrene, chrysene, phenanthrene and pyrene). This value has also been suggested as the threshold limit value (TLV) by the American Conference of Governmental Industrial Hygienists (ACGIH) in the USA. China has set a STEL of 0.6 mg/m^3 for this parameter. Also in the

Figure 1.4. Percentage of total daily benzo[*a*]pyrene (BaP) intake from selected food groups among 228 subjects in Maryland, USA[a]



From Kazerouni *et al*. (2001)
[a] Data read from graph

USA, the National Institute of Occupational Safety and Hygiene (NIOSH) has suggested a recommended exposure limit of a 0.1-mg/m^3 TWA for the cyclohexane-extractable fraction of CTPVs; this value also is applied in Hong Kong, Ireland and South Africa. The Netherlands report a value of 0.2 mg/m^3, but without a designation of soluble fraction. Mexico has set a limit for CTPVs at 0.002 mg/m^3, without a designation of soluble fraction.

Mexico has set the limit for PAH particulates at a 0.2-mg/m^3 TWA and a 0.3-mg/m^3 STEL; the corresponding values for Canada (Alberta) are a 0.2-mg/m^3 TWA and a 0.6-mg/m^3 STEL as benzene solubles; Norway has set a limit of 0.04 mg/m^3 for total PAHs (ACGIH, 2005; OSHA, 2005a).

In Germany, benzo[*a*]pyrene is the indicator for external exposure to PAHs. The German technical guiding concentration for benzo[*a*]pyrene in the air is 0.005 mg/m^3 for the production, loading and unloading of pencil pitch and in the area near the ovens in coking plants and 0.002 mg/m^3 for all other workplaces (Bundesministerium für Arbeit und Sozialordnung, 2000; ACGIH, 2005). The Czech Republic has set the limit for benzo[*a*]pyrene at a 0.005-mg/m^3 TWA (with a skin irritation notation) and a 0.025-mg/m^3 STEL; Canada (Quebec) has set a limit of 0.005 mg/m^3; Poland, Sweden and Switzerland have set a limit of 0.002 mg/m^3; and Finland has set a limit of 0.01 mg/m^3 (ACGIH, 2005; Suva, 2005).

The US Occupational Safety and Hygiene Administration (OSHA) has established a permissible exposure limit for workers exposed to coke oven emissions; the concentration cannot exceed 0.15 mg CTPV/m^3 of air, averaged over any 8-h period (ACGIH, 2005; OSHA, 2005b,c,d). China has set the limit for coke-oven emissions at a 0.1-mg/m^3 TWA and a 0.3-mg/m^3 STEL (ACGIH, 2005).

While not determining an actual biological exposure index value, the US ACGIH® recommends that a benchmark value of 1 μg/L 1-hydroxypyrene/L (0.49 μmol/mol creatinine) in urine be considered as a postshift level that indicates occupational exposure to PAHs. The benchmark level is based on exposures to PAHs that result in urinary levels of 1-hydroxypyrene that are greater than those of at least 99% of the population with no occupational or significant environmental exposure. The biological exposure index is applicable to all exposures that involve PAHs, and is designed to assist industrial hygienists and other occupational health professionals to control occupational exposure to PAHs from all routes (ACGIH, 2005).

On the basis of the measured levels of 1-hydroxypyrene in the urine of workers exposed in the various industries surveyed, the Health and Safety Executive adopted a biological monitoring guidance value of 4 μmol/mol creatinine (Health and Safety Executive, 2005). This value represents the 90th percentile of measurements taken from industries that are deemed to have good controls, which includes the CTPV industries but excludes data from one timber impregnation site. This value was also approved by WATCH (Pedersen, 2003).

PAH mixtures are very variable qualitatively and quantitatively. The relative amounts of pyrene and benzo[*a*]pyrene in the applicable exposure matrix (air and/or dermal/surface contamination) should be determined; the benchmark level of 1 μg/L can then be adjusted to take into account different pyrene:benzo[*a*]pyrene ratios in the specific exposure matrix. The adjustment should be based on data collected from air, surface or skin samples, as follows:

$$\text{adjusted recommended level} = 1\ \mu\text{g 1-hydroxypyrene/L} \times \left(\frac{(\text{pyrene measured/benzo[}a\text{]pyrene measured})}{2.5} \right)$$

The ratio of 2.5 (pyrene:benzo[*a*]pyrene) was selected as the default ratio to provide a realistic, although in some cases conservative, estimate of the proportion of carcinogens in the mixture. Some reports of the relative amounts of pyrene:benzo[*a*]pyrene are summarized in Table 1.17 (ACGIH, 2005).

The 1993 WHO *Guidelines for Drinking-water Quality* concluded that there were insufficient data available to derive drinking-water guidelines for PAHs other than benzo[*a*]pyrene, for which the guideline value was calculated to be 0.7 μg/L and remains the current guideline value (WHO, 2004).

The European Union has set the drinking-water guideline for PAHs at 0.1 μg/L for the sum of four compounds (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene

Table 1.17. Reported ratios of pyrene:benzo[*a*]pyrene

Mixture	Pyrene: benzo[*a*]-pyrene ratio	Reference
Aluminum workers: Anode factory (air)	3.7	van Schooten *et al.* (1995)
Aluminum workers: Bake oven (air)	4.3	van Schooten *et al.* (1995)
Aluminum workers: Electrolysis (air)	4.0	van Schooten *et al.* (1995)
Aluminum workers: Foundry (air)	2.0	van Schooten *et al.* (1995)
Aluminum workers: Pot relining (air)	30.8	van Schooten *et al.* (1995)
Bitumens (bulk) average of 8 samples	1.5	IARC (1985)
Chisellers of creosoted rails (air)	1.3	Heikkila *et al.* (1995)
Coal tar asphalt pavers (air)	9	Jongeneelen *et al.* (1988b)
Coal tar asphalt pavers (bulk)	4.0	Jongeneelen *et al.* (1988b)
Coal tar asphalt pavers (hand skin)	6	Jongeneelen *et al.* (1988b)
Coal tar asphalt pavers (wrist pad contamination)	8	Jongeneelen *et al.* (1988b)
Coke oven (air samples)	2.4	IARC (1985)
Creosote	7.2	Elovaara *et al.* (1995)
Creosote (bulk)	38	Elovaara *et al.* (1995)
Electrode manufacturing high exposure (air)	2.0	van Delft *et al.* (1998)
Electrode manufacturing intermediate exposure (air)	1.4	van Delft *et al.* (1998)
Electrode paste plant: all workers (air)	1.9	Bentsen *et al.* (1998)
Electrode paste plant: mixing (air)	11.5	Bentsen *et al.* (1998)
Electrode paste plant: mold filling (air)	2.5	Bentsen *et al.* (1998)
Electrode paste plant: truck drivers (air)	1.3	Bentsen *et al.* (1998)
Graphite electrode production (air)	4.0	IARC (1985)
Medicinal coal tar (bulk)	1.2	Godschalk *et al.* (1998)
Medicinal coal tar (bulk)	1.1	VanRooij *et al.* (1993c)
Protective paints (bulk)	3.6	IARC (1985)
Range of bulk samples in petrochemical industries	0.4–220	Boogaard & van Sittert (1994)
Road tars (bulk) average of 2 samples	3.4	IARC (1985)
Roofers (air)	3.2	Wolff *et al.* (1989)
Roofers (skin wipes)	2.1	Wolff *et al.* (1989)
Shale oils (bulk) average of 5 samples	3.8	IARC (1985)
Soots (bulk) average of 3 sites hardwood fireplace	2.2	IARC (1985)
Tobacco smoke	5	Guérin (1980)

From ACGIH (2005)

and indeno[1,2,3-*cd*]pyrene), and at 0.01 μg/L for benzo[*a*]pyrene (European Union, 1998).

Canada has set a guideline for the maximum acceptable concentration of benzo[*a*]pyrene in drinking-water at 0.01 μg/L (Health Canada, 1986, 2004).

The Environmental Protection Agency of the USA (2004) has set a drinking-water standard (maximum contaminant level) for benzo[*a*]pyrene at 0.2 μg/L.

In the USA, coal tar may be used as an active ingredient in external drug products at a concentration of 0.5–5% for control of dandruff, seborrheic dermatitis and psoriasis (Food

and Drug Administration, 2005). When a coal-tar solution, derivative or fraction is used as the source of the coal tar, the labelling must specify the identity and concentration of the source of the coal tar used and the concentration of coal tar present in the final product.

1.4 References

ACGIH (2005) Documentation of the TLVs® and BEIs® with Other Worldwide Occupational Exposure Values CD-ROM – 2005, Cincinnati, OH, American Conference of Governmental Industrial Hygienists

Anastasio, A., Mercogliano, R., Vollano, L., Pepe, T. & Cortesi, M.L. (2004) Levels of benzo[a]pyrene (BaP) in 'mozzarella di bufala campana' cheese smoked according to different procedures. J. agric. Food Chem., 52, 4452–4455

Andersson, K., Levin, J.-O. & Nilsson, C.-A. (1983) Sampling and analysis of particulate and gaseous polycyclic aromatic hydrocarbons from coal tar sources in the working environment. Chemopshere, 12, 197–207

Angerer, J., Mannschreck, C. & Gündel, J. (1997) Occupational exposure to polycyclic aromatic hydrocarbons in a graphite-electrode producing plant: Biological monitoring of 1-hydroxypyrene and monohydroxylated metabolites of phenanthrene. Int. Arch. occup. environ. Health, **69**, 323–331

Arey, J. & Atkinson, R. (2003) Photochemical reactions of PAHs in the atmosphere. In: Douben, P.E.T., ed., PAHs: An Ecological Perspective, New York, John Wiley & Sons, pp. 47–63

Assennato, G., Ferri, G.M., Foà, V., Strickland, P., Poirier, M., Pozzoli, L. & Cottica, D. (1993) Correlation between PAH airborne concentration and PAH–DNA adducts levels in coke-oven workers. Int. Arch. occup. environ. Health, **65**, S143–S145

ATSDR (2002) Toxicological Profile for Wood Creosote, Coal Tar Creosote, Coal Tar, Coal Tar Pitch, and Coal Tar Pitch Volatiles, Atlanta US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry

ATCO Power (2008) Coal-fired Thermal Generating Plants. Available at: http://www.atcopower.com/technology/coal_fired.htm

Bagchi, N.J. & Zimmerman, R.E. (1980) An industrial hygiene evaluation of chimney sweeping. Am. ind. Hyg. Assoc. J., **41**, 297–299

Beckman Sundh, O., Thuvander, A. & Andersson, C. (1998) Preview of PAHs in Food: Potential Health Effects and Contents in Food, (Report 8), Uppsala, Swedish National Food Administration

Behrens, V. & Liss, G. (1984), Fisher Body Plant, Roofing Site, Hamilton, Ohio (Health Hazard Evaluation Report, No. HETA-84-062-1552), Cincinnati, OH, National Institute of Occupational Safety and Health

Benke, G., Abramson, M. & Sim, M. (1998) Exposures in the alumina and primary aluminium industry: An historical review. Ann. occup. Hyg., **42**, 173–189

Benner, B.A., Jr (1998) Summarizing the effectiveness of supercritical fluid extraction of polycyclic aromatic hydrocarbons from natural matrix environmental samples. Anal. Chem., **70**, 4594–4601

Bentsen, R.K., Notø, H., Halgard, K. & Øvrebø, S. (1998) The effect of dust-protective respirator mask and the relevance of work category on urinary 1-hydroxypyrene concentration in PAH exposed electrode paste plant workers. Ann. occup. Hyg., **42**, 135–144

Bentsen-Farmen, R.K., Botnen, I.V., Notø, H., Jacob, J. & Øvrebø, S. (1999) Detection of polycyclic aromatic hydrocarbon metabolites by high-pressure liquid chromatography after purification on immunoaffinity columns in urine from occupationally exposed workers. Int. Arch. occup. environ. Health, **72**, 161–168

Betts, W.D. (1997) Tar and pitch. In: Kirk-Othmer Encyclopedia of Chemical Technology, 5th Ed., New York, John Wiley & Sons [online edition]

Bjorseth, A., ed. (1983) Handbook of Polycyclic Aromatic Hydrocarbons, New York, Marcel Dekker

Bjorseth, A. & Ramdahl, T., eds (1985) Handbook of Polycyclic Aromatic Hydrocarbons, Vol. 2, Emission Sources and Recent Progress in Analytical Chemistry, New York, Marcel Dekker

Boden, A.R. & Reiner, E.J. (2004) Development of an isotope-dilution gas chromatographic–mass spectrometric method for the analysis of polycyclic aromatic compounds in environmental matrices. Polycyc. aromat. Compds, **24**, 309–323

Bolt, H.M. & Golka, K. (1993) Cases of lung cancer and tar-related skin changes in an aluminium reduction plant. Med. Lav., **84**, 178–181

Boogaard, P.J. & van Sittert, N.J. (1994) Exposure to polycyclic aromatic hydrocarbons in petrochemical industries by measurement of urinary 1-hydroxypyrene. Occup. environ. Med., **51**, 250–258

Boström, C.E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Rannug, A., Törnqvist, M., Victorin, K. & Westerholm, R. (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect., **110** (Suppl. 3), 451–488

Brescia, G., Celotti, L., Clonfero, E., Neumann, H.G., Forni, A., Foà, V., Pisoni, M., Ferri, G.M. & Assennato, G. (1999) The influence of cytochrome P450 1A1 and glutathione S-transferase M1 genotypes on biomarker levels in coke-oven workers. Arch. Toxicol., **73**, 431–439

Buchet, J.P., Gennart, J.P., Mercado-Calderon, F., Delavignette, J.P., Cupers, L. & Lauwerys, R. (1992) Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: Assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. Br. J. ind. Med., **49**, 761–768

Bundesministerium für Arbeit und Sozialordung (2000) [Technical Rules for Hazardous Substances: Air Limit Values at the workplace 'Air Limit Values'] (TRGS 900), Berlin, Bundesarbeitsblatt Ausgabe (In German)

Burstyn, I., Boffetta, P., Kauppinen, T., Heikkilä, P., Svane, O., Partanen, T., Stücker, I., Frentzel-Beyme, R., Ahrens, W., Merzenich, H., Heederik, D., Hooiveld, M., Langard, S., Randem, B.G., Järvholm, B., Bergdahl, I., Shaham, J., Ribak, J. & Kromhout, H. (2003) Estimating exposures in the asphalt industry for an international epidemiological cohort study of cancer risk. Am. J. ind. Med., **43**, 3–17

Carstensen, U., Yang, K., Levin, J.O., Østman, C., Nilsson, T., Hemminki, K. & Hagmar, L. (1999) Genotoxic exposures of potroom workers. Scand. J. Work Environ. Health, **25**, 24–32

CDC (2005) Third National Report on Human Exposure to Environmental Chemicals, Atlanta, GA, Department of Health and Human Services, Center for Disease Control and Prevention, National Center for Environmental Health

Chen, M.L., Mao, I.F., Wu, M.T., Chen, J.R., Ho, C.K., Smith, T.J., Wypij, D. & Christiani, D.C. (1999) Assessment of coke oven emissions exposure among coking workers. Am. ind. Hyg. Assoc. J., **60**, 105–110

Chuang, J.C., Hannan, S.W. & Wilson, N.K. (1987) Field comparison of polyurethane foam and XAD-2 resin for air sampling for polynuclear aromatic hydrocarbons. Environ. Sci. Technol., **21**, 798–804

Clonfero, E., Granella, M., Marchioro, M., Barra, E.L., Nardini, B., Ferri, G. & Foà, V. (1995) Urinary excretion of mutagens in coke oven workers. Carcinogenesis, **16**, 547–554

Colmsjö, A. (1998) Concentration and extraction of PAHs from environmental samples. In: Neilson, A.H., ed., The Handbook of Environmental Chemistry, Vol. 3, PAHs and Related Compounds, Part I, Chemistry, Berlin, Springer-Verlag, pp. 55–76

Coutant, R.W., Brown, L., Chuang J.C., Riggin, R.M. & Lewis R.G. (1988) Phase distribution and artifact formation in ambient air sampling for polynuclear aromatic hydrocarbons. Atmos. Environ., **22**, 403–409

Crelling, J.C., Hagemann, H.W., Sauter, D.H., Ramani, R.V., Vogt, W., Leininger, D., Krzack, S., Meyer, B., Orywal, F., Reimert, R., Bonn, B., Bertmann, U., Klose, W. & Gatzka, W. (2005) Coal. In: Ullmann's Encyclopedia of Industrial Chemistry, 5th Ed., Wiley-VCH Verlag GmbH & Co. KGaA [online version]

Criscione, J.M., Reddy, R.L., Fulgenzi, C.F., Page, D.J., Fisher, F.F., Dzermejko, A.J. & Hedge, J.B. (1992) Graphite, application of artificial. In: Kirk-Othmer Encyclopedia of Chemical Technology, 5th Ed., New York, John Wiley & Sons [online edition]

Darby, F.W., Willis, A.F. & Winchester, R.V. (1986) Occupational health hazards from road construction and sealing work. Ann. occup. Hyg., **30**, 445–454

van Delft, J.H.M., Steenwinkel, M.J.S.T., van Asten, J.G., van Es, J., Kraak, A. & Baan, R.A. (1998) Monitoring of occupational exposure to polycyclic aromatic hydrocarbons in a carbon-electrode manufacturing plant. Ann. occup. Hyg., **42**, 105–114

van Delft, J.H.M., Steenwinkel, M.J.S.T., van Asten, J.G., de Vogel, N., Bruijntjes-Rozier, T.C.D.M., Schouten, T., Cramers, P., Maas, L., van Herwijnen, M.H., van Schooten, F.J. & Hopmans, P.M.J. (2001) Biological monitoring the exposure to polycyclic aromatic hydrocarbons of coke oven workers in relation to smoking and genetic polymorphisms for GSTM1 and GSTT1. Ann. occup. Hyg., **45**, 395–408

Dennis, M.J., Massey, R.C., Cripps, G., Venn, I., Howarth, N. & Lee, G. (1991) Factors affecting the polycyclic aromatic hydrocarbon content of cereals, fats and other food products. Food Addit. Contam., **8**, 517–530

Ding, Y.S., Trommel, J.S., Yan, X.J., Ashley, D. & Watson, C.H. (2005) Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from domestic cigarettes. Environ. Sci. Technol., **39**, 471–478

Dor, F., Dab, W., Empereur-Bissonnet, P. & Zmirou, D. (1999) Validity of biomarkers in environmental health studies: The case of PAHs and benzene. Crit. Rev. Toxicol., **29**, 129–168

Dufresne, A., Loosereewanich, P., Armstrong, B., Thériault, G. & Bégin, R. (1996) Inorganic particles in the lungs of five aluminum smelter workers with pleuro-pulmonary cancer. Am. ind. Hyg. Assoc. J., **57**, 370–375

Elovaara, E., Heikkilä, P., Pyy, L., Mutanen, P. & Riihimäki, V. (1995) Significance of dermal and respiratory uptake in creosote workers: Exposure to polycyclic aromatic hydrocarbons and urinary excretion of 1-hydroxypyrene. Occup. environ. Med., **52**, 196–203

Environmental Protection Agency (2004) 2004 Edition of the Drinking Water Standards and Health Advisories (EPA 822-R-04-005), Washington DC, Office of Water

European Union (1998) Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off. J. Eur. Communities, **L 330**, 32–43

Falćo, G., Domingo, J.L., Llobet, J.M. Teixidó, A., Casas, C. & Müller, L. (2003) Polycyclic aromatic hydrocarbons in foods: Human exposure through the diet in Catalonia, Spain. J. Food Prot., **66**, 2325–2331

Ferreira, M., Jr, Buchet, J.P., Burrion, J.B., Moro, J., Cupers, L., Delavignette, J.P., Jacques, J. & Lauwerys, R. (1994) Determinants of urinary thioethers, D-glucaric acid and mutagenicity after exposure to polycyclic aromatic hydrocarbons assessed by air monitoring and measurement of 1-hydroxypyrene in urine: A cross-sectional study in workers of coke and graphite-electrode-producing plants. Int. Arch. occup. environ. Health, **65**, 329–338

Fetzer, J.C. & Biggs, W.R. (1993) Use of full-spectrum absorbance and emission detectors in environmental analyses. J. Chromatogr., **642**, 319–327

Food and Drug Administration (2005) Miscellaneous external drug products for over-the-counter human use. Code fed. Regul., **Title 21**, Part 358, Section 700, Subpart H (21CFR358)

Frank, W.B., Haupin, W.E., Dawless, R.K., Granger, D.A., Wei, M.W., Calhoun, K.J. & Bonney, T.B. (2005) Aluminum. In: Ullmann's Encyclopedia of Industrial Chemistry, 5th Ed., Wiley-VCH Verlag GmbH & Co. KGaA [online version]

García Falcón, M.S., González Amigo, S., Lage Yusty, M.A. & Simal Lozano, J. (1999) Determination of benzo[a]pyrene in some Spanish commercial smoked products by HPLC-FL. Food Addit. Contam., **16**, 9–14

Gendre, C., Lafontaine, M., Morele, Y., Payan, J.-P. & Simon, P. (2002) Relationship between urinary levels of 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene for workers exposed to polycyclic aromatic hydrocarbons. Polycyc. aromat. Compds, **22**, 761–769

Godschalk, R.W.L., Ostertag, J.U., Moonen, E.J., Neumann, H.A.M., Kleinjans, J.C.S. & van Shooten, F.J. (1998) Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. Cancer Epidemiol. Biomarkers Prev., **7**, 767–773

Gomaa, E.A., Gray, J.I., Rabie, S., Lopez-Bote, C. & Booren, A.M. (1993) Polycyclic aromatic hydrocarbons in smoked food products and commercial liquid smoke flavourings. Food Addit. Contam., **10** , 503–521

Government of Canada (1994) Priority Substances List Assessment Report. Polycyclic Aromatic Hydrocarbons (Canadian Environmental Protection Act), Ottawa

Grimmer, G., Brune, H., Deutsch-Wenzel, R., Dettbarn, G., Misfeld, J., Abel, U. & Timm, J. (1984) The contribution of polycyclic aromatic hydrocarbons to the carcinogenic impact of emission condensate from coal-fired residential furnaces evaluated by topical application to the skin of mice. Cancer Lett., **23**, 167–176

Grimmer, G., Dettbarn, G., Naujack, K.-W. & Jacob, J. (1991) Excretion of hydroxy derivatives of polycyclic aromatic hydrocarbons of the masses 178, 202, 228 and 252 in the urine of coke and road workers. Int. J. environ. anal. Chem., **43**, 177–186

Grimmer, G., Dettbarn, G. & Jacob, J. (1993) Biomonitoring of polycyclic aromatic hydrocarbons in highly exposed coke plant workers by measurement of urinary phenanthrene and pyrene metabolites (phenols and dihydrodiols). Int. Arch. occup. environ. Health, **65**, 189–199

Guérin, M.R. (1980) Chemical composition of cigarette smoke. In: Gori, G.B. & Bock, F.G., eds, A Safe Cigarette? (Banbury Report 3), Cold Spring Harbor, NY, CSH Press, pp. 191–204

Guillén, M.D. (1994) Polycyclic aromatic compounds: Extraction and determination in food. Food Addit. Contam., **11**, 669–684

Gundel, J., Mannschreck, C., Büttner, K., Ewers, U. & Angerer, J. (1996) Urinary levels of 1-hydroxypyrene, 1-, 2-, 3-, and 4-hydroxyphenanthrene in females living in an industrial area of Germany. Arch. environ. Contam. Toxicol., **31**, 585–590

Gustavsson, P. & Reuterwall, C. (1990) Mortality and incidence of cancer among Swedish gas workers. Br. J. ind. Med., **47**, 169–174

Hattemer-Frey, H.A. & Travis, C.C. (1991) Benzo(a)pyrene. Environmental partitioning and human exposure. Toxicol. ind. Health, **7**, 141–157

Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G.E., Newman, M.J. & Harris, C.C. (1986) Determination of polycyclic aromatic hydrocarbons in the urine, benzo(a)pyrene diol epoxide–DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. Cancer Res., **46**, 4178–4183

Health and Safety Executive (2005) Workplace Exposure Limits (EH40/2005), London, HSE Books

Health Canada (1986) Benzo[a]pyrene. Available at: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/doc_sup-appui/benzo_a_pyrene/index_e.htm

Health Canada (2004) Summary of Guidelines for Canadian Drinking Water Quality. Available at: http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/doc_sup-appui/sum_guide-res_recom/index_e.htm

Heikkilä, P.R., Hämeilä, M., Pyy, L. & Raunu, P. (1987) Exposure to creosote in the impregnation and handling of impregnated wood. Scand. J. Work Environ. Health, **13**, 431–437

Heikkilä, P., Luotamo, M., Pyy, L. & Riihimäki, V. (1995) Urinary 1-naphthol and 1-pyrenol as indicators of exposure to coal tar products. Int. Arch. occup. environ. Health, **67**, 211–217

Hemminki, K., Grzybowska, E., Chorazy, M., Twardowska-Saucha, K., Sroczynski, J.W., Putman, K.L., Randerath, K., Phillips, D.H. & Hewer, A. (1990) Aromatic DNA adducts in white blood cells of coke workers. Int. Arch. occup. environ. Health, **62**, 467–470

Huang, W., Grainger, J., Patterson, D.G., Jr, Turner, W.E., Caudill, S.P., Needham, L.L., Pirkle, J.L. & Sampson, E.J. (2004) Comparison of 1-hydroxypyrene exposure in the US population with that in occupational exposure studies. Int. Arch. occup. environ. Health, **77**, 491–498

IARC (1973) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 3, Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds, Lyon

IARC (1983) IARC Monographs on the Evaluation of the Carcinogenicity of Chemicals to Humans, Vol. 32, Polynuclear Aromatic Compounds, Part 1: Chemical, Environmental and Experimental Data, Lyon

IARC (1984) IARC Monographs on the Evaluation of the Carcinogenicity of Chemicals to Humans, Vol. 34, Polynuclear Aromatic Compounds, Part 3: Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding, Lyon

IARC (1985) IARC Monographs on the Evaluation of the Carcinogenicity of Chemicals to Humans, Vol. 35, Polynuclear Aromatic Compounds, Part 4: Bitumens, Coal-Tars and Derived Products, Shale-Oils and Soots, Lyon

IARC (1987) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supp.7, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42, Lyon

IARC (2004) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 83, Tobacco Smoke and Involuntary Smoking, Lyon

IPCS (1998) Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons (Environmental Health Criteria 202), Geneva, International Programme on Chemical Safety, WHO

IPCS (2004) Asphalt (Bitumen) (Concise International Chemical Assessment Document 59), Geneva, International Programme on Chemical Safety, WHO

IUPAC (International Union of Pure and Applied Chemistry) (1979) Nomenclature of Organic Chemistry, Sections A, B, C, D, E, F and H, Oxford, Pergamon Press

Jakszyn, P., Agudo, A., Ibáñez, R., García-Closas, R., Pera, G., Amiano, P. & González, C.A. (2004) Development of a food database of nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. J. Nutr., **134**, 2011–2014

JEFCA (Joint FAO/WHO Expert Committee on Food Additives) (2005) Sixty-fourth Meeting (JECFA/64/SC), Rome

Jongeneelen, F.J. (2001) Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. Ann. occup. Hyg., **45**, 3–13

Jongeneelen, F.J., Anzion, R.B.M., Leijdekkers, C.M., Bos, R.P. & Henderson, P.T. (1985) 1–Hydroxypyrene in human urine after exposure to coal tar and a coal tar derived product. Int. Arch. occup. environ. Health, **57**, 47–55

Jongeneelen, F.J., Bos, R.P., Anzion, R.B.M., Theuws, J.L.G. & Henderson, P.T. (1986) Biological monitoring of polycyclic aromatic hydrocarbons. Metabolites in urine. Scand. J. Work Environ. Health, **12**, 137–143

Jongeneelen, F.J., Scheepers, P.T.J., Groenendijk, A., Van Aerts, L.A.G.J.M., Anzion, R.B.M., Bos, R.P. & Veenstra, S.J. (1988a) Airborne concentrations, skin contamination, and urinary metabolite excretion of polycyclic aromatic hydrocarbons among paving workers exposed to coal tar derived road tars. Am. ind. Hyg. Assoc. J., **49**, 600–607

Jongeneelen, F.J., Anzion, R.B.M., Scheepers, P.T.J., Bos, R.P., Henderson, P.T., Nijenhuis, E.H., Veenstra, S.J., Brouns, R.M.E. & Winkes, A. (1988b) 1-Hydroxypyrene in urine as a biological indicator of exposure to polycyclic aromatic hydrocarbons in several work environments. Ann. occup. Hyg., **32**, 35–43

Jongeneelen, F.J., van Leeuwen, F.E., Oosterink, S., Anzion, R.B.M., van der Loop, F., Bos, R.P. & van Veen, H.G. (1990) Ambient and biological monitoring of cokeoven workers: Determinants of the internal dose of polycyclic aromatic hydrocarbons. Br. J. ind. Med., **47**, 454–461

Kaegi, D., Addes, V., Valia, H. & Grant, M. (1993) Coal conversion processes, carbonization. In: Kirk-Othmer Encyclopedia of Chemical Technology, 5th Ed., New York, John Wiley & Sons [online edition]

Kauppinen, T., Toikkanen, J., Pedersen, D., Young, R., Ahrens, W., Boffetta, P., Hansen, J., Kromhout, H., Maqueda, B.J., Mirabelli, D.D., de la Orden-Rivera, V., Pannett, B., Plato, N., Savela, A., Vincent, R. & Kogevinas, M. (2000) Occupational exposure to carcinogens in the European Union. Occup. environ. Med., **57**, 10–18

Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A. & Rothman, N. (2001) Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. Food chem. Toxicol., **39**, 423–436

Kenny, L.C., Aitken, R., Chalmers, C., Fabriès, J.F., Gonzalez-Fernandez, E., Kromhout, H., Lidén, G., Mark, D., Riediger, G. & Prodi, V. (1997) A collaborative European study of personal inhalable aerosol sampler performance. Ann. occup. Hyg., **41**, 135–153

Knecht, U. & Woitowitz, H.J. (1989) Risk of cancer from the use of tar bitumen in road works. Br. J. ind. Med., **46**, 24–30

Knecht, U., Bolm-Audorff, U. & Woitowitz, H.-J. (1989) Atmospheric concentrations of polycyclic aromatic hydrocarbons during chimney sweeping. Br. J. ind. Med., **46**, 479–482

Larsson, B.K. (1986) Formation of polycyclic aromatic hydrocarbons during the smoking and grilling of food. Prog. clin. biol. Res., **206**, 169–180

Larsson, B.K., Sahlberg, G.P., Eriksson, A.T. & Busk, L.Å. (1983) Polycyclic aromatic hydrocarbons in grilled food. J. agric. Food Chem., **31**, 867–873

Lee, M.L., Novotny, M.V. & Bartle, K.D. (1981) Analytical Chemistry of Polycyclic Aromatic Compounds, New York, Academic Press

Letzel, S., Schaller, K.H., Elliehausen, H.-J., Gissibl, R., Hoffmann, G., Schmittner, H., Paur, R., Angerer, J. & Lehnert, G. (1999) Study on the internal exposure of chimney sweeps to hazardous substances. Occup. Hyg., **5**, 59–71

Levin, J.O., Rhén, M. & Sikström, E. (1995) Occupational PAH exposure: Urinary 1-hydroxypyrene levels of coke oven workers, aluminium smelter pot-room workers, road pavers, and occupationally non-exposed persons in Sweden. Sci. total Environ., **163**, 169–177

Lijinsky, W. (1991) The formation and occurrence of polynuclear aromatic hydrocarbons associated with food. Mutat. Res., **259**, 251–261

Lindstedt, G. & Sollenberg, J. (1982) Polycyclic aromatic hydrocarbons in the occupational environment. Scand. J. Work Environ. Health, **8**, 1–19

Lioy, P.J. & Greenberg, A. (1990) Factors associated with human exposures to polycyclic aromatic hydrocarbons. Toxicol. ind. Health, **6**, 209–223

Lioy, P.L., Waldman, J.M., Greenberg, A., Harkov, R. & Pietarinen, C. (1988) The Total Human Environmental Exposure Study (THEES) to benzo(a)pyrene: Comparison of the inhalation and food pathways. Arch. environ. Health., **43**, 304–312

Lodovici, M., Dolara, P., Casalini, C., Ciappellano, S. & Testolin, G. (1995) Polycyclic aromatic hydrocarbon contamination in the Italian diet. Food Addit. Contam., **12**, 703–713

Lu, P.L., Chen, M.L. & Mao, I.F. (2002) Urinary 1-hydroxypyrene levels in workers exposed to coke oven emissions at various locations in a coke oven plant. Arch. environ. Health, **57**, 255–261

Mahler, B.J., Van Metre, P.C., Bashara, T.J., Wilson, J.T. & Johns, D.A. (2005) Parking lot sealcoat: An unrecognized source of urban polycyclic aromatic hydrocarbons. Environ. Sci. Technol., **39**, 5560–5566

Malkin, R., Kiefer, M. & Tolos, W. (1996) 1-Hydroxypyrene levels in coal-handling workers at a coke oven. J. occup. environ. Med., **38**, 1141–1144

Marczynski, B., Rihs, H.P., Rossbach, B., Hölzer, J., Angerer, J., Scherenberg, M., Hoffmann, G., Bruning, T. & Wilhelm, M. (2002) Analysis of 8-oxo-7,8-dihydro-2'-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: Comparison with

ambient monitoring, urinary metabolites and enzyme polymorphisms. Carcinogenesis, **23**, 273–281

Marriott, P.J., Haglund, P. & Ong, R.C.Y. (2003) A review of environmental toxicant analysis by using multidimensional gas chromatography and comprehensive GC. Clin. chim. Acta, **328**, 1–19

Menzie, C.A., Potocki, B.B. & Santodonato, J. (1992) Exposure to carcinogenic PAHs in the environment. Environ. Sci. Technol., **26**, 1278–1284

Mielzyńska, D., Braszcyńska, Z., Siwińska, E., Smolik, E., Bubak, A. & Sokal, J.A. (1997) Exposure of coke-oven workers to polycyclic aromatic hydrocarbons based on biological monitoring results. Am. ind. Hyg. Assoc. J., **58**, 661–666

Neilson, A.H., ed. (1998) The Handbook of Environmental Chemistry, Vol. 3, Anthropogenic compounds: PAHs and Related Compounds, Part I, Chemistry, Berlin, Springer-Verlag

NIOSH (National Institute for Occupational Safety and Health) (2000) Hazard Review, Health Effects of Occupational Exposure to Asphalt, Atlanta, GA, Center for Disease Control and Prevention

Noble, C.A. & Prather, K.A. (2000) Real-time single particle mass spectrometry: A historical review of a quarter century of the chemical analysis of aerosols. Mass Spectrom. Rev., **19**, 248–274

Notø, H., Halgard, K., Daae, H.L., Bentsen, R.K. & Eduard, W. (1996) Comparative study of an inhalable and a total dust sampler for personal sampling of dust and polycyclic aromatic hydrocarbons in the gas and particulate phase. Analyst, **121**, 1191–1196

OSHA (2005a) Regulations (Standards - 29 CFR) Air contaminants. - 1910.1000: Table Z-1 Limits for Air Contaminants. Available at: http://www.osha.gov

OSHA (2005b) Safety and Health Topics: Coal Tar Pitch Volatiles - Hazards and Solutions. Available at: http://www.osha.gov

OSHA (2005c) Safety and Health Topics: Coal Tar Pitch Volatiles - Hazards and Solutions. Available at: http://www.osha.gov

OSHA (2005d) Safety and Health Topics: Coal Tar Pitch Volatiles. Available at: http://www.osha.gov/SLTC/coaltarpitchvolatiles/

Øvrebø, S., Haugen, A., Fjeldstad, P.E., Hemminki, K. & Szyfter, K. (1994) Biological monitoring of exposure to polycyclic aromatic hydrocarbon in an electrode paste plant. J Occup Med., **36**, 303-310

Øvrebø, S., Haugen, A., Farmer, P.B. & Anderson, D. (1995) Evaluation of biomarkers in plasma, blood, and urine samples from coke oven workers: Significance of exposure to polycyclic aromatic hydrocarbons. Occup. environ. Med., **52**, 750–756

Pan, G., Hanaoka, T., Yamano, Y., Hara, K., Ichiba, M., Wang, Y., Zhang, J., Feng, Y., Shujuan, Z., Guan, D., Gao, G., Liu, N. & Takahashi, K. (1998) A study of multiple biomarkers in coke oven workers—A cross-sectional study in China. Carcinogenesis, **19**, 1963–1968

Partanen, T., Chaves, J., Wesseling, C., Chaverri, F., Monge, P., Ruepert, C., Aragón, A., Kogevinas, M., Hogstedt, C. & Kauppinen, T. (2003) Workplace carcinogen and pesticide exposures in Costa Rica. Int. J. occup. environ. Health., **9**, 104–111

Pavanello, S., Genova, A., Foà, V. & Clonfero, E. (2000) [Assessment of occupational exposure to polycyclic aromatic hydrocarbons by analysis of urinary 1-pyrenol]. Med. Lav., **91**, 192–205 (in Italian)

Pavanello, S., Siwinska, E., Mielzynska, D. & Clonfero, E. (2004) GSTM1 null genotype as a risk factor for anti-BPDE–DNA adduct formation in mononuclear white blood cells of coke-oven workers. Mutat. Res., **558**, 53–62

Pedersen, R. (2003) Polycyclic Aromatic Hydrocarbons. A Control Approach (Paper No. ACTS/26/2003), Merseyside, Health and Safety Commission, Advisory Committee on Toxic Substances

Petry, T., Schmid, P. & Schlatter, C. (1996) Airborne exposure to polycyclic aromatic hydrocarbons (PAHs) and urinary excretion of 1-hydroxypyrene of carbon anode plant workers. Ann. occup. Hyg., **40**, 345–357

Pfannhauser, W. (1991) Polynuclear aromatic hydrocarbons (PAH) in food and selected vegetables in Austria. Mitt. Geb. Lebensmittel. Hyg., **82**, 66–79

Phillips, D.H. (1999) Polycyclic aromatic hydrocarbons in the diet. Mutat. Res., **443**, 139–147

Popp, W., Vahrenholz, C., Schell, C., Kraus, R., von Bülow, J., Müller, G. & Norpoth, K. (1995) Risk estimation in coke-oven workers by determining some biomarkers of carcinogen exposure. Exp. Toxicol. Pathol., **47**, 440–442

Popp, W., Vahrenholz, C., Schell, C., Grimmer, G., Dettbarn, G., Kraus, R., Brauksiepe, A., Schmeling, B., Gutzeit, T., von Bülow, J., & Norpoth, K. (1997) DNA single strand breakage, DNA adducts, and sister chromatid exchange in lymphocytes and phenanthrene and pyrene metabolites in urine of coke oven workers. Occup. environ. Med., **54**, 176–183

Poster, D.L., Sander, L.C. & Wise, S.A. (1998) Chromatographic methods of analysis for the determination of PAHs in environmental samples. In: Neilson, A.H., ed., The Handbook of Environmental Chemistry, Vol. 3, PAHs and Related Compounds, Part I, Chemistry, Berlin, Springer-Verlag, pp. 77–135

Pyy, L., Makela, M., Hakala, E., Kakko, K., Lapinlampi, T., Lisko, A., Yrjanheikki, E. & Vahakangas, K. (1997) Ambient and biological monitoring of exposure to polycyclic aromatic hydrocarbons at a coking plant. Sci Total Environ., **199**, 151-158

Quinlan, R., Kowalczyk, G., Gardiner, K., Hale, K., Walton, S. & Calvert, I. (1995a) Urinary 1-hydroxypyrene: A biomarker for polycyclic aromatic hydrocarbon exposure in coal liquefaction workers. Occup. Med., **45**, 63–68

Quinlan, R., Kowalczyk, G., Gardiner, K., Calvert, I.A., Hale, K. & Walton, S.T. (1995b) Polycyclic aromatic hydrocarbon exposure in coal liquefaction workers: The value of urinary 1-hydroxypyrene excretion in the development of occupational hygiene control strategies. Ann. occup. Hyg., **39**, 329–346

Quinlan, R., Kowalczyk, G., Gardiner, K. & Calvert, I. (1995c) Exposure to polycyclic aromatic hydrocarbons in coal liquefaction workers: Impact of a workwear policy on excretion of urinary 1-hydroxypyrene. Occup. environ. Med., **52**, 600–605

Ramesh, A., Walker, S.A., Hood, D.B., Guillén, M.D., Schneider, K. & Weyand, E.H. (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. Int. J. Toxicol., **23**, 301–333

Reed, L.D. (1982), Anchor Hocking Glass Company, Roofing Site, Lancaster, Ohio, (Health Hazard Evaluation Report, No. HETA-82-067-1253), Cincinnatti, OH, National Institute of Occupational Safety of Health

Reisen, F. & Arey, J. (2005) Atmospheric reactions influence seasonal PAH and nitro-PAH concentrations in the Los Angeles Basin. Environ. Sci. Technol., **39**, 64–73

Reuterwall, C., Aringer, L., Elinder, C.G., Rannug, A., Levin, J.O., Juringe, L. & Onfelt, A. (1991) Assessment of genotoxic exposure in Swedish coke-oven work by different methods of biological monitoring. Scand. J. Work Environ. Health, **17**, 123–132

Romundstad, P.R., Ronneberg, A., Leira, H.L. & Bye, T. (1998) Health survey of former workers in a Norwegian coke plant: Part. 1. Estimation of historical exposures. Occup. environ. Med., **55**, 616–621

Romundstad, P., Haldorsen, T. & Rønneberg, A. (1999) Exposure to PAH and fluoride in aluminum reduction plants in Norway: Historical estimation of exposure using process parameters and industrial hygiene measurements. Am. J. ind. Med., **35**, 164–174

Roth, M.J., Strickland, K.L., Wang, G.Q., Rothman, N., Greenberg, A. & Dawsey, S.M. (1998) High levels of carcinogenic polycyclic aromatic hydrocarbons present within food from Linxian, China may contribute to that region's high incidence of oesophageal cancer. Eur. J. Cancer, **34**, 757–758

Roth, M.J., Qiao, Y.-L., Tangrea, J.A., Dawsey, S.M., Wang, G.-Q., Cho, S.-H., Kang, D., Taylor, P.R. & Strickland, P.T. (2001) High urine 1-hydroxypyrene glucuronide concentrations in Linxian, China, an area of high risk for squamous oesophageal cancer. Biomarkers, **6**, 381–386

Sander, L.C. & Wise, S.A. (1997) Polycyclic Aromatic Hydrocarbon Structure Index (NIST Special Publication 922), Washington DC, US Government Printing Office

Sanders, R.E., Jr (2002) Aluminum and aluminum alloys. In: Kirk-Othmer Encyclopedia of Chemical Technology, 5th Ed., New York, John Wiley & Sons [online edition]

Sangster Research Laboratories (2005) LOGKOW© A Databank of Evaluated Octanol-Water Partition Coefficients (Log P), ICSU-CODATA. Available at: http://logkow/cisti.nrc.ca/logkow (last accessed September, 2005)

Santodonato, J., Howard, P. & Basu, D. (1981) Health and ecological assessment of polynuclear aromatic hydrocarbons. J. environ. Pathol. Toxicol., **5**, 1–364

Schauer, C., Niessner, R. & Pöschl, U. (2003) Polycyclic aromatic hydrocarbons in urban air particulate matter: Decadal and seasonal trends, chemical degradation, and sampling artifacts. Environ. Sci. Technol., **37**, 2861–2868

van Schooten, F.J., Jongeneelen, F.J., Hillebrand, M.J.X., van Leeuwen, F.E., de Looff, A.J.A., Dijkmans, A.P.G., van Rooij, J.G.M., den Engelse, L. & Kriek, E. (1995) Polycyclic aromatic hydrocarbon–DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: Relation with job category and synergistic effect of smoking. Cancer Epidemiol. Biomarkers Prev., **4**, 69–77

Schubert, P., Schantz, M.M., Sander, L.C. & Wise, S.A. (2003) Determination of polycyclic aromatic hydrocarbons with molecular weight 300 and 302 in environmental–matrix standard reference materials by gas chromatography/mass spectrometry. Anal. Chem., **75**, 234–246

Shadle, L.J., Berry, D.A. & Syamlal, M. (2002) Coal conversion processes, gasification. In: Kirk-Othmer Encyclopedia of Chemical Technology, 5th Ed., New York, John Wiley & Sons [online edition]

Sinha, R., Cross, A., Curtin, J., Zimmerman, T., McNutt, S., Risch, A. & Holden, J. (2005) Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol. Nutr. Food Res., **49**, 648–655

Sonnefeld, W.J., Zoller, W.H., May, W.E. & Wise, S.A. (1982) On-line multidimensional liquid chromatographic determination of polynuclear aromatic hydrocarbons in complex samples. Anal. Chem., **54**, 723–727

State Committee for Air Pollution Control (1992) [Cancer Risk from Air Pollution. Development of Evaluation Criteria for Carcinogenic Pollutants]. Minister for Environment and Agriculture for Northrhine-Westfalia, Düsseldorf (in German)

Strickland, P., Kang, D. & Sithisarankul, P. (1996) Polycyclic aromatic hydrocarbon metabolites in urine as biomarkers of exposure and effect. Environ. Health Perspect., **104**, 927–932

Strickland, P.T., Qian, Z., Friesen, M.D., Rothman, N. & Sinha, R. (2002) Metabolites of 2-amino-1-methyl-6-phenylimidazo(4, 5-b)pyridine (PhIP) in human urine after consumption of charbroiled or fried beef. Mutat. Res., **506–507**, 163–173

Strunk, P., Ortlepp, K., Heinz, H., Rossbach, B. & Angerer, J. (2002) Ambient and biological monitoring of coke plant workers—Determination of exposure to polycyclic aromatic hydrocarbons. Int. Arch. occup. environ. Health, **75**, 354–358

Suva (2005) [Limit values at the workplace] Luzern. Available at: arbeitsmedizin@suva.ch (in German)

Terjung, J. (2000) World coal and coke market, presence and future. In: 4th European Coke and Ironmaking Congress, Paris, June 2000

Tjoe Ny, E., Heederik, D., Kromhout, H. & Jongeneelen, F. (1993) The relationship between polycyclic aromatic hydrocarbons in air and in urine of workers in a Söderberg potroom. Am. ind. Hyg. Assoc. J., **54**, 277–284

Tolos, W.P., Shaw, P.B., Lowry, L.K., MacKenzie, B.A., Deng, J.-F. & Markel, H.L. (1990) 1-Pyrenol: A biomarker for occupational exposure to polycyclic aromatic hydrocarbons. Appl. occup. environ. Hyg., **5**, 303–309

Van Hummelen, P., Gennart, J.P., Buchet, J.P., Lauwerys, R. & Kirsch-Volders, M. (1993) Biological markers in PAH exposed workers and controls. Mutat. Res., **300**, 231–239

VanRooij, J.G.M., Bodelier-Bade, M.M., de Looff, A.J.A., Dijkmans, A.P.G. & Jongeneelen, F.J. (1992) Dermal exposure to polycyclic aromatic hydrocarbons among primary aluminium workers. Med. Lav., **83**, 519–529

VanRooij, J.G.M., Bodelier-Bade, M.M. & Jongeneelen, F.J. (1993a) Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. Br. J. ind. Med., **50**, 623–632

VanRooij, J.G.M., van Lieshout, E.M.A., Bodelier-Bade, M.M. & Jongeneelen, F.J. (1993b) Effect of the reduction of skin contamination on the internal dose of creosote workers exposed to polycyclic aromatic hydrocarbons. Scand. J. Work Environ. Health, **19**, 200–207

VanRooij, J.G.M., de Roos, J.H.C., Bodelier-Bade, M.M. & Jongeneelen, F.J. (1993c) Absorption of polycyclic aromatic hydrocarbons through human skin: Differences between anatomical sites and individuals. J. Toxicol. environ. Health, **38**, 355–368

van de Ven, C. & Nossent, C. (1984) [Who deals with pitch... Occupational hygiene study in a coal-tar processing plant.] 195. Gezondheidsleer, V146 Luchthygiene en –verontreiniging. Health education, air quality and air pollution (in Dutch)

Viau, C. & Vyskocil, A. (1995) Patterns of 1-hydroxypyrene excretion in volunteers exposed to pyrene by the dermal route. Sci. total Environ., **163**, 187–190

de Vos, R.H., van Dokkum, W., Schouten, A. & de Jong-Berkhout, P. (1990) Polycyclic aromatic hydrocarbons in Dutch total diet samples (1984–1986). Food chem. Toxicol., **28**, 263–268

Voutsa, D. & Samara, C. (1998) Dietary intake of trace elements and polycyclic aromatic hydrocarbons via vegetables grown in an industrial Greek area. Sci. total Environ., **218**, 203–216

Waidyanatha, S., Zheng, Y. & Rappaport, S.M. (2003) Determination of polycyclic aromatic hydrocarbons in urine of coke oven workers by headspace solid phase microextraction and gas chromatography–mass spectrometry. Chem.-biol. Interact., **145**, 165–174

Watson, A.Y. & Valberg, P.A. (2001) Carbon black and soot: Two different substances. Am. ind. Hyg. Assoc. J., **62**, 218–228

WHO (2004) Guidelines for Drinking-water Quality, Vol. 1, Recommendations, 3rd Ed., Geneva, pp. 428–430 [www.who.int]

Winker, N., Tuschl, H., Kovac, R. & Weber, E. (1996) Immunological investigations in a group of workers exposed to various levels of polycyclic aromatic hydrocarbons. J. appl. Toxicol., **17**, 23–29

Wise, S.A., Sander, L.C. & May, W.E. (1993) Review: Determination of polycyclic aromatic hydrocarbons by liquid chromatography. J. Chromatogr., **642**, 329–349

Wolff, M.S., Herbert, R., Marcus, M., Rivera, M., Landrigan, P.J. & Andrews, L.R. (1989) Polycyclic aromatic hydrocarbon (PAH) residues on skin in relation to air levels among roofers. Arch. environ. Health, **44**, 157–163

Wright, C.W., Later, D.W., Pelroy, R.A., Mahlum, D.D. & Wilson, B.W. (1985) Comparative chemical and biological analysis of coal tar-based therapeutic agents to other coal-derived materials. J. appl. Toxicol., **5**, 80–88

Wu, M.T., Mao, I.F., Ho, C.K., Wypij, D., Lu, P.L., Smith, T.J., Chen, M.L. & Christiani, D.C. (1998) Urinary 1-hydroxypyrene concentrations in coke oven workers. Occup. environ. Med., **55**, 461–467

Yabiku, H.Y., Martins, M.S. & Takahashi, M.Y. (1993) Levels of benzo[a]pyrene and other polycyclic aromatic hydrocarbons in liquid smoke flavour and some smoked foods. Food Addit. Contam., **10**, 399–405

Yu, S. & Campiglia, A.D. (2004) Laser-excited time-resolved Shpol'skii spectroscopy for the direct analysis of dibenzopyrene isomers in liquid chromatography fractions. Appl. Spectrosc., **58**, 1385–1393

Zey, J.N. (1983), Roofing Construction, Houston, Texas (Health Hazard Evaluation Report No. HETA-83-210-1887), Cincinnati, OH, National Institute of Occupational Safety and Health

Zhang, J., Ichiba, M., Hara, K., Zhang, S., Hanaoka, T., Pan, G., Yamano, Y., Takahashi, K. & Tomokuni, K. (2001) Urinary 1-hydroxypyrene in coke oven workers relative to exposure, alcohol consumption, and metabolic enzymes. Occup. environ. Med., **58**, 716–721

2. Studies of Cancer in Humans

2.1 Cohort and nested case–control studies by industry

2.1.1 *Coal gasification* (Table 2.1)

Kennaway and Kennaway (1947) analysed the mortality from cancer of the lung and larynx by occupation in England and Wales in 1921–38 that had been reported earlier (Kennaway & Kennaway, 1936). Data on occupation were obtained from death certificates; the population at risk in each occupation was estimated from national census data, which allowed the calculation of age-adjusted expected numbers of cancers by occupation. [Since different sources were used to obtain occupational titles for the numerator and denominator, this may have caused a bias in the estimated risks.] Among gas works labourers, 96 deaths from lung cancer (standardized mortality ratio [SMR], 1.29; 95% confidence interval [CI], 1.04–1.57) and 43 deaths from laryngeal cancer (SMR, 0.90; 95% CI, 0.65–1.22) occurred; among male gas producers, 12 deaths from lung cancer (SMR, 2.03; 95% CI, 1.05–3.55) and two deaths from laryngeal cancer (SMR, 0.59; 95% CI, 0.07–2.12) occurred. No data on tobacco smoking habits were available.

Bruusgaard (1959) analysed causes of death among current and former employees at a Norwegian gas producing plant over a 15-year period, during which 125 deaths occurred. Of these, 12 were from cancers of the respiratory tract, four of which were lung cancers. Among all cancer deaths, the proportion of cancer of the respiratory tract was higher than that in the general population. [This comparison was made on crude death rates that were not adjusted for the age distribution among the gas workers.]

Kawai *et al*. (1967) reported the findings of a cohort study of all 504 generator gas production workers who were employed at a Japanese steel factory until it was closed down in 1953. Earlier publications from the plant included a report of a series of 12 cases of lung cancer among the generator gas workers during 1931–35 (Kuroda & Kawahata, 1936). Mortality in the cohort was followed prospectively until 1965, up to a maximum age of 55 years. Causes of death were obtained from medical records from the two hospitals that served the area. Expected numbers of deaths were obtained by the person–year method that used mortality in a reference cohort of 25 760 steel workers who had not worked at the generator gas plant. Six cases of lung cancer occurred among the gas workers in comparison with 0.18 expected (SMR, 33.33; [95% CI, 12.20–72.60]). [Only eight deaths from lung cancer occurred in the reference cohort, but the authors stated that the age-specific mortality from lung cancer in the reference group did not differ from that among Japanese men in general. However, the precision of the estimates of the expected numbers of cases among the gas workers was low.]

Table 2.1. Cohort and linkage studies of coal gasification workers

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Kennaway & Kennaway (1947), United Kingdom	Register-based national mortality analysis of all deaths in England and Wales, 1921–38	Occupational titles	Lung	Gas works labourers	96	1.29 [1.04–1.57]	No data available on tobacco smoking
				Gas producers (male)	12	2.03 [1.05–3.55]	
			Larynx	Gas works labourers	43	0.90 [0.65–1.22]	
				Gas producers (male)	2	0.59 [0.07–2.12]	
Kawai *et al*. (1967), Japan	503 workers at a generator gas plant in a steel industry followed from 1953 to 1965; the plant was closed down in 1953.	Occupational titles	Lung	Gas generator plant	6	33.3 [12.2–72.6]	Precision in the estimation of expected numbers was low.
Doll *et al.* (1972), United Kingdom	3023 gas manufacturing workers aged 40–65 years employed at, or in recpt of pension from, four gas boards ('original gas boards') followed for mortality from 1953 to 1965; minimum duration of employment, 5 years	Occupational titles at start of study	Lung	Heavy exp. to coal gas (A)	99	1.79 [1.46–2.18]	Tobacco smoking habits studied in a 10% sample of the cohort indicated no excess. No excess of atheros-clerotic heart disease supports that smoking habits were not excessive among the gas workers.
				Low or no exp. (C1)	11	0.75 [0.37–1.34]	
			Bladder	Heavy exp. to coal gas (A)	10	2.35 [1.13–4.33]	
				Low or no exp. (C1)	1	[0.77 (0.02–4.29)]	
			Skin	Heavy exp. to coal gas (A)	3	6.0 [1.24-17.5]	
				Low or no exp. (C1)	0 (not possible to calculate expected number)	–	
	4687 men from four additional gas boards followed for mortality from 1957 (one gas board from 1959) to 1965		Lung	Heavy exp. to coal gas (A)	23	1.34 [0.85–2.01]	
				Intermittent exp. (B)	40	1.72 [1.23–2.35]	
				Low or no exp. (C2)	16	0.53 [0.30–0.86]	
			Bladder	Heavy exp. to coal gas (A)	2	1.53 [0.19–5.54]	
				Intermittent exp. (B)	2	1.07 [0.13–3.85]	
				Low or no exp. (C2)	1	[0.40 (0.01–2.23)]	

Table 2.1 (Contd)

Hansen *et al.* (1986), Esbjerg, Denmark	47 gas production workers employed >1 year any time between 1911 and 1970.; an age-matched reference cohort of 141 persons selected from population registers		Lung		7	**OR** 3.94 (p <0.05)	No data on tobacco smoking habits available; analytical method may not have been appropriate. A shorter time to death from lung cancer was noted among the gas wokers than among the referent cohort (p = 0.01)
Wu (1988), China	3107 workers active in 1971 at any of six coal gas plants followed for mortality until 1982		Lung		[not stated]	**SRR** (90% CI) 3.66 (2.36–5.43)	The short report does not allow an assessment of the validity of the study.
Gustavsson & Reuterwall (1990), Stockholm, Sweden	295 male blue-collar workers from a gas production plant in Stockholm employed > 1 year between 1965 and 1972; followed for mortality from 1966 to 1986 and for cancer incidence from 1966 to 1983	Department	Lung	Entire cohort	4	1.35 (0.36–3.46)	
				Coke-oven department	0 (0.9 expected)	–	
			Nose and sinuses	Entire cohort	2	29.57 (3.57–106.89)	
Berger & Manz (1992), Hamburg, Germany	4908 male employees from a gas- producing plant, employed >10 years between 1900 and 1989 were followed for mortality from 1953 to 1989	Department	Lung	Gas furnace workers	78	2.88 (2.28–3.59)	Data on tobacco smoking available for about 80% of the cohort; no smoking- adjusted SMR for lung cancer was presented. Causes of death were obtained by different methods for the cohort and the national reference group.
				Other labourers	102	0.96 (0.78–1.17)	
				White-collar workers	12	0.45 (0.23–0.79)	
			Stomach	Gas furnace workers	31	1.77 (1.20–2.51)	
				Other labourers	72	1.13 (0.88–1.42)	
				White-collar workers	10	0.57 (0.27–1.05)	
			Colon and rectum	Gas furnace workers	13	1.84 (0.98–3.15)	
				Other labourers	48	1.70 (1.25–22.5)	
				White-collar workers	7	0.92 (0.37–1.90)	

Table 2.1 (Contd)

Martin *et al.* (2000), France	Case–control study nested within a cohort of male workers employed >1 year at a company producing gas and electricity. 310 lung cancer cases occurring between 1978 and 1989 were included, 1225 referents were selected from the cohort.	Industry-specific job–exposure matrix with index of cumulative exposure	Lung	Coal gas production Unexposed Q1 Q2 Q3 Q4	 298 7 7 7 5	 1.0 1.02 (0.21–4.94) 1.59 (0.39–6.49) 0.55 (0.07–4.57) 3.87 (1.15–12.9)	Risks adjusted for exposure to asbestos and socioeconomic status. There may be residual confounding from tobacco smoking.

CI, confidence interval; exp., exposed; OR, odds ratio; Q, quartile; SMR, standardized mortality ratio; SRR, standardized rate ratio

Doll (1952) and Doll *et al*. (1965, 1972) reported mortality among a cohort of British gas workers that originally comprised 11 499 men employed at, or in receipt of a pension from, four British gas boards (North Western, West Midlands, South Eastern and North Thames) in 1953. Men aged 40–65 years with a minimum duration of employment of 5 years were included. Job titles at the start of employment were used to classify the degree of contact with coal gas: coal carbonizing process workers (heavy exposure, Class A); intermittent exposure in gas-producing plants or process work other than in retort houses (intermittent exposure, Class B); process and maintenance workers in chemical and by-product plants (low exposure, Class C1); or pre-payment meter collectors, credit meter readers and gas fitters (low or no exposure, Class C2). For the final follow-up, the cohort was restricted to 3023 men who were classified into Class A or C1. In addition, employees at four other gas boards were included (South Western, North Eastern, Southern and East Midlands), using similar inclusion criteria and exposure classification processes, and thereby increased the number of men in the cohort by 4687. The original cohort was followed for mortality from 1953 to 1965, and workers from the additional gas boards were followed from 1957 (one gas board from 1959) to 1965. Follow-up was nearly 100% complete, and data were missing for only one individual. Underlying causes of death were obtained from death certificates. Age-standardized death rates were calculated and compared within the cohort as well as with the general death rates for England and Wales. Local death rates were also used for some analyses to take into account geographical differences in cancer rates. In the four original gas boards, there was an increased risk for lung cancer among men who were heavily exposed to coal gas (Class A) [SMR, 1.79; 95% CI, 1.46–2.18]. In Class C (with low or no exposure), there was no excess risk for lung cancer [SMR, 0.75; 95% CI, 0.37–1.34]. Ten deaths from bladder cancer occurred in Class A, which was in excess of the expected number [SMR, 2.35; 95% CI, 1.13–4.33], and only one death in Class C1 versus 1.3 expected. Three deaths from skin cancer were observed in Class A, which indicated an excess risk [SMR, 6.00; 95% CI, 1.24–17.50]. In the four additional gas boards, there was increased mortality from lung cancer among both Class A [SMR, 1.34; 95% CI, 0.85–2.01] and Class B [SMR, 1.72; 95% CI, 1.23–2.35] workers. Thus, the excess was higher among intermittently exposed and not statistically significant among regularly exposed workers. [The authors discussed various explanations for this unexpected finding, the most plausible of which appeared to be random variation.] Only two cases of bladder cancer occurred in Class A and two in Class B, which were within the limits of those expected by chance. An internal analysis based on a matched case–control approach indicated that work as a top man or hydraulic mains attendant, both associated with high exposure to coal gas, was especially associated with an increased risk for lung cancer. Smoking habits were studied in a 10% sample of the cohort from the original four gas boards and indicated no excess among the gas workers (Doll *et al*., 1965). [The Working Group noted that the findings of the analysis of atherosclerotic and degenerative heart disease did not indicate that smoking habits were in excess among the gas workers.]

Hansen *et al*. (1986) studied mortality among 47 gas workers in Esbjerg, Denmark, who had been employed for >1 year at any time during 1911–70. A reference cohort was formed by selecting age-matched referents from the population registers of the Municipality of Esbjerg. Three referents were selected per cohort member (141 persons). Dates of death were obtained from population registers, and causes of death were obtained from death certificates. The mortality of gas workers was compared with that of the reference cohort using a Kaplan-Meier survival analysis. Odds ratios were also calculated. Thirty-three of the gas workers and 78 of the referent cohort had died at the end of follow-up. [The Working Group noted that the analytical methods were not sufficiently described. The end of follow-up was not specified but a maximum survival of 60 years was noted.] Mortality from all causes did not differ between gas workers and the reference cohort. However, mortality from cancer, and especially from lung cancer, was higher among the gas workers than the referent cohort (odds ratio, 3.84; p <0.01; and odds ratio, 3.94; p <0.05, respectively). Restriction of the analysis to those with at least 10 years of employment and allowing for a latency of 10 years gave similar results. No data on tobacco smoking were available.

Wu (1988) reported summary results for a number of occupational epidemiological studies in the People's Republic of China. Mortality in a cohort of 3107 workers from six coal gas plants was compared with that in a cohort of steel workers. [The Working Group noted that an increased risk for lung cancer has been observed among steel workers, which may underestimate the true risk.] Workers employed in 1971 were followed for mortality up to 1981, and 234 deaths occurred among gas workers (standardized rate ratio [SRR], 1.29; 90% CI, 1.16–1.44). The SRR among workers in the gas departments of the coal gas plant was increased (SRR, 3.66; 90% CI, 2.36–5.43). [It is not possible to assess the validity of the study from this short report.]

Gustavsson and Reuterwall (1990) studied mortality and cancer incidence among gas workers employed by the Stockholm Gas Company in Sweden. All men who had been employed for at least 1 year between 1965 and 1972 were followed for mortality from 1966 to 1986 and for cancer incidence from 1966 to 1983. In total, 300 individuals were identified from company records; four had emigrated and one could not be traced, and the analyses were based on the remaining 295 workers. Follow-up was carried out via computerized population registers, underlying causes of death were obtained from death certificates and incident cases of cancer were obtained from the national cancer register. Expected numbers of deaths were calculated from gainfully employed men in Stockholm using the person–year method; expected numbers of cancers were based on national cancer rates. Overall mortality was slightly higher among gas workers than in the reference population (84 deaths; SMR, 1.27; 95% CI, 1.01–1.57). Four incident cases of lung cancer occurred which was close to the expected number (standardized incidence ratio [SIR], 1.35; 95% CI, 0.36–3.46). A significant increase in the incidence of cancer of the nose and nasal sinuses was found (SIR, 29.57; 95% CI, 3.57–106.89). [The Working Group noted that this is a rare tumour, and that the observed increase was based on only two cases.]

Berger and Manz (1992) studied mortality among 4908 male workers at a gas production plant in Hamburg, Germany. All male blue- and white-collar workers who had been employed for >10 years during 1900–89 were included and were followed for mortality from 1952 until 1989. Three exposure groups were formed: 789 gas furnace workers (Group I), 3401 workmen in other parts of the plant (Group II) and 718 white-collar workers (Group III). The average duration of employment was 26.7, 26.1 and 38.0 years, respectively. Causes of death were obtained from autopsy records, medical records from hospital or family doctors and statements from relatives. SMRs were computed using the person–year method and two reference cohorts: the general population of Germany and Group III as an internal comparison. Tobacco smoking habits were obtained from occupational health service registers, colleagues, next of kin and the subjects themselves when alive. Of the initial 4928 men, 20 could not be traced and the analyses were based on the remaining 4908. A total of 2240 cohort members had died at the end of follow-up, which was somewhat below the expected number based on national death rates (SMR, 0.83; 95% CI, 0.80–0.87). Gas furnace workers (Group I) showed a highly increased rate of mortality from lung cancer (SMR, 2.88; 95% CI, 2.28–3.59) and from stomach cancer and cancer of the colon and rectum. Mortality from lung cancer was close to the expected rate in Group II (SMR, 0.96; 95% CI, 0.78–1.17), whereas it was lower than that expected among the white-collar workers (SMR, 0.45; 95% CI, 0.23–0.79). A clear relation with duration of employment was observed for lung cancer but not for stomach or colorectal cancer. [The analysis of mortality in relation to duration of employment was published in a non-peer reviewed report (Manz *et al*., 1983).] The authors recognized the methodological problem raised by the fact that causes of death were obtained using different methods within the cohort, including data from next of kin, and national reference rates. An internal analysis using white-collar workers as a reference cohort addressed this question and showed a higher SMR for lung cancer (SMR, 7.28; 95% CI, 5.79–9.03) than that obtained with external rates. [It is questionable whether white-collar workers constitute a suitable reference group.] The analysis of tobacco smoking habits showed a higher rate of smokers among the gas furnace workers (69.2%) than among other labourers (64.1%) and white-collar workers (48.7%). It should be noted that smoking habits could not be obtained for 18, 16 and 22% of Groups I–III, respectively. [It is improbable that all of the high excess of lung cancer among gas furnace workers could be explained by tobacco smoking.]

Martin *et al.* (2000) reported a case–control study of lung cancer nested within a cohort of all men who had been employed for at least 1 year at a French electricity and gas producing company (EDF–GDF) between January 1978 and December 1989. Cases of lung cancer were identified from the company's cancer registry. Using incidence density sampling, four individually age-matched referents per case were selected from the cohort, which resulted in a total of 310 cases and 1225 referents in the study. Occupational exposure to over 20 agents or exposure situations was assessed by a industry-specific job–exposure matrix. The relative risk for lung cancer was estimated by conditional logistic regression with adjustment for exposure to asbestos and

socioeconomic status, which served as a proxy for tobacco smoking habits. The relative risk for lung cancer was increased among workers in coal gas production (odds ratio, 1.64; 95% CI, 0.80–3.40). An analysis of exposure–response based on an index of cumulative exposure showed a statistically significant excess risk in the highest quartile of exposure (odds ratio, 3.87; 95% CI, 1.15–12.9). [There may be residual confounding from tobacco smoking since socioeconomic status is not a good proxy for tobacco smoking habits. Also, adjustment for socioeconomic status may lead to overmatching and result in underestimation of relative risks.]

2.1.2 *Coke production* (Table 2.2)

In the study by Kennaway and Kennaway (1947) (reported in detail in Section 2.1.1), 85 deaths from lung cancer (SMR, 2.84; [95% CI, 2.27–3.52]) and 37 deaths from laryngeal cancer (SMR, 2.13; [95% CI, 1.50–2.93]) occurred among gas stokers and coke-oven chargers. No data on tobacco smoking habits were available.

Reid and Buck (1956) reported a mortality study of British coke workers, in which deaths among coke plant workers from 1949 to 1954 were identified from the Carbonization Department of the National Coal Board. Causes of death were ascertained either from claims to the trade union's funeral fund that required death certificates, or from the General Registrar's Office. The population at risk was estimated from a census in 1952, which allowed estimation of person–years by job category and age group, and was approximately 8000 persons. Expected numbers of deaths were calculated from death rates obtained from 'a large industrial organization' [It was not stated that the death rates were said to be similar to those of the General Post Office]. During the study period, 14 deaths from respiratory cancer occurred [SMR, 1.40; 95% CI, 0.77–2.35]. A proportionate mortality analysis was performed for retired workers but was uninformative due to the small numbers of deaths. [Insufficient details were given regarding the data sources, the methods used to estimate the population at risk and the reference cohort to assess the validity of the study.]

Sakabe *et al.* (1975) reported mortality among 2178 coke-oven workers in Japan who retired (at age 55 years) between 1947 and 1973 from 11 companies, and who were followed for mortality from 1949 to 1973. Expected numbers of deaths were calculated from the general Japanese male population, with adjustment for age. Overall mortality was not reported. Fifteen deaths from lung cancer were observed in comparison with 11.67 expected [SMR, 1.29; 95% CI, 0.72–2.12]. [The methods for tracing the vital status of the retired workers was not reported.]

Davies (1977) reported mortality among 610 coke production workers who were actively employed in May 1954 at two coke plants located at steel industries in South Wales, United Kingdom, and who were followed for mortality until June 1965. The cohort was identified from personnel registers of the companies that also kept information on job departments. Follow-up was achieved through company records, through mail and National Insurance numbers, and underlying causes of death were obtained from death

Table 2.2. Cohort and record linkage studies of coke production workers

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Kennaway & Kennaway (1947), United Kingdom	Register-based national mortality analysis of all deaths in England and Wales 1921–38	Occupational title	Lung Larynx	Gas stokers & coke-oven chargers	85 37	2.84 [2.27–3.52] 2.13 [1.50–2.93]	
Reid & Buck (1956), United Kingdom	Deaths among coke workers of the National Coal Board from 1949 to 1954 obtained from local or national registers; population at risk estimated from a census.	Job department	Respiratory cancer	Coke-oven workers	14	[1.40 (0.77–2.35)]	The validity of the study design and methods is questionable
Sakabe *et al.* (1975), Japan	2178 coke-oven workers who retired 1947–73 followed for mortality from 1949 to 1973		Lung		15	1.29 [0.72–2.12]	The methods for tracing vital status among the retired workers was not reported

Table 2.2 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Davies (1977), Wales, United Kingdom	601 coke production workers in active employment in 1954 at two plants followed for mortality until 1965	None	Lung		8	[0.82 (0.35–1.62)]	Follow-up period limited
Hurley *et al.* (1983), United Kingdom	6767 male manual workers from 14 BSC works and 13 NSF Ltd works in active employment in 1966–67 followed for mortality until 1979	Oven, part-oven, non-oven work; duration of employment	Lung	Non-oven work Part-oven work Oven work	65 33 66	[0.93 (0.71–1.18)] [1.22 (0.84–1.72)] [0.99 (0.76–1.26)]	The period of follow-up was short
Wu (1988), China	21 995 coke plant workers in the steel industry employed before 1965 followed for mortality from 1971 to 1982	Job department	Lung	Entire cohort Coke oven workers	93 40	[2.55 (2.06–3.13)] [4.97 (3.55–6.77)]	Description of methods insufficient

Table 2.2 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Swaen *et al.* (1991), Netherlands	5659 male workers employed for at least 6 months at any of three plants in 1945–69 followed for mortality up to 1984	Job department (coke plant or by-product plant)	Lung	By-product plant	104	1.0 [0.82–1.21]	
				Coke oven	62	1.29 [0.99–1.65]	
			Bladder	By-product plant	20	1.37 [0.84–2.12]	
				Coke oven	7	0.98 [0.39–2.02]	
			Skin	By-product plant	4	15.5 [0.42–3.97]	
				Coke oven	0	–	
Chau *et al.* (1993), Lorraine, France	536 manual workers who retired from two coke plants between 1963 and 1982 followed for mortality up to 1987	Work area	Lung	*All areas*	25	2.38 [1.54–3.51]	Adjustment for tobacco smoking by an indirect method gave ambiguous results
				Coke ovens	2	1.75 [0.21–6.32]	
				Near ovens	8	2.52 [1.09–4.97]	
				By-product area	2	2.37 [0.29–8.56]	
				Workshops	6	4.33 [1.59–9.42]	
				Unexposed areas	6	2.28 [0.84–4.96]	
Franco *et al.* (1993), Carrara, Italy	538 male production workers employed >1 year in 1960–85 followed for mortality up to 1990		Lung		19	1.70 (1.02–2.65)	

Table 2.2 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Costantino *et al.* (1995); Lloyd & Ciocco (1969); Lloyd *et al.* (1970); Lloyd (1971); Redmond *et al.* (1972); Redmond (1983)	5321 coke-oven workers from 12 plants in the USA and Canada in employment any time in 1951–55 (or for two plants, in 1953) followed for mortality up to 1982	Information on job histories, department, work tasks and occupational hygiene measurements used to calculate the individual cumulative exposure to CTPV	Lung	*Years as coke-oven worker* 0 1–5 5–9 10–15 15–19 >20	 203 39 18 26 47 125	 1 (–) 1.33 (0.92–1.89) 1.37 (0.92–2.51) 1.82 (1.26–2.99) 2.91 (2.27–4.52) 2.71 (1.76–2.85)	The use of other steel workers as a reference cohort may reduce the potential for bias due to tobacco smoking, and the clear exposure–response relationship precludes tobacco smoking as an explanation. Especially high risks were noted among non-whites, who also held the most highly exposed jobs

Table 2.2 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Bye *et al.* (1998), Norway	888 male workers employed >1 year in 1962–88 were followed for mortality and cancer incidence up to 1993.	Individual job histories and personal exposure measurements for PAH	Lung	**Lung**		**SIR**	Lagged results
				Unexposed	7	0.82 (0.33–1.70)	
				Cumulative PAH ($\mu g/m^3 \times y$)	5	0.76 (0.25–1.77)	
				<50	0	0 (1.1 expected)	
				50–149	0	0 (0.5 expected)	
				≥150	2	4.82 (0.58–17.41)	
			Stomach	**Stomach**			
				Unexposed	9	2.22 (1.01–4.21)	
				Cumulative PAH ($\mu g/m^3 \times y$)	6	1.72 (0.63–3.74)	
				<50	1	2.74	
				50–149	1	7.40	
				≥150	1	12.61	

BSC, British Steel Corporation; CI, confidence interval; CTPV, coal-tar pitch volatiles; NSF, National Smokeless Fuels; PAH, polycyclic aromatic hydrocarbon; SIR, standardized incidence ratio; SMR, standardized mortality ratio

certificates. Follow-up was complete for all 610 workers. [The follow-up period was short.] Expected numbers of deaths for the entire follow-up period were computed by the person–year method using death rates for England and Wales in 1958–61. A total of 82 deaths occurred in the cohort [SMR, 0.86; 95% CI, 0.68–1.06], eight of which were from lung cancer [SMR, 0.82; 95% CI, 0.35–1.62].

Hurley *et al*. (1983) reported mortality in two cohorts that together constituted a large part of all British coke workers. One cohort comprised all male manual workers at coke production plants owned by the British Steel Corporation (BSC), who were actively employed for the full period 1 January 1966 to 31 July 1967. The second cohort comprised all male manual workers in coke production plants owned by National Smokeless Fuels Limited (NSF) who were employed on 1 January 1967. Information on work histories, including job departments, was obtained from company registers. In total, 6767 men from 14 BSC and 13 NSF works in England, Scotland and Wales were included and followed up to 1979. Follow-up was achieved through population registers. SMRs were calculated by the person–year method using national death rates to calculate expected numbers of deaths. Surveys of tobacco smoking habits were performed among those employed by the BSC in 1971–73 and by the NSF in 1968 and 1973. Follow-up was completed for all but 159 men (2.3%) who were excluded from the analyses which were performed on the remaining 6608 men. Four hundred and three deaths occurred in the BSC cohort and 734 deaths in the NSF cohort, giving a total of 1137 deaths during the follow-up period; 480.2 and 802.5 deaths were expected, respectively, giving a combined SMR for both cohorts of 0.89 (95% CI, 0.84–0.94); 167 deaths were from lung cancer (SMR, 1.17; 95% CI, 1.00–1.37). A subdivision of the cohort into the categories 'non-oven work' (SMR, 0.93; 95% CI, 0.71–1.18; n=65 deaths), 'part oven work' (SMR, 1.22; 95% CI, 0.84–1.72; n=33 deaths) and 'oven work' (SMR, 0.99; 95% CI, 0.76–1.26; n=66 deaths) showed no evidence of a dose–response pattern. Based on a small subset, no trend in the SMR for lung cancer was observed with duration of employment. [The limited data on tobacco smoking did not permit an assessment of the contribution of smoking habits to the increased risk for lung cancer.]

Wu (1988) reported summary results for a number of occupational epidemiological studies in the People's Republic of China. Mortality in a cohort of 21 995 workers employed before 1965 in 19 coke plants in the metallurgical industry in China was compared with that among workers in a primary rolling mill in the steel industry from 1971 to 1982. Among the coke plant workers, 837 deaths occurred (SMR, 1.33; 95% CI, 1.24–1.42); 93 deaths from lung cancer (SMR, 2.55; 95% CI, 2.06–3.13) occurred in the cohort and 40 occurred among the coke-oven workers (SMR, 4.97; 95% CI, 3.55–6.77). The highest risk was observed in workers on top of the oven. [It is not possible to assess the validity of the study from this short report.]

Swaen *et al*. (1991) reported the findings of a cohort study of 5659 male workers who had been employed for at least 6 months at any of three Dutch coke plants at any time between 1945 and 1969; 5740 workers who had been employed at a fertilizer plant during the same period were used as a reference cohort. Information on job histories was

obtained from company records, and study and reference cohorts were followed for mortality up to 1984, using company registers, registers of retired workers or municipal population registers. Although access to causes of death for individuals in the cohort was denied for reasons of confidentiality, numbers of deaths from different causes were obtained from the Central Bureau of Statistics. Mortality in both the study and reference cohort was compared with that of the general male Dutch population by calculation of SMRs, using the person–year method. Of the 5659 coke plant workers, 1374 had died, 127 had emigrated and four were lost to follow-up. The coke plant workers had worked either at the coke ovens or at the by-product plant. There were 520 deaths among the coke-oven workers (SMR, 1.19; [95% CI, 1.09–1.29]) and 854 among the by-product workers (SMR, 0.97; [95% CI, 0.91–1.04]). Among the coke-oven workers, 62 deaths from lung cancer (SMR, 1.29; 95% CI, 0.99–1.65), seven deaths from bladder cancer (SMR, 0.98; 95% CI, 0.39–2.02) and no deaths from skin cancer occurred. The SMR for lung cancer among the coke by-product workers was 1.00 [95% CI, 0.82–1.21] and that among the fertilizer plant workers was 0.87 [95% CI, 0.71–1.05].

Chau *et al*. (1993) investigated mortality among male manual workers who had retired from two coke plants in Lorraine, France, between 1 January 1963 and 31 December 1982. The 536 workers identified were followed for mortality from retirement until 31 December 1987. A report by Bertrand *et al*. (1987) included follow-up of the same cohort until 1982. Underlying causes of death were sought from medical records from hospitals, occupational physicians and general practitioners. Work histories were obtained from company and occupational health records, as well as from foremen. SMRs were calculated by the person–year method using the French male population to obtain expected numbers. Data on tobacco smoking (ever smoker/nonsmoker) were collected from medical records, next of kin or from the individuals themselves and were obtained for 87% of the cohort. By the end of follow-up, 182 deaths had occurred (SMR, 1.41; 95% CI, 1.21–1.63), of which 25 were from lung cancer (SMR, 2.38; 95% CI, 1.54–3.51). A subdivision by work area showed that two lung cancer cases had worked at the ovens (SMR, 175; 95% CI, 21–632) and eight cases had worked near the ovens (SMR, 2.52; 95% CI, 1.09–4.97). Workshop workers had an SMR of 4.33 (95% CI, 1.59–9.42; six cases), by-product workers had an SMR of 2.37 (95% CI, 0.29–8.56; two cases) and non-exposed workers had an SMR of 2.28 (95% CI, 0.84–4.96; six cases). The authors noted that the Lorraine region had a 40% higher rate of death from lung cancer than the national average, on which the expected numbers were based. [The Working Group noted that an indirect adjustment for tobacco smoking habits gave ambiguous results; the proportion of smokers among coke plant workers (74%) was lower than the national average (80% smokers), but adjustment for tobacco smoking habits reduced the SMR for lung cancer to close to unity. It remains unclear whether the findings were confounded by tobacco smoking.]

Franco *et al.* (1993) investigated mortality among 538 male production workers who had been employed for at least 1 year between January 1960 and December 1985 at a coke plant in Carrara, Italy, which first started to operate in 1943 and was closed down in

1989. The cohort was identified from company records and was followed for mortality from January 1960 to December 1990. Vital status and causes of death were traced via municipal registers. SMRs were calculated according to the person–year method, using national, regional (Tuscany) and provincial (Massa Carrara) mortality rates to calculate the expected numbers. Follow-up was complete for 98.1% of the cohort. At the end of follow-up, 118 persons had died (SMR, 1.04; 95% CI, 0.86–1.25) (using regional reference rates), and 19 deaths from lung cancer occurred (SMR, 1.70; 95% CI, 1.02–2.65) (regional rates). Applying reference rates obtained from the local area (Massa Carrara), the SMR was reduced to 1.47 (95% CI, 0.89–2.30). Work histories were complete only for a subset of the cohort, and it was not possible to investigate SMRs by department in the plant. No data on tobacco smoking habits were available.

A cohort study of over 59 000 steel workers from seven plants in Allegheny County, OH (USA) was first reported by Lloyd and Ciocco (1969) and included all steel workers who were employed in 1953. Subdivision by work area showed a high risk for lung cancer among the 2552 coke-oven workers (Lloyd *et al.*, 1970; Lloyd, 1971). An update and extension of the study that focused on coke plant workers was reported by Redmond *et al*. (1972), and included 1356 workers from the two coking facilities of the original cohort and 4661 coke plant workers (all workers who had been employed any time between 1951 and 1955) in 10 plants in the USA and Canada. A further update was published by Redmond (1983). The most recent report (Costantino *et al*., 1995) covered all coke-oven workers from the original study (two coke-oven facilities) as well as those from the new cohort to give a total cohort of 5321 persons. Work histories and vital status were updated through to 1982. Similar procedures were used for cohort identification and follow-up for both cohorts. Company registers were used to identify individuals and work histories and company and federal registers were used to assess vital status. Underlying causes of death were obtained from death certificates. Follow-up for the original cohort of steel workers (in 1966) was complete for all but 61 workers (0.1%), while 40 of 3305 men (1.2%) were lost to follow-up in the new cohort (Redmond *et al*., 1972). Mortality among coke-oven workers was compared with a reference cohort, individually matched (2:1) with regard to race, age, plant and start of hire, that was selected from among non-oven workers at the steel industries involved. The relative risk for death among coke-oven workers versus the reference cohort was estimated by the Mantel-Haenszel procedure. There were 2291 deaths among coke-oven workers (relative risk, 1.08; 95% CI, 1.02–1.14), of whom 255 had died from lung cancer (relative risk, 1.95; 95% CI, 1.59–2.33). Excess mortality was also noted for prostatic cancer (58 deaths; relative risk, 1.57; 95% CI, 1.09–2.30). The earlier report by Redmond *et al*. (1972) regarding the original Allegheny cohort identified that work on top of the ovens was especially hazardous with regard to risk for lung cancer. The extended study that included follow-up through to 1982 showed a positive association between the risk for lung cancer and number of years as a coke-oven worker. The relative risks for lung cancer in categories of increasing duration of employment as a coke-oven worker are shown in Table 2.2 (p for trend <0.001). An even more pronounced trend was noted for duration of

work in top-side jobs: 1.0 (reference), 1.67 (95% CI, 1.41–2.51), 2.58 (95% CI, 1.75–4.23), 4.25 (95% CI, 2.91–7.14), 4.45 (95% CI, 2.79–7.56) and 4.34 (95% CI, 2.89–6.97) for categories of duration of 0 (reference), 1–5, 5–9, 10–14, 15–19 and >20 years (p for trend <0.001). A positive trend in relative risk was also noted for cumulative exposure to coal-tar pitch volatiles (CTPV). An analysis of mortality from lung cancer in relation to the period of follow-up indicated that the high risks noted among the coke-oven workers were greater in the period up to 1965 and lower, but still significantly elevated, for the periods 1966–75 and 1976–82. The relative risk for lung cancer was higher among non-white coke-oven workers (173 deaths; relative risk, 2.22; 95% CI, 1.70–2.84) than among white coke-oven workers (82 deaths; relative risk, 1.62; 95% CI, 1.20–2.17). Further analysis of risk for prostatic cancer was reported only for all coke oven workers by weighted exposure index and indicated no clear exposure–response pattern (p for trend, 0.11).

An analysis of exposure–response within this cohort was published by Mazumdar *et al.* (1975), using follow-up data up to 1966. Data on hygiene measurements were used to classify jobs with regard to exposure to CTPV, and a personal cumulative exposure of CTPV–years was calculated based on work histories. A positive association between cumulative exposure to CTPV and risk for lung cancer was found for non-white, but not for white, coke-oven workers. Similar findings were made when exposure–response was further investigated by Moolgavkar *et al*. (1998) using follow-up data until 1982 and based on more detailed work histories. There was a positive association between cumulative exposure to CTPV and risk for lung cancer among non-whites, both in the Allegheny and non-Allegheny part of the cohort, while no evident exposure–response relationship was found among the whites. However, the risk for lung cancer was clearly elevated amongst both white and non-white coke-oven workers in general in comparison with the steelworker reference cohort. The life-time unit risk associated with an exposure to 1 $\mu g/m^3$ CTPV was estimated to be 1.5×10^{-4} (95 % CI, 1.2×10^{-4}–1.8×10^{-4}).

Bye *et al.* (1998) followed mortality and cancer incidence among 888 male workers employed for >1 year in a Norwegian coke plant in 1962–88 until 1993. Exposure to polycyclic aromatic hydrocarbons (PAHs) was assessed based on individual job histories and time-weighted average exposures using personal measurements. Vital status, causes of death and cancer diagnoses were obtained from national registries and expected numbers were computed based on national statistics. During follow-up, 122 deaths occurred (SMR, 0.87; 95% CI, 0.72–1.04); overall, an excess risk for stomach cancer was observed (nine cases; SIR, 2.22; 95% CI, 1.01–4.21), but not for lung cancer (seven cases; SIR, 0.82; 95% CI, 0.33–1.70). In dose–response analyses, the highest SIRs for cancers of the lung and stomach were observed in categories of high cumulative exposure, and the test for trend was statistically significant for stomach cancer after taking into account a latency period of 20 years.

2.1.3 *Coal-tar distillation* (Table 2.3)

Relatively few studies distinguish tar distillation workers in particular.

An early study by Henry *et al.* (1931) examined 11 429 male deaths from cancer of the urinary bladder and prostate in relation to last recorded occupation (as given on the death certificate) of 13 965 cases that occurred in England and Wales from 1921 to 1928. Expected numbers of deaths were calculated from death rates for cancers of the urinary bladder and prostate in 5-year age groups and were applied to the population of workers in each 5-year age group according to census occupation tables. Among 2665 tar-distillery workers, four deaths from urinary bladder cancer were observed with 1.2 expected, and one death from prostatic cancer with 1.2 expected. [Different sources were used to obtain occupational titles for the numerator and denominator of the occupational death rates, which may have caused bias in the estimated risks.]

Henry (1946) described a large series of epitheliomatous ulcerations or cancers of the skin that were notified as occupational diseases during 1920–43 in England and Wales. The second largest occupational category cited was tar distillers that comprised 767 cases among a total of 3333 notifications. Of these, 451 were on the head and neck, 195 on the hands and arms and 152 on the scrotum. Mortality rates for scrotal cancer were estimated using deaths over the period 1911–38 and the total working population in the relevant job categories taken from the 1921 and 1931 censuses. Tar-distillery workers showed one of the higher rates, with a mortality rate of 213 per million based on 31 cases, compared with a rate in the general population of 4.2 per million. [The Working Group noted that these rates were not age-adjusted, that there may be better case identification and diagnosis in jobs with known tar exposure and that job classification data differed for the numerator and denominator. However, in view of the 50-fold difference in rates, many of these cases can reasonably be attributed to exposure to coal-tar pitch.]

Schunk (1979) reported on the mortality of 76 male blue-collar tar-distillation workers in Gotha, German Democratic Republic. Between 1962 and 1972, on average 38 were employed on an annual basis and, by 1972, a total of 38 had retired. Eight deaths, including seven deaths from cancer (four from lung cancer and two from stomach cancer), were observed. The duration of employment of the deceased workers varied between 7.9 and 15.5 years. In comparison with workers at a local rubber manufacturing plant or with the national reference population, the mortality from cancer and lung cancer was increased approximately threefold. [The Working Group noted that only crude mortality rates were reported.]

Maclaren and Hurley (1987) followed 259 British tar-distillery workers, who represented all those employed at four tar distillation plants on 1 January 1967, until 1983. Occupational histories were extracted from employment records from date of hire to 1975. All but four were successfully traced through the national health service central register, which left 255 for analysis. Comparisons were made with appropriate regional mortality rates to compute SMRs. Twelve lung cancers were observed (7.5 expected; SMR, 1.60 [95% CI, 0.83–2.79]) and three urinary bladder cancers (0.7 expected; SMR,

Table 2.3. Cohort and record linkage studies of coal-tar distillation workers

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Henry *et al.* (1931), England and Wales	11 429 male deaths from cancer of the urinary bladder and prostate that occurred in 1921–28, among whom 2665 had been tar distillery workers	Last recorded occupation as given by the death certificate	Urinary bladder	Tar-distillery workers	4	[3.33 (0.91–8.53)]	Occupational titles for the numerator and denominator came from different sources.
			Prostate		1	[0.83 (0.02–4.64)]	
Henry (1946), England and Wales	1638 male deaths from scrotal cancer that occurred in 1911–38	Last recorded occupation as given by the death certificate	Scrotal cancer			*Crude mortality rates*	Crude mortality rates for the period 1911–38
				General population	1638	4.2/million	
				Tar-distillery workers	31	213/million	
Maclaren & Hurley (1987), England	259 British tar-distillery workers employed at 4 tar distillation plants on 1 January 1967, followed from 1967 to 1983; 4 lost to follow-up		Lung	Tar-distillery workers	12	1.60 [0.83–2.79]	
			Bladder		3	4.29 [0.88–12.5]	

Table 2.3 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Moulin *et al.* (1988), France	963 men employed on or after 1 January 1970 in a tar distillery and who had worked >1 year followed up to 1984; follow-up 100% complete		Lung Larynx Buccal cavity and pharynx	Tar-distillery workers	5 0 10	0.70 (0.23–1.64) (2.4–3.5 expected) 2.94 (1.41–5.41)	Causes of death were not similarly retrieved for people included in the cohort (contact with treating physicians) and for referent rates (death certificates).
Swaen *et al.* (1991), The Netherlands	5659 male workers employed for at least 6 months at any of three plants in 1945–69 followed for mortality up to 1984	Job department (coke plant or by-product plant)	Lung	Tar-distillery workers	NA	1.54 (not significant)	
Swaen & Slangen (1997), The Netherlands	907 male tar-distillery workers employed >6 months between January 1947 and January 1980, identified from pension records; followed up to 1988; 4.2% lost to follow-up for roofers and tar distillers together	Job histories	Lung Urinary bladder Kidney	Tar-distillery workers	48 2 0	1.18 (0.87–1.57) 0.55 (0.06–2.01) (2.4 expected)	No exposure data were reported.

CI, confidence interval; NA, not available; SMR, standardized mortality ratio

4.29 [95% CI, 0.88–12.5]). The SMRs for lung cancer increased with increasing time since hire (p for trend = 0.25). Nested case–control studies were carried out to investigate associations with specific job categories, but these were reported to be non-significant. [The Working Group noted that numbers were small and that the power to subdivide these small case groups was low.]

Moulin *et al.* (1988) studied the mortality of a cohort of 963 men who had worked for at least 1 year in a tar distillery and who had been employed on or after 1 January 1970. The population was followed up to 1984 by contacting the company, physicians, family and registers from the place of birth. Follow-up was 100% successful. Information was sought from next of kin so that treating physicians could be contacted to establish the cause of death. Proportionate mortality ratios (PMRs) and SMRs were computed using national reference rates; SMRs were also computed using local ('département') rates; and a nested case–control study was carried out. Five deaths from lung cancer occurred and expected rates varied from 10.7 in the PMR analysis to 7.1 and 9.4 in the SMR analyses (SMR relative to national rates, 0.70; 95% CI, 0.23–1.64). There were no cases of laryngeal cancer (2.4–3.5 expected in the various analyses), but a significant excess of cancer of the buccal cavity and pharynx was observed (10 deaths; SMR relative to national rates, 2.94; 95% CI, 1.41–5.41). [The Working Group noted that the method of retrieving cause of death for the cohort may have led to some misclassification of deaths relative to the assignment of cause by death certificate for cause-specific reference rates.]

In a study of the mortality of coke plant workers (described in detail in Section 2.1.2), Swaen *et al.* (1991) reported results for a group of tar-distillery workers. An SMR of 154 was reported for lung cancer among the tar-distillery workers; however, this was stated to be non-significant [no observed or expected numbers were given; p-value not stated].

Swaen and Slangen (1997) reported on a cohort 907 tar-distillery workers who were identified from pension records and met the criterion that they had been employed for at least 6 months between January 1947 and January 1980. Women and workers with foreign nationality were excluded. Subjects were traced through municipal registers, with a no-trace rate of 4.2% (for roofers and tar distillers combined). Follow-up was carried out through to 1988. Expected deaths were calculated using Dutch national death rates. Job histories were available for all tar-distillery workers. Forty-eight deaths from lung cancer occurred (SMR, 1.18; 95% CI, 87–157), two deaths from urinary bladder cancer (3.6 expected; SMR, 0.55; 95% CI, 0.06–2.01) and no death from kidney cancer (2.4 expected).

Letzel and Drexler (1998) described an extended case series of skin tumours among German tar-refinery workers. The occupational health records of 606 workers who had been recorded as having had tar dermatitis between 1946 and 1996 were searched for a mention of tumours; 4754 skin tumours had been identified and surgically removed up to the end of 1996, of which 90% (4280) had histological diagnoses. Most (2456) were keratoses that were classified as precancerous lesions. Among the various histologies, 380 squamous-cell carcinomas, 218 basal-cell carcinomas and 182 keratoacanthomas were reported. Some cases had multiple tumours, and one individual was reported to have

had 88 squamous-cell carcinomas over the period of observation. The 598 squamous-cell and basal-cell carcinomas occurred in 207 persons (34.2% of the total). The authors noted that the ratio of squamous- to basal-cell carcinomas was 1.7:1 in contrast to a ratio of 1:10 in the German population. Most of the tumours occurred in areas that had been in contact with the tar or tar fumes, notably the facial area, forearms and hands. In addition, 20 squamous-cell carcinomas of the scrotum were reported. Latency from first exposure to diagnosis ranged up to 69 years with a median latency of 28 years for squamous-cell carcinoma and 35 years for basal-cell carcinoma. [The size of the population within which these 606 individuals with dermatitis were reported was not given. However, the large proportion of subjects with at least one malignant tumour, the high proportion of squamous-cell carcinomas and the occurrence on exposed areas suggest that occupational exposures at this tar refinery represented a risk factor for these tumours.]

2.1.4 *Roofing and paving* (Table 2.4)

Roofing and flooring, and paving of roads have involved the use of bitumen and coal-tar pitch. Coal-tar pitch has been phased out in most countries and, in some areas, bitumen has been used exclusively because of local availability. Some studies of road pavers and roofers include workers who have been only exposed to coal-tar pitch only or to both bitumen and coal-tar pitch and both are considered here. Other studies which are largely or completely restricted to bitumen exposure are not reviewed here and they will be addressed in a later monograph on bitumen (Maizlish *et al*., 1988; Bender *et al*., 1989; Hansen, 1989a,b, 1991; Watkins *et al*., 2002).

Kennaway and Kennaway (1947) analysed mortality from lung and laryngeal cancer in 1921–38 in England and Wales using the occupation recorded on the death certificate and occupational data from the census to calculate expected deaths by occupation. In the category ‘paviours, street masons, concretors, asphalters’, 26 deaths from lung cancer occurred (SMR, 1.64; [95% CI, 1.07–2.40]) and 13 deaths from laryngeal cancer occurred (SMR, 1.40; [95% CI, 0.75–2.39]). [The Working Group noted that this SMR is vulnerable to some numerator/denominator bias. While the proportion of asphalters in this group was unknown, it was believed that, during those years, coal tar would have been used in asphalting tasks.]

A 12-year mortality study was carried out on 5939 members of a roofers’ union in the USA who were alive in 1960 and who had had at least 9 years of union membership; 5788 were traced through to 1971 (Hammond *et al*., 1976). Employment as a roofer in the USA entails the application of hot pitch or hot bitumen. Coal-tar pitch was used more frequently than bitumen in the past but bitumens are now more commonly used. Most of the men were reported to have worked with both substances. Expected numbers of deaths were computed on the basis of US life-tables specific for age and single calendar year. The SMR for all deaths was 1.03 in the first 6 years of the study and 1.10 in the second 6 years. The SMRs for lung cancer generally increased with increasing time since

Table 2.4. Cohort studies of asphalting and roofing workers

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Kennaway & Kennaway (1947), United Kingdom	Register-based national mortality analysis of all deaths in England and Wales, 1921–38	Occupational titles from death certificates	Lung	Paviours, street masons	26	1.64 [1.07–2.40]	
			Larynx	Concretors, asphalters	13	1.40 [0.75–2.39]	
Hammond *et al.* (1976), USA	5939 members of a roofer's union alive in 1960 with >9 years of union membership, among whom 5788 were followed up to 1971		Lung	*Years since joining the union*			No data on tobacco smoking
				9–19	22	0.92 [0.58–1.39]	
				20–29	66	1.52 [1.17–1.93]	
				30–39	21	1.50 [0.93–2.29]	
				≥40	12	2.47 [1.28–4.32]	
			Oral cavity, larynx and oesophagus	≥20 years since joining the union	31	1.95 [1.32–2.76]	
			Stomach	≥20 years since joining the union	24	1.67 (1.07–2.49)	
			Leukemia	≥20 years since joining the union	13	1.68 (0.89–2.87)	
			Urinary bladder	≥20 years since joining the union	13	1.68 (0.90–28.8)	
			Non-melanoma skin cancer	Entire cohort	5	[4.24] [1.38–9.89]	

Table 2.4 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Menck & Henderson (1976), Los Angeles County, USA	3938 white men who had died from or were carrying a lung cancer selected from the county cancer registry for the periods 1968–70 for mortality and 1972–73 for morbidity data	Last recorded occupation and industry of employment from death certificates for mortality data and hospital records for incident cases	Lung	Roofers	6	4.96 [1.82–10.80]	No data on tobacco smoking
Milham (1982), Washington, USA	Proportionate mortality analysis of deaths among white male residents in the State of Washington during 1950–79		Bronchus and lung Larynx	Roofers and slaters	53 4	**PMR** 1.61 [1.21–2.11] 2.70 [0.74–6.91]	No data on tobacco smoking. It is not known if workers in Washington State were likely to use coal tar in these jobs
Engholm *et al.* (1991), Sweden	704 male roofers employed in 1971–79, followed for mortality until 1985 and cancer incidence until 1984	Job titles obtained from the registries of Bygghälsan	Lung	Roofers Road paving asphalt workers	4 8	**SIR** 3.62 [0.99–9.27] 1.24 [0.54–2.44]	

Table 2.4 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Hrubec *et al.* (1991), USA	Cohort assembled from a roster of approximately 300 000 veterans who served in the US Armed Forces some time between 1917 and 1940 and who held active government life insurance policies	Mailed questionnaire that inquired about occupation, industry of employment and tobacco use	Respiratory system	Roofers and slaters	4	3.00 [1.16–7.73]	Relative risk was adjusted for age, calendar time, smoking group and amount of smoking
Pukkala (1995), Finland	Finnish 1970 Population Census file (which consisted of 98% of the inhabitants as of 31 December 1970 who completed a questionnaire on occupation, family structure, living conditions) was linked to the Finnish Cancer Registry and followed for cancer incidence in 1971–85	Job histories	Lung	Asphalt roofers	18	**SIR** 3.25 (1.92–5.13)	Social class; crude SIR was not modified significantly after adjustment for social class

Table 2.4 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Swaen & Slangen (1997), Netherlands	866 male roofers employed >6 months between January 1947 and January 1980, followed up to 1988 (4.2% lost to follow-up for roofers and tar distillers combined)	Job histories	Lung	Roofers	39	1.31 (0.93–1.80)	No exposure data reported
			Urinary bladder		3	1.15 (0.23–3.37)	
			Larynx		1	1.36 (0.02–7.95)	
Stern *et al.* (2000), USA	Proportionate mortality analysis of 11 370 male deaths identified among unionized roofers and waterproofers; 224 were excluded because of missing death certificate or missing date of entry		Lung			**PMR**	
				Entire cohort	1071	1.39 (1.31–1.48)	
				Decade of first membership in the union:			
				<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)	
				≥1975		1.69 (1.16–2.39)	
			Larynx	Entire cohort	46	1.45 (1.06–1.93)	
			Urinary bladder	Entire cohort	89	1.38 (1.11–1.70)	

Table 2.4 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Boffetta *et al.* (2003a,b), European study	Cohorts from road paving companies were assembled in Denmark, Finland, France, Germany, Israel, the Netherlands and Norway. In addition, a Swedish cohort drawn from a national surveillance programme for the building industry was included, which was used as a referent population in internal comparisons. Overall, 79 822 workers were included (1 287 209 person–years)	Information on exposure to coal tar was assembled from the published literature, unpublished industrial hygiene measurements and from questionnaires sent to the participating plants used to reconstruct exposure histories	Lung Head and neck	Coal tar	308 104	1.05 (0.93–1.17) 1.11 (0.91–1.34)	Information on workers' smoking habits was not systematically collected

CI, confidence interval; PMR, proportionate mortality ratio; SIR, standardized incidence ratio; SMR, standardized mortality ratio

joining the union: 9–19 years: 22 deaths observed; SMR, 0.92; [95% CI, 0.58–1.39]; 20–29 years: 66 deaths observed; SMR, 1.52; [95% CI, 1.17–1.93]; 30–39 years: 21 deaths observed; SMR, 1.50; [95% CI, 0.93–2.29]; and ≥40 years: 12 deaths observed; SMR, 2.47; [95% CI, 1.28–4.32]. In the group with 20 or more years of membership in the union, increases in SMRs were reported for cancer of the oral cavity, pharynx, larynx and oesophagus (31 deaths observed; SMR, 1.95; [95% CI, 1.32–2.76]), cancer of the stomach (24 deaths observed; SMR, 1.67; [95% CI, 1.07–2.49]), leukaemia (13 deaths observed; SMR, 1.68; [95% CI, 0.89–2.87]) and cancer of the urinary bladder (13 deaths observed; SMR, 1.68; [95% CI, 0.90–28.8]). In the total cohort, five deaths from skin cancer other than melanoma occurred (1.18 expected [SMR, 4.24; 95% CI, 1.38–9.89]). [No data on tobacco smoking were available.]

In a study by Menck and Henderson (1976) of occupational differences in lung cancer rates among white men in Los Angeles County, USA, mortality data for 1968–70 and morbidity data from the county cancer registry for 1972–73 were pooled. The 3938 men under study were classified by their last recorded occupation and the industry in which they were employed. This information was obtained from death certificates for mortality and from hospital records for incident cases. Age-adjusted expected numbers of deaths and incident cases were calculated on the basis of a 2% sample of 1970 census records for Los Angeles County classified by occupation and industry. Among roofers, a significantly increased risk for lung cancer was observed (six deaths observed; SMR, 4.96; [95% CI, 1.82–10.80]). [No data on tobacco smoking were available.]

Milham (1982) conducted a proportionate mortality analysis of deaths among white male residents in the State of Washington during 1950–79. Among roofers and slaters, four deaths from laryngeal cancer (PMR, 2.70; [95% CI, 0.74–6.91]) and 53 deaths from cancer of the bronchus and lung (PMR, 1.61; [95% CI, 1.21–2.11]) occurred. [No data on tobacco smoking were available. It is not known whether workers in Washington State were likely to use coal tar in these jobs.]

Engholm *et al.* (1991) followed 704 roofers and 2572 road-paving asphalt workers who were part of the larger Swedish Bygghälsan cohort (the Swedish Construction Industry's Organization for Working Environment, Safety and Health). These men had had at least one annual medical examination during 1971–79 and were followed for mortality and cancer incidence for an average of 11.5 years. The SIR for lung cancer among roofers and asphalt workers was 3.62 ([95% CI, 0.99–9.27]; four cases) and 1.24 ([95% CI, 0.54–2.44]; eight cases), respectively.

Hrubec *et al.* (1992) reported a smoking-adjusted relative risk of 3.00 ([95% CI, 1.16–7.73]; four cases) for respiratory cancer in a cohort of US veterans who had an occupation as 'roofer and slater'. Follow-up for mortality was conducted from 1954 to 1980.

Pukkala (1995) reported a cancer incidence follow-up study from the Finnish 1970 census (which consisted of 98% of inhabitants as at 31 December 1970 who completed a questionnaire on occupation, family structure, living conditions, etc.). Eighteen lung

cancers occurred among asphalt roofers (unadjusted SIR, 3.50; 95% CI, 2.07–5.53; SIR adjusted for social class, 3.25; 95% CI, 1.92–5.13).

Swaen and Slangen (1997) reported a cohort of 866 roofers who had been employed for at least 6 months between 1947 and 1980 in The Netherlands and who were followed up until 1988. They were identified from pension records and traced through municipal registers; the no-trace rate was 4.2% (for roofers and tar distillers combined). Women and workers of foreign nationality were excluded, and no exposure data were reported. During follow-up, 39 deaths from cancer of the lung (SMR, 1.31; 95% CI, 0.93–1.80), three deaths from urinary bladder cancer (SMR, 1.15; 95% CI, 0.23–3.37) and one death from laryngeal cancer (SMR, 1.36; 95% CI, 0.02–7.95) were observed.

Stern *et al.* (2000) carried out a proportionate mortality analysis of unionized roofers and waterproofers in the USA. A total of 11 370 male deaths were identified. Death certificates could not be retrieved for 72 (0.6%) and date of entry into the union was missing for a further 152; consequently, 11 144 men were available for analysis. PMRs were calculated using race-, age- and calendar year (5-year groups)-specific data for US men. The PMR for lung cancer was 1.39 (95% CI, 1.31–1.48; based on 1071 deaths). PMRs for other sites were: laryngeal cancer, 1.45 (95% CI, 1.06–1.93; based on 46 deaths); and urinary bladder cancer, 1.38 (95% CI, 1.11–1.70; based on 89 deaths). Results were subdivided by decade of first membership in the union (up to 1935, 1935–44, 1945–54, 1955–64, 1965–74 and in 1975 and thereafter). The PMRs for lung cancer were 1.41, 1.70, 1.39, 1.42, 1.53 and 1.69, respectively. [The Working Group noted the absence of a reduction in trend over a time when exposure to coal tar relative to that to bitumen was believed to have a downward trend.]

The largest study of exposure to asphalt/bitumen is a European multicentre cohort study of workers engaged in road paving, asphalt mixing and roofing (Boffetta *et al.*, 2003a,b). Cohorts were assembled in Denmark, Finland, France, Germany, Israel, the Netherlands and Norway from road paving and asphalt mixing companies; a further cohort in Sweden was drawn from a national surveillance programme for the building industry. The study was designed to include workers exposed to bitumen and to minimize the inclusion of those with exposure to coal-tar pitch, although some longer-term workers were exposed to both. The cohort included 29 820 workers employed in paving, roofing, waterproofing and asphalt mixing, 32 245 workers employed in building or ground construction work only and other bitumen-exposed jobs, and 17 757 workers who were not classifiable as bitumen workers. The mean follow-up was for 16.7 years which yielded a total of 1 287 209 person–years of observation. The cohort was also analysed in relation to bitumen, coal tar and other exposures. Exposure histories were developed by assembling information from the literature, unpublished industrial hygiene reports and from questionnaires sent to the participating plants. Exposure to the various agents was classified as ever/never. The expected numbers of deaths used to calculate SMR were computed based on national rates for each country and by Poisson regression for internal comparisons. Of the population, 27.5% was classified as having had some exposure to coal tar (334 509 person–years among those exposed and 861 102 person–years among

those not exposed). Exposure to coal tar was assessed from information on the use of coal tar collected from company questionnaires and from expert judgement. The SMR for head and neck cancer (oral cavity, pharynx and larynx) was 1.11 (95% CI, 0.91–1.34), the SMR for lung cancer for those exposed to coal tar was 1.05 (95% CI, 0.93–1.17; based on 308 cases), while the SMR for those who were not exposed to coal tar was 1.08 (95% CI, 0.98–1.19; based on 431 cases).

2.1.5 *Creosote* (Table 2.5)

O'Donovan (1920) reported three cases of skin cancer in men exposed occupationally to creosote. One of them had been applying creosote to timber for 40 years, and warts had appeared on his hands, legs and behind his ears for 7 years. Additional reports of individual cases have also been published (Cookson, 1924; Shimauchi *et al.*, 2000; Carlsten *et al.*, 2005).

Henry (1946) also calculated crude mortality rates for scrotal cancer for some groups that were considered to be occupationally exposed to creosote. The crude mortality rate for scrotal cancer during 1911–38 for brickmakers exposed to 'creosote oil' was 29 per million men, based on nine verified cases compared with a rate of 4.2 per million for the national average in the United Kingdom and rates of one per million or less for groups not exposed to suspected skin carcinogens. In addition, there were several other creosote-exposed groups who had increased rates based on fewer cases.

Henry (1947) reviewed 3753 (3921 sites) cases of cutaneous epitheliomata reported to the British Medical Inspector of Factories from 1920 to 1945 and reported that 35 cases (39 sites), 12 of which were of the scrotum, had been exposed to creosote. Of these 35 cases, 14 (15 sites) occurred among workers who had treated timber with creosote, nine (11 sites) among people who had handled creosote in storage and 10 (11 sites) among people who had used creosote as a releasing agent for brick moulds.

Axelson and Kling (1983) reported a cohort study of 123 Swedish workers who applied creosote to wood between 1950 and 1980. Fifty of these men were exposed to arsenic in addition to creosote. Among the entire cohort, eight workers died from tumours, whereas 6.0 deaths from tumours were expected from national statistics [SMR, 1.33; 95% CI, 0.58–2.63]. In a subgroup of 21 workers who had been exposed to creosote only for 5 years or longer, three deaths from cancer (leukaemia, pancreas and stomach) were observed compared with 0.8 expected deaths [SMR, 3.75; 95% CI, 0.77–10.96]. [Levels of exposure were not reported.]

Tornqvist *et al.* (1986) conducted a cohort study of 3358 power linesmen and 6703 power station operators in Sweden who were identified in the 1960 census and followed up for cancer incidence between 1961 and 1979. SIRs were calculated in relation to the expected number of cancers for all blue-collar workers in the census. The SIRs for power linesmen showed a deficit for lung cancer (SIR, 0.7; 95% CI, 0.4–1.0; based on 17 cases). Some cancer sites showed non-significant excesses: urinary organs

Table 2.5. Cohort and record linkage studies of creosote workers

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Henry (1946), United Kingdom	Brickmakers in the period 1911–38, considered to be exposed to 'creosote oil' occupationally	Job histories	Scrotum			**Crude mortality rate**	Crude mortality rates, calculated from 1911–38, are approximate as there was no adjustment for age distribution or other confounders
				Brickmakers	9	$29/10^6$	
				General population	1631	$4.2/10^6$	
				Workers not exposed to suspected skin carcinogens	Varies by job title	$\leq 1/10^6$	
Axelson & Kling (1983), Sweden	123 workers who applied creosote to wood followed for mortality between 1950 and 1980	Job histories	Any cancer	Entire cohort	8	[1.33] [0.58–2.63]	Levels of exposure not reported. No adjustment for tobacco smoking
				Workers exposed only to creosote >5 years	3	[3.75] [0.77–10.96]	
Tornqvist *et al.* (1986), Sweden	3358 power linesmen and 6703 power station operators identified in the 1960 Swedish census and followed up for cancer incidence between 1961 and 1979 through linkage to the national cancer registry	Job histories				**SIR (90% CI)**	Stratification for age (5-year groups) and county; findings could be due to chance and there may be other relevant exposures. No adjustment for tobacco smoking. The study population overlaps substantially with that included in Steineck *et al.* (1989)
			Lung	Power linesmen	17	0.7 (0.4–1.0)	
			Urinary organs, excl. kidney		18	1.2 (0.8–1.8)	
			Kidney		15	1.3 (0.8–2.0)	
			Non-melanoma skin cancers		8	1.5 (0.7–2.6)	

Table 2.5 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Steineck *et al.* (1989), Sweden	Swedish 1960 census population was linked to national cancer registry and followed up for cancer incidence between 1961 and 1979	Job–exposure matrix	Urinary bladder	Line workers	48	**RR** 1.35 (1.10–1.79)	Age, socioeconomic group, degree of urbanization; non-differential exposure misclassification was probable therefore may have underestimated the magnitude of association. The authors adjusted for socioeconomic group and degree of urbanization as a proxy for smoking. The study population overlaps substantially with that included in Tornqvist *et al.* (1986)
			Renal pelvis		6	2.13 (0.94–4.80)	
Karlehagen *et al.* (1992), Norway and Sweden	All impregnators exposed to creosote employed >1 year in 1950–75 at 13 plants known to have used creosote regularly followed from 1958 to 1985 in Sweden and from 1953 to 1987 in Norway; 992 workers included in analysis		Lung	Entire cohort	13	**SIR** 0.79 (0.42–1.35)	Authors discussed that a joint effect of sunlight and creosote may be relevant to the excess for non-melanoma skin cancer. Information on individual smoking habits could not be obtained
			Urinary bladder		10	1.11 (0.53–2.04)	
			Non-melanoma skin cancer		9	2.37 (1.08–4.50)	

Table 2.5 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Pukkala (1995), Finland	Finnish 1970 Population Census file (which consisted of 98% of inhabitants as of 31 December, 1970 who completed a questionnaire on occupation, family structure, living conditions) was linked to the Finnish Cancer Registry and followed for cancer incidence in 1971–85	Job histories	Non-melanoma skin cancer	Timber workers	5	**SIR** 4.64 (1.51–10.8)	Social class; crude SIR was not modified significantly after adjustment for social class

Table 2.5 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Martin *et al.* (2000), France	Case–control study nested in a cohort of male workers employed >1 year at a gas and electricity company; 310 lung cancer cases occurring between 1978 and 1989 included, 1225 referents selected from the cohort	An industry-specific job–exposure matrix was used. An index of cumulative exposure was developed and used to categorize study subjects in quartiles	Lung	Exposed to creosote	114	**OR** 1.56 (1.08–2.27)	Socioeconomic status and asbestos exposure; socioeconomic status was used as a proxy for tobacco consumption

Table 2.5 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Wong & Harris (2005), USA	2179 individuals employed at some point between 1 January 1979 and 31 December 1999 in 11 plants in the USA that used creosote to treat wood, followed up to December 2001 for mortality (follow-up 100% complete) Two nested case–control studies of cohort members who died of lung cancer and multiple myeloma. Controls were cohort members who died of other causes and matched to cases by age, plant and gender	Detailed employment histories abstracted from employment records; tobacco use ascertained from fellow workers in the case–control studies	Pancreas Lung Larynx Kidney Multiple myeloma Lung	Hourly-paid employees Routine exposure to creosote-treated products	4 34 2 3 6 38	1.18 (0.32–3.02) 1.34 (0.93–1.87) 1.83 (0.22–6.62) 1.88 (0.39–5.49) 4.0 (1.47–8.73) **OR** 1.45 (0.28–7.51)	Results from the nested case–control studies for the different exposure variables were not significant with wide confidence intervals

CI, confidence interval; excl., excluding; OR, odds ratio; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio

excluding kidney (SIR, 1.2; 95% CI; 0.8–1.8; based on 18 cases), kidney (SIR, 1.3; 95% CI, 0.8–2.0; based on 15 cases) and non-melanoma skin cancer (SIR, 1.5; 95% CI, 0.7–2.6, based on eight cases). As power linesmen are exposed to creosote and other chemicals in addition to electrical and magnetic fields, the authors discussed the possible role of exposure to creosote. [The Working Group noted that the findings could be due to chance and that there may be other relevant exposures.]

Steineck *et al.* (1989) applied a job–exposure matrix to the Swedish 1960 census population, which was linked to the national Swedish Cancer Registry to identify incident cases of urothelial cancer during 1961–79. When subjects classified as having been exposed to creosote were compared with men who had no exposure to any industry-related urothelial carcinogen, there was an increased risk for renal pelvic cancer (relative risk, 2.13; 95% CI; 0.94–4.80, based on six cases) and urinary bladder cancer (relative risk, 1.35; 95% CI; 1.10–1.79, based on 48 cases;). Non-differential exposure misclassification was probable and therefore may have underestimated the magnitude of association. [It should be noted that there is substantial overlap in the study populations of Steineck *et al.* (1989) and Tornqvist *et al.* (1986).]

Karlehagen *et al.* (1992) reported on a follow-up of impregnators exposed to creosote at 13 plants in Norway and Sweden that were known to have used creosote regularly. All those who had been employed for at least 1 year between 1950 and 1975 were included; 34 were excluded because of missing information (mainly loss to follow-up) which left 992 eligible subjects. Cancers were identified through linkage to national cancer registries, with follow-up covering 1958–85 in Sweden and 1953–87 in Norway. In the cohort, 129 cancers occurred (SIR, 0.94; 95% CI, 0.78–1.10), of which 13 were lung cancers (SIR, 0.79; 95% CI, 0.42–1.35), 10 were bladder cancers (SIR, 1.11; 95% CI, 0.53–2.04) and nine were non-melanoma skin cancers (SIR, 2.37; 95% CI, 1.08–4.50). The authors discussed the possibility that a joint effect of sunlight and creosote may be relevant to the excess mortality from non-melanoma skin cancer.

Pukkala (1995) analysed the Finnish cancer registry data during 1971–85 in relation to occupational categories in the 1970 census and found a significant excess of non-melanoma skin cancer among timber workers (SIR, 4.64; 95% CI, 1.51–10.8; based on five cases; adjusted for social class). Heikkilä (2001) commented that these job categories include workers exposed to creosote.

Martin *et al.* (2000) (described in detail in Section 2.1.1) estimated the risk for lung cancer for exposures assessed by a job–exposure matrix in a case–control study nested in the French cohort of gas and electricity workers. For creosotes, the odds ratio was 1.56 (95% CI, 1.08–2.27; based on 50 cases; adjusted for socioeconomic status and exposure to asbestos). [The Working Group noted that the published values of case and control numbers have been transposed in the published paper.]

Wong and Harris (2005) reported a cohort study and nested case–control studies of workers in 11 plants in the USA that used creosote to treat wood, particularly railway ties and utility poles. Individuals employed at some point between 1 January 1979 and 31 December 1999 were included and vital status up to December 2001 was established

from company records and linkage to national death indices. Vital status was established for 100% of the cohort and SMRs were computed on the basis of national rates. In the cohort of 2179 individuals, most (92.2%) were male and most were hourly paid employees (87.2%), whose potential exposure to creosote was much higher than that of salaried employees. For hourly paid employees, the SMR for all causes of death was 0.90 (95% CI, 0.80–1.02) based on 260 deaths; 34 deaths were from lung cancer (SMR, 1.34; 95% CI, 0.93–1.87), two deaths were from laryngeal cancer (SMR, 1.83; 95% CI, 0.22–6.62), three deaths were from kidney cancer (SMR, 1.88; 95% CI, 0.39–5.49), four deaths were from pancreatic cancer (SMR, 1.18; 95% CI, 0.32–3.02) and no deaths from urinary bladder cancer occurred. Furthermore, the SMR for lung cancer did not show a trend with latency. There was a non-significant excess of 'cancer of other lymphopoeitic tissue' (primarily non-Hodgkin lymphoma and multiple myeloma) among hourly paid employees (SMR, 1.79; 95% CI, 0.66–3.90; based on six cases); however, all six cases were multiple myeloma, for which the SMR was somewhat higher (SMR, 4.01; 95% CI, 1.47–8.73).

Two case–control studies nested within this cohort were carried out on lung cancer and multiple myeloma. Up to five deceased controls were selected for each case and matched on plant, 5-year age group and gender. Detailed employment histories were abstracted from employment records and tobacco use was ascertained from fellow workers. Exposure to creosote was investigated by classifying jobs in terms of degree and intermittency of routine exposure to creosote directly or indirectly through handling creosote-treated wood. Results from conditional logistic regression in the nested case–control study of lung cancer found no significant results for variables of exposure to creosote, with non-significant and wide confidence intervals for all odds ratios. Routine exposure to creosote preservatives had an odds ratio of 0.58 (95% CI, 0.11–3.03) for lung cancer cancer while routine exposure to creosote-treated products had an odds ratio of 1.45 (95% CI, 0.28–7.51). The nested case–control study of multiple myeloma involved too few numbers to be informative.

2.1.6 *Aluminium production* (Table 2.6)

Konstantinov and Kuz'minykh (1971) compared cancer mortality rates in two aluminium production plants in the former USSR, one of which used the Söderberg process and the other pre-bake anodes. Expected figures were computed from regional rates. Excesses of all cancers and of lung cancer were reported for the Söderberg-process workers over the 10-year period 1956–66; an increase in the incidence of skin cancer was also reported, particularly in young workers. [No detailed figures were given.]

Konstantinov *et al.* (1974) reported an investigation of mortality from cancer among pot-room workers in three aluminium plants in the former USSR, two of which used the Söderberg process and the other pre-bake anodes. Mortality from cancer in the plants was compared with that of the general population of the cities and provinces in which the aluminium plants were located. Elevated ratios were reported for lung cancer in the two

Table 2.6. Epidemiological studies of aluminium production workers

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Wigle (1977), Québec, Canada	163 350 people from the 1971 Chicoutimi census division of Québec followed for mortality during 1969–73	Aluminium production in the area	Urinary bladder		81	1.59 (1.26–1.97)	Hypothesis that this finding was related to the presence of the aluminium production industry that mainly used the Söderberg process in this area
Thériault *et al.* (1981), Québec, Canada	Case–control study; 81 cases identified in 1970–75 in Chicoutimi district; 81 controls identified in case neighbourhood, matched by age and sex		Urinary bladder			**OR**	Tumours classified on the basis of provincial and hospital records
				Aluminium production workers	25	2.83 (1.06–7.54)	
				Non-smoking aluminium Production workers	NA	1.9 (0.51–7.00)	
				Smoking aluminium Production workers	NA	5.7 (2.00–12.30)	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Gibbs (1985), Canada	5406 men employed on 1 January 1950 in 2 aluminium production plants that used the Söderberg process followed for mortality from 1950 to 1977	Expert assessment of exposure to condensed pitch volatiles ('tar') by occupation	Lung	**Ever exposed**	101	1.43 [1.16–1.74]	
				Years			
				0	30	1.01 [0.68–1.45]	
				1–10	42	0.97 [0.70–1.31]	
				11–20	27	1.72 [1.13–2.50]	
				≥21	32	2.71 [1.85–3.82]	
			Urinary bladder	**Ever exposed**	12	1.60 [0.83–2.79]	
				Years			
				0	1	0.28 [0.01–1.55]	
				1–10	3	0.61 [0.13–1.79]	
				11–20	3	1.88 [0.39–5.48]	
				≥21	6	6.67 [2.45–14.51]	
			Oesophagus and stomach	**Ever exposed**	50	1.52 [1.13–2.01]	
				Years			
				0	17	1.11 [0.65–1.78]	
				1–10	31	1.42 [0.96–2.01]	
				11–20	10	1.43 [0.69–2.63]	
				≥21	9	2.37 [1.08–4.50]	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Thériault *et al.* (1984); Armstrong *et al.* (1986), Québec, Canada	Case–control study in different regions of Province of Québec where 5 aluminium plants were operating using the Söderberg process; 488 cases identified through the tumour registry and local hospitals during 1970–79, 85 of whom had been employed in the aluminium industry >12 months; three controls matched on age, year of hire in same plant and length of employment		Urinary bladder			**OR**	Similar results after adjustment for smoking
				Söderberg reactor room workers (≥1 year)	45	2.70 (1.64–4.43)	
				Duration (years)			
				<1	40	1.0	
				1–9	12	1.86	
				10–19	12	3.00	
				20–29	18	3.29	
				≥30	3	4.5	
						p for trend <0.05	
				Benzene-soluble material ($mg/m^3 \times years$)			
				0–9	39	1.0	
				10–19	14	2.75 (1.3–5.7)	
				20–29	15	4.81 (2.2–10.6)	
				≥30	17	6.30 (2.8–14.1)	
				Benzo[a]pyrene ($\mu g/m^3 \times years$)			
				0–99	45	1.0	
				100–199	17	5.86 (2.4–14.3)	
				200–299	13	3.78 (1.7–8.4)	
				≥300	10	5.89 (2.2–15.9)	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Tremblay *et al.* (1995), Québec, Canada	Case–control study in aluminium plant using the Söderberg process; 138 incident cases identified from Québec tumour registry and records of one regional hospital; 3 controls matched to each case on age, year of hire at plant and duration of employment	Job–exposure matrix based on estimated exposure to benzene-soluble matter and benzo[*a*]pyrene	Urinary bladder	*Benzene-soluble matter (mg/m^3–years)*		**OR**	Smoking
				0–0.9	22	1.0	
				1.0–9.9	32	1.67 (0.89–3.16)	
				10.0–19.9	23	3.93 (1.85–8.49)	
				20.0–29.9	35	7.31 (3.56–14.99)	
				≥30.0	26	5.18 (2.47–10.89)	
				Benzo[a]pyrene ($\mu g/m^3$–years)			
				0–9.9	35	1.0	
				10.0–99.9	29	1.97 (1.10–3.51)	
				100.0–199.9	26	6.24 (3.00–12.97)	
				200.0–299.9	30	6.66 (3.42–12.99)	
				≥300	18	4.36 (2.10–9.17)	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Armstrong *et al.* (1994), Québec, Canada	Case–cohort study of workers employed >1 year in one plant in 1950–79, followed up 1950–98, ~98% complete; 1138 controls selected from the 16 297 aluminium workers eligible for inclusion	Job–exposure matrix based on estimated exposure to benzene-soluble matter and benzo[*a*]pyrene	Lung	*Benzene-soluble matter (mg/m^3–years)*			
				<1	82	1.00	
				1–9	123	1.15 (0.84–1.59)	
				10–19	54	2.25 (1.50–3.38)	
				20–29	42	1.90 (1.22–2.97)	
				≥30	37	2.08 (1.30–3.33)	
				Benzo[a]pyrene (μg/m^3–years)			
				<10	138	1.00	
				10–99	94	1.48 (1.09–2.00)	
				100–199	41	2.23 (1.46–3.39)	
				200–299	46	2.10 (1.40–3.15)	
				≥300	19	1.87 (1.05–3.33)	
Giovanazzi & D'Andrea (1981), Italy	494 workers in an aluminium production plant using the Söderberg process, during 1965–79 followed up to 1979		Lung	Pot-room workers	4	1.74 [0.47–4.46]	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Rockette & Arena (1983), USA	21 829 workers with >5 years employment in 14 plants between 1946 and 1977 followed up for mortality to 1977	Job histories and process; pre-bake process (2 plants) and Söderberg process (6 plants)	Lung	*Entire cohort*	272	0.96 [0.85–1.06]	Milham (1979) study included; separate analysis of plant 3 which used the pre-bake and the Söderberg process, but job titles did not allow to distinguish between the different processes; plant 11 excluded from analyses by duration of employment because 16% missing data.
				Pre-bake process	161	1.00 [0.82–1.13]	
				Söderberg process	64	0.87 [0.67–1.11]	
				Plant 3 – carbon department >25years of employment	6	3.66 [1.34–7.96]	
				Carbon departments of other pre-bake plants			
			Urinary bladder	*Entire cohort*	19	0.83 [0.50–1.30]	
				Pre-bake process	19	0.78 [0.46–1.18]	
				Söderberg process	11	0.73 [0.35–1.26]	
				>5 years of employment	8	1.62 [0.69–3.15]	
				All workers	6	2.36 (0.46–2.73)	
				White workers	6	4.08 [1.50–8.88]	
			Lymphatic and haemato-poietic system	*Entire cohort*	94	1.09 [0.86–1.30]	
			Lympho-sarcoma and reticulo-sarcoma	*Entire cohort*	22	1.11 [0.68–1.65]	
				1 plant with employment in potroom or carbon department	NA	3.40 (p <0.05)	
			Pancreas	*Entire cohort*	63	1.25 [0.96–1.60]	
				Men employed >15 years			
				Pre-bake process	12	2.22 [1.15–3.88]	
				Söderberg process	17	2.30 [0.92–4.73]	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Mur *et al.* (1987), France	6455 workers who worked >1 year in one of 11 plants between 1950 and 1976 followed up for mortality to 1976; follow-up 95% complete, cause of deaths known for 71.3%		Lung	*Entire cohort*	37	1.14 (0.85–1.48)	Tobacco smoking for workers still employed in 1976 similar between work areas and length of employment *Process-specific data available for only 31% of pot-room workers
				Söderberg process	4	1.36 (0.39–3.46)*	
				Pre-bake process	0	0 (0–5.27)*	
			Urinary bladder	*Entire cohort*	7	2.09 (0.96–3.68)	
Moulin *et al.* (2000), France	2133 men employed >1 year in 1950–94 followed for mortality 1968–94. The plant used both Söderberg and pre-bake processes, but only pre-bake process since 1982	Ever employment in pot-room and other departments with PAH exposure	Lung	*Entire cohort*	19	0.63 (0.38–0.98)	Extended follow-up of one of the 11 plants studied by Mur *et al.* (1987). Analyses by time since first or duration of employment did not indicate any trend.
				Workers with probable PAH exposure	15	0.69 (0.39–1.15)	
			Urinary bladder	*Entire cohort*	7	1.77 (0.71–3.64)	
				Workers with probable PAH exposure	6	2.15 (0.79–4.68)	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Spinelli *et al.* (1991), British Columbia, Canada	4213 men who worked >5 years at a Söderberg plant between 1954 and 1985 followed up from 1959 to 1985	Expert assessment of cumulative exposure to CTPV based on benzene-soluble material	Lung			**SIR**	
				Entire cohort	37	0.97 [0.69–1.34]	
				CTPV (Benzene-soluble matter × years)			
				<1	11	0.72 [0.36–1.29]	
				1–4.9	9	1.02 [0.47–1.94]	
				5–9.9	7	1.14 [0.45–2.33]	
				10–19.9	7	1.24 [0.50–2.58]	
				≥20	3	1.43 [0.29–4.17]	
						p for trend >0.05	
			Urinary bladder	**Entire cohort**	16	1.69 [0.97–2.74]	
				CTPV (Benzene-soluble matter × years)			
				<1	4	1.03 [0.28–2.62]	
				1–4.9	1	0.44 [0.01–2.42]	
				5–9.9	2	1.31 [0.16–4.82]	
				≥10	9	5.0 [2.29–9.49]	
						p for trend <0.01	

Table 2.6 (contd)

Reference, location	Study description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Romunstad *et al.* (2000a), Norway	11 103 men employed >3 years between 1953 and 1996 in 6 aluminium plants in Norway followed up 1953–96	Job–exposure matrix to estimate cumulative exposure to PAH and fluorides	Lung	**Entire cohort**	189	**SIR/rate ratio*** 1.0 (0.9–1.2)	Adjustment for tobacco smoking in 5107 workers (3 of 6 plants) produced similar results.
				Cumulative exposure to PAHs (μg/m³–years)			
				0	93	1.0	
				0–499	38	1.4 (0.9–2.0)	*Lagged 20 years; 'weak association' with exposure to fluorides and bladder cancer, possibly due to some correlation with PAH exposures
				500–1999	30	0.9 (0.6–1.3)	
				≥2000	28	1.0 (0.6–1.5)	
						p for trend >0.5	
			Urinary bladder	**Entire cohort**	130	1.3 (1.1–1.5)	
				Cumulative exposure to PAHs (μg/m³–years)			
				0	52	1.0	
				0–499	20	1.3 (0.8–2.1)	
				500–1999	27	1.3 (0.8–1.9)	
				≥2000	31	1.8 (1.1–2.8)	
						p for trend = 0.02	
Carta *et al.* (2004), Italy	1152 men employed for >1 year between 1972 and 1980 in a pre-bake aluminium smelter and followed up to 2001	Job history and PAH measurements by task and department	Lung		11	0.70 (0.39–1.26)	Increased smoking-adjusted risk for pancreatic cancer and employment in anodes factory (4 exposed deaths)
			Urinary bladder		3	0.79 (0.26–2.44)	
			Pancreas		6	2.41 (1.11–5.23)	
			Lymphomas and leukaemias		8	2.03 (1.03–4.00)	

CI, confidence interval; CTPV, coal-tar pitch volatiles; OR, odds ratio; PAHs, polycyclic aromatic hydrocarbons; PMR, proportionate mortality ratio; SIR, standardized incidence ratio; SMR, standardized mortality ratio

plants that used the Söderberg process and for skin cancer in one of the two. [The absence of information on both the study population and the reference population precludes evaluation of these results. It was not possible to establish whether any of the factories included in the study had already been surveyed in the earlier report by the same authors.]

A series of overlapping studies assessed cancer risk related to aluminium production in Québec, Canada. In a letter, Wigle (1977) reported a high incidence of bladder cancer in the Chicoutimi census division of Québec, where aluminium is produced. During the 5-year period 1969–73, in a population of 163 350 (1971), 81 newly diagnosed cases of urinary bladder cancer were recorded; 51 were expected on the basis of incidence rates for the Province of Québec [SMR, 1.59; 95% CI, 1.26–1.97]. The hypothesis that this finding was related to the presence in that area of an aluminium production industry which is mainly based on the Söderberg process was tested in a case–control study (Thériault *et al*., 1981). In the Chicoutimi census division, 96 cases of urinary bladder cancer were diagnosed between 1970 and 1975 and were identified from records of the Province Tumor Registry and the Regional Hospital. The case–control study was carried out on the 81 men in the group, who were matched by age and sex with neighbours as controls. There was no difference between cases and controls with regard to previous urinary tract disease (22:19), alcohol intake (45:46) or coffee drinking (61:60). The tobacco smoking-adjusted odds ratio for urinary bladder cancer for men who worked in the aluminium production plant was 2.83 (25 exposed cases; 95% CI, 1.06–7.54).

The mortality of 5881 men employed in three Canadian aluminium production plants in Québec that used the Söderberg process was examined between 1950 and 1977 and compared with the pertinent rates for the Province of Québec (Gibbs & Horowitz 1979; Simonato, 1981; Gibbs, 1983, 1985). Cohort 1 consisted of all 5406 workers employed on 1 January 1950 at plant A or C, and cohort 2 included all 485 men employed on 1 January 1951 at plant B. In cohort 1, workers ever exposed to condensed pitch volatiles ('tar') exhibited significantly increased mortality from all cancers (SMR, 1.23; [95% CI, 110–138]; 304 observed) and from oesophageal and stomach cancer (SMR, 1.52; [95% CI, 1.13–2.01]; 50 observed), lung cancer (SMR, 1.43; [95% CI, 1.16–1.74]; 101 observed) and other malignancies (60 observed, 45.3 expected). Analysis of mortality from lung cancer by increasing years of exposure, tar–years of exposure and years since first exposure to tar revealed a statistically significant, increasing trend. Deaths from cancer of the urinary organs (20 observed; SMR, 1.46; [95% CI, 0.89–2.25]) and urinary bladder (12 observed, 7.5 expected; SMR, 1.60; [95% CI, 0.83–2.79]) were more numerous than expected. When mortality from cancer at each of these sites was analysed according to tar–years of exposure, significantly increasing trends were noted. For cancers of the oesophagus or stomach; SMRs increased with increasing tar–years of exposure. Among workers classified as never exposed to tar, mortality was not elevated above that expected for any cancer site.

The risk for urinary bladder cancer was further investigated in a population-based case–control study based on 488 male cases of bladder cancer that occurred in 1970–79 in different regions of the Province of Québec where five aluminium plants were operating

using the Söderberg production process (Thériault *et al.*, 1984). The aluminium companies identified 96 of these men as being current or former employees. After exclusion of 11 cases who were employed for less than 12 months; 85 remained. For each case, three controls were selected matched on age, year of hire in the same company and length of work. Of the 85 cases, 73 came from one plant in Arvida. A statistically significant odds ratio of 2.70 (95% CI, 1.64–4.43; 45 exposed cases) was found for employment in Söderberg reactor rooms. The risk increased steadily with length of time worked in this department; odds ratios ranged from 1.86 for those who had worked for 1–9 years up to 4.50 for those who had worked in the department for over 30 years, a trend that was statistically significant. The risk also increased steadily with increasing estimated exposure to 'tar' and PAHs and remained almost unchanged after adjustment for cigarette smoking and length of employment. This set of data was later re-analysed in an attempt to quantify better the noted exposure–response relationship (Armstrong *et al.*, 1986). More refined quantitative estimates of historical workplace exposure and more complete information on tobacco smoking habits were used. Estimates of risk for urinary bladder cancer were statistically significantly related to three exposure indices: years spent in the Söderberg pot-room, cumulative exposure to benzene-soluble material, an indicator of overall exposure to tar volatiles, and cumulative exposure to benzo[*a*]pyrene, an indicator of exposure to PAHs. It was estimated that an aluminium smelter worker who had been exposed to 0.2 mg/m^3 benzene-soluble material for 40 years had a likelihood of developing urinary bladder cancer that was approximately 2.5-fold that of an unexposed person. Workers who had been exposed to 5 μg/m^3 benzo[*a*]pyrene for 40 years had a likelihood of developing urinary bladder cancer that was approximately fivefold that of an unexposed person. Tobacco smoking did not confound the relationship. Tremblay *et al.* (1995) expanded the previous study and included 138 cases of bladder cancer, 66 cases of the previous study, 69 cases diagnosed between 1980 to 1988 and three additional cases diagnosed before 1980. Three matched controls were taken from the previous studies or selected from a subcohort of 1138 men enumerated by Armstrong *et al.* (1994, see below) drawn mainly from the same subcohort. The smoking adjusted odds ratios increased with years of employment in Södberg rooms and with estimated exposure to benzo[*a*]pyrene and benzene-soluble matter.

Mortality from lung cancer up to 1988 was analysed for exposure–response relationships using a case–cohort design (Armstrong *et al.*, 1994) in a population of men who had worked for at least 1 year between 1950 and 1979 at one plant in Québec, Canada. Cases were identified from the Québec tumour registry, from records of the single local hospital and from the study by Gibbs (1985). A total of 338 deaths from lung cancer (including 131 from the previous study) were observed and were compared with a random sample (subcohort) of 1138 men drawn from the 16 297 aluminium workers eligible for inclusion. Exposure to benzo[*a*]pyrene and total PAHs measured as benzene-soluble material was estimated and relative risks by cumulative exposure were computed. The smoking-adjusted relative risks for lung cancer rose with increasing duration of

employment in Söderberg rooms: rate ratios were 1.0, 1.32, 1.37 and 2.00 for <1, 1–9, 10–19 and 20–41 years of employment, respectively.

Giovanazzi and D'Andrea (1981) examined the mortality of 494 workers in a primary aluminium production plant in Trentino, Italy, that mainly used the Söderberg process during the period 1965–79. Among 212 pot-room workers, 40 deaths (2115 person–years) occurred and, among 282 workers in other departments, 13 deaths (3191 person–years) occurred. Expected figures were computed on the basis of national mortality data and municipal data. In pot-room workers, more deaths than expected were observed for all tumours (14 deaths; SMR, 1.75; [95% CI, 0.96–2.94]) and for liver cirrhosis (seven deaths; SMR, 3.89; [95% CI, 1.56–8.01]); a statistically non-significant excess of lung cancer (four deaths; SMR, 1.74; [95% CI, 0.47–4.46]) was also observed.

Milham (1976) noted an elevated PMR in aluminium workers in Washington State, USA, during the period 1950–71 for all neoplasms and for cancer of the pancreas (PMR, 204) and lymphoma (PMR, 250) among pot-room workers. Mortality was subsequently investigated in a cohort of 2103 men who had worked for at least 3 years, and at least for 1 year between 1946 and 1962, at a pre-bake aluminium plant in Washington State (Milham, 1979). The cohort was followed up for 30 years from 1946 to 1976. Expected figures were computed on the basis of national rates. The SMR for respiratory cancer was 1.17 ([95% CI, 0.82–1.63]; 35 cases). An excess was noted for lymphosarcoma and reticulosarcoma (SMR, 3.16; [95% CI, 1.27–6.51]; 17 cases) and for pancreatic cancer (SMR, 1.80; [95% CI, 82–342]; nine cases). The cohort was divided into exposed and unexposed workers: exposure was defined as those incurred in carbon plants, rodding, pot-lining, pot-rooms and quality control; 'unexposed' workers were defined as mechanics, maintenance workers, electrical workers, yard workers, metal product workers, guards, janitors, storeroom workers, south plant workers, exempt workers, welders, carpenters, pipe-shop workers and masons. The SMR for respiratory cancer was 1.29 ([95% CI, 0.74–2.10]; 16 cases) for exposed and 1.09 ([95% CI, 0.66–1.71]; 19 cases) for non-exposed workers. No cases of urinary bladder cancer were observed (1.0 expected in exposed, 1.5 expected in unexposed). The SMR for lymphosarcoma/reticulosarcoma was 6.43 ([95% CI, 2.36–14.0]; six cases) among 'exposed' workers. [There were no data on tobacco smoking.]

Rockette and Arena (1983) reported a cohort of 21 829 workers who had had 5 or more years of employment in 14 aluminium production plants in the USA between 1946 and 1977, and who were followed up to 1977. The plant studied by Milham (1979) was included (plant 11). The earliest date when production started was 1903. Three types of process were used in the plants: pre-bake (seven plants), vertical-stud Söderberg (one plant) and horizontal-stud Söderberg (five plants). In addition to studying overall patterns of mortality for selected causes of death, a more detailed analysis was made for individual processes in relation to years of cumulative employment. SMRs were used to compare cause-specific mortality of the workers with that of the US male population. Data from plant 3 were analysed separately, since all three processes were used in this plant but, in most cases, no indication was given in the job history of the process in which an

employee worked. Overall, the SMR for lung cancer was 0.96 ([95% CI, 0.83–1.06]; 272 deaths;); among workers in plants using the Söderberg process, 64 deaths from lung cancer occurred (SMR, 0.87; [95% CI, 0.67–1.11]); among workers in plants using the pre-bake process, 161 deaths from lung cancer occurred (SMR, 1.0; [95% CI, 0.82–1.13]); and among the remainder, 47 deaths from lung cancer occurred (SMR, 0.99; [95% CI, 0.72–1.30]). Analyses by plant, work area and cumulative duration of employment were carried out and the authors noted that the only significant excess of lung cancer was for plant 3 in the carbon department for workers with ≥25 years of employment (1.64 expected; SMR, 3.66; [95% CI, 1.34–7.96]; six deaths). The excess was concentrated in the pre-bake process which is reported to have one of the highest exposures to CTPV. However, the SMR for lung cancer for the same area in the remainder of the plants was 0.83 [95% CI, 0.50–1.30], based on 19 observed deaths. For men employed in the pot-room or carbon department of the Söderberg plants for 5 or more years, the SMR for urinary bladder cancer was found to be 2.36 (95% CI, 0.46–2.73; six deaths). This excess occurred in whites, for whom the SMR was 4.08 ([95% CI, 1.50–8.88]; six deaths). Among the 94 deaths from haematolymphopoietic cancers, 22 were from lymphosarcoma and reticulosarcoma; 13 of these occurred in plants 1 and 11 (pre-bake) for which the SMRs were 1.77 and 2.82, respectively, but were not statistically significant. For men in plant 11 who had been employed in the pot-room or carbon department, the SMR was 3.40 (p <0.05). The largest SMR in the haematolymphopoietic category was for leukaemia and aleukaemia (1.28 [95% CI, 0.90–1.68]; 43 deaths). For men who had less than 15 years of cumulative employment in the pot-room or carbon department in plants using the Söderberg process, there was a statistically significant excess (SMR, 2.75; [95% CI, 1.32–5.05]; 10 deaths). Although it was not statistically significant, there was a more than 20% excess of stomach cancer in white workers in both the pre-bake and Söderberg processes. Of the 55 deaths observed in the cohort, 22 occurred in plant 1 (pre-bake) (SMR, 1.74; p <0.05). This excess occurred in men with exposure in the pot-room and time since first employment of at least 30 years (SMR, 2.96; p <0.01). Examination of the work histories revealed no common factor that might explain the excess. Men employed for 15 or more years in the pre-bake and horizontal-stud Söderberg process and in plant 3 had an increased risk for pancreatic cancer (SMR, 2.22; p <0.05; 2.71; p <0.05; and 1.68, respectively). Because an estimated 16% of the cohort from plant 11 (pre-bake) was lost to follow-up, it was not included in the analysis that was based on duration of employment. However, an excess of pancreatic cancer was also seen in the pot-rooms in this plant (SMR, 1.97), as noted by Milham (1979) in a different cohort from the same plant.

Mur *et al.* (1987) reported mortality in a cohort of 6455 aluminium smelter workers who had worked for at least 1 year in one of 11 plants in France between 1950 and 1976, during which time they were also followed up for mortality, giving a total of 113 671 person–years. The earliest date of exposure was 1907, and several of the plants used both pre-bake and Söderberg processes. The majority of workers (about two-thirds) had been employed for more than 10 years and one-third for over 20 years. Vital status was

established for about 95% of the cohort by consulting registry offices at cohort members' birthplaces (for French-born workers) and consulates for foreign workers. Cause of death was ascertained by contacting attending physicians or consulting hospital records. Cause was established for only 71.3% of deaths. Cause-specific deaths were 'corrected' by the ratio of the total number to deaths with a known cause. Expected deaths were based on national rates. Confidence intervals were calculated on the basis of these corrected observed deaths, rounded to nearest integer. [The Working Group noted that the use of different sources of cause of death other than death certificates may lead to misclassification. This may be compounded by the method of compensating for missing information on cause of death.] Tobacco smoking habits were surveyed in workers who were still employed in 1976. The overall SMR for all causes was 0.85, based on 996 deaths, with 37 deaths from lung cancer (SMR, 1.14; 95% CI, 0.85–1.48) and seven from urinary bladder cancer (SMR, 2.09; 95% CI, 0.96–3.68).

In one of the French plants, Moulin *et al.* (2000) updated the study of Mur *et al.* (1987) to cover mortality among 2133 male workers who had been employed in the plant for at least 1 year between 1950 and 1994. The cohort was followed up for mortality for an average of 16.5 years from 1968 to 1994, giving a total of 35 145 person–years. Cause of death was obtained by matching with the national file of all causes of death given on French death certificates. Risks for lung and urinary bladder cancer were reported by time since first employment and duration of exposure and were based on comparison with regional rates. Both pre-bake and Söderberg anodes were used in this plant, although only pre-bake had been used since 1982. Overall, 335 deaths occurred (SMR, 0.81), with 19 deaths from lung cancer (SMR, 0.63; 95% CI, 0.38–0.98) and seven from urinary bladder cancer (SMR, 1.77; 95% CI, 0.71–3.64). Among a subset of workers in workshops where exposure to PAHs was judged to be probable, six urinary bladder cancers occurred (SMR, 2.15; 95% CI, 0.79–4.68). Analyses by time since first employment and duration of employment did not indicate any trend.

Spinelli *et al.* (1991) reported morbidity risks for both lung and urinary bladder cancer by cumulative exposure to CTPV (measured as benzene-soluble material) in a cohort of 4213 men who had worked for at least 5 years at a Söderberg aluminium plant in British Columbia, Canada, between 1954 and 1985. A total of 60 590 person–years were observed, with 2.9% accounted for by 204 men with more than 20 benzene-soluble material–years of exposure. Risks were compared with regional rates, with a 3-year lag period and follow-up from 1959 to 1985 for mortality and from 1970 to 1985 for cancer incidence. Overall, the SMR for all causes was 0.77 (based on 337 deaths), with 32 deaths from lung cancer (SMR, 0.93; [95% CI, 0.64–1.31]) and 37 incident cases (SIR, 0.97; [95% CI, 0.69–1.34]). For bladder cancer, three deaths (SMR, 1.37; [95% CI, 0.28–4.00]) and 16 incident cases (SIR, 1.69; [95% CI, 0.97–2.74]) occurred. There was a significant increasing trend in the incidence of urinary bladder cancer with cumulative exposure to CTPV ($p < 0.01$), and the SIR rose to 5.0 based on nine cases with ≥10 benzene-soluble material–years of exposure. No significant trend was observed for lung cancer, although there was some suggestion of an increase with increasing cumulative exposure; the

highest SMRs of 1.24 (seven cases) and 1.43 (three cases) were found for the highest categories of 10–19.9 and ≥ 20 benzene-soluble material–years. When a lag of 10 years was applied, the corresponding SIRs were 1.53 (seven cases) and 2.23 (two cases). This study was updated and results were available as an abstract (Friesen *et al.*, 2005). Among 6444 men employed at least 3 years between 1954 and 2000, patterns of the incidence of lung cancer and urinary bladder cancer were reported to be more consistently, monotonically increased with cumulative exposure than in the earlier reports, with a significant trend for both cancer outcomes with or without a 20-year lag. There was a twofold risk for lung and bladder in the highest versus lowest category of exposure expressed as CTPV or benzo[*a*]pyrene.

Romundstad *et al.* (2000a) updated the results from previous cohort studies of workers in six aluminium plants in Norway (Andersen *et al.*, 1982; Rønneberg & Andersen, 1995; Rønneberg *et al.*, 1999; Romundstad *et al.*, 2000b,c). Cancer incidence was investigated among 11 103 men who had been employed for more than 3 years between 1953 and 1996, during which time they were also followed up to provide 272 554 person–years. The earliest date at which the plants started production was 1914. A job–exposure matrix was constructed to estimate cumulative exposure to PAHs and fluorides. Information on smoking habits was available for 5107 workers from three of the six plants. No increase in the risk for lung cancer was observed (SIR, 1.0; 95% CI, 0.9–1.2; 189 cases) though an increased risk for bladder cancer was observed (SIR, 1.3; 95% CI, 1.1–1.5; 130 cases). Poisson regression analysis in relation to estimated cumulative exposure to PAHs showed evidence of an increasing trend for cancer of the urinary bladder but not of the lung. The rate ratio for bladder cancer increased with increasing dose (1.0, 1.3, 1.3 and 1.6; p for trend = 0.05) and with 30 years of latency (1.0, 1.0, 1.3 and 2.0; p for trend = 0.03). Results were similar after adjustment for smoking. Further analyses were conducted using a cumulative fluoride measurement and only a 'weak association' with urinary bladder cancer was reported for lag times of <20 years, possibly due to some correlation with exposures to PAHs. [Fluoride has been shown to be associated with urinary bladder cancer in a Danish cryolite mill (Grandjean & Olsen, 2004); however, the evidence for a higher risk in pot-room areas where exposure to PAHs but not necessarily to fluoride is higher points to PAHs as the causal factor rather than fluoride.] Positive exposure–response associations were also reported for cumulative exposure to PAHs and pancreatic and kidney cancer.

Carta *et al.* (2004) followed mortality from cancer up to 2001 in a population of 1152 men who had been employed at a pre-bake aluminium smelter in Sardinia, Italy, for at least 1 year between 1972 and 1980. Results were presented for various sites including the lung (SMR, 0.70; 95% CI, 0.39–1.26; 11 deaths), urinary bladder (SMR, 0.79; 95% CI, 0.26–2.44; three deaths), lymphoma and leukaemia (SMR, 2.03; 95% CI, 1.03–4.00; eight deaths) and pancreas (SMR, 2.41; 95% CI, 1.11–5.23; six deaths). Analyses by increasing rank of exposure to PAHs showed an increasing trend only for pancreatic cancer among these four sites. In a small nested case–control study, employment in the

anodes factory was associated with an increased risk for pancreatic cancer (four exposed cases) after adjustment for smoking.

2.1.7 *Carbon electrode manufacture* (Table 2.7)

Teta *et al*. (1987) reported the findings of a mortality surveillance system at the carbon product department of the Union Carbide Corporation; 2219 white men who were employed at the beginning of 1974 and who had a prior service of ≥10 years were followed for mortality from 1974 to 1983; six of these men were lost to follow up. To assess the potential impact of differences in socioeconomic status and job exposures on the healthy worker effect, three exposure groups were formed based on job tasks: supervisory, office and research (A), crafts (B) and operations, labour and service (C). Vital status was ascertained annually from payroll registers, the company's benefits department and from the Social Security Administration. Underlying causes of deaths were obtained from death certificates. SMRs were calculated according to the person–year method, using death rates among US white men to calculate the expected numbers. In all, 223 of the cohort members had died at the end of follow-up (SMR, 0.67; 95% CI, 0.55–0.72). The number of deaths was small for all types of cancer (SMR, 0.93; 95% CI, 0.74–1.17; 78 deaths) and for respiratory cancer (SMR, 0.85; 95% CI, 0.57–1.21; 29 deaths). An internal analysis of mortality by job category showed that mortality from respiratory cancer was lowest among the low-exposed workers (group A; three deaths; 40% of the average risk in the cohort), highest among the craftsmen (group B; six deaths; 1.3 times the average risk in the entire group) and intermediate among labourers (group C; 20 deaths, 1.2 times the average risk); SMRs were not calculated. There was a non-significant trend of increasing mortality with increasing time since entry into employment. High mortality from respiratory cancer was noted in a particular location (SMR, 3.36; significant at the 5% level; five deaths) where asbestos had been used and where use of coal-tar pitch had been abandoned in 1928. [The Working Group noted a strong healthy worker effect in the cohort.] These five men who had died from respiratory cancers had smoked cigarettes, had been hired after 1934 and had worked at that location for at least 25 years; however, these data are insufficient to confirm that exposure to asbestos and tobacco were involved in the etiology of their death.

Moulin *et al*. (1989) reported the findings of an epidemiological study of two carbon electrode manufacturing plants in France. In plant A (which produced graphite electrodes), all male workers employed at the beginning of 1975 were followed for cancer incidence in 1975–85. In plant B, all workers employed at the beginning of 1957 were followed for mortality in 1957–84. Cohort definitions and outcome (mortality or incidence) were chosen depending on the availability of data. Causes of death were obtained from attending physicians. SIRs for plant A were computed by the person–year method. Local cancer rates were not available, and cancer rates for the population from an adjacent district were used, after verification that cancer mortality did not differ substantially between the two districts. SMRs for plant B were computed using national

Table 2.7. Cohort studies of carbon electrode manufacturing workers

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Teta *et al.* (1987), USA	2213 white men employed at a carbon product department in 1974 and who had a prior service of at least 10 years followed for mortality from 1974 to 1983	Job task (three groups) and job location (11 locations)	Respiratory system	Entire cohort	29	0.85 (0.57–1.21)	Strong healthy worker effect expected with current study design; no data on tobacco smoking
Moulin *et al.* (1989), France	Two carbon electrode plants: plant A: 1302 male workers in employment on 1 January 1975 followed for cancer incidence 1975–85; plant B: 1115 workers in employment on 1 January 1957 followed for mortality 1957–84	Contact with PAH (yes/no) based on job tasks				**SIR**	Stratification by job type and age at hire; an internal analysis taking exposure status and tobacco smoking into account gave low numbers, wide CI and was uninformative
			Lung	Plant A	7	0.79 (0.32–1.63)	
			Urinary bladder		0	0 (0–1.92)	
			Kidney	Plant B	2	1.71 (0.21–6.17)	
			Lung		13	1.18 (0.63–2.01)	
			Urinary bladder		3	1.94 (0.40–5.66)	
			Kidney		0	Not calculated	

Table 2.7 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Gustavsson *et al.* (1995), Sweden	901 blue- and white-collar workers from a graphite electrode manufacturing plant employed >3 months from 1968 to 1988 followed for mortality 1969–89 and for cancer incidence 1969–88	Cumulative exposure to BaP ($\mu g/m^3$ BaP × years) calculated from job histories and hygiene investigations; tobacco smoking habits investigated by means of a questionnaire given to workers employed in 1990	Lung Larynx	Entire cohort	2 deaths 2 cases 2 cases	1.68 (0.20–6.07) **SIR** 1.80 (0.22–6.51) 10 (0.25–55.64)	Local death and cancer rates were used. Survey of smoking habits indicated lower level of smoking than in the general population in the area
Liu *et al.* (1997), China	6635 male carbon electrode workers from six carbon electrode manufacturing companies and one aluminum smelter employed for more than 15 years since January 1971 followed for mortality up to 1985	Degree of contact with carbon compounds was coded from occupational histories and smoking habits were obtained from a questionnaire	Lung	**Entire cohort** *Contact with carbon compounds* None Low Moderate High	50 13 6 5 26	2.16 [1.61–2.85] 1.49 [0.79–2.54] 1.20 [0.44–2.60] 1.52 [0.49–3.54] 4.30 [2.81–6.30]	This study included some aluminium reduction plant workers in addition to carbon electrode manufacturing workers

Table 2.7 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Donato *et al.* (2000), Umbria, Italy	1006 male graphite electrode manufacturing workers employed ≥1 year in 1945–71 followed for mortality in 1955–96	Duration of employment was used as a proxy for cumulative PAH exposure	Lung Larynx Urinary bladder Skin		34 4 7 3	0.77 (0.53–1.08) 0.79 (0.21–2.02) 2.29 (0.47–6.69) 1.04 (0.42–2.14)	Local death rates showed slightly higher SMRs. Detailed data on job titles and smoking habits were not available. Smoking survey in subgroup indicated a somewhat lower proportion of smokers in the cohort than in the population in central Italy.
Mori (2002), Japan	332 male blue-collar workers employed for ≥5 years in 1951–74 at a Japanese graphite electrode manufacturing plant were followed for mortality in 1951–88.	Department and duration of employment	Lung Multiple myeloma	Entire cohort	9 2	2.62 (1.20–4.98) 13.4 (1.62–48.3)	Analysis by time period, department, time since first exposure or duration of employment gave no further information.

Table 2.7 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Merlo *et al.* (2004), Brescia, Italy	1291 male blue-collar workers employed ≥1 year at a graphite electrode plant in 1950–89 and followed for mortality in 1950–97	Duration of employment	Lung		32	0.97 (0.67–1.37)	Very large number of deaths from silicosis was found (79 observed versus 1.19 expected). Information on smoking and drinking habits was not available
			Urinary bladder		5	1.08 (0.35–2.53)	
			Larynx		5	1.32 (0.43–3.07)	
			Lip, mouth, pharynx		8	2.10 (0.91–4.14)	
			Oesophagus		5	2.19 (0.80–4.77)	
			Liver		24	4.19 (2.68–6.23)	
			Lung	1–10 years	2	2.37 (0.29–8.57)	
				10–19 years	5	1.35 (0.44–3.14)	
				>19 years	25	0.88 (0.57–1.3)	

BaP, benzo[*a*]pyrene; CI, confidence interval; PAH, polycyclic aromatic hydrocarbon; SIR, standardized incidence ratio; SMR, standardized mortality ratio

mortality rates, stratified by cause, sex, 5-year groups and calender year. In plant A, 1302 workers were identified and 11.8% were lost to follow-up. In plant, B 1115 workers were identified and 13.5% were lost to follow-up. Those lost to follow-up were assumed to be alive. [The Working Group noted that this procedure would lead to an underestimation of the risk.] Tobacco smoking habits for plant A were obtained from the personnel office. In plant A, 38 cases of cancer were observed during follow-up (SIR, 0.87; 95% CI, 0.61–1.19), seven cases of lung cancer (SIR, 0.79; 95% CI, 0.32–1.63), no case of urinary bladder cancer and two cases of kidney cancer occurred (ICD-8 189; SIR, 1.71; 95% CI, 0.21–6.17). In plant B, 164 deaths were observed (SMR, 0.82; 95% CI, 0.70–0.96); 13 deaths from lung cancer (SMR, 1.18; 95% CI, 0.63–2.01) and three deaths from urinary bladder cancer (SMR, 1.94; 95% CI, 0.40–5.66) occurred, but none from kidney cancer. Nested case–control studies of lung cancer and cancer of the pharynx, larynx and buccal cavity were performed for both plants in relation to occupational exposure to PAHs; however, the numbers of cases were too small and the confidence intervals were too wide to allow for meaningful interpretation.

Gustavsson *et al*. (1995) reported the mortality from and incidence of cancer among 901 employees at a Swedish graphite electrode manufacturing company. The plant started operations in 1968 and was closed down in 1990. All employees who had been employed for at least 3 months between 1968 and 1988 were included in the study, regardless of age, gender or work task; 914 individuals were identified, but 13 could not be traced so the analysis was based on the remaining 901. Follow-up was carried out via national population registers: underlying causes of death were obtained from death certificates and cancer incidence was obtained from the national cancer registry. The cohort was followed from 1969 to 1989 for mortality and from 1969 to 1988 for cancer incidence. SMRs and SIRs were calculated by the person–year method, using national death and cancer incidence rates (standardized for age, gender, calendar time and county) to calculate the expected numbers. Aggregate personal historical exposure to benzo[*a*]pyrene was calculated from assessments of job-specific exposure, based on current and previous hygiene measurements and personal work histories obtained from company records. The number of benzo[*a*]pyrene–years ($\mu g/m^3$ benzo[*a*]pyrene × years) was computed by multiplying the task-specific level of benzo[*a*]pyrene and the duration of exposure, aggregated over the entire work history. At the end of follow-up, 15% of the cohort members had no exposure to benzo[*a*]pyrene, 48% had >0–5 benzo[*a*]pyrene–years, 15% had 5–10 benzo[*a*]pyrene–years and 22% had >10 benzo[*a*]pyrene–years of exposure. Tobacco smoking habits were investigated by means of a questionnaire given to workers who were employed in 1990. Forty-two deaths occurred in the cohort (SMR, 1.30; 95% CI, 0.94–1.76), and 14 incident cases of cancer were observed (SIR, 1.13; 95% CI, 0.62–1.90). Two cases of lung cancer (SIR, 1.80; 95% CI, 0.22–6.51) and one case of laryngeal cancer (SIR, 10; 95% CI, 25–55.64) were observed. The investigation of tobacco smoking habits among those in employment in 1990 showed that graphite electrode manufacturing workers smoked slightly less than the general population in the same region.

Liu *et al.* (1997) reported the mortality among 6635 male workers from various carbon plants in the People's Republic of China. The cohort included workers from six carbon plants that mainly manufactured carbon electrodes and a carbon department and pot-room in an aluminium reduction plant. All workers who had been employed for more than 15 years since January 1971 were followed for mortality up to 1985. Information on occupational history and tobacco smoking habits were obtained from a questionnaire that was completed by the individuals themselves or by next-of-kin for deceased subjects. SMRs were calculated by the person–year method, using the mortality in a cohort of 11 470 Chinese steel workers to calculate the expected numbers of deaths. The level of exposure to 'carbon compounds' was coded from the occupational title of the job in which the employees had worked the longest. In the cohort, 390 deaths occurred (SMR, 1.10; [95% CI, 1.00–1.22]) and 50 persons had died from lung cancer (SMR, 2.16; [95% CI, 1.61–2.85]). There was a positive trend in SMR with increasing contact with carbon compounds (see Table 2.7). An elevated risk for lung cancer remained in the exposed and highly exposed workers when the study was limited to nonsmokers. [The Working Group noted that this study included an unspecified number of aluminum reduction plant workers in addition to carbon electrode manufacturing workers.]

Donato *et al*. (2000) studied the mortality of workers at a plant manufacturing carbon (graphite) electrodes in Umbria, Italy, that had started to operate in 1901. A cohort of 1006 male workers who had been employed for ≥ 1 year in 1945–71 was identified from company registers and followed up for mortality in 1955–96. Vital status was assessed from municipal registers, and 44 (4.4%) of the cohort members were lost to follow-up. SMRs were calculated by the person–year method, using both national and local death rates to compute expected numbers. Poisson regression was used to study the effect of duration of employment and time since first exposure. During follow-up, 424 deaths occurred, giving an SMR of 0.85 (95% CI, 0.77–0.93), and 125 deaths from cancer gave an SMR of 0.84 (95% CI, 0.70–1.00). Thirty-four deaths were from lung cancer (SMR, 0.77; 95% CI, 0.53–1.08), four deaths were from laryngeal cancer (SMR, 0.79; 95% CI, 0.21–2.02), three deaths were from skin cancer (including melanoma) (SMR, 2.29; 95% CI, 0.47–6.69) and seven deaths were from urinary bladder cancer (SMR, 1.04; 95% CI, 0.42–2.14); SMRs were based on national death rates because the Umbria region has slightly lower death rates than Italy as a whole. However, use of the regional reference rates did not change the results substantially. Analysis by duration of employment showed no trend for lung cancer or urinary bladder cancer, but an increased risk for gastric cancer was observed among those who had been employed for >20 years compared with those who had been employed for 1–9 years (based on seven deaths; relative risk, 7.06; 95% CI, 1.27–39.3; *p* value for trend, 0.03). Analysis by time since first exposure showed a positive trend for mortality from all causes and cancer of the lung (10–19 years since first employment: relative risk, 1.38; 95% CI, 0.39–4.80; >20 years since first employment: relative risk, 2.06; 95% CI, 0.49–8.72).

Mori (2002) reported the findings of a cohort mortality study at a graphite electrode manufacturing plant in Japan, which had started to operate in 1934 and was relocated in

1974. All male workers who had been employed for ≥5 years from 1951 to 1974 in the manufacturing, transportation and maintenance divisions of the plant were identified from its labour union registers; 336 workers were identified and four were excluded due to incomplete identification data; 21 of the remaining 332 were lost to follow-up but were assumed to be alive at the end follow-up. The cohort was followed for mortality from 1951 to 1988 via population registers. Underlying causes of death were obtained from death certificates, the attending physician, the families of the deceased or from the municipal cancer register. SMRs were calculated by the person–year method, using Japanese national death rates as well as approximate local rates to compute expected numbers. During follow-up, 52 deaths occurred in the cohort (SMR, 0.68; 95% CI, 0.51–0.89), 22 of which were from cancer (SMR, 1.01; 95% CI, 0.63–1.53) and nine from lung cancer (SMR, 2.62; 95% CI, 1.20–4.98). Analyses by department, time period, time since first exposure and duration of employment gave little additional information due to the small number of workers in these subgroups. A survey of smoking habits in a subset of the cohort, based on 65 skilled workers, was reported. [However, the Working Group questioned the representativity of this survey and noted the possibility that incomplete ascertainment of individuals in the cohort for the early years may have contributed to the low overall SMR.]

Merlo *et al*. (2004) investigated mortality among 1291 male workers at a graphite electrode manufacturing plant in Brescia, Italy, which had started to operate in 1929 and was closed down in 1994. All male blue-collar workers who had been employed for >1 year from 1950 to 1989 were identified from company registers and followed up for mortality in the period 1950–97 through municipal and local health unit registers. SMRs were calculated by the person–year method. Expected numbers were calculated from national death rates, supplemented by analyses based on local death rates for selected causes which were only available for 1970–97. At the end of follow-up, 747 (57.9%) members of the cohort were still alive, 541 (41.9%) had died and three (0.2%) people were lost to follow-up. The 541 deaths gave an SMR for all causes of 1.44 (95% CI, 1.32–1.56); 141 of the deaths were from cancer (SMR, 1.27; 95% CI, 1.07–1.50). There was a marked excess of cancers of the liver (24 deaths; SMR, 4.19; 95% CI, 2.68–6.23). It was noted that numbers of observed deaths were greater than those expected for cancers of the lip, mouth and pharynx as well as oesophagus, but SMRs for these sites were not significantly increased. Thirty-two deaths from lung cancer gave an SMR of 0.97 (95% CI, 0.67–1.37). Five deaths were from urinary bladder cancer (SMR, 1.08; 95% CI, 0.35–2.53) and five were from laryngeal cancer (SMR, 1.32; 95% CI, 0.43–3.07). A very large number of deaths from silicosis occurred (79 observed versus 1.19 expected; SMR, 66.39; 95% CI, 52.56–82.74). Silicosis was mentioned as a contributing cause of death on another 21 death certificates. The use of local death rates to calculate expected numbers generally produced lower SMRs, but these were still significantly elevated for mortality from all causes and from liver cancer. No clear exposure–response pattern was observed for lung cancer. [The Working Group noted that several potential causes of liver cancer, including viral infections (the rates of which are known to be high in the area),

alcohol consumption and other occupational exposures may have contributed to the excess mortality from liver cancer.]

2.1.8 *Calcium carbide production*

Kjuus *et al.* (1986) reported on the incidence of cancer in a cohort of 790 male Norwegian calcium carbide production workers. All workers who had been employed for at least 18 months between 1953 and 1970 were followed for cancer incidence and mortality until 1983. SMRs and SIRs were calculated by the person–year method and expected numbers were calculated from national statistics. Söderberg electrodes were used in the plant, but PAHs were not considered to be a main occupational hazard by the authors. The workers were exposed to calcium carbide and asbestos among other substances. In the cohort, 234 deaths occurred (SMR, 0.93; 95% CI, 0.81–1.06). Excesses of prostatic cancer (25 cases; SIR, 1.78; 95% CI, 1.16-2.63) and colon cancer (12 cases; SIR, 2.09; 95% CI, 1.08–3.66) were observed and 10 lung cancers occurred (SIR, 1.15; 95% CI, 0.55–2.11). No significant excesses were noted at other sites. A subdivision by department showed that the excess of prostatic cancer was mostly found among furnace workers. A subdivision by duration of employment showed that the excess of prostatic and colonic cancer occurred in workers who had been employed for ≥25 years. [The Working Group noted that there were few cases of either tumour who had less than 25 years of employment.]

2.1.9 *Chimney sweeps and other exposures to soot* (Table 2.8)

As early as 1775, Sir Percival Pott documented the occurrence of scrotal cancer among chimney sweeps in London (Pott, 1775). Several subsequent cases of skin or scrotal cancer among chimney sweeps have been reported (e.g. Earle, 1808; Butlin, 1892; Henry & Irvine, 1936). A report to the Registrar General for England and Wales (Henry, 1937) indicated a clear excess of skin cancer among chimney sweeps.

Kennaway and Kennaway (1947) reported mortality among chimney sweeps in England and Wales; 21 deaths from lung cancer occurred compared with 17.6 expected (SMR, 1.19; [95% CI, 0.74–1.82]). [Different sources were used to obtain occupational titles for the numerator and denominator of the occupational death rates, which may have caused bias in the estimated risks.] (see IARC (1985) for further details)

Kupetz (1966) reported analyses of mortality and morbidity among chimney sweeps in Berlin, Germany. Age-adjusted expected numbers of deaths were calculated using 5-year age groups and the male population of Berlin as the reference. Among an average of 255 chimney sweeps, 31 died (33.8 expected) between 1954 and 1963. Among the nine deaths from cancer, seven were histologically confirmed lung cancers (2.0 expected; [SMR, 3.50; 95% CI, 1.41–7.21]); the average duration of exposure was 49 years. The incidence rate for respiratory cancer among chimney sweeps in former East Germany between 1956 and 1958 was 13.7/10 000 (eight observed, 6.7/10 000 expected).

Table 2.8. Cohort studies of chimney sweeps and other exposures to soot

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SMR (95% CI)	Adjustment for potential confounders/Comments
Hansen *et al.* (1982), Copenhagen, Denmark	106 chimney sweeps active any time between 1958 and 1977 followed for mortality up to 1977	–	Cancer		11	1.77 [0.88–3.16]	No
Hansen (1983), Denmark	713 chimney sweeps active in 1970 followed for mortality 1970–75	–	Cancer		12	[2.27 (1.17–3.97)]	No
Evanoff *et al.* (1993), Sweden	5313 chimney sweeps active any time between 1917 and 1980 followed for mortality up to 1990 and cancer incidence up to 1987	Employment duration	Incidence			**SIR**	An analysis of asbestos exposure levels and dose–response calculations showed that asbestos could contribute to only a minor part of the lung cancer excess. A survey of tobacco smoking habits in 1972 was used to calculate adjusted SIRs. SIRs for lung and urinary bladder cancer were still significantly elevated after adjusting for smoking.
			Lung	*All*	50	2.09 (1.55–2.76)	
				1–9 years of employment	4	0.89 [0.24–2.28]	
				10–19 years of employment	16	2.16 [1.24–3.51]	
				20–29 years of employment	15	2.68 [1.50–4.42]	
				>30 years of employment	15	2.34 [1.31–3.87]	
			Oesophagus	All	11	3.87 (1.93–6.93)	
			Urinary bladder	All	37	2.53 (1.78–3.49)	
			Skin (non-melanoma)	All	4	0.61 (0.17–1.57)	
			Haemato-lymphatic	All	36	1.51 (1.06–2.09)	

Table 2.8 (contd)

Reference, location	Cohort description	Exposure assessment	Organ site	Exposure categories	No. of cases/ deaths	SIR (95% CI)	Adjustment for potential confounders/Comments
Pukkala (1995), Finland	The Finnish 1970 Census file was linked to the national cancer registry to follow men for cancer incidence in 1971–85	Occupational titles	Lung		23	1.35 (0.86–2.03)	Age, time period, social class
Haldorsen *et al.* (2004), Norway	All men aged 25–64 years in the Norwegian census 1970 followed for cancer incidence 1971–91	Occupational titles	Lung	Chimney sweeps	14	1.19 (0.7–2.0)	Adjusted for tobacco smoking habits at the group level by indirect method

CI, confidence interval; SIR, standardized incidence ratio; SMR, standardized mortality ratio

Hansen *et al*. (1982) investigated mortality from cancer and non-cancerous diseases among all male chimney sweeps in Copenhagen, Denmark, who were also members of the Copenhagen Chimney Sweeps' Pension Fund from 1958 to 1977. Membership in the pension fund was mandatory both for employed and self-employed chimney sweeps. A total of 106 chimney sweeps were identified and followed for mortality until 1977. Follow-up was complete and underlying causes of death were obtained from death certificates. A life-table survival analysis was performed, which compared mortality from cancer and non-cancerous diseases among chimney sweeps with that of the male population of Copenhagen, adjusted for age and calendar period. The ratio of observed to expected number of deaths was calculated; 33 deaths occurred in the cohort (SMR, 1.32; [95% CI, 0.91–1.86]), of which 11 were from cancer (SMR, 1.77; [95% CI, 0.88–3.16]). Of these, four deaths were from lung cancer but the expected number was not given.

Cadez (1983) reported a study on all chimney sweeps in Slovenia. Tar concentrations were reported to exceed the maximum accepted concentration (0.2 mg/m^3) by 30–96-fold. Of 479 chimney sweeps employed in 1969, 54 died between 1969 and 1981, 23% of whom died from cancer — 11 from lung cancer, eight from laryngeal cancer and four from stomach cancer but none from cancer of the scrotum. [The Working Group noted that only crude numbers of deaths were reported.]

Hansen (1983) investigated mortality among all male Danish chimney sweeps identified from the national census in 1970, and 713 were followed for mortality in 1970–75 by linkage with national population registries. Expected numbers of deaths were calculated by the person–year method, based on the death rates among all employed Danish men. The study group partly overlapped with a previous study (Hansen *et al*., 1982). During follow-up, 38 deaths were observed [SMR, 2.08; 95% CI, 1.47–2.85], 12 of which were from cancer [SMR, 2.27; 95% CI, 1.17–3.97].

Evanoff *et al*. (1993) investigated mortality and cancer incidence among a cohort of 5313 Swedish chimney sweeps from previous studies (Hogstedt *et al*., 1982; Gustavsson *et al*., 1988). All male members of the Swedish chimney sweeps' trade union from 1918 to 1980 and who were still alive in the beginning of 1951 were included. This trade union represented approximately 95% of all Swedish chimney sweeps. The cohort was followed for mortality from 1951 to 1990 (restricted to persons aged <80 years at death) and for cancer incidence from 1958 to 1987. Underlying causes of death were obtained from death certificates and cancer diagnoses from the national cancer register. SMRs were calculated by the person–year method, using national mortality and cancer rates to calculate expected numbers of deaths. Tobacco smoking habits were available for 1040 chimney sweeps employed in 1972 and were used to additionally calculate smoking-adjusted SIRs and SMRs. Of the cohort members, 919 had died at the end of follow-up, which was complete for 98.2%. The SMR for all causes of death was 1.35 (95% CI, 1.27–1.44) and was 1.46 for all cancers (95% CI, 1.27–1.67). Three hundred and thirty-five incident cases of cancer were observed (SIR, 1.43; 95% CI, 1.28–1.59). Significant excess risks were noted for lung cancer (SIR, 2.09; 95% CI, 1.55–2.76; based on 50 cases), urinary bladder cancer (SIR, 2.53; 95% CI, 1.78–3.49; based on 37 cases),

oesophageal cancer (SIR, 3.87; 95% CI, 1.93–6.93; based on 11 cases) and haematolymphatic malignancies (SIR, 1.51; 95% CI, 1.06–2.09; based on 36 cases). Three pleural mesotheliomas were observed versus 0.6 expected (SIR, 4.69; 95% CI, 0.94–13.70). No excess incidence of skin cancer was noted. Analysis of cancer incidence by duration of employment (categories 1–9, 10–19, 20–29 and ≥ 30 years) showed statistically significant positive trends for all cancer and for lung cancer. The recalculated effect estimates adjusted for smoking habits in 1972 showed somewhat higher expected numbers of lung (SMR) and urinary bladder (SIR) cancers, but the SMRs and SIRs still remained significantly elevated. Three cases of pleural mesothelioma indicated that the cohort had been exposed to asbestos. [The ratio of the excess number of lung cancers to the number of mesotheliomas (27:3) does not suggest that asbestos was an important confounder for lung cancer.]

A study in Finland (Pukkala, 1995) linked the occupations of individuals in the 1970 census with cancer incidence during 1971–85, as ascertained through the Finnish Cancer Registry. SIRs were calculated and adjusted for age, time period and social class. The entire national economically active population was used as the reference population. The SIR for lung cancer was 1.35 (95% CI, 0.86–2.03; based on 23 incident cases) for male chimney sweeps.

Haldorsen *et al.* (2004) investigated the incidence of lung cancer among all Norwegian men aged 25–64 years included in the census of 1970, who were followed from 1971 to 1991 and for whom occupational titles were obtained from censuses. Deaths and cancer cases were identified from national registers. Adjustment was made for tobacco smoking habits at the group level by an indirect method. Fourteen lung cancers occurred among chimney sweeps (SIR, 1.19; 95% CI, 0.7–2.0; adjusted for smoking).

2.2 Case–control studies by cancer site

2.2.1 *Lung cancer* (Table 2.9)

Blot *et al*. (1983) conducted a case–control study in Pennsylvania, USA, of all deaths from primary lung cancer among white men aged 30–79 years resident in Lehigh and Northampton counties during 1976–77 and in Carbon county during 1974–77. These counties had a substantial population that was employed in the steel industry. A total of 360 cases were identified at the files of the Pennsylvania Division of Vital Statistics. Three hundred and sixty deceased controls matched to cases on sex, race, age, county of residence and year of death were selected at random from among deaths from causes other than respiratory cancer, chronic respiratory disease or suicide: the majority of the controls had acute heart conditions or non-respiratory cancer as the cause of death. Personal interviews were conducted by trained interviewers, blinded to case/control status, to obtain information from the next of kin on residential, occupational and smoking histories and demographic data. Usual industry, adapted partly from the Standard Industrial Classification system, and usual job title were defined as those in which largest

Table 2.9. Case–control and case–cohort studies of lung cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Lung cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Blot *et al*. (1983), Pennsylvania, USA	335 white male cases (deceased), 332 matched white male population-based controls (deceased)	Coke worker in the steel industry as longest held job	Any	2	1.2 (0.2–6.9)	Odds ratios adjusted for sex, race, age, county and year of death, relative to men never employed in the steel industry, based on two cases among coke-oven workers. Odds ratios were not adjusted for tobacco smoking
Schoenberg *et al.* (1987), New Jersey, USA	763 white male cases identified in 1980–81, 900 population-based controls	Roofer or slater	Any	13	1.7 (0.68–4.40)	Adjusted for tobacco smoking
Zahm *et al.* (1989), Missouri, USA	4431 white male cases 1980–85, 11 326 white male cancer controls	Roofer	Any Adenocarcinoma Squamous-cell Other/mixed	6 1 3 2	2.1 (0.6–8.2) 1.5 (0.1–13.3) 2.6 (0.5–12.7) 2.9 (0.4–18.0)	Adjusted for tobacco smoking and age; latest job available for 52% of cases and 45% of controls
Morabia *et al.* (1992), USA	1793 male cases and 3228 male cancer and non-cancer hospital-based controls identified from 24 US hospitals in 1980–89	Roofer and slater	Any	7	2.1 (0.7–6.2)	Adjusted for race, age, geographical location, version of questionnaire and tobacco smoking

Table 2.9 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Lung cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Partanen & Boffetta (1994)	Meta-analysis of Schoenberg *et al*. (1987), Zahm *et al.* (1989), Morabia *et al.* (1992)	Roofer and slater	Any	26	1.88 (1.23–2.76)	Adjustments as in the original studies: age, gender, geographical area, questionnaire version and cigarette/tobacco smoking
Bovenzi *et al*. (1993), Trieste, Italy	756 male cases (deceased), 756 male matched population-based controls (deceased) identified from local registries	Gas workers Asphalt workers	Any Any	7 7	1.43 (0.45–4.47) 2.27 (0.50–10.3)	Adjusted for tobacco smoking
Wu-Williams *et al*. (1993), People's Republic of China	965 female incident cases identified from local cancer registries between 1985–87; 959 randomly selected age-matched, female population-based controls	**Coke-oven emissions** *Years of exposure* 1–10 years 11–20 years ≥21 years *p* trend	Any	51	1.5 (0.9–2.5) 1.2 1.4 3.0 0.04	Adjusted for tobacco smoking, study area, age and education

Table 2.9 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Lung cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Nadon *et al*. (1995), Montréal, Canada	857 male cases rapidly ascertained from all major Montréal hospitals, 2056 male population controls (identified from electoral lists and RDD) and cancer controls identified from the same hospitals as cases	*Total PAHs*	Any			All analyses adjusted for age, ethnic group, family income, cumulative tobacco smoking index and exposure to asbestos, silica and chromium, nickel and arsenic compounds
		Low		418	1.0 (0.8–1.3)	
		High		209	1.0 (0.8–1.3)	
		BaP				
		Low		160	0.9 (0.7–1.2)	
		High		75	1.0 (0.7–1.4)	
		Total PAHs	Small-cell (oat-cell)			
		Low		78	1.0 (0.6–1.5)	
		High		40	1.0 (0.6–1.7)	
		BaP				
		Low		28	0.8 (0.5–1.3)	
		High		14	0.6 (0.3–1.3)	
		Total PAHs	Squamous-cell			
		Low		184	1.2 (0.9–1.7)	
		High		93	1.2 (0.8–1.7)	
		BaP				
		Low		75	1.0 (0.8–1.5)	
		High		33	1.0 (0.7–1.7)	
		Total PAHs	Adenocarcinoma			
		Low		85	1.1 (0.7–1.7)	
		High		37	1.0 (0.6–1.7)	
		BaP				
		Low		29	0.9 (0.6–1.4)	
		High		10	0.5 (0.3–1.2)	

Table 2.9 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Lung cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Nadon *et al*. (1995) (contd)		**<800 cigarettes–years**	All			For all analyses stratified by smoking status, the reference category was the unexposed among light smokers.
		Total PAHs				
		Unexposed		41	1.0 (ref)	
		Low		80	1.4 (0.9–2.1)	
		High		31	1.8 (1.1–3.0)	
		BaP				
		Unexposed		112	1.0 (ref)	
		Low		24	1.1 (0.7–1.7)	
		High		16	2.4 (1.3–4.4)	
		≥800 cigarettes–years	All			
		Total PAHs				
		Unexposed		189	6.7 (4.6–9.8)	
		Low		341	6.1 (4.2–8.7)	
		High		175	6.0 (4.1–8.9)	
		BaP				
		Unexposed		510	5.2 (4.1–6.6)	
		Low		142	5.3 (3.9–7.2)	
		High		53	3.9 (2.6–5.8)	
van Loon *et al*. (1997), Netherlands	524 male cases identified through national registries; cohort subsample of 1688 men used to calculate person–years at risk	*Cumulative occupational exposure to PAHs*	All		**Rate ratio**	Adjusted for age, other occupational exposures, tobacco smoking, dietary intake of vitamin C, β-carotene and retinol; response rate to baseline questionnaire was low (36%).
		Ever versus never		34	0.53 (0.26–1.07)	
		I tertile		10	0.53 (0.13–2.14)	
		II tertile		12	0.83 (0.32–2.20)	
		III tertile		12	0.28 (0.09–0.89)	
					p for trend <0.01	

Table 2.9 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Lung cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Grimsrud *et al*. (1998), Rana, Norway	86 male cases identified from the national cancer registry, 196 male population controls matched by year of birth and selected from the national registry	Exposed to PAHs at steel, iron and coke plant only	All	13	1.9 (0.8–4.6)	Adjusted for tobacco smoking
		Any occupational exposure to PAHs (see text for details)		18	2.9 (1.2–6.7)	Adjusted for tobacco smoking
Droste *et al*. (1999), Belgium	478 male cases and 536 male hospital controls identified during 1995–97 from hospitals that served the entire Antwerp area	Self-reported exposure to PAHs	All	77	1.1 (0.7–1.7)	A lag time of 10 years was applied to all exposure variables to allow for an induction latency period before diagnosis of disease. [The job–exposure for PAHs included several occupations in which exposure to PAHs is questionable]. All odds ratios were adjusted for age, smoking history, marital and socioeconomic status
		Matrix-based exposure to PAHs		235	1.2 (0.9–1.6)	

Table 2.9 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Lung cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Brüske-Hohlfeld *et al*. (2000), Germany	2 pooled case–control studies; 4184 male cases, 4253 male controls	*Occupational PAH exposure*	All			Adjusted for smoking and exposure to asbestos
		Ever versus never		181	1.53 (1.14–2.04)	
		Up to 20 BaP–years		80	1.15 (0.77–1.71)	
		>20 BaP–years		101	2.09 (1.36–3.22)	
Gustavsson *et al*. (2000), Sweden	1042 male cases, 2364 male population controls matched by 5-year age group and year of inclusion (1985–90)	**Combustion products**	All	824	1.00 (ref)	All odds ratios adjusted for age, year of inclusion, tobacco smoking, residential radon level and environmental exposure to nitrogen dioxide
		Unexposed				
		μg/m^3 BaP				
		0.05–0.4 (>1 year)		46	1.07 (0.72–1.60)	
		0.5–4.9 (>1 year)		48	1.33 (0.89–2.00)	
		≥5 (>1 year)		35	2.10 (1.25–3.53)	
		μg/m^3–years of BaP				
		>0–2.9		47	1.20 (0.80–1.80)	
		3.0–6.6		51	1.05 (0.71–1.57)	
		6.7–23.8		47	1.05 (0.69–1.59)	Additionally adjusted for diesel exhaust and exposure to asbestos
		≥23.9		73	1.60 (1.09–2.34)	
		Duration (years)				
		>0–9		Not stated	1.42 (0.96–2.10)	
		10–29			1.37 (1.01–1.85)	
		≥30			1.37 (0.98–1.91)	

BaP, benzo[*a*]pyrene; CI, confidence interval; RDD, random digit dialling

number of years were spent. After exclusions, most of whom were non-respondents (response rate, 96% in cases and 94% in controls), the effective numbers of cases and controls were 335 and 332, respectively. The odds ratio (adjusted for the matching variables) for coke-oven worker in the steel industry as the longest job held was 1.2 (95% CI, 0.2–6.9; based on two cases and two controls in that job).

Schoenberg *et al*. (1987) conducted a case–control study among 763 white male cases of lung cancer who were identified in 1980–81 in New Jersey (USA) municipalities that have high mortality from lung cancer and 900 population controls matched to cases by race, age, area of residence and closest date of death (for deceased persons). Occupational histories were obtained from the subjects or their next of kin. The odds ratio for roofers and slaters, adjusted for tobacco smoking, was 1.7 (95% CI, 0.68–4.40; based on 13 exposed cases).

Zahm *et al*. (1989) conducted a case–control study of 4431 male residents in Missouri (USA) who were identified as lung cancer cases in 1980–85 at the Missouri Cancer Registry. Controls were selected from this Registry during the same period and comprised 11 326 white male Missouri residents who did not have cancer of the lip, oral cavity, oesophagus, lung, urinary bladder, ill-defined sites or unknown sites. Occupation at the time of cancer diagnosis was abstracted and coded from the Cancer Registry records; this information was available for only 52% of the cases of 45% of the controls. Data on tobacco smoking were coded into seven categories, including 'unknown smoking history'. The odds ratio for roofers, adjusted for age and tobacco smoking, was 2.1 (95% CI, 0.6–8.2; based on six exposed cases).

Morabia *et al*. (1992) conducted a case–control study of 1793 male cases of lung cancer who were identified in 24 hospitals in the USA and 3228 cancer and non-cancer hospital controls matched to cases on race, age, hospital and cigarette smoking history. All subjects were identified between 1980 and 1989. Data on usual occupation, tobacco smoking and other potential confounders were obtained by interviews of the subjects. Job titles were coded according to the standard of the United States Bureau of Census codes. The odds ratio for roofers and slaters, adjusted for race, age, geographical location, version of the questionnaire (I and II) and tobacco smoking, was 2.1 (95% CI, 0.7–6.2; based on seven exposed cases). [The Working Group noted that the control group included some cancer sites that are possibly related to exposure to PAHs, and thereby the odds ratio is potentially understimated.]

Partanen and Boffetta (1994) conducted a meta-analysis of the results of Schoenberg *et al*. (1987), Zahm *et al*. (1989) and Morabia *et al*. (1992) for roofers and slaters. The overall relative risk was 1.88 (95% CI, 1.23–2.76; based on 26 exposed cases).

Bovenzi *et al*. (1993) conducted a case–control study of 756 cases among 938 male deaths from primary lung cancer who were identified at the records of the cancer registry of the province of Trieste, Italy, during 1979–81 and 1985–86, and for whom next of kin could be located and interviewed. For each case, one male control of similar age, who had died within the same 6-month period, was randomly selected at the registry of the Department of Pathology that holds the autopsy records of 70% of all deaths of the

province. The controls represented causes of death other than chronic lung diseases or cancers of the upper aerodigestive tract, urinary tract, pancreas, liver or gastrointestinal system; the majority died from circulatory or gastrointestinal disease. Telephone interviews were conducted with the next of kin of the subjects at least 1 year after their death to obtain information on residential, occupational (jobs, industries, places of work, employers and durations of employment in each job) and tobacco smoking histories and demographic data. The principal source of occupational histories was the subjects' employment cards which were carefully kept by the families for social security purposes. No next of kin refused the interview. The industries/occupations were classified into two lists by an occupational physician: those that were considered to be causally associated with lung cancer and those suspected of being related to an increased risk for lung cancer. Of the single occupational titles, gas workers had a smoking-adjusted odds ratio of 1.43 (95% CI, 0.45–4.47; seven cases) for risk for lung cancer and asphalt workers had an odds ratio of 2.27 (95% CI, 0.50–10.3; based on seven cases) compared with subjects who had presumably not been exposed to occupational lung carcinogens. [It was unclear whether 'gas workers' referred to coal gasification and whether coal tar was used in the asphalt mix of asphalt workers.].

In a case–control study in the People's Republic of China (Wu-Williams *et al*., 1993), 965 confirmed cases of primary incident lung cancer among women aged 29–70 years in the general population of Shenyang and Harbin (the major industrial cities of northern China) were identified, located and interviewed during 1985–87 through the cancer registries of the two cities. The refusal rate among cases was 0.3%. The 959 control women were randomly selected from the general populations of the two cities, frequency matched to the cases by 5-year age group and interviewed. Subjects were interviewed at the hospital, at home or at work with a structured questionnaire that addressed occupational histories and exposures, tobacco smoking histories, indoor and outdoor sources of pollution, medical and dietary histories and demographic data. The odds ratio for self-reported exposure to coke-oven emissions, adjusted by unconditional logistic regression for tobacco smoking, study area, age and education, was 1.5 (95% CI, 0.9–2.5; based on 51 exposed cases). An increase in trend for mortality from lung cancer (p=0.04) was found for increasing years of exposure to coke-oven emissions (odds ratios, 1.2, 1.4 and 3.0 for 1–10, 11–20 and >20 years of exposure, respectively). The point estimates of the odds ratios were similar when analyses were restricted to nonsmokers only.

A population-based case–control study of occupational cancer in Montréal, Canada (Nadon *et al*. 1995), included 857 histologically confirmed male cases of lung cancer, aged 35–70 years, who were newly diagnosed between 1979 and 1986 and ascertained in all large hospitals that serve Montréal, as well as 533 population controls and 1523 non-lung cancer controls. The two control series were pooled for the analyses because parallel analyses showed little difference between results based on each control group used separately as the referent. The cases included 159 small-cell (oat-cell) cancers, 359 squamous-cell cancers and 162 adenocarcinomas. Cases were rapidly ascertained and personally interviewed to obtain information on each job held during their lifetime and on

potential confounders. The interviewers were trained to probe for as much information as the subjects could supply on the employer's or company's activities, the raw materials used, the type of room or building in which he had worked, the activities of workmates, the presence of gases, fumes or dusts and other pertinent data. The exposure of each subject to approximately 300 occupational agents was assessed by a group of hygienists and chemists on the basis of job descriptions and lung cancer cases were compared with the rest of the study population, after adjustment for age, ethnic group, family income, cumulative tobacco smoking index and exposure to asbestos, silica and chromium, nickel and arsenic compounds. Occupational exposure was defined as levels that exceeded general environmental levels; 68% of all subjects were classified as ever having been exposed to PAHs from any source, 24% were thought to have been exposed to benzo[*a*]pyrene and 9% had been exposed to PAHs from coal. Cumulative exposures at low, medium or high levels were also computed, based on the sum product of duration, frequency and concentration of exposure. Table 2.9 summarizes the odds ratios and confidence intervals for exposure to total PAHs, benzo[*a*]pyrene and coal-derived PAHs for all lung cancers and small-cell (oat-cell) and squamous-cell lung cancers and lung adenocarcinomas. None of the associations with type of PAH were significant and the odds ratios were generally close to unity. However, effect modification was observed between tobacco smoking and exposure to PAHs. Among heavy smokers (800 cigarette–years or more), there was no effect on the risk for lung cancer with different levels of exposure to PAHs. However, among 'light smokers and nonsmokers' (<800 cigarette–years), there was evidence of an increasing risk for lung cancer with increasing exposure to total PAHs and benzo[*a*]pyrene. The odds ratio for exposure to high levels of total PAH was 1.8 (95% CI, 1.1–3.0; based on 31 exposed cases) for 'light smokers and nonsmokers' and 6.0 (95% CI, 4.1–8.9; based on 175 exposed cases) for heavy smokers compared with those not exposed to total PAHs. For high-level exposure to benzo[*a*]pyrene, the odds ratio was 2.4 (95% CI, 1.3–4.4; based on 16 exposed cases) for 'light smokers and nonsmokers' and 3.9 (95% CI, 2.6–5.8; based on 53 exposed cases) for heavy smokers compared with those not exposed to benzo[*a*]pyrene.

In 1986, the Netherlands Cohort Study was initiated as a prospective follow-up for cancer incidence in relation to lifestyle factors within a cohort of 340 439 men and women aged 55–69 years selected from the general population of the Netherlands (van Loon *et al*., 1997). Prevalent cases of cancer were excluded. A self-administered questionnaire was sent to all cohort members regarding job history, diet, lifestyle and other sociodemographic factors. Only 36% of the cohort responded and after comparing several variables between the cohort and Dutch population, it was considered that the remaining members of the cohort were still representative of the Dutch population. A case–cohort study of lung cancer was designed in the remaining cohort of 58 279 men, and 677 cases of incident lung cancer were determined between September 1986 and December 1990 through the national pathology register and the regional cancer registers. Person–years at risk were estimated from a subsample of 1630 men who were followed up biennially for vital status. A self-administered baseline questionnaire enquired about

lifetime job history with job titles, type of company, periods and duration of employment as well as the company's products up to September 1986. Exposure assessment was conducted with no knowledge of health outcomes. Experts classified the job histories for probabilities of exposure to asbestos, paint dust, PAHs and welding fumes into four categories: no exposure, possible exposure (probability of exposure <30%), probable exposure (30–90% probability) and nearly certain exposure (>90% probability). Cumulative exposure to each agent was calculated as the product of the midpoint of the probability in each of the four exposure categories (scored as 0, 0.15, 0.6 and 0.95, respectively) and the duration of exposure. Distributions of cumulative probability were then compared between the cases and the subcohort. Case–cohort analyses were completed by calculating rate ratios for each occupational cumulative probability, adjusted for age, other occupational exposures, tobacco smoking and dietary intake of vitamin C, β-carotene and retinol. The adjusted rate ratio for ever versus never exposure to PAHs was 0.53 (95% CI, 0.26–1.07; based on 34 exposed cases); thus, exposure to PAHs was inversely associated with the risk for lung cancer. The adjusted rate ratios for the non-zero tertiles of cumulative probability of exposure to PAHs were all below unity: the highest tertile was associated with a risk ratio of 0.28 (95% CI, 0.09–0.89; based on 12 cases) and the *p* value associated with this negative trend was <0.01. The odds ratios were considerably higher (but not significantly so) when adjusted for age and other occupational exposures or age only.

Another population-based case–control study was conducted in Norway (Grimsrud *et al*., 1998); the national cancer registry was used for the ascertainment of 88 incident cases of lung cancer registered among male residents of the municipality of Rana during 1980–92. Rana is located in northern Norway and had had an iron and steel plant (since 1955) and a separate coke plant (since 1964) that were in operation until the late 1980s. Three male population controls were selected for each case, were matched on year of birth, were free of lung cancer and lived in the municipality at the time of diagnosis of the case. Occupational histories (period of employment, department, details on section and job) were gathered from personnel files of the iron, steel and coke plants and by personal interviews with subjects or their next of kin. All interviews were performed by one interviewer who addressed occupational and tobacco smoking histories, dietary habits, education and residential history. After refusals, exclusions because of insufficient data and replacement of controls, 86 cases and 196 controls remained for data analysis. Subjects exposed to PAHs only at the iron, steel and coke plants had a smoking-adjusted odds ratio of 1.9 (95% CI, 0.8–4.6; 13 exposed cases).

Droste *et al*. (1999) conducted a hospital-based case–control study of lung cancer and exposure to occupational carcinogens in the Antwerp region of Belgium. A total of 478 histologically confirmed male cases and 536 male controls without cancer or primary lung disease (mainly admitted from cardiovascular surgery wards) were identified and interviewed personally in 10 hospitals during 1995–97. The catchment area of the participating hospitals had to include the total Antwerp area so that there would be no selection bias for socioeconomic status, occupational conditions or potential exposures.

The interviews covered information on occupation (job tasks, industries and duration of employment), exposure to 16 potential carcinogens, hobbies and tobacco smoking histories. Job titles were coded into a standard classification, and exposures were assessed by self-reporting and through an exposure matrix that converted data on jobs and tasks into selected exposures. Exposure data were reviewed by occupational hygienists. Odds ratios were calculated with logistic regression models, adjusting for age, tobacco smoking history, marital status and socioeconomic status. The time lag between the onset of exposure and diagnosis of disease was set at 10 years; the same time lag was also used for other covariates that may have acted as confounders. Relative to no exposure, the odds ratio for self-reported exposure to PAHs was 1.1 (95% CI, 0.7–1.7; 77 exposed cases). For exposure matrix-based exposure to PAHs, the odds ratio was 1.2 (95% CI, 0.9–1.6; 235 exposed cases). [The Working Group noted that the job–exposure matrix for PAHs included several occupations in which exposure to PAHs is questionable.]

Data from two German case–control studies of lung cancer were pooled for joint analysis (Brüske-Hohlfeld *et al*., 2000). One study was carried out by the Bremen Institute for Prevention Research and Social Medicine in Bremen, the area surrounding Bremen and the Frankfurt/Main area to determine the association between lung cancer and occupational risk factors among 1004 cases and 1004 population controls randomly selected from mandatory community registries and matched on age, sex and region of residence in 1988–93. The other study was carried out to determine the risk for lung cancer from exposure to indoor radon in West and East Germany (covering the regions of Nordrhein-Westfalen, Rheinland-Pfalz, Bayern, Saarland, Thüringen and Sachsen) among 3180 cases and 3249 controls matched by sex, age and region in 1990–94. A standardized questionnaire was administered by trained interviewers in both studies to collect data on occupational exposure, residential history, tobacco smoking and other risk factors. The response rates were 69% for cases and 68% for controls (in the Bremen BIPS study) and 77% for cases and 41% for controls (in the 'indoor radon study'). The final pooled analyses were restricted to 3541 male controls and 3498 male cases that were cytologically and/or histologically verified, excluding metastases secondary to other primary tumours. Using 'never exposed' as the reference category, odds ratios were calculated for workers who had been 'ever exposed', for duration of exposure and for cumulative exposures through a job–exposure matrix. The 'ever' category of exposure to PAHs was associated with an odds ratio of 1.53 (95% CI, 1.04–3.95), adjusted for tobacco smoking and exposure to asbestos. Duration of exposure to PAHs did not show a clear trend, while job–exposure matrix-based cumulative exposure to benzo[*a*]pyrene did, yielding risk ratios of 1.15 (95% CI, 0.77–1.71) for 0–20 benzo[*a*]pyrene–years and 2.09 (95% CI, 1.36–3.22) for 20 or more benzo[*a*]pyrene–years. The risk was highest for workers in coking plants; no increased risk was seen for chimney sweeps or those working in smelters, after adjustment for tobacco smoking and exposure to asbestos. [TheWorking Group noted the low response rate among controls of the indoor radon study.]

Gustavsson *et al*. (2000) conducted a study among male residents of Stockholm County, Sweden, aged 40–75 years: 1042 lung cancer cases and 2364 population controls, matched on age and year of inclusion (1985–90). Questionnaires were mailed to the subjects or their next of kin on detailed lifetime occupational history, residential history, tobacco smoking habits and other potential risk factors for lung cancer. Response rates were 87% in cases and 85% in controls. Intensity, probability and duration of exposure were assessed for seven specific occupational exposures for each work period by an occupational hygienist who was blinded to case/control status. For combustion products, odds ratios were elevated for the highest categories of exposure intensity, assessed as ≥5 μg/m^3 benzo[*a*]pyrene (odds ratio, 2.10; 95% CI, 1.25–3.53; based on 35 exposed cases), cumulative exposure of ≥23.9 μg/m^3–years benzo[*a*]pyrene (odds ratio, 1.60; 95% CI, 1.09–2.34; based on 73 exposed cases; adjusted additionally for exposure to diesel exhaust and asbestos) and duration of exposure of 10–29 years (odds ratio, 1.37; 95% CI, 1.01–1.85) and ≥30 years (odds ratio, 1.37; 95% CI, 0.98–1.91). The odds ratios were adjusted for age, year of inclusion, tobacco smoking, residential radon level and environmental exposure to nitrogen oxide.

2.2.2 *Cancer at sites other than the lung*

(a) Renal-cell carcinoma (Table 2.10)

A population-based case–control study was conducted in the seven-county Minneapolis–St Paul Standard Metropolitan Statistical Area (USA): eligible cases were white residents of the area who were diagnosed with renal-cell carcinoma (International Classification of Diseases (ICD) 8, 189.00) in 1974–79 (McLaughlin *et al*., 1984). A total of 506 cases, aged 30–85 years, were ascertained; 495 interviews were conducted, more than half of which were with the next of kin because 237 (47%) cases were deceased and 14 were too ill to be interviewed. A group of 714 population controls was identified, who were an age- and sex-stratified random sample of white residents of the area. Those who were 30–64 years of age were chosen at random from a complete listing of telephone subscribers that included 98% of the households in this area; those over 65 years of age were chosen at random from a health care financial administrative list that included 95% of US residents in this age group. A total of 697 controls were interviewed. To account for the possible influence of interviews with next of kin, a second control group of 495 of subjects who had died from causes other than urinary tract cancer during the period 1974–79 was constituted from death certificates and were frequency-matched to deceased cases on the age and year of death and gender. For this group, 493 next of kin answered the questionnaire, which included a category on occupation, although occupational exposures were not reported in detail and exposure to specific PAHs was not described. A very high response rate was observed (approximately 95% in the different groups of cases and controls). Statistical models were systematically adjusted for age, and separate odds ratios were estimated to take into account the type of respondent (index subject or next of kin). A final model included all different exposures studied in the analysis to adjust simultaneously

Table 2.10. Case–control studies of kidney cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Kidney cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
McLaughlin *et al.* (1984), Minneapolis, USA	495 cases (1974–79), 1190 population controls (697 alive and directly interviewed, 493 deceased) matched by age and sex	Petroleum, tar, pitch Exposure ≥20 years	Renal-cell carcinoma	36 Not given	1.7 (1.0–2.9) 2.6 (1.2–5.7)	Occupational history was not described in detail; the number of controls used in odds ratio estimations was not clear (total 1190 controls or 697 alive controls); the results were adjusted for tobacco smoking
Sharpe *et al.* (1989), Canada	164 cases (1982–87), 161 hospital controls matched by age, sex and urologist	Coke Tar or pitch	Renal-cell carcinoma	6 9	2.00 (0.49–8.14) 9.29 (1.16–74.20)	Detailed occupational history not described; no description of the exposure evaluation: the exposures studied were derived from job titles; adjusted for matching variables but not for smoking; a statistically significant trend (p <0.025) of increasing odds ratios was seen with increasing intensity of exposure to burning coal
Partanen *et al.* (1991), Finland	408 cases (1977–78), 819 population controls matched by year of birth, sex and vital status	PAH (men and women) PAH (men only) Blue-collar workers (men and women) Male blue-collar workers	Renal-cell carcinoma	7 NA NA	1.1 (0.4–3.1) 1.2 (0.4–3.4) 4.4 (0.4–43.1) 5.5 (0.5–58.9)	Whole occupational history for the period 1920–68; adjusted for smoking, coffee consumption and obesity; the number of women was not given, therefore could not evaluated the number of male exposed cases. Increase in association after exclusion of white-collar workers

Table 2.10 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Kidney cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Mandel *et al.* (1995), Australia, Denmark, Germany, Sweden and USA	1732 cases (1989–91), 2309 population controls	Blast furnace/coke-oven industries	Renal-cell carcinoma	57	1.7 (1.1–2.7)	Information on specific occupations and industries of interest and whole occupational history reported in Germany; these results were for men only.
Nadon *et al.* (1995), Canada	181 male cases (1979–85), 2196 male population and cancer controls	*PAHs derived from coal* Low High	Renal-cell carcinoma	 11 4	 1.0 (0.5–1.9) 0.9 (0.3–2.6)	Lifetime occupational history evaluated on a case-by-case basis; adjusted for smoking, age, socioeconomic status and ethnic group; diagnoses of lung and urinary bladder cancers were excluded from the control group.
Pesch *et al.* (2000), Germany	935 cases (1991–95), 4298 population controls	**Tar, pitch, mineral oil** *Men* Medium High Substantial *Women* Medium High Substantial	Renal-cell carcinoma	 86 96 34 15 16 10	 1.1 (0.9–1.5) 1.2 (0.9–1.6) 1.4 (0.9–2.1) 1.0 (0.6–1.7) 1.2 (0.7–2.0) 2.1 (1.0–4.5)	Whole occupational history using English job–exposure matrix; adjusted for tobacco smoking; exposure categories were defined by the 30th, 60th and 90th percentiles of the distribution of the exposure among controls; adjusted for age, centre and smoking.

Table 2.10 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Kidney cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Pesch *et al.* (2000) (contd)		**PAH**				
		Men				
		Medium		71	0.9 (0.7–1.2)	
		High		96	1.3 (1.0–1.6)	
		Substantial		32	1.2 (0.8–1.9)	
		Women				
		Medium		17	1.1 (0.6–1.8)	
		High		21	1.5 (0.9–2.4)	
		Substantial		6	1.3 (0.5–3.3)	
		Men				Job–task–exposure matrix
		Medium		80	0.9 (0.7–1.2)	
		High		67	0.8 (0.6–1.0)	
		Substantial		26	0.9 (0.6–1.4)	

CI, confidence interval; NA, not applied

for age, tobacco smoking, body mass index, use of phenacetin, kidney infection, kidney stones, nationality of the parents, consumption of tea, beer and meat and exposure to petroleum, tar and pitch. Few results concerned occupational exposure to PAHs. The results were presented according to the industry in which the index subject worked the greatest number of years but did not include sectors that are of interest to this monograph. The authors studied some occupational exposures that included the group petroleum, tar and pitch and found a significant relationship with renal-cell carcinoma (odds ratio, 1.7; 95% CI, 1.0–2.9; based on 36 exposed cases). This relationship was reinforced when persons who had been exposed for 20 years or more (odds ratio, 2.6; 95% CI, 1.2–5.7) were compared with those who had been exposed for less than 20 years (odds ratio, 1.1; 95% CI, 0.5–2.5). No other estimation was made (i.e. level or latency). In the final model that included all items studied, the odds ratio for renal-cell carcinoma in relation to petroleum, tar and pitch was 1.6 (95% CI, 0.9–2.7) for men and 4.6 (95% CI, 0.4–51) for women.

In a hospital-based case–control study in Canada, cases who were diagnosed between 1982 and 1987 were determined retrospectively from the medical records of nine hospitals in the Montréal area (Sharpe *et al*., 1989). Eligible cases were alive at the time of the review and had a histologically confirmed diagnosis (ICD-8, 189.00). Of the 403 cases identified, only 168 were able to participate; 163 had died, 43 could not be traced, the physician refused to participate for 25 and four were too ill to participate. Controls were patients who attended the same hospital departments for haematuria during the same 5-year interval as the cases, and for whom a non-neoplastic diagnosis had been given. One control per case was selected and matched for sex, 5-year age group and urologist. A total of 164 cases and 161 controls completed a mailed questionnaire (97% overall response rate); a telephone interview was conducted to clarify and complete missing information. No information was given on the content of the questionnaire, particularly on the occupational section. The authors highlighted that information on exposure to hydrocarbons was requested for regular and repeated exposure (at least once a week). Statistical analysis was carried out using unconditional logistic regression. All models were adjusted for the matching variables and the other exposures studied; however, it is unclear which variables were included in the final models. Although tobacco smoking was studied, the authors did not consider it to be a significant confounder and therefore excluded it from the final models. Exposure to coke was associated with a non-significant increase in risk for renal-cell carcinoma (odds ratio, 2.00; 95% CI, 0.49–8.14; based on six exposed cases and three exposed controls). Those considered to be occupationally exposed to coke were persons who had been exposed to burning fuels (e.g. janitors, foundry workers, locomotive engineers, forced labourers, sailors and those involved in heating buildings). Exposure to tar or pitch was related to a large increase in risk for renal-cell carcinoma (odds ratio, 9.29; 95% CI, 1.16–74.20; based on nine exposed cases (three in roofing, three in the construction industry, one laboratory worker, one petrochemical salesman and one prisoner of war) and one control

exposed through road paving). A statistically significant trend ($p < 0.025$) of increasing odds ratios was seen with increasing intensity of exposure to burning coal.

A population-based case–control study carried out in Finland included men and women aged 20 years or above who were diagnosed in 1977–78 as having renal adenocarcinoma (ICD-7, 189.0) registered with the Finnish Cancer Registry (Partanen *et al*., 1991). Two controls per case were identified at random from the Population Register Centre, and matched by year of birth, gender and vital status. A total of 672 cases and 1344 controls were thus identified. Information was collected by questionnaire sent to the index person for live subjects or to a next of kin for deceased subjects. Response rates were almost identical between cases and controls, and were slightly higher for subjects who were alive (78%) than for those who were deceased (66%). After exclusion of subjects without employment, the study included 408 cases (98 alive and 310 deceased) and 819 controls (195 alive and 624 deceased). Information included in the questionnaire concerned the whole occupational history (jobs, time period, employers) as well as information on tobacco smoking history, coffee consumption and obesity. Occupational histories from the period 1920–68 were reconstructed on an annual basis to examine occupational exposure to PAHs and eight other agents. An industrial hygienist scored each annual exposure into three classes: background (<0.01 μg/m^3 benzo[*a*]pyrene), low level (0.01–1 μg/m^3 benzo[*a*]pyrene if exposed for more than 30% of the normal annual worktime) and high level (>1 μg/m^3 benzo[*a*]pyrene if exposed for more than 30% of the normal annual worktime). Statistical analysis was carried out using a dichotomous exposure indicator: 0 (background exposure) and 1 (≥5 years at a low or high level or ≥1 year at a high level). Odds ratios were estimated by conditional logistic regression, adjusted for tobacco smoking (never/ever), coffee consumption (no/medium/heavy) and obesity (five classes) as potential confounders. Odds ratios were estimated for selected industries, job titles and the nine occupational exposures. Very few subjects were classified as having been exposed to PAHs (seven cases). The estimated odds ratio for the whole population was 1.10 (95% CI, 0.39–3.09). Exclusion of women did not modify the result (odds ratio, 1.21; 95% CI, 0.43–3.45; based on seven exposed cases). Restriction to blue-collar workers increased the association (for men and women combined: odds ratio, 4.4; 95% CI, 0.4–43; for men only: odds ratio, 5.5; 95% CI, 0.5–58.9).

An international population-based case–control study was carried out in Australia, Denmark, Germany, Sweden and the USA using a similar protocol in the participating centres (Mandel *et al*., 1995). Cases were patients with incident renal-cell carcinoma [ICD-9, 189.0], histologically confirmed and diagnosed between 1989 and 1991. Cancer registries were the main source of ascertainment of cases, except in Germany, where cases were identified through active surveillance. Controls were chosen at random from different population sources (registries, electoral rolls, residential lists) and frequency-matched to cases on sex and age. Personal interviews were conducted at home for most centres except Germany, where subjects were interviewed at the hospital. Most centres requested information on selected occupations or industries of interest to the study (German centres collected complete occupational histories) in addition to information on

demographics, personal and family medical history, lifestyle factors, and use of tobacco, alcohol and medication. All centres coded occupations and industries according to standard occupational and industrial classification codes. The response rates were 72% for cases and 75% for controls, resulting in 1732 cases and 2309 controls available for analysis. Statistical analysis was carried out using logistic regression separately for men and women, adjusted for age, smoking status, body mass index, education and study centre. A minimum of 1 year in a specific job or exposure was considered as being exposed. Working at least 1 year in the 'blast furnace and coke ovens' industry was associated with an increased risk for renal-cell carcinoma (odds ratio, 1.7; 95% CI, 1.1–2.7; based on 57 exposed male cases). The exposures incurred in other industries were not relevant to the topic of this monograph. Duration of exposure in the blast furnace industry was studied, but no dose–response was seen (odds ratio for 1–2 years of exposure: 1.9; 95% CI, 0.9–4.0; 21 exposed cases; 3–7 years: 1.6; 95% CI, 0.7–3.3; 18 exposed cases; 8–41 years: 1.6; 95% CI, 0.7–3.4); 14 exposed cases.

A large population-based case–control study was carried out in Canada (Nadon *et al*., 1995) as part of a larger study (described in detail in Section 2.2.1). A total of 181 male renal-cell carcinoma patients [ICD, 189.0] were selected from the database of all cancer patients registered during 1979–85. and 2196 male population and cancer controls were selected after exclusion of those with lung and urinary bladder cancer. Odds ratios derived from unconditional logistic regression were adjusted for age, family income, ethnicity and cumulative tobacco smoking index. Results related to PAHs derived from coal did not show any association with renal-cell carcinoma.

A population-based case–control study was carried out in five regions of Germany (two in East Germany and three in West Germany) (Pesch *et al*., 2000). Cases were male and female patients at large German hospitals and of German nationality, with no restriction on age, who had had histologically confirmed renal-cell carcinoma diagnosed between 1991 and 1995. Local residency registries provided controls, who were frequency-matched by region, sex and age. A total of 935 incident cases of renal-cell carcinoma (570 men and 365 women) and 4298 controls (2650 men and 1648 women) were included in the study. The response rate was 88% for cases and 71% for controls. Cases and controls were interviewed personally using a questionnaire to obtain full occupational histories, as well as supplemental information on job tasks with suspected exposure to different agents. Job titles were coded according to the International Standard Classification of Occupations (ISCO) code. Retrospective evaluation of exposure was made using a job–exposure matrix (English or German). In addition, German experts created a job–task–exposure matrix that accounted for the probability and intensity of exposure to a specific agent. To assess the effect of occupational exposures, the ISCO code (three-digit) of the longest job held was used for statistical analysis. The total number of years worked in a job title represented a subject's lifetime exposure, whereas the quantification of exposures in job tasks involved summing of the number of years worked, weighted by the percentage of time spent at the specific task ('duration'). The results according to duration were given for job groups that represented an aggregate of

job titles or job tasks with similar exposure circumstances. The job groups constituted were not relevant to the topic of this monograph. The job–exposure matrix (English or German) or the job–task–exposure matrix allowed the construction of an index that corresponded to the product of the duration × intensity × probability of exposure. This exposure index was divided into four categories according to the 30th, 60th and 90th percentiles. All odds ratios were adjusted for age, region and sex. Results given for the job title (three-digit ISCO code) included only those with a significant odds ratio. The English job–exposure matrix included a category entitled 'tar, pitch and mineral oil' that showed a non-significant association that increased with the level of the exposure index (odds ratio: medium, 1.1; 95% CI, 0.9–1.5; based on 86 exposed cases; high, 1.2; 95% CI, 0.9–1.6; based on 96 exposed cases; substantial, 1.4; 95% CI, 0.9–2.1; based on 34 exposed cases). The same trend was observed for women and was of borderline significance with 'substantial' exposure (odds ratio: medium, 1.0; 95% CI, 0.6–1.7; based on 15 exposed cases; high, 1.2; 95% CI, 0.7–2.0; based on 16 exposed cases; substantial, 2.1; 95% CI, 1.0–4.5; based on 10 exposed cases). Similar estimates could not be made with the German job–exposure matrix. The English job–exposure matrix also considered exposure to PAHs. The results were similar to those for 'tar, pitch and mineral oil'. Results for PAHs using the German job–task matrix were available only for men and did not show any increased risk (odds ratio, 0.9; 95% CI, 0.7–1.2; 0.8; 95% CI, 0.6–1.0; 0.9; 95% CI, 0.6–1.4 for medium, high and substantial exposure; based on 80, 67 and 26 exposed cases, respectively).

(b) Urinary bladder cancer (Table 2.11)

Baxter and McDowall (1986) conducted a case–control study in London, United Kingdom, based on death certificates. Cases were all deaths from urinary bladder cancer (ICD-8, 188) from 1968 to 1978 among male residents of six London boroughs. Two controls per case were selected, one from among male deaths from all other cancers and one from among all deaths including cancer, and matched on borough, age and year of death. A total of 1080 cases were included [the number of controls was not given but was assumed to be 2160, given two per case]. Occupational information was abstracted from death certificates by one of the authors. The occupation of 'gas worker' had odds ratios of 0.8 and 1.4 using the control groups of deaths from all other cancers and deaths from all other causes including cancer, respectively [confidence intervals were not given and the results were not statistically significant].

A hospital-based case–control study was carried out in 27 hospitals in the Copenhagen area, Denmark (Jensen *et al*., 1988). Cases were 97 incident cancers of the renal pelvis and ureter diagnosed between 1979 and 1982. The authors estimated that their case population represented approximately 80% of all Danish incident cases. Three hospital controls per case were matched for hospital, sex and age. Patients with urinary tract or tobacco smoking-related diseases were excluded from the controls, leaving 288 controls enrolled. Trained interviewers used a detailed questionnaire to obtain information on tobacco smoking, consumption of beverages, use of analgesics and occupational

Table 2.11. Case–control studies of urinary tract cancers and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Urinary cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Baxter & McDowall (1986), United Kingdom	1080 deceased male cases (1968–78), 2160 deceased male population controls	Gas workers	Bladder	11	0.8 [not given] 1.4 [not given]	Controls: deaths from all other cancers Controls: included deaths from all other causes, including cancer Job category extracted from death certificates; data of too poor quality to be considered; number of exposed deaths (cases + controls)
Jensen *et al.* (1988), Denmark	97 cases (1979–82), 288 hospital controls	Coke, coal Asphalt, tar	Renal pelvis, ureter	8 9	4.0 (1.2–13.6) 5.5 (1.6–19.6)	Only men were employed in these occupations and occupational history was not described in detail; adjusted for tobacco smoking
Risch *et al.* (1988), Canada	835 cases (1979–82), 792 population controls	*Aluminium smelting* Ever/never 8–28 years before diagnosis *Tar, asphalt* Ever/never 8–28 years before diagnosis	Bladder, including tumours of borderline malignancy	14 46 	1.91 (0.64–6.43) 2.61 (0.70–12.5) 1.44 (0.78–2.74) 3.11 (1.19–9.68)	From a list of industries/occupations and specific agents; only men were exposed to these occupations/agents; adjusted for smoking
Bonassi *et al.* (1989), Italy	144 male cases (1972–82), 405 male population controls	*Road menders* Definite exposure to PAHs Possible exposure to PAHs	Bladder, including papillomas	2 25 74	1.40 (0.27–7.28) 2.14 (0.82–5.60) 1.05 (0.45–2.44)	Job–exposure matrix for >1 year of exposure developed; adjusted for tobacco smoking and aromatic amines

Table 2.11 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Urinary cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Schumacher *et al.* (1989), Utah, USA	417 cases (1977–83), 877 population controls	**Coal tar, pitch**	Bladder			A job–exposure matrix was applied to code the complete lifetime occupational history collected; adjusted for tobacco smoking, age, religion, and education
		Men		92		
		Ever/never			1.08 (0.78–1.49)	
		<10 years/never			1.10 (0.76–1.60)	
		≥10 years/never			1.04 (0.64–1.68)	
		Women				
		Ever/never		3	2.30 (0.30–17.5)	
Clavel *et al.* (1994), France	658 male cases (1984–87), 658 male hospital controls	*Exposure to PAHs*	Bladder			Exposure assessed on case-by-case basis; adjusted for matching variables, tobacco smoking and coffee consumption; excluding subjects possibly exposed to aromatic amines; results unchanged after inclusion of subjects with possible exposure to aromatic amines
		Ever/never		231	1.3 (1.0–1.7)	
		Maximum intensity of exposure to PAHs				
		Low			1.2 (0.9–1.7)	
		Medium			1.3 (0.9–2.1)	
		High			1.8 (0.8–3.3)	
					p for trend <0.05	
Nadon *et al.* (1995), Canada	486 male cases, 2196 male population and cancer controls	*PAHs derived from coal*	Bladder			Case-by-case evaluation of lifelong occupational history; adjusted for tobacco smoking, age, socioeconomic status, ethnic group, proxy respondent and aromatic amines
		Low		154	0.8 (0.6–1.3)	
		High		67	0.6 (0.3–1.2)	

Table 2.11 (contd)

Reference, location	Effective no. of subjects	Job/exposure category	Urinary cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Grimsrud *et al.* (1998), Norway	52 male cases (1980–92), 156 male population controls	ISCP >1 year and exposed to PAHs	Bladder	3	0.5 (0.1–1.9)	Exposure was obtained from personnel files at the ISCP; no interview of the subjects, no data available for work outside these plants or for tobacco smoking; considered exposed to PAHs if worked in pig iron smelter department or >1 year in coke plant, according to an industrial hygienist
		ISCP >1 year without exposure to PAHs		20	1.4 (0.7–2.9)	

CI, confidence interval; ISCP, iron, steel and coke plants

and related exposures; 99% of cases and 100% of controls were enrolled and most (94%) of these subjects were interviewed at the hospital. Results were listed by occupation; however, no details were given on which jobs were concerned [i.e. the longest job held, the job held at the time of diagnosis]. Significant associations were obtained for occupational exposure to: coke and coal (odds ratio, 4.0; 95% CI, 1.2–13.6; based on eight exposed cases and seven exposed controls) and asphalt and tar (odds ratio, 5.5; 95% CI, 1.6–19.6; based on nine exposed cases and six exposed controls); only male subjects worked in these industries. All of the results were adjusted for lifetime tobacco consumption. [This study is qualified by the authors as an "explorative study with regard to occupational exposure". Neither temporal associations (duration, latency) nor the effect of the level of exposure was tested.]

Risch *et al*. (1988) conducted a population-based case–control study in Canada. Incident cases of urinary bladder cancer, diagnosed between 1979 and 1982 and aged 35–79 years, were included. Identification of the cases differed according to their residential region (registry, active review of medical records in main hospitals). A total of 1251 cases were identified, and a response rate of 67% resulted in a group of 835 cases. Controls were randomly selected through population listings, matched for year of birth, sex and area of residence; 1483 controls were selected and 792 accepted to participate (53% response rate). Questionnaires were completed at the subjects' home by trained interviewers. The occupational component included a list of 26 specific industries/occupations selected *a priori* by the authors, and subjects were asked whether they had ever worked in such industries. In the case of a positive answer, duration of employment was also requested. In addition, a list of substances was proposed to the subjects. Data were analysed in terms of ever versus never and duration of exposure, for which the authors defined a window of exposure from 8 to 28 years [the Working Group queried the choice of the time window] before case diagnosis in which they investigated an association with industries/occupations and with occupational exposure to agents. Statistical analysis was performed using conditional logistic regression models that systematically included tobacco smoking history. Among the industries/occupations studied, the odds ratio for ever working in aluminium smelting was 1.91 (95% CI, 0.64–6.43; based on 14 exposed cases). Interestingly, this association was reinforced when exposure occurring between 8 and 28 years before diagnosis was considered (odds ratio, 2.61; 95% CI, 0.70–12.50) as well as the trend with duration of exposure (odds ratio, 5.92; 95% CI, 1.07–3.20; for a unit of 10 years of duration). With regard to occupational exposure to substances, the odds ratio for urinary bladder cancer for the group 'tar asphalt' was 1.44 (95% CI, 0.78–2.74; based on 46 exposed cases) for subjects ever exposed versus never exposed, 3.11 (95% CI, 1.19–9.68) for subjects exposed 8 to 28 years before diagnosis and 2.02 (95% CI, 1.08–4.97) for a trend with a 10-year duration of exposure. [Some (n = 64) of the total (n = 826) cases that were included in the analysis were of borderline malignancy. However, exclusion of these cases and their matched controls did not modify the results.]

Bonassi *et al*. (1989) carried out a population-based case–control study in the Bormida valley (Italy) that focused on urinary bladder cancer and occupational exposure to PAHs. Cases were 150 histologically confirmed bladder cancers that were identified through hospital admission discharges and diagnosed between 1972 and 1982; 52% of cases were deceased at the time of interview. Population controls were randomly selected from the demographic registries of the region at the time of the diagnosis of the case, and were matched to cases on sex and age; 5% of the controls were deceased. The response rate among subjects (or the next of kin for the deceased) was 96% among cases and 90% among controls. A questionnaire was administered in 1983. Women were finally excluded from the analysis because of their small number and absence of exposure to PAHs, which left 144 male cases and 405 matched controls for the analysis. An a-priori list of 11 occupational categories of interest for bladder cancer was devised based on previous literature. A subject was classified as exposed if he had worked for more than 1 year in one of these categories (none of the subjects had worked in more than one category). In addition, a job–exposure matrix was constructed specifically for this study. According to the literature, some occupations were classified as having definite exposure to PAHs: coke worker, mechanic, railroad worker, glass worker, road mender, stoker (in a distillery), welder, coalman and mason (in a kiln). Imprecise occupational exposure to PAHs was classified as possible exposure. Odds ratios were adjusted for tobacco smoking using unconditional logistic regression. Among the list of the nine occupational categories, the odds ratio for road menders was 1.40 (95% CI, 0.27–7.28; based on two exposed cases and six exposed controls). Analysis for definite exposure to PAHs showed a non-significant increase in the risk for bladder cancer (odds ratio, 2.14; 95% CI, 0.82–5.60; based on 25 exposed cases and 48 exposed controls; also adjusted for exposure to aromatic amines). This increase in risk was not observed for subjects with possible exposure to PAHs (odds ratio, 1.05; 95% CI, 0.45–2.44; based on 74 exposed cases and 199 exposed controls; also adjusted for exposure to aromatic amines). In addition, an analysis stratified for exposure to aromatic amines showed that the odds ratio related to definite exposure to PAHs among subjects not exposed to aromatic amines was 2.53 (95% CI, 0.56–11.50; based on three exposed cases and five exposed controls). [The 'possible' category included jobs with imprecise occupational histories that are very heterogeneous with regard to exposure to PAHs (craftsman, carpenter, blacksmith) and therefore suffered from non-differential exposure misclassification, biasing the odds ratios towards the null value.]

A population-based case–control study was conducted in Utah, USA (Schumacher *et al*., 1989). Eligible cases were Utah residents, aged 21–84 years, who had had a urinary bladder cancer diagnosed between 1977 and 1983, and were identified through the Utah Cancer Registry. Controls, matched to cases by sex and age, were identified through random-digit dialling (for those aged 21–64 years) or through the Health Care Financial Administrative list (for those aged 65–84 years). The response rate was 76% for cases and 79% for controls, leading to a final sample of 417 cases and 877 controls after exclusion of subjects who did not have any occupation listed. Interviews were conducted at home

and provided information on several items including tobacco smoking, coffee consumption and complete lifetime occupational history (with the time period worked for each job). Occupations were coded using the 1970 US Bureau of Census Alphabetic Index of Industries and Occupations and a job–exposure matrix was applied (Hoar *et al*, 1980). Duration of exposure was classified as ever/never exposed or less than 10 or more than 10 years. Odds ratios and confidence intervals were generated using logistic regression models that were stratified by sex, smoking status and/or age. Men that had been ever exposed to coal tar and pitch did not have a significantly increased risk for bladder cancer (odds ratio, 1.08; 95% CI, 0.78–1.49; based on 92 exposed cases and 160 exposed controls). Taking duration of exposure into account did not modify the results (odds ratio, 1.10; 95% CI, 0.78–1.49; and 1.04; 95% CI, 0.64–1.68, respectively, for the categories <10 years and ≥10 years). These associations were adjusted for age, tobacco smoking, religion and education. Very few women were assessed as ever having been exposed to coal tar and pitch (odds ratio, 2.30; 95% CI, 0.30–17.5; based on three exposed cases and three exposed controls).

Clavel *et al*. (1994) conducted a multicentre, hospital-based case–control study in France to investigate occupational risk factors and urinary bladder cancer. A total of 765 histologically confirmed bladder cancers, diagnosed between 1984–1987 and aged <80 years, were identified as well as 765 controls who had been admitted to the same hospitals for different diseases, excluding cancer, haematuria and work accidents. The controls were matched to cases on sex, age, hospital, place of residence and ethnicity. Analysis was restricted to men (658 cases and 658 controls), since few women had been exposed to PAHs. A questionnaire was developed specifically for occupational history in which each job held by the subject was described. Job titles were further coded with the International Labour Office code and the French code of industrial sectors. Occupational exposure was assessed on a case-by-case basis by a hygienist team following the procedure developed by Gérin *et al*. (1985). Exposure to PAHs was considered in three categories (<10 ng/m^3, 10–999 ng/m^3 and ≥1000 ng/m^3), in terms of maximum dose, average or cumulative exposure to PAHs and according to time-related variables (total duration, age at start, time since starting, time since cessation of exposure). Statistical analysis was performed using unconditional logistic regression models adjusted for the matching variables, tobacco smoking and/or coffee consumption. Associations were also re-estimated after exclusion of subjects who had possibly been exposed to aromatic amines. When all job titles with exposure to PAHs were considered, regardless of the level, an odds ratio of 1.3 (95% CI, 1.0–1.7; adjusted for the matching variables, cumulative smoking and coffee consumption; excluding subjects possibly exposed to aromatic amines) was observed. A more restrictive definition of exposure to PAHs (>30% of workers exposed to the medium or high level) resulted in an association of 1.6 (95% CI, 1.0–2.5; based on 64 exposed cases; adjusted for matching variables and smoking status). Significant dose–response relationships (p-value for trend <0.05) were observed with average and maximum exposure to PAHs (odds ratio for categories of maximum exposure to PAHs compared to unexposed: low exposure, 1.2; 95% CI, 0.9–1.7; medium

exposure, 1.3; 95% CI, 0.9–2.1; high exposure, 1.8; 95% CI, 0.9–3.6). The dose–response relationship with cumulative exposure was more heterogeneous and therefore the test for trend was not significant (odds ratio: <100 ng/m^3, 1.7; 95% CI, 1.2–2.4; 100–499 ng/m^3, 0.8; 95% CI, 0.5–1.3; 500–14 999 ng/m^3, 1.3; 95% CI, 0.8–2.0; ≥ 15 000 ng/m^3, 1.8; 95% CI, 0.8–3.9; adjusted for matching variables, cumulative smoking, coffee consumption; excluding subjects possibly exposed to aromatic amines). No significant trend was seen with total duration of exposure or time since start or cessation of the exposure. Taking latency into account, the highest association was observed for the category 20–29 years since start of exposure (odds ratio, 1.8; 95% CI, 0.9–3.5; adjusted for matching variables, cumulative smoking, coffee consumption; excluding subjects possibly exposed to aromatic amines).

The study by Nadon *et al*. (1995) (described in detail in Section 2.2.2(*a*)) also reported results for urinary bladder cancer among 486 male cases and 2196 male population and cancer controls (excluding cancers of the lung and kidney). Overall, there were no significant associations between bladder cancer and exposure to different types of PAHs; however, high exposure to benzo(*a*)pyrene was associated with a significantly reduced risk for bladder cancer (odds ratio, 0.5; 95% CI, 0.3–0.8; based on 176 exposed cases). The odds ratios for low and high exposure to coal were 0.8 (95% CI, 0.6–1.3; based on 154 cases) and 0.6 (95% CI, 0.3–1.2; based on 67 exposed cases), respectively. There was no interaction between exposure to PAHs and smoking status in the risk for bladder cancer.

A population-based case–control study was carried out in the municipality of Rana, a town in Norway where iron, steel and coke plants are located (Grimsrud *et al*., 1998), and was aimed at determining why the incidences of lung and urinary bladder cancer in this area increased steadily during 1980–92 whereas the national incidence rates stabilized during the late 1980s. Lung cancer was the main end-point studied, although bladder cancer was included in the protocol. Fifty-two incident cases of bladder cancer diagnosed among male residents of Rana in 1980–92 and registered at the cancer registry constituted the case group. A group of 156 controls (three per case), matched to cases on sex and year of birth, was identified from the national registrar and selected from among residents living in Rana on January 1993 and deceased persons who had Rana as their last place of residence. Information on job titles and work area were taken from personnel files of the plants studied. Evaluation of exposure to PAHs was based on measurements in the job–exposure matrix used for lung cancer. Positive exposure was assigned for those who had worked for more than 1 year in the pig iron department of the iron or steel plants or those who had worked at the coke plants and had been exposed to PAHs according to environmental measurements made previously by industrial hygienists. Odds ratios and confidence intervals for urinary bladder cancer were derived from unadjusted conditional logistic regression models. Information on smoking habits and occupations outside the industry was not available. There was no increased risk for bladder cancer related to employment in the iron, steel and coke plants: the odds ratio for exposure to PAHs was 0.5 (95% CI, 0.1–1.9; based on three exposed cases and 19 exposed controls), and was

1.4 (95% CI, 0.7–2.9; based on 20 exposed cases and 45 exposed controls) for non-exposed workers at the same plants. The authors mentioned that the highest level of exposure to PAHs for top-side coke-oven workers was <300 μg/m^3 in the late 1970s, which was less than reported levels from American coke plants. The PAH level in the pig iron department of the iron and steel foundries was 25 μg/m^3, which was less than the national occupational exposure limit.

(c) Skin cancer (Table 2.12)

Kubasiewicz *et al*. (1989, 1991) conducted a population-based case–control study among men in Poland which also included a hospital-based control group. A total of 534 incident cases of skin cancer were identified from a hospital registry during 1983–88 and 376 of these were enrolled. Two age-matched (2:1) control groups comprised 752 subjects each, one from the general population and one from hospital clinics in the same area, which included dermatological outpatients who were treated for conditions other than skin cancer. A personal interview was conducted in the subjects' homes, in which detailed lifetime occupational history was collected using a questionnaire. Substances that contain PAHs were extracted from the occupational history (grease, tar, pitch, soot, mineral oil, creosote oil, anthracene oil, coke, petrol, paraffin, gasoline, paraffine oil, soft asphalt, mazout, bituminous mass, petroleum and asphalt). Only significant confidence intervals were reported. The frequency of exposure among cases, population controls and hospital controls was 57%, 54% and 57%, respectively. The association between exposure to PAHs and skin cancer was non-significant, regardless of the control group used (odds ratios, 1.15 and 1.14 for population and hospital controls, respectively; based on 216 exposed cases). There was also no significant association with duration of exposure. The study of each PAH considered separately, ignoring potential concomitant exposure to other PAHs, showed a statistically significant relation only for mineral oils (odds ratio, 1.46; 95% CI, 1.06–2.05; based on 99 exposed cases). This association was significant with the population control group but not with the hospital controls and was confirmed when a latency of 30 years or more was considered (1.60; 95% CI, 0.98–2.63; based on 36 exposed cases; odds ratio). [The Working Group noted that the description of the population was poor for both the cases and the controls. The histology of the skin cancer was not described and was not confirmed (except for the fact that they were selected from the registry). The assessment of exposure was not well described, and statistical analysis was also poor. Confounding factors were not taken into account, and confidence intervals were not given, except when significant. Therefore, the results of this study should be considered with caution.]

Gallagher *et al*. (1996) conducted a population-based case–control study among men in Canada. Eligible cases were men aged 20–79 years who had been diagnosed with a first primary basal-cell carcinoma (*n*=314) or a first primary squamous-cell carcinoma (*n*=225) and who were selected through the cancer registry. The group with basal-cell cancer included every fourth man registered with a head and neck basal-cell cancer and all men with a basal-cell cancer at any other anatomical site. Response rates were slightly

Table 2.12. Case–control studies of skin cancers and exposure to polycylic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Skin cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Kubasiewicz *et al.* (1991), Poland	374 cases (1983–88), 752 population and 752 hospital controls	*Exposure to PAHs* Tar Pitch Soot Coke	Any	216 28 15 29 32	1.15 [NA, p >0.05] 1.09 [NA, p >0.05] 0.93 [NA, p >0.05] 1.22 [NA, p >0.05] 1.29 [NA, p >0.05]	Lifetime occupational history. [poor description of study population; the results should be interpreted with caution].
Gallagher *et al.* (1996), Canada	226 BCC cases and 180 SCC cases (1983–84), 406 population controls	Pitch tar and tar products Coal dust	BCC SCC BCC SCC	32 27 67 69	1.2 (0.7–2.1) 0.9 (0.5–1.7) 1.4 (0.9–2.1) 1.6 (1.0–2.4)	Lifetime occupational history; adjusted for skin and hair colour and mother's ethnicity

BCC, basal-cell carcinoma; CI, confidence interval; NA, not applied; SCC, squamous-cell carcinoma

higher for the group with squamous-cell carcinoma (80%) than for the basal-cell cancer patients (72%). A total of 226 basal-cell and 180 squamous-cell carcinomas were finally included. Eligible controls were 573 age-matched subjects randomly selected from the files of Alberta Health care insurance plan subscribers, who had had no prior basal-cell cancer or squamous-cell carcinoma. A 71% response rate resulted in 406 enrolled subjects. Subjects were interviewed at home by trained interviewers who were unaware of the objectives of the study and were blinded to case–control status. The questionnaire included questions on phenotype and pigmentation factors, factors related to the medical history, smoking history and detailed lifetime occupational history (job titles, periods). Detailed information on exposure assessment was not given. A list of specific substances to which subjects might have been exposed was created that included 'coal dust' and 'pitch tar and tar products'. To test for a dose–response effect, a 'duration' variable was constructed from the duration of the jobs held weighted with the source of exposure (direct job, job environment, hobby, home) and intensity of exposure (<1 h/week, 1–4 h/week, 5–19 h/week, ≥20 h/week). Subjects were dichotomized to compare exposed with unexposed subjects. The same control group was used for comparisons between the two case groups (basal-cell cancer and squamous-cell carcinoma). Statistical analysis was carried out using conditional logistic regression models adjusted for age, skin and hair colour, mother's ethnic origin and exposure to sunlight as potential confounding factors. No association was noted with pitch tar and tar products (odds ratio, 1.2; 95% CI, 0.7–2.1; 32 exposed cases of basal-cell cancer; odds ratio, 0.9; 95% CI, 0.5–1.7; 27 exposed cases of squamous-cell carcinoma), but an association of borderline significance was noted with exposure to coal dust (odds ratio, 1.4; 95% CI, 0.9–2.1; 67 exposed basal-cell cancer cases; odds ratio, 1.6; 95% CI, 1.0–2.4; 69 exposed squamous-cell carcinoma cases). All the results were adjusted for age, mother's ethnicity, and skin and hair colour. [The frequency of exposure was 16% for pitch tar and 43% for coal dust. Job titles that were considered to entail exposure to these substances were not specified. The duration–effect relationship with squamous-cell carcinoma, observed for exposure to petroleum products and coal dust, increased the credibility of the association.]

(d) Laryngeal cancer (Table 2.13)

Ahrens *et al*. (1991) conducted a hospital-based case–control study in a main general hospital of the Bremen region, Germany. Cases included in the study were 55 incident male patients who were diagnosed with histologically confirmed laryngeal cancer in 1986 and 30 prevalent male cases diagnosed during 1984–85. Age, localization of the tumours and region of last residence were distributed equally between the two groups of cases, and all but one were squamous-cell carcinomas. Controls were selected from among the admission list of the same hospital, excluding neoplastic diseases and tobacco smoking-related diseases. Patients were interviewed with a standardized questionnaire that included questions on smoking and drinking habits as well as lifetime occupational history (industry, job titles, period). Industries and job titles were coded blinded to case–control status using standard German codes. Analysis was performed by grouping industries and

Table 2.13. Case–control studies of laryngeal cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Head and neck cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Gustavsson *et al.* (1998), Sweden	157 cases (1988–91), 641 population controls	*Exposure to PAHs*	Larynx			Lifetime occupational history; adjusted for region, age group and tobacco and alcohol consumption
		Low		26	0.77 (0.46–1.28)	
		High		53	1.47 (0.96–2.24)	
Elci *et al.* (2003), Turkey	940 cases (1979–84), (227 glottic cancer, 438 supraglottic cancer, 275 subglottic or unspecified cancer), 1519 hospital controls	Exposure to PAHs	All larynx	376	1.3 (1.1–1.6)	Lifetime occupational history; adjusted for age, tobacco smoking and alcohol consumption; no participation rates given
			Supraglottis	174	1.3 (1.1–1.7)	
			Glottis	94	1.4 (1.0–1.9)	
			Other sites	108	1.3 (0.9–1.7)	
		Intensity of exposure to PAHs	All larynx			
		Low		189	1.4 (1.1–1.7)	
		Medium		138	1.3 (1.0–1.6)	
		High		49	1.5 (1.0–2.2)	
Becher *et al.* (2005), Germany	257 cases (1998–2000), 769 population controls	**Road construction worker**	Larynx	22	6.4 (2.4–17.3)	Lifetime occupational history; adjusted for cigarette smoking and alcohol consumption
		Exposure to PAHs				
		Based on supplementary job questionnaire		19	2.3 (1.15–5.2)	
		Based on exposure checklist		25	1.6 (0.9–3.1)	

CI, confidence interval

job titles into 31 occupations and 21 branches of industry. In addition, the subjects declared at the end of the occupational history if they had been exposed to any substance on a list of 19 occupational exposures. Statistical analysis was performed using unconditional logistic regression, adjusting for age, tobacco smoking and alcohol drinking as potential confounders. With regard to self-reported occupational exposures, there was no association with coal tar/bitumen (odds ratio, 0.6; 95% CI, 0.2–1.9 [the number of exposed subjects was not given]). The coal tar/bitumen group included subjects who had been employed in the following industries: manufacture of machinery and transport equipment, general construction and interior construction; and for the following occupations: bricklayers and reinforced concreters, stone masons, road and other construction workers, stationery engine, crane and hoist operators, earth-moving machine operators, managers and accountants. [The Working Group considered that the category coal tar/bitumen was too wide to be considered in the scope of this monograph.]

Gustavsson *et al.* (1998) conducted a population-based case–control study of oral cancer in Sweden. Incident male cases of oral cancer (oral cavity, oropharynx, hypopharynx, oesophagus and larynx; ICD-9: 141, 143, 144, 145, 146, 148, 150 and 161), aged 40–79 years, who were registered in the cancer registry as being from Stockholm or living in the southern health care region and who were diagnosed between 1988 and 1991 were included. Controls were selected from population registers, and were frequency-matched to cases for region and age group. The response rates were 90% in cases and 85% in controls. A total of 157 cases of laryngeal cancer and 641 controls were included in the analysis. A standardized questionnaire included questions on lifestyle, tobacco and alcohol consumption as well as lifetime occupational history. Occupational history was reviewed blinded to the case–control status by an occupational hygienist who coded job titles according to a standard Swedish classification as well as the intensity and probability of exposure to 17 specific occupational exposure factors, including PAHs. Intensity of exposure to PAHs was assessed on a four-level scale, using benzo[*a*]pyrene as an indicator of exposure; level 3 represented exposures that were greater than one-fifth of the threshold limit value (TLV) for benzo[*a*]pyrene. The ratio between levels 1, 2 and 3 was 1, 10 and 100 for PAHs. Probability of exposure was also assessed on three levels: >70%, 33–70% and <33%. Cumulative exposure was calculated as the product of intensity, probability and duration of exposure; the contribution over the entire work history was also added. Statistical analysis was performed using unconditional logistic regression, adjusted for region, age group, tobacco smoking (current/former/never) and alcohol consumption (average level of alcohol intake in the past 5 years in grams of ethanol per week). Exposure to PAHs was first dichotomized in two classes (low, high), using the median of the cumulative index for the exposed controls as the cut-off point. Low exposure was not significantly related to laryngeal cancer (odds ratio, 0.77; 95% CI, 0.46–1.28; 26 exposed cases). For high exposure to PAHs, the odds ratio increased to 1.47 (95% CI, 0.96–2.24; 53 exposed cases). Among controls, the authors assessed the level of exposure to PAHs to be high for 1 year of employment as a coke or gas worker or 10 years as a steel or foundry worker and to be low for car repairers, garage workers and

iron miners because they would never accumulate enough exposure to be included in the high-exposure group.

Elci *et al*. (2003) carried out a hospital-based case–control study among men in Turkey. A total of 940 laryngeal cancer patients (ICD 161.0, 161.1, 161.2 and 161.9), diagnosed between 1979 and 1984 in the oncology treatment centre, and a control group of 1308 other cancer patients and 211 non-cancer subjects were analysed. During a personal interview, subjects were asked about tobacco smoking, alcohol drinking and lifetime occupational history. Each job held was coded for industry and occupation according to standard US codes by an industrial hygienist who created a job–exposure matrix that allowed assessment of exposure to several agents including PAHs and diesel exhaust. Exposure was assessed in terms of probability (lower than 25%, 25–75% and >75%) and intensity (<TLV, between TLV and 2 TLV and >2 TLV). Probability and intensity were then combined to achieve a final score that was categorized into unexposed, and low, medium and high exposure. Statistical analysis was performed using unconditional logistic regression, adjusted for age, tobacco smoking and alcohol drinking. Data on tobacco smoking and alcohol drinking were included in the models as ever/never exposure. Results were given only according to the occupational exposure assigned by the job–exposure matrix. The most prevalent exposure among cases was to PAHs (40%) followed by diesel exhaust (32%). The association between exposure to PAHs and all laryngeal cancers was significant (odds ratio, 1.3; 95% CI, 1.1–1.6; 376 exposed cases and 486 exposed controls), and was similar for the individual subsites (supraglottic, glottic and others). However, these results were mainly influenced by exposure to diesel exhaust since all subjects exposed to diesel exhaust were also exposed to PAHs. After exclusion of the subjects exposed to diesel exhaust, the association with PAHs was no longer significant (odds ratio, 0.8; 95% CI, 0.6–1.1). The association was further explored by intensity and probability of exposure. For intensity, no dose–response relationship was observed with exposure to PAHs in general (odds ratio: low, 1.4; 95% CI, 1.1–1.7; medium, 1.3; 95% CI, 1.0–1.6; high, 1.5; 95% CI, 1.0–2.2), although a dose–response was observed for supraglottic cancers only (odds ratio: low, 1.2; 95% CI, 0.9–1.6; medium, 1.4; 95% CI, 1.0–1.9; high, 1.7; 95% CI, 1.1–2.8). [The Working Group noted the poor adjustment for tobacco smoking and alcohol consumption, which are strong risk factors for laryngeal cancer. In addition, consideration of exposure to these factors with a covariable 'ever use' may lead to an underadjustment. The analysis clearly shows that the association with PAHs is actually governed by exposure to diesel exhaust, which is outside of the scope of this monograph.]

Becher *et al*. (2005) conducted a population-based case–control study in Germany among citizens aged up to 80 years. Cases were selected from the clinics of the different cities, and had been diagnosed with a histologically confirmed laryngeal tumour during 1998–2000. Local practitioners were also contacted to verify complete case ascertainment. Controls were randomly selected from population registries, and were frequency matched to cases (1:3) on age and gender. Personal interviews were carried out on 257 cases (89% response rate) and 769 controls (62% response rate). Occupational

exposure was assigned using three sources of the questionnaire: a detailed lifetime occupational history, an exposure checklist and supplementary job questionnaires. Industries and occupations were coded using standard German and International Labour Organization codes. Job titles were grouped into broader categories, which were analysed for their association with laryngeal cancer. Exposure to PAHs was assessed from selected jobs known to entail such exposures: roofer and installer of house siding, insulation installer, and workers in road construction, civil engineering, building construction, agriculture and forestry. In addition, the following substances were also listed in the questionnaire: coal tar, pitch, carbolineum and coal or graphite electrodes. Odds ratios were obtained using conditional logistic regression, adjusting for tobacco smoking and alcohol consumption and stratifying by age and gender. The category of road construction worker (on an 'ever held job' basis) was strongly associated with laryngeal cancer (odds ratio, 6.4; 95% CI, 2.4–17.3; based on 22 exposed cases and nine exposed controls). [It was not clear to the Working Group the type of exposures that were covered by the definition of road construction.] Exposure to PAHs assessed by the supplementary job questionnaire was statistically related to laryngeal cancer (odds ratio, 2.3; 95% CI, 1.1–5.2; based on 19 exposed cases and 15 exposed controls), although the relation diminished when assessment was based on the checklist of the questionnaire (odds ratio, 1.6; 95% CI, 0.9–3.1; based on 25 exposed cases and 29 exposed controls). Duration of exposure was explored in three categories, and the relation was significant only for the highest class (odds ratio, 3.8; 95% CI, 1.3–11.1; corresponding to >1300 h of exposure; 15 exposed cases and seven exposed controls). The odds ratio for the intermediate class (>0–1300 h) was 1.06 (95% CI, 0.3–4.0; four exposed cases and eight exposed controls).

(e) Pancreatic cancer (Table 2.14)

Kauppinen *et al.* (1995) conducted a population-based case–control study in Finland, on 1419 deceased cases of pancreatic cancer (ICD-9, 157), aged 40–74 years, who were identified from the Finnish Cancer Register files during 1984–87. Controls comprised 3150 subjects who had died from cancer at other sites (stomach, colon, rectum), at the same age and time period as the cases. Lifetime occupational histories were collected by a postal questionnaire sent to the next of kin, for which the response rates were 47% and 50% for cases and controls, respectively. After exclusions, mainly non-respondents (n=2497), the study included 595 cases and 1622 controls. Occupational histories were reviewed by industrial hygienists. Statistical analysis was performed using unconditional logistic regression models, adjusted for age, gender, history of diabetes mellitus, and smoking and alcohol use in the 1960s. The odds ratio for exposure to PAHs, based on industrial hygienist expertise of work histories, was 1.33 (95% CI, 0.69–2.57; based on 14 exposed cases).

Alguacil *et al*. (2000) conducted a hospital-based case–control study in Spain. Cases were incident pancreatic cancer patients who were diagnosed in one of the five hospitals included in the study. A total of 185 cases were identified, and 164 (96 men and 68 women) were included in the analysis (participation rate, 89%). Hospital controls were

Table 2.14. Case–control studies of pancreatic cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Pancreatic cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Kauppinen *et al.* (1995), Finland	595 deceased cases (1984–87), 1622 hospital controls	PAH exposure as assessed by an industrial hygienist	Any	14	1.33 (0.69–2.57)	Adjusted for age, gender, history of diabetes, smoking and alcohol use
Alguacil *et al.* (2000), Spain	164 cases (1992–95), 238 hospital controls	**Exposure to PAHs** *Intensity (for duration >10 years, lag of 10 years before diagnosis)*	Any	13	0.81 (0.37–1.76)	List of 10 activities, and jobs held for more than 6 years; lifetime occupational history obtained; Finnish job–exposure matrix used
		Low		8	1.08 (0.39–3.00)	
		High		2	1.73 (0.22–13.8)	
		>35μg/m^3 PAH		2	0.78 (0.12–5.18)	
Nadon *et al.* (1995), Canada	117 cases, 2741 population and hospital controls	*PAHs derived from coal*	Any			Other cancers among controls were lymphoma, periampullary cancer, and cancers of the lung, stomach, colon, liver, small intestine and oesophagus
		Low		7	1.1 (0.5–1.5)	
		High		5	2.1 (0.8–5.4)	

CI, confidence interval

selected from the same hospital as the case, and were free of pancreatic cancer, but an initial diagnosis was suspicious of pancreatic cancer, biliary cancer or chronic pancreatitis. Controls were diagnosed with acute or chronic pancreatitis, benign biliary pathology as well as lymphoma, periampullary neoplasms, abdominal digestive neoplasms of unspecified origin, metastasis and cancers of the colon, lung, liver, small intestine and oesophagus. Of the 264 eligible controls, 238 patients (167 men and 71 women) accepted to participate (90%). A panel of experts reviewed the diagnoses of all patients. Interviews took place during hospitalization by trained monitors who recorded occupation and lifestyle histories. A list of 10 work-related activities, defined *a priori* as being potentially related to pancreatic cancer, was proposed to the subjects. Subjects were also asked to describe jobs held for more than 6 years. Two industrial hygienists assessed exposure to 22 substances and classified them by level of exposure (exposed, unexposed or unknown) as well as by intensity of exposure (low, medium, high or unknown). In addition, the Finnish job–exposure matrix was used to assess exposure to 21 substances (unexposed, low, substantial). The cut-off point between low and substantial was at the 75th percentile of the distribution of the product of probability × intensity. Statistical analysis was performed by unconditional logistic regression, adjusted for age, gender, tobacco smoking and alcohol use. An assessment of exposure to PAHs by industrial hygienists was associated with an odds ratio of 0.81 (95% CI, 0.37–1.76; 13 exposed cases (8%) and 34 exposed controls (13%)). Although the results were non-significant, the relation increased when intensity, duration and latency were accounted for: low exposure for at least 10 years, 10 years before diagnosis (odds ratio, 1.08; 95% CI, 0.39–3.00; eight cases (5%) and 17 controls (7%)) and high exposure for at least 10 years, 10 years before diagnosis (odds ratio, 1.73; 95% CI, 0.22–13.8; two cases (1%) and two controls (<1%)). The association between exposure to PAHs (>0.35 μg/m^3) and pancreatic tumours using the Finnish job–exposure matrix as a method of assessment was not significant (odds ratio, 0.78; 95% CI, 0.12–5.18; two cases (1%) and three controls (2%) exposed for at least 10 years, 10 years before the diagnosis).

In the case–control study conducted by Nadon *et al*. (1995) in Canada (described in detail in Section 2.2.2*(a)*), 117 pancreatic cancer patients were included, and 2741 population and cancer controls were enrolled after exclusion of lung cancers. No significant association was observed between pancreatic cancer and exposure to PAHs.

A meta-analysis on occupational exposures and pancreatic cancer (Ojajärvi *et al.*, 2000) identified four informative studies of occupational exposures to PAH compounds (Cammarano *et al.*, 1986; Moulin *et al.*, 1989; Siemiatycki, 1991; Kauppinen *et al.*, 1995). The meta-risk ratio for pancreatic cancer associated with exposure to PAHs was 1.5 (95% CI, 0.9–2.5).

(f) Stomach cancer (Table 2.15)

In the case–control study conducted by Nadon *et al*. (1995) in Canada (described in detail in Section 2.2.2*(a)*), 250 stomach cancers were included, together with a total of

Table 2.15. Case–control studies of stomach cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Stomach cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Nadon *et al.* (1995), Canada	250 male cases (1979–86), 2514 male population and cancer controls	*PAHs derived from coal*	Any			Lifetime occupational history; case-by-case evaluation; adjusted for tobacco smoking, age, socioeconomic status and ethnicity; cancers of the lung and oesophagus were excluded from the control group
		Low		17	1.1 (0.7–1.9)	
		High		9	1.5 (0.8–3.1)	
Cocco *et al.* (2005), USA	41 957 cases (1984–94), 83 914 population controls	*Intensity of exposure to PAHs (white men)*	Any			Study subjects were obtained from death certificates. Occupation and industry reported on the death certificate; crude occupational data; results are given in terms of number of exposed subjects.
		Low		4047	1.00 (0.95–1.04)	
		Medium		1983	1.08 (1.02–1.15)	
		High		616	1.00 (0.90–1.10)	
		Probability of exposure to PAHs (white men)				
		Low		1356	1.04 (0.97–1.12)	
		Medium		1896	1.06 (1.00–1.13)	
		High		3394	0.99 (0.95–1.05)	

CI, confidence interval

2514 population and cancer control patients (excluding cancers of the lung and oesophagus). Results are presented in Table 2.15 but no significant results were observed.

Cocco *et al*. (2005) carried out a case–control study in 24 states of the USA based on death certificates. The authors obtained deaths from stomach cancer for the period 1984–96 among persons aged ≥25 years for 20 878 white men, 14 125 white women, 4215 African–American men and 2739 African–American women. Controls were deaths from non-malignant disease, matched (2:1) on region, gender, age and race. Occupation and industry, coded routinely since 1984 according to the 1980 US census occupation and industry codes, were extracted from the certificates. A job–exposure matrix was developed by two of the authors, which assessed a probability of exposure and an intensity level for each three-digit occupation and industry code. A probability and intensity score was then built. This score was based on the product of the subject's probability (and intensity) score attributed to the occupation with that of industry, if industry was the main contributor to exposure assessment. If the information on exposure came from occupation irrespective of the industry (i.e. plumber, welder), the score was based on the square of the occupational score. Odds ratios derived from logistic regression models included the following covariables: age, ethnic origin, marital status, urban/non-urban residence and socioeconomic status (based on Green's standardized score for specific occupation). Among white men, there was no association with exposure to PAHs, regardless of the intensity of exposure: low (odds ratio, 1.0; 95% CI, 0.95–1.04; 4047 exposed cases), medium (odds ratio, 1.08; 95% CI, 1.02–1.15; 1983 exposed cases) or high (odds ratio, 1.0; 95% CI, 0.90–1.10; 616 exposed cases). A weak association among white women was found at the low (odds ratio, 1.29; 95% CI, 1.06–1.59; 159 exposed cases) and medium levels of intensity (odds ratio, 1.39; 95% CI, 1.13–1.70; 158 exposed cases) but not at the high intensity level of exposure (odds ratio, 0.89; 95% CI, 0.67–1.18; 72 exposed cases). There were no significant associations with different exposure intensities observed among the African–American men and women. Probability of exposure gave similar results in white men for low (odds ratio, 1.04; 95% CI, 0.97–1.12; 1356 exposed cases), medium (odds ratio, 1.06; 95% CI, 1.00–1.13; 1896 exposed cases) and high probability (odds ratio, 0.99; 95% CI, 0.95–1.05; 3394 exposed cases).

(g) Oesophageal cancer (Table 2.16)

Gustavsson *et al.* (1998) (described in detail in Section 2.2.2(*d*)), included 122 cases of oesophageal cancer and 641 age- and region-matched population controls. Results for exposure to PAHs showed a significant relation at both low levels (odds ratio, 2.01; 95% CI, 1.16–3.48; based on 32 exposed cases) and high levels (odds ratio, 1.87; 95% CI, 1.11–3.16; based on 37 exposed cases) of exposure. However, no increase in the effect was observed with increasing exposure.

Parent *et al*. (2000) studied male oesophageal cancer patients (63 squamous-cell, 23 adenocarcinoma and 13 of uncertain morphology) aged 35–70 years, selected from a database of all cancer patients registered during 1979–85, who were included in a large

Table 2.16. Case–control studies of oesophageal cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference and country	Effective no. of subjects	Job/exposure category	Oesophageal cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Gustavsson *et al.* (1998), Sweden	122 cases (1988–91), 641 controls	*Exposure to PAHs*	Any			Lifetime occupational history
		Low		32	2.01 (1.16–3.48)	
		High		37	1.87 (1.11–3.16)	
Parent *et al.* (2000), Canada	99 cases (1979–85) (63 squamous-cell, 23 adenocarcinoma, 13 uncertain origin), 1066 population and cancer controls	*PAHs from coal*	Any			Lifetime occupational history; case-by-case exposure assessment
		Ever/never		10	1.2 (0.6–2.5)	
		Non-substantial		4	0.7 (0.2–2.1)	
		Substantial		6	2.0 (0.8–5.3)	
		Benzo[a]pyrene				
		Ever/never		24	1.1 (0.7–1.9)	
		Non-substantial		19	1.0 (0.5–1.7)	
		Substantial		5	2.3 (0.8–6.5)	

CI, confidence interval

Canadian case–control study. Controls were selected from electoral lists by random digit dialling. The response rates were 82% for cases and 75% for controls, which resulted in a final sample size of 99 cases and 1066 age-matched controls (533 population controls and 533 cancer controls) for analysis. Odds ratios were estimated by unconditional logistic regression, adjusted for age, birthplace, education, respondent (self or proxy), tobacco smoking, alcohol consumption and β-carotene intake. Branches of occupation and industry were coded using standard classification systems and were analysed by duration of employment, excluding the 5 years preceding diagnosis or enrolment into the study. The odds ratio for exposure to PAHs from coal in the ever-exposed category for all histological types was 1.2 (95% CI, 0.6–2.5; 10 exposed cases) and that for a substantial level of exposure was 2.0 (95% CI, 0.8–5.3; six exposed cases). Results were also non-significant for exposure to benzo[*a*]pyrene. When squamous-cell carcinoma was considered separately, the results were not significantly modified. When other potential exposures to carcinogens (sulfuric acid, toluene, chrysotyle asbestos, other paints and varnishes, iron compounds) were taken into account, the results remained similar for exposure to carbon black (odds ratio, 2.1; 95% CI, 0.8–5.3; nine exposed cases). [No industrial branches or occupations for which odds ratios were estimated were within the scope of this monograph.]

(h) Prostatic cancer (Table 2.17)

Aronson *et al*. (1996) studied occupational risk factors for prostatic cancer within a large Canadian case–control study (Gérin *et al*, 1985). Male patients with histologically confirmed prostatic cancer were selected from a database of all cancer patients registered in Montréal during 1979–85 (557 eligible patients) and 449 were interviewed (response rate, 81%). The statistical analysis was carried out with a pooled control group of 533 population-based (response rate, 72%) and 1550 other cancer controls for 17 occupations, 11 industries and 27 substances using unconditional logistic regression, adjusted for age, family income, ethnicity, Quetelet (body mass) index and respondent status. Each occupation, industry or substance was included in separate models. Exposure to the substances was assessed as non-substantial/substantial according to an index that combined confidence of assessment, latency, duration and the product of the concentration of and frequency of exposure to the substance (both scored as low, medium and high). Results by industry or occupation were reported according to duration (dichotomized into greater or less than 10 years of employment). Results for the assessment of exposure to PAHs from any sources showed no relation with either non-substantial or substantial exposure (odds ratio for non-substantial, 0.84; 95% CI, 0.63–1.12; odds ratio for substantial, 1.21; 95% CI, 0.68–2.17). With regard to exposure to PAHs from coal, a significant relation was observed at the non-substantial level (odds ratio, 1.99; 95% CI, 1.24–3.20) which was not confirmed at the substantial level (odds ratio, 1.08; 95% CI, 0.40–2.95). The relationship between prostatic cancer and exposure to PAHs from coal was further explored according to confidence of assessment,

Table 2.17. Case–control studies of prostatic cancer and exposure to polycyclic aromatic hydrocarbons (PAHs)

Reference, location	Effective no. of subjects	Job/exposure category	Prostatic cancer subtype	No. of exposed cases	Odds ratio (95% CI)	Comments
Aronson *et al.* (1996), Canada	449 cases (1979–85), 2083 population and hospital controls	**PAH from any source**	Any			Lifetime occupational history; case-by-case expertise; categories were based on an index of cumulative exposure.
		By level of exposure				
		Non-substantial		238	0.84 (0.63–1.12)	
		Substantial		62	1.21 (0.68–2.17)	
		PAH from coal				
		By level of exposure				
		Non-substantial		40	1.99 (1.24–3.20)	
		Substantial		20	1.08 (0.40–2.95)	
		By confidence				
		Probable		13	1.3 (0.7–2.5)	
		Definite		47	1.6 (1.1–2.3)	
		By concentration				
		Low		21	2.0 (1.2–3.5)	
		Medium-high		39	1.3 (0.9–2.0)	
		By frequency				
		Low-medium		35	1.4 (0.9–2.1)	
		High		25	1.7 (1.0–2.7)	
		By duration				
		<10 years		31	1.6 (1.0–2.4)	
		>10 years		29	1.4 (0.9–2.3)	
Krstev *et al.* (1998), USA	981 incident cases (1986–89), 1315 population controls	**Power plant operators**	Any			Lifetime occupational history
		By ethnicity				
		African–American		9	3.96 (1.05–14.90)	
		White		7	1.68 (0.55–5.08)	
		By duration of employment				
		<5 years		7	1.51 (0.50–4.55)	
		5–19 years		6	3.68 (0.74–18.40)	
		≥20 years		3	4.04 (0.41–39.8)	
		p for trend			0.03	

CI, confidence interval

concentration of exposure and duration. Results are included in Table 2.17 and are of moderate magnitude (range, 1.3–2.0) and mostly of borderline significance.

Krstev *et al.* (1998) conducted a population-based case–control study in the USA that included 981 incident cases of prostatic cancer (479 African–American and 502 white men) diagnosed between 1986–89 and 1315 controls (594 African–American and 721 white men category matched by age and race), aged 40–79 years, who resided in Atlanta, Detroit and 10 counties in New Jersey that were covered by population-based cancer registries. Lifetime occupational history was obtained from the subjects and job titles were coded according to standard occupational codes. The odds ratio for power plant operators adjusted for age, study site and race was 2.28 (95% CI, 1.00–5.21; based on 16 exposed cases). When stratified by race, the odds ratio was 3.96 (95% CI, 1.05–14.90; based on nine exposed cases) among African–Americans and 1.68 (95% CI, 0.55–5.08) among whites. A trend (p = 0.03) was observed for duration of exposure (odds ratios, 1.51, 3.68 and 4.04 for <5, 5–19 and ≥20 years of exposure, respectively).

2.3 Meta-analyses of studies of workers exposed to PAHs

Partanen and Boffetta (1994) reviewed 20 studies of asphalt workers and roofers that included 11 cohort and nine case–control studies of exposure to asphalt/bitumen or coal tar. All of the cohort studies addressed lung cancer and some provided data on additional sites. The case–control studies addressed cancers of the lung, urinary bladder and stomach, leukaemia and non-melanoma skin cancer. The meta-analysis excluded some studies that specified occupational categories poorly. Pooled relative risks were presented for the cohort and case–control studies separately, as well as for all cohort and case–control studies combined; the results were presented together with a test for heterogeneity between individual studies. Results were presented for the whole population and separately for three categories of occupational exposure: pavers and highway maintenance workers, roofers and miscellaneous or unspecified workers. For roofers, the overall pooled relative risk was 1.76 (95% CI, 1.46–2.11; based on 118 cases) for lung cancer in four cohort studies and 1.88 (95% CI, 1.23–2.76; based on 26 cases) in three case–control studies. The pooled relative risk across both designs was 1.78 (95% CI, 1.50–2.10; based on 144 cases; p-value for heterogeneity, 0.03). For pavers and highway maintenance workers, the overall pooled relative risk for lung cancer in three cohort studies was 0.87 (95% CI, 0.74–1.01; based on 167 cases). For stomach cancer, the pooled relative risks in three cohort studies were 1.33 (95% CI, 1.05–1.66; based on 77 cases) for all asphalt workers, 1.71 (95% CI, 1.12–2.51; based on 26 cases) for roofers and 1.14 (95% CI, 0.83–1.53; based on 44 cases) for pavers and highway maintenance workers. [The potential separate contributions of exposure to coal tar and bitumen to the observed excess risks could not be distinguished. The potential for exposure to coal tar was probably higher for roofers than for pavers.] For urinary bladder cancer, the pooled relative risks in cohort studies were 1.38 (95% CI, 1.06–1.78; based on 60 cases) for all asphalt workers, 1.68 (95% CI, 0.90–2.88; based on 13 cases in one study) for roofers and

1.20 (95% CI, 0.74–1.83; based on 21 cases in two studies) for pavers and highway maintenance workers. Only cohort studies investigated non-melanoma skin cancer and the pooled relative risks were 1.74 (95% CI, 1.07–2.65; based on 21 cases in four studies) for all asphalt workers, 4.0 (95% CI, 0.83–11.7; based on three cases in one study) for roofers and 1.20 (95% CI, 1.19–3.66; based on 14 cases in one study) for pavers and highway maintenance workers. Only cohort studies investigated leukaemia and the pooled relative risks were 1.41 (95% CI, 1.05–1.85; based on 51 cases in six studies) for all asphalt workers, 1.73 (95% CI, 0.94–2.90; based on 14 cases in two studies) for roofers and 1.31 (95% CI, 0.92–1.81; based on 36 cases in three studies) for pavers and highway maintenance workers.

Armstrong *et al.* (2002, 2004) performed a meta-analysis of published cohort studies in which PAHs were judged to be the predominant carcinogens implicated in lung (Armstrong *et al*., 2004) or urinary bladder (Armstrong *et al.*, 2002) cancer; 39 cohorts met these criteria. For each study, a unit relative risk for lung cancer was calculated in relation to cumulative exposure to benzo[*a*]pyrene. Eleven studies provided measurements of exposure to PAHs indexed as benzo[*a*]pyrene, five provided measurements of exposure to PAHs that were converted to benzo[*a*]pyrene from benzene-soluble matter, total PAHs or carbon black and, in 20 studies, benzo[*a*]pyrene was estimated by industrial hygienists from published measurements in comparable workplaces (Armstrong *et al.*, 2002). These latter estimations were carried out blind to the relative risks in the studies to which they were applied. To calculate cumulative exposure (as benzo[*a*]pyrene concentration–years), duration of employment was taken from the studies or, for those studies that did not provide duration of employment, a default value of 20 years was chosen, which was close to the average duration reported in other studies. Unit relative risks for each study were calculated by Poisson regression, assuming a log-linear relative risk model, and these were presented with 95% confidence intervals for the risk per 100 μg/m^3 benzo[*a*]pyrene–years of cumulative exposure. In studies that provided more than one contrast, internal comparisons were used rather than external comparisons, and higher contrasts in estimated exposure were used rather than lower ones. For all the studies combined and for groupings of results by industrial sector, heterogeneity of unit relative risk was estimated, and pooled unit relative risk values were estimated by meta-regression, using a log-linear random effects model with restricted maximum likelihood. Twenty-eight (72%) of the unit relative risks were >1, with the lower confidence limit >1 (p <0.05) in 14 of these. The pooled unit relative risk estimates were 1.17 (95% CI, 1.12–1.22; based on 10 studies) for coke ovens, 1.15 (95% CI, 1.11–1.20; based on four studies) for gas distillation and 1.16 (95% CI, 1.05–1.28; based on eight studies) for aluminium reduction. The test for heterogeneity between the three latter estimates was not significant (p >0.2). Higher values were found for other sectors: the pooled unit relative risk was 17.50 (95% CI, 4.21–72.78; based on three studies) for asphalt workers, 12.28 (95% CI, 0.48–314.4; based on three studies) for tar distillers, 16.24 (95% CI, 1.64–160.7; based on two studies) for chimney sweeps and 4.30 (95% CI, 0.81–22.79; based on four studies) for carbon electrode manufacturers. In addition, estimates were made for workers

in thermoelectric power plants that resulted in a very high but unstable unit relative risk (>1000; lower CI, 0; based on three studies) and in carbon black plants that gave a unit relative risk of 0 (upper CI, >1000; based on two studies). [The latter two industries had very low estimated levels of of benzo[*a*]pyrene and, as they do not use or process coal-tar derivatives, were not considered to be directly relevant to this monograph. Two of the three studies of thermoelectric power plants used fuel oil or both coal and fuel oil combustion. The one study of coal-fired plants (Petrelli *et al.*, 1989) was of limited power; six lung cancer deaths were observed whereas 4.39 were expected (SMR, 1.36; 95% CI, 0.50–2.97).]

In the meta-analysis of urinary bladder cancer (Armstrong *et al.*, 2002), 27 eligible cohorts were identified and meta-regression results were more heterogeneous between industries than those for lung. Only the overall result for the aluminium industry was statistically significant, with a unit relative risk of 1.42 (95% CI, 1.23–1.65; based on six studies) at 100 μg/m^3–years of benzo[*a*]pyrene. Other unit relative risks were 1.04 (95% CI, 0.79–1.37; based on six studies) for coke ovens, 8.80 (95% CI, 0.08–967.8; based on two studies) for gas works, 4.40 (95% CI, 0.27–70.67; based on three studies) for asphalt and >1000 (95% CI, 0.04–>1000; based on three studies) for tar distillery.

[The studies included in the meta-analyses have been summarized separately in this monograph. The Working Group noted that a number of exposures had to be imputed by the authors. However, it was considered that these meta-analyses added to the information available by providing a comparable exposure metric and unit relative risks across these studies and industrial sectors. Those derived for gasworks, aluminium works and coke ovens were very similar and each was statistically significant in the analysis of lung cancer.]

Ojajärvi *et al.* (2000) conducted a meta-analysis of pancreatic cancer and environmental or occupational exposures. The meta-risk ratio for occupational exposure to PAHs was 1.50 (95% CI, 0.90–2.50) based on the results of two cohort studies (Cammarano *et al.*, 1986; Moulin *et al.*, 1989) and two case–control studies (Siemiatycki, 1991; Kauppinen *et al.*, 1995).

2.4 Dietary exposure to PAHs and cancer

PAHs are formed during the grilling, barbecueing, curing or smoking of meats and other foods (Kazerouni *et al.*, 2001). Few epidemiological studies have investigated directly the association between dietary intake of PAHs and cancer at different sites; however, with the development of targeted meat-cooking questionnaires and PAH databases (CHARRED http://charred.cancer.gov/), results from epidemiological studies are becoming increasingly available (Kazerouni *et al*., 2001; Sinha *et al.*, 2005a).

Three studies of colorectal adenoma and one of colonic cancer have investigated the association between PAH and colonic tumours. A hospital-based case–control study in Maryland, USA, that included 146 cases and 228 negative screening (by sigmoidoscopy or colonoscopy) controls was specifically designed to address the hypothesis that dietary

intake of PAHs is associated with risk for colorectal adenoma (Sinha *et al*., 2005b). The study used a food-frequency questionnaire with a module that contained detailed questions on method and degree of cooking of meat in conjunction with the benzo[*a*]-pyrene database (as a surrogate for total and carcinogenic PAHs) that was derived from the collection and analysis of a wide range of food samples and referred to the time period 1 year before the interview (Sinha *et al*., 2005a). Odds ratios were adjusted for age, gender, total caloric intake, reason for screening (routine or other), level of physical activity, pack–years of cigarette smoking and use of non-steroidal anti-inflammatory drugs. Additional adjustment for consumption of total fat, saturated fat, fruit, vegetables, fibre or alcohol, education, race, body mass index, frequency of bowel movements and family history of colorectal cancer did not alter the findings substantially. The estimated median, 10th and 90th percentiles of benzo[*a*]pyrene intake in controls were 5, 0.2 and 66 ng/day, respectively, from meat and 73, 35 and 140 ng/day, respectively, from all foods. The estimated median, 10th and 90th percentiles of benzo[*a*]pyrene intake in cases were 17, 0.5 and 101 ng/day, respectively, from meat and 76, 44 and 163 ng/day, respectively from all foods. The odds ratios for exposure to dietary benzo[*a*]pyrene from meat with the 1st quintile of intake as the referent group were: 1.19 (95% CI, 0.51–2.80) for the 2nd quintile, 1.71 (95% CI, 0.76–3.83) for the 3rd quintile, 2.16 (95% CI, 0.96–4.86) for the 4th quintile and 2.82 (95% CI, 1.24–6.43) for the 5th quintile (*p* for trend, 0.01). The increased risk for colorectal adenomas was more strongly associated with benzo[*a*]pyrene intake estimated from all foods (odds ratio, 2.61; 95% CI, 1.08–6.29 for the 2nd quintile; odds ratio, 4.21; 95% CI, 1.79–9.91 for the 3rd quintile; odds ratio, 2.45; 95% CI, 0.98–6.12 for the 4th quintile; and odds ratio, 5.60; 95% CI, 2.20–14.20 for the 5th quintile; *p* for trend = 0.002).

[The Working Group noted that one of the strengths of this study was that the questionnaire was designed to investigate the role of carcinogens found in cooked meats. Other strengths were that the cases had adenomas rather than cancer and were thus less likely to have changed their current dietary habits following diagnosis. Furthermore, their responses to questions about usual dietary habits were less likely to be influenced by medical treatment. Finally, cases and controls were recruited from a well-defined base of individuals and the study had high participation rates. One of the limitations for this and other case–control studies is the potential for recall bias because the subjects were interviewed after diagnostic and treatment procedures; this is probably less of a problem when studying pre-malignant tumours compared with malignant lesions. Cases had a colonoscopy while the controls had only a sigmoidoscopy; as a consequence, some controls might have had undetected adenomas in the right-side of the colon. However, when analysis was restricted to cases with left-sided colonic adenomas that were detectable by sigmoidoscopy, the results were essentially unchanged.]

A second study investigated whether the intake of benzo[*a*]pyrene was associated with colorectal adenoma in the context of a polyp-screening study conducted in southern California, USA (Gunter *et al*., 2005). Benzo[*a*]pyrene intake was estimated using a meat-cooking module and CHARRED database. Covariates included were study centre,

age, gender, ethnicity, total calorific intake, consumption of fruit, vegetables, saturated fat and alcoholic beverages, physical activity, past and current tobacco smoking (using separate indicators), use of non-steroidal anti-inflammatory drugs, body mass index and family history of colorectal cancer. There was a 6% increase in risk for large (>1 cm) adenoma per 10 ng benzo[*a*]pyrene consumed per day (odds ratio, 1.06; 95% CI, 1.00–1.12; p for trend = 0.04). Consistent with this finding, an incremental increase of 10 g barbecued red meat per day was associated with a 29% increase in risk for large adenoma (odds ratio, 1.29; 95% CI, 1.02–1.63; p for trend = 0.04). Individuals in the top quintile of barbecued red meat intake were at increased risk for large colorectal adenoma (odds ratio, 1.90; 95% CI, 1.04–3.45) compared with those who never consumed barbecued red meat.

[The Working Group noted that one of the strengths of this investigation was that the study population had participated in a polyp-screening trial; hence, the control group was free of neoplastic lesions in the left-side of the colon. However, the possibility that polyps in the right-side of the colon were undetected cannot be excluded, thus attenuating the results through the assumption that benzo[*a*]pyrene is a risk factor for both left- and right-sided adenomas. Another potential concern is that, although detailed information on meat-cooking practices was given, responses were provided retrospectively, which introduces the potential for recall bias; however, there was no change in the risk estimates when analyses were restricted to those who reported no dietary change. One further limitation of the questionnaire is that participants reported only habitual diet from the past year. This fails to account for dietary patterns in the past, which may be more relevant for the initiating stages of carcinogenesis. Although detailed data on cooking practices were available, the participation rate was low (56% for the cases and 59% for the controls) which raises the possibility of selection bias.]

A large case–control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial in the USA investigated the role of benzo[*a*]pyrene in colorectal adenoma in 3696 cases of left-sided (descending and sigmoid colon and rectum) adenoma and 34 817 endoscopy-negative controls (Sinha *et al.*, 2005c). Dietary intake was assessed using a 137-item food-frequency questionnaire with additional questions on meats and meat-cooking practices which was linked to CHARRED to estimate benzo[*a*]pyrene intake. The analyses controlled for ethnicity (American Indian/Alaskan Native, Asian, Hispanic, non-Hispanic black, non-Hispanic white or Pacific Islander), educational attainment (<8 years at school, 8–11 years at school, 12 years at school/high school equivalent, post-high school other than college, some college, college graduate or post-graduate), tobacco use (never, smoked cigar or pipe, quit smoking ≥20 years and smoked ≤1 pack/day, quit ≥20 years and smoked >1 pack/day, quit <20 years and smoked ≤1 pack/day, quit <20 years and smoked >1 pack/day or unknown), alcohol use (<1, ≥1–15, >15–30 or >30 g/day), use of aspirin and ibuprofen separately (no regular use, <2, 2–3, 4, 8, 12–16, 30 or 60 per month), vigorous physical activity (none, <1, 1, 2, 3 or ≥4 h/week), body mass index (calculated as kilograms per square metre), total folate intake (micrograms per day), calcium intake (milligrams per day) and dietary fibre intake

(grams per day). When the 5th quintile was compared with the 1st quintile, benzo[*a*]pyrene resulted in a marginally elevated risk for colorectal adenoma (odds ratio, 1.15; 95% CI, 1.02–1.29). In investigating the subtypes, an increase in risk for adenomas in the descending colon and sigmoid colon were observed for benzo[*a*]pyrene (odds ratio, 1.18; 95% CI, 1.02–1.35) but not in the rectum. In addition, benzo[*a*]pyrene was associated with an increased risk for non-advanced colorectal adenoma (odds ratio, 1.18; 95% CI, 1.02–1.37) and for single adenomas (odds ratio, 1.17; 95% CI, 1.03–1.34).

[The Working Group noted that the Prostate, Lung, Colorectal, and Ovarian Cancer Trial contained a very large number of well-characterized cases of colorectal adenoma, which provided good power to investigate the role of meat and meat-related mutagens as risk factors for colorectal adenoma in relation to various end-points, including location and number of adenomas. This study included detailed questions on meat and meat-cooking methods in the dietary questionnaire which were linked to a database of meat-related mutagens. Although these methods of dietary assessment are the most comprehensive available, they are still probably associated with a degree of measurement error. Random measurement error can lead to attenuated risk estimates and, therefore, the actual risks may be higher. This cross-sectional study may be subject to dietary recall bias, although most participants (89%) completed the questionnaire before or on the same day as sigmoidoscopy screening, and hence before diagnosis. This potential bias was investigated and no appreciable differences were noted in risk estimates for meat intake among participants who completed the dietary questionnaire before, on the day of, or subsequent to the day of the sigmoidoscopy screening, which suggests that the time when the questionnaire was completed did not affect the associations between meat intake and adenoma.]

A population-based case–control study of colon cancer that included 701 African–Americans (274 cases, 427 controls) and 957 whites (346 cases, 611 controls) in North Carolina, USA. This study also used the meat-cooking module and CHARRED database. Covariates included consumption of fruit, vegetables, dietary fibre, total fat and dietary folate, total energy intake, physical activity, height, weight and body mass index (kilograms per square metre); fat intake was adjusted for total caloric intake using the residual method. No overall relationship was observed between benzo[*a*]pyrene intake and colon cancer; however, when stratified by race, an association was seen among African–Americans (odds ratio, 1.7; 95% CI, 0.9–3.2) but not among whites (odds ratio, 0.9; 95% CI, 0.6–1.5) (Butler *et al*., 2003).

[The Working Group noted that the strength of this study was the inclusion of an ethnically diverse population. In this population, African–Americans tended to consume higher levels of total and white meat. However, most notably, no differences in the association were observed between categories of meat intake and colonic cancer by race. Rapid ascertainment was used in an attempt to reduce recall difficulties, and cases were identified within 3 months of diagnosis. Selection bias represents another potential source of error, as indicated by an overall response rate of 61%, with a 10% greater response among cases than among controls.]

A population-based case–control study in Minnesota (USA) of 192 cases of exocrine pancreatic cancer and 670 controls collected information using the meat-cooking module; intake of benzo[*a*]pyrene was estimated using the CHARRED database (Anderson *et al*., 2002, 2005). The median intake of benzo[*a*]pyrene for each quintile was 0.3, 0.8, 1.8, 10.4 and 53.7 ng/day for the 1st, 2nd, 3rd, 4th and 5th quintiles, respectively. The corresponding odds ratios for benzo[*a*]pyrene intake were: 1.6 (95% CI, 0.9–2.8), 1.4 (95% CI, 0.8–2.6); 2.0 (1.1–3.7) and 2.2 (1.2–4.0) with a *p*-value for trend of 0.05. These estimates were adjusted for age, sex, tobacco smoking, education, race and diabetes. Grilled/barbecued red meat as a proxy for PAH intake was also a statistically significant predictor of risk for pancreatic cancer (Anderson *et al.*, 2002).

[The Working Group noted that this study was designed to address the hypothesis that dietary benzo[*a*]pyrene intake is associated with risk for pancreatic cancer. Detailed information on cooking practices and degree of cooking for specific types of commonly consumed meats was collected from direct interviews. This is essential to estimate most accurately the carcinogen intake and mutagenicity index associated with meat consumption. However, similarly to estimates of dietary nutrient intakes, estimates of dietary carcinogen intakes are imperfect and may lead to misclassification of exposure. Because approximately half of all pancreatic cancer cases die within 3 months of diagnosis, case–control studies of this disease are particularly challenging. The proportion of all eligible cases enrolled was low (approximately 30%), thus creating the potential for selection bias. In addition, pancreatic cancer cases who do enroll are usually quite ill and as a result may report their food intake history differently from controls.]

Other studies have investigated the relationship between benzo[*a*]pyrene and prostatic cancer and non-Hodgkin lymphoma but have found no association. In a prospective analysis of prostatic cancer in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, benzo[*a*]pyrene intake was estimated for 29 361 men. During follow-up, 1338 cases of prostatic cancer were ascertained but no association between prostatic cancer and benzo[*a*]pyrene intake was observed (Cross *et al.*, 2005). No association between benzo[*a*]pyrene intake and non-Hodgkin lymphoma was seen in a population-based case–control study in Iowa, Detroit, Seattle and Los Angeles (USA) that included 458 cases and 383 controls (Cross *et al*., 2006).

2.5 References

Ahrens, W., Jockel, K.H., Patzak, W. & Elsner, G. (1991) Alcohol, smoking, and occupational factors in cancer of the larynx: A case–control study. *Am. J. ind. Med*., **20**, 477–493

Alguacil, J., Kauppinen, T., Porta, M., Partanen, T., Malats, N., Kogevinas, M., Benavides, F.G., Obiols, J., Bernal, F., Rifa, J. & Carrato, A. (2000) Risk of pancreatic cancer and occupational exposures in Spain. PANKRAS II Study Group. *Ann. occup. Hyg*., **44**, 391–403

Andersen, A., Dahlberg, B.E., Magnus, K. & Wannag, A. (1982) Risk of cancer in the Norwegian aluminium industry. *Int. J. Cancer,* **29***,* 295–298

Anderson, K.E., Sinha, R., Kulldorff, M., Gross, M., Lang, N.P., Barber, C., Harnack, L., DiMagno, E., Bliss, R. & Kadlubar, F.F. (2002) Meat intake and cooking techniques: associations with pancreatic cancer. Mutat. Res., 506-507, 225-231

Anderson, K.E., Kadlubar, F.F., Kulldorff, M., Harnack, L., Gross, M., Lang, N.P., Barber, C., Rothman, N. & Sinha, R. (2005) Dietary intake of heterocyclic amines and benzo(a)pyrene: Associations with pancreatic cancer. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2261–2265

Armstrong, B.G., Tremblay, C.G., Cyr, D. & Thériault, G.P. (1986) Estimating the relationship between exposure to tar volatiles and the incidence of bladder cancer in aluminum smelter workers. *Scand. J. Work Environ. Health*, **12**, 486–493

Armstrong, B.G., Tremblay, C., Baris, D. & Thériault, G. (1994) Lung cancer mortality and polynuclear aromatic hydrocarbons: A case–cohort study of aluminum production workers in Arvida, Quebec, Canada. *Am. J. Epidemiol*., **139**, 250–262

Armstrong, B.G., Hutchinson, E. & Fletcher, T. (2002) *Cancer Risk Following Exposure to Polycyclic Aromatic Hydrocarbons (PAHs): A Meta-Analysis* (Rep. No. 068), Sudbury, Health and Safety Executive. Available at: http://.hse.gov.uk/research.rrhtm/rr068.htm [accessed 19 May 2004]

Armstrong, B.G., Hutchinson, E., Unwin, J. & Fletcher, T. (2004) Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: A review and meta-analysis. *Environ. Health Perspect.*, **112**, 970–978

Aronson, K.J., Siemiatycki, J., Dewar, R. & Gérin, M. (1996) Occupational risk factors for prostate cancer: Results from a case–control study in Montréal, Québec, Canada. *Am. J. Epidemiol.*, **143**, 363–373

Axelson, O. & Kling, H. (1983) [Mortality among wood preservers with creosote exposure] (Abstract). In: *32nd Nordic Occupational Hygiene Conference,* Solna, Arbetarskyddsstyrelsen (National Board of Occupational Safety and Health), pp. 125–126 (in Swedish)

Baxter, P.J. & McDowall, M.E. (1986) Occupation and cancer in London: An investigation into nasal and bladder cancer using the Cancer Atlas. *Br. J. ind Med*., **43**, 44–49

Becher, H., Ramroth, H., Ahrens, W., Risch, A., Schmezer, P. & Dietz, A. (2005) Occupation, exposure to polycyclic aromatic hydrocarbons and laryngeal cancer risk. *Int. J. Cancer*, **116**, 451–457

Bender, A.P., Parker, D.L., Johnson, R.A., Scharber, W.K., Williams, A.N., Marbury, M.C. & Mandel, J.S. (1989) Minnesota Highway Maintenance Worker Study: Cancer mortality. *Am. J. ind. Med.*, **15**, 545–556

Berger, J. & Manz, A. (1992) Cancer of the stomach and the colon–rectum among workers in a coke gas plant. *Am. J. ind. Med.*, **22**, 825–834

Bertrand, J.P., Chau, N., Patris, A., Mur, J.M., Pham, Q.T., Moulin, J.J., Morviller, P., Auburtin, G., Figueredo, A. & Martin, J. (1987) Mortality due to respiratory cancers in the coke oven plants of the Lorraine coalmining industry (Houillères du Bassin de Lorraine). *Br. J. ind. Med*., **44**, 559–565

Blot, W.J., Brown, L.M., Pottern, L.M., Stone, B.J. & Fraumeni, J.F., Jr (1983) Lung cancer among long-term steel workers. *Am. J. Epidemiol*., **117**, 706–716

Boffetta, P., Burstyn, I., Partanen, T., Kromhout, H., Svane, O., Langård, S., Järvholm, B., Frentzel-Beyme, R., Kauppinen, T., Stücker, I., Shaham, J., Heederik, D., Ahrens, W., Bergdahl, I.A., Cenée, S., Ferro, G., Heikkilä, P., Hooiveld, M., Johansen, C., Randem, B.G. & Schill, W. (2003a) Cancer mortality among European asphalt workers: An international

epidemiological study. II. Exposure to bitumen fume and other agents. *Am. J. ind. Med.*, **43**, 28–39

Boffetta, P., Burstyn, I., Partanen, T., Kromhout, H., Svane, O., Langard, S., Jarvholm, B., Frentzel-Beyme, R., Kauppinen, T., Stucker, I., Shaham, J., Heederik, D., Ahrens, W., Bergdahl, I. A., Cenee, S., Ferro, G., Heikkila, P., Hooiveld, M., Johansen, C., Randem, B. G. & Schill, W. (2003b) Cancer mortality among European asphalt workers: An international epidemiological study. I. Results of the analysis based on job titles. *Am. J. ind. Med.*, **43**, 18–27

Bonassi, S., Merlo, F., Pearce, N. & Puntoni, R. (1989) Bladder cancer and occupational exposure to polycyclic aromatic hydrocarbons. *Int. J. Cancer*, **44**, 648–651

Bovenzi, M., Stanta, G., Antiga, G., Peruzzo, P. & Cavallieri, F. (1993) Occupational exposure and lung cancer risk in a coastal area of northeastern Italy. *Int. Arch. occup. environ. Health*, **65**, 35–41

Brüske-Hohlfeld, I., Mohner, M., Pohlabeln, H., Ahrens, W., Bolm-Audorff, U., Kreienbrock, L., Kreuzer, M., Jahn, I., Wichmann, H.E. & Jockel, K.H. (2000) Occupational lung cancer risk for men in Germany: Results from a pooled case–control study. *Am. J. Epidemiol.*, **151**, 384–395

Bruusgaard, A. (1959) [Incidence of certain forms of cancer in gas workers.] *Tidsskr. Nor. Laegeforen.*, **79**, 755–756 (in Norwegian)

Butler, L.M., Sinha, R., Millikan, R.C., Martin, C.F., Newman, B., Gammon, M.D., Ammerman, A.S. & Sandler, R.S. (2003) Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. *Am. J. Epidemiol.*, **157**, 434–445

Butlin, H.T. (1892) Three lectures on cancer of the scrotum in chimney sweeps and others. III. Tar and paraffin cancer. *Br. med. J.*, **iii**, 66–71

Bye, T., Romundstad, P.R., Rønneberg, A. & Hilt, B. (1998) Health survey of former workers in a Norwegian coke plant: Part 2. Cancer incidence and cause specific mortality. *Occup. environ. Med.*, **55**, 622–626

Cadez, E. (1983) [Ergebnisse langjähriger Untersuchungen über den Einfluss von Schadstoffen auf die Gesundheit von Schornstein-fegern]. *Staub. Reinhalt Luft*, **43**, 116–117 (in German)

Cammarano, G., Crosignani, P., Berrino, H. & Berra, G. (1986) Additional follow-up of cancer mortality among workers in a thermoelectric power plant. *Scand. J. Work Environ. Health*, **12**, 631–632

Carlsten, C., Hunt, S.C. & Kaufman, J.D. (2005) Carcinoma of the skin and coal tar creosote exposure in a railroad worker. *Environ. Health Perspect*., **113**, 96–97

Carta, P., Aru, G., Cadeddu, C., Gigli, G., Papi, G., Carta, F. & Nurchis, P. (2004) Mortality for [sic] pancreatic cancer among aluminium smelter workers in Sardinia, Italy. *G. Ital. Med. lav. Ergon*., **26**, 83–89

Chau, N., Bertrand, J.P., Mur, J.M., Figueredo, A., Patris, A., Moulin, J.J. & Pham, Q.T. (1993) Mortality in retired coke oven plant workers. *Br. J. ind. Med*., **50**, 127–135

Clavel, J., Mandereau, L., Limasset, J.C., Hemon, D. & Cordier, S. (1994) Occupational exposure to polycyclic aromatic hydrocarbons and the risk of bladder cancer: A French case–control study. *Int. J. Epidemiol*., **23**, 1145–1153

Cocco, P., Ward, M.H. & Dosemeci, M. (1999) Risk of stomach cancer associated with 12 workplace hazards: Analysis of death certificates from 24 states of the United States with the aid of job exposure matrices. *Occup. environ. Med*., **56**, 781–787

Cookson, H.A. (1924) Epithelioma of the skin after prolonged exposure to creosote. *Br. med. J.*, **i**, 368

Costantino, J.P., Redmond, C.K. & Bearden, A. (1995) Occupationally related cancer risk among coke oven workers: 30 years of follow-up. *J. occup. environ. Med.*, **37**, 597–604

Cross, A.J., Peters, U., Kirsh, V.A., Andriole, G.L., Reding, D., Hayes, R.B. & Sinha, R. (2005) A prospective study of meat and meat mutagens and prostate cancer risk. *Cancer Res.*, **65**, 11779–84

Cross, A.J., Ward, M.H., Schenk, M., Kulldorff, M., Cozen, W., Davis, S., Colt, J.S., Hartge, P., Cerhan, J.R. & Sinha, R. (2006) Meat and meat-mutagen intake and risk of non-Hodgkin lymphoma: Results from a NCI-SEER case–control study. *Carcinogenesis*, **27**, 293–297

Davies, G.M. (1977) A mortality study of coke oven workers in two South Wales integrated steelworks. *Br. J. ind. Med.*, **34**, 291–297

Doll, R. (1952) The causes of death among gas-workers with special reference to cancer of the lung. *Br. J. ind. Med.*, **9**, 180–185

Doll, R., Fisher, R.E.W., Gammon, E.J., Gunn, W., Hughes, G.O., Tyrer, F.H. & Wilson, W. (1965) Mortality of gasworkers with special reference to cancers of the lung and bladder, chronic bronchitis, and pneumoconiosis. *Br. J. ind. Med.*, **22**, 1–12

Doll, R., Vessey, M.P., Beasley, R.W.R., Buckley, A.R., Fear, E.C., Fisher, R.E.W., Gammon, E.J., Gunn, W., Hughes, G.O., Lee, K. & Norman-Smith, B. (1972) Mortality of gasworkers — Final report of a prospective study. *Br. J. ind. Med.*, **29**, 394–406

Donato, F., Monarca, S., Marchionna, G., Rossi, A., Cicioni, C., Chiesa, R., Colin, D. & Boffetta, P. (2000) Mortality from cancer and chronic respiratory diseases among workers who manufacture carbon electrodes. *Occup. environ. Med.*, **57**, 484–487

Droste, J.H., Weyler, J.J., Van Meerbeeck, J.P., Vermeire, P.A. & van Sprundel, M.P. (1999) Occupational risk factors of lung cancer: A hospital based case–control study. *Occup. environ. Med.*, **56**, 322–327

Earle, J. (1808) *The Chirurgical Works of Percivall Pott, F.R.S.*, Vol. III, London, Wood & Innes, pp. 176–183

Elci, O.C., Akpinar-Elci, M., Blair, A. & Dosemeci, M. (2003) Risk of laryngeal cancer by occupational chemical exposure in Turkey. *J. occup. environ. Med.*, **45**, 1100–1106

Engholm, G., Englund, A. & Linder, B. (1991) Mortality and cancer incidence in Swedish road paving asphalt workers and roofers. *Eur. Asphalt Mag.*, **1**, 62–67

Evanoff, B.A., Gustavsson, P. & Hogstedt, C. (1993) Mortality and incidence of cancer in a cohort of Swedish chimney sweeps: An extended follow up study. *Br. J. ind. Med.*, **50** 450–459

Franco, F., Chellini, E., Seniori Costantini, A., Giola, A., Carra, G., Paolinelli, F., Martelli, C. & Vigotti, M. (1993) Mortality in the coke oven plant of Carrara, Italy. *Med. Lav.*, **84**, 443–447

Friesen, M.C., Demers, P.A., Spinelli, J.J., Lorenzi, M. & Le, N.D. (2005) Specificity of exposure metric in dose–response relations in a retrospective aluminum smelter cohort. *Occup. environ. Med.*, **62** [http://www.occenvmed.com/cgi/content/full/62/11/e22]

Gallagher, R.P., Bajdik, C.D., Fincham, S., Hill, G.B., Keefe, A.R., Coldman, A. & McLean, D.I. (1996) Chemical exposures, medical history, and risk of squamous and basal cell carcinoma of the skin. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 419–424

Gérin, M., Siemiatycki, J., Kemper, H. & Bégin, D. (1985) Obtaining occupational exposure histories in epidemiologic case–control studies. *J. occup. Med.*, **27**, 420–426

Gibbs, G.W. (1983) Mortality experience in Eastern Canada. In: Hughes, J.P., ed., *Health Protection in Primary Aluminium Production,* Vol. 2, *Proceedings of a Seminar, Montreal, 22–24 September, 1981;* London, International Primary Aluminium Institute, pp. 59–69

Gibbs, G.W. (1985) Mortality of aluminum reduction plant workers, 1950 through 1977. *J. occup. Med*., **27**, 761–770

Gibbs, G.W. & Horowitz, I. (1979) Lung cancer mortality in aluminum reduction plant workers. *J. occup. Med*., **21**, 347–353

Giovanazzi, A. & D'Andrea, F. (1981) [Causes of death among workers in an aluminium electrolytic reduction plant.] *Med. Lav*., **72**, 277–282 (in Italian)

Grandjean, P. & Olsen, J.H. (2004) Extended follow-up of cancer incidence in fluoride-exposed workers. *J. natl Cancer Inst*., **96**, 802–803

Grimsrud, T.K., Langseth, H., Engeland, A. & Andersen, A. (1998) Lung and bladder cancer in a Norwegian municipality with iron and steel producing industry: Population based case–control studies. *Occup. environ. Med*., **55**, 387–392

Gunter, M.J., Probst-Hensch, N.M., Cortessis, V.K., Kulldorff, M., Haile, R.W. & Sinha, R. (2005) Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case–control study. *Carcinogenesis*, **26**, 637–642

Gustavsson, P. & Reuterwall, C. (1990) Mortality and incidence of cancer among Swedish gas workers. *Br. J. ind. Med.*, **47**, 169–174

Gustavsson, P., Gustavsson, A. & Hogstedt, C. (1988) Excess of cancer in Swedish chimney sweeps. *Br. J. ind. Med*., **45**, 777–781

Gustavsson, P., Bellander, T., Johansson, L. & Salmonsson, S. (1995) Surveillance of mortality and cancer incidence among Swedish graphite electrode workers. *Environ. Res*., **70**, 7–10

Gustavsson, P., Jakobsson, R., Johansson, H., Lewin, F., Norell, S. & Rutkvist, L.E. (1998) Occupational exposures and squamous cell carcinoma of the oral cavity, pharynx, larynx, and oesophagus: A case–control study in Sweden. *Occup. environ. Med*., **55**, 393–400

Gustavsson, P., Jackobsson, R., Nyberg, F., Pershagen, G., Järup, L. & Schéele, P. (2000) Occupational exposure and lung cancer risk: A population-based case–referent study in Sweden. *Am. J. Epidemiol*., **152**, 32–40

Haldorsen, T., Andersen, A. & Boffetta, P. (2004) Smoking-adjusted incidence of lung cancer by occupation among Norwegian men. *Cancer Causes Control*, **15**, 139–147

Hammond, E.C., Selikoff, I.J., Lawther, P.L. & Seidman, H. (1976) Inhalation of benzpyrene and cancer in man. *Ann. N.Y. Acad. Sci.*, **271**, 116–124

Hansen, E.S. (1983) Mortality from cancer and ischemic heart disease in Danish chimney sweeps: A five-year follow-up. *Am. J. Epidemiol.*, **117**, 160–164

Hansen, E.S. (1989a) Cancer mortality in the asphalt industry: A ten year follow up of an occupational cohort. *Br. J. ind. Med.*, **46**, 582–585

Hansen, E.S. (1989b) Cancer incidence in an occupational cohort exposed to bitumen fumes. *Scand. J. Work Environ. Health*, **15**, 101–105

Hansen, E.S. (1991) Mortality of mastic asphalt workers. *Scand. J. Work Environ. Health*, **17,** 20–24

Hansen, E.S., Olsen, J.H. & Tilt, B. (1982) Cancer and non-cancer mortality of chimney sweeps in Copenhagen. *Int. J. Epidemiol*., **11**, 356–361

Hansen, K.S., Viskum, S. & Pedersen, M.S. (1986) [Mortality among gas workers.] *Ugeskr. Laeger*, **148**, 610–612 (in Danish)

Henry, S.A. (1937) The study of fatal cases of cancer of the scrotum from 1911 to 1935 in relation to occupation, with special reference to chimney sweeping and cotton mule spinning. *Am. J. Cancer*, **31**, 28–57

Henry, S.A. (1946) *Cancer of the Scrotum in Relation to Occupation*, New York, Oxford University Press

Henry, S.A. (1947) Occupational cutaneous cancer attributable to certain chemicals in industry. *Br. med. Bull*., **4**, 389–401

Henry, S.A. & Irvine, E.D. (1936) Cancer of the scrotum in the Blackburn registration district, 1837–1929. *J. Hyg.*, **36**, 310–340

Henry, S.A., Kennaway, N.M. & Kennaway, E.L. (1931) The incidence of cancer of the bladder and prostate in certain occupations. *J. Hyg.*, **31**, 125–137

Hoar, S.K., Morrison, A.S., Cole, P. & Silverman, D.T. (1980) An occupation and exposure linkage system for the study of occupational carcinogenesis. *J. occup. Med*., **22**, 722–726

Hogstedt, C., Andersson, K., Frenning, B. & Gustavsson, A. (1982) A cohort study on mortality among long-time employed Swedish chimney sweeps. *Scand. J. Work Environ. Health*, **8** (Suppl. 1), 72–78

Hrubec, Z., Blair, A.E., Rogot, E. & Vaught, J. (1992) *Mortality Risks by Occupation among U.S. Veterans of Known Smoking Status 1954–80* (NIH Publication No. 92-3407), Bethesda, MD, National Institutes of Health

Hurley, J.F., Archibald, R.M., Collings, P.L., Fanning, D.M., Jacobsen, M. & Steele, R.C. (1983) The mortality of coke workers in Britain. *Am. J. ind. Med*., **4**, 691–704

IARC (1985) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 35, *Polynuclear Aromatic Compounds, Part 4, Bitumens, Coal-tars and Derived Products, Shale-oils and Soots*, Lyon, p. 233

Jensen, O.M., Knudsen, J.B., McLaughlin, J.K. & Sorensen, B.L. (1988) The Copenhagen case–control study of renal pelvis and ureter cancer: Role of smoking and occupational exposures. *Int. J. Cancer*, **41**, 557–561

Karlehagen, S., Andersen, A. & Ohlson, C.G. (1992) Cancer incidence among creosote-exposed workers. *Scand. J. Work Environ. Health*., **18**, 26–29

Kauppinen, T., Partanen, T., Degerth, R. & Ojajärvi, A. (1995) Pancreatic cancer and occupational exposures. *Epidemiology*, **6**, 498–502

Kawai, M., Amamoto, H. & Harada, K. (1967) Epidemiologic study of occupational lung cancer. *Arch. environ. Health*, **14**, 859–864

Kazerouni, N., Sinha, R., Hsu, C.H., Greenberg, A. & Rothman, N. (2001) Analysis of 200 food items for benzo[a]pyrene and estimation of its intake in an epidemiologic study. *Food chem. Toxicol.*, **39**, 423–436

Kennaway, N.M. & Kennaway, E.L. (1936) A study of the incidence of cancer of the lung and larynx. *J. Hyg.*, **36**, 236–267

Kennaway, E.L. & Kennaway, N.M. (1947) A further study of the incidence of cancer of the lung and larynx. *Br. J. Cancer*, **1**, 260–298

Kjuus, H., Andersen, A. & Langård, S. (1986) Incidence of cancer among workers producing calcium carbide. *Br. J. ind. Med.*, **43**, 237–242

Konstantinov, V.G. & Kuz'minykh, A.I. (1971) Tarry substances and 3, 4-benzpyrene in the air of electrolysis shops of aluminum works and their carcinogenic significance. *Hyg. Sanit*., **36**, 368–373

Konstantinov, V.G., Simakhina, P.G., Gotlib, E.V. & Kuz'minykh, A.I. (1974) [Problem of the carcinogenic hazard in aluminium electrolysis halls.] In: *Professional'nyi Rak*, Moscow, Academy of Medical Sciences, pp. 87–91 (in Russian)

Krstev, S., Baris, D., Stewart, P.A., Hayes, R.B., Blair, A. & Dosemeci, M. (1998) Risk for prostate cancer by occupation and industry: A 24-state death certificate study. *Am. J. ind. Med*., **34**, 413–420

Kubasiewicz, M. & Starzynski, Z. (1989) Case–referent study on skin cancer and its relation to occupational exposure to polycyclic aromatic hydrocarbons. I. Study design. *Pol. J. occup. Med*., **2**, 221–228

Kubasiewicz, M., Starzynski, Z. & Szymczak, W. (1991) Case–referent study on skin cancer and its relation to occupational exposure to polycyclic aromatic hydrocarbons. II. Study results. *Pol. J. occup. Med. environ. Health*., **4**, 141–147

Kupetz, G.-W. (1966) [*Tumour of the Respiratory Tract and Chimney Sweep Occupation*], Inaugural Dissertation, Berlin, Humboldt University (in German)

Kuroda, S. & Kawahata, K. (1936) [On the occupational origin of lung cancer among generator gas workers.] *Z. Krebsforsch*., **45**, 36–39 (in German)

Letzel, S. & Drexler, H. (1998) Occupationally related tumors in tar refinery workers. *J. Am. Acad. Dermatol.*, **39**, 712–720

Liu, N., Wang, Z., Dong, D., Chen, K. & Quin, L. (1997) Cancer mortality among carbon workers in China: Retrospective cohort study. *J. occup. Health*, **39**, 325–330

Lloyd, J.W. (1971) Long-term mortality study of steelworkers. V. Respiratory cancer in coke plant workers. *J. occup. Med*., **13**, 53–68

Lloyd, J.W. & Ciocco, A. (1969) Long-term mortality study of steelworkers. I. Methodology. *J. occup. Med*., **11**, 299–310

Lloyd, J.W., Lundin, F.E., Redmond, C.K. & Geiser, P.B. (1970) Long-term mortality study of steelworkers. IV. Mortality by work area. *J. occup. Med*., **12**, 151–157

van Loon, A.J., Kant, I.J., Swaen, G.M., Goldbohm, R.A., Kremer, A.M. & van den Brandt, P.A. (1997) Occupational exposure to carcinogens and risk of lung cancer: Results from the Netherlands cohort study. *Occup. environ. Med*., **54**, 817–824

Maclaren, W.M. & Hurley, J.F. (1987) Mortality of tar distillation workers. *Scand. J. Work Environ. Health*, **13**, 404–411

Maizlish, N., Beaumont, J. & Singleton, J. (1988) Mortality among California highway workers. *Am. J. ind. Med.*, **13**, 363–379

Mandel, J.S., McLaughlin, J.K., Schlehofer, B., Mellemgaard, A., Helmert, U., Lindblad, P., McCredie, M. & Adami, H.O. (1995) International renal-cell cancer study. IV. Occupation. *Int. J. Cancer*, **61**, 601–605

Manz, A., Berger, J. & Waltsgott, H. (1983) [*Occupational Cancer in Gas Industry Worker.*], (Wirtschaftsverlag, NW, Report No. 352) Bremerhaven, German Federal Department for Worker Safety and Accident Research, (in German)

Martin, J.C., Imbernon, E., Goldberg, M., Chevalier, A. & Bonenfant, S. (2000) Occupational risk factors for lung cancer in the French electricity and gas industry. *Am. J. Epidemiol.*, **151**, 902–912

Mazumdar, S., Redmond, C., Sollecito, W. & Sussman, N. (1975) An epidemiological study of exposure to coal tar pitch volatiles among coke oven workers. *J. Air Pollut. Control Assoc*., **25**, 382–389

McLaughlin, J.K., Mandel, J.S., Blot, W.J., Schuman, L.M., Mehl, E.S. & Fraumeni, J.F., Jr (1984) A population-based case–control study of renal cell carcinoma. *J. natl. Cancer Inst*., **72**, 275–284

Menck, H.R. & Henderson, B.E. (1976) Occupational differences in rates of lung cancer. *J. occup. Med*., **18,** 797–801

Merlo, D.F., Garattini, S., Gelatti, U., Simonati, C., Covolo, L., Ceppi, M. & Donato, F. (2004) A mortality cohort study among workers in a graphite electrode production plant in Italy. *Occup. environ. Med*., **61**, e9

Milham, S. (1982) *Occupational Mortality in Washington State 1950–1979*, Prepared under Contract No. 210-80-0088, Cincinnati, OH, National Institute for Occupational Safety and Heath

Milham, S., Jr (1976) Cancer mortality pattern associated with exposure to metals. *Ann. N.Y. Acad. Sci*., **271**, 243–249

Milham, S., Jr (1979) Mortality in aluminum reduction plant workers. *J. occup. Med*., **21**, 475–480

Moolgavkar, S.H., Luebeck, E.G. & Anderson, E.L. (1998) Estimation of unit risk for coke oven emissions. *Risk Anal*., **18**, 813–825

Morabia, A., Markowitz, S., Garibaldi, K. & Wynder, E.L. (1992) Lung cancer and occupation: Results of a multicentre case–control study. *Br. J. ind. Med.*, **49**, 721–727

Mori, I. (2002) Cancer mortality among man-made graphite electrode manufacturing workers: Results of a 38 year follow up. *Occup. environ. Med*., **59**, 473–480

Moulin, J.J., Mur, J.M., Wild, P., Demonchy, A., Eloy, E., Jeannot, A. (1988) [Epidemiological study of mortality among coal tar distillery workers]. *Rev. epidemiol. Santé publique,* **36**, 99–107 (in French)

Moulin, J.J., Wild, P., Mur, J.M., Lafontaine, M., Lefer, M., Mercier-Gallay, M., Villemot, P., Whebi, V. & Coulon, J.P. (1989) Risk of lung, larynx, pharynx and buccal cavity cancers among carbon electrode manufacturing workers. *Scand. J. Work Environ. Health*, **15**, 30–37

Moulin, J.J., Clavel, T., Buclez, B. & Laffitte-Rigaud, G. (2000) A mortality study among workers in a French aluminium reduction plant. *Int. Arch. occup. environ. Health*, **73**, 323–330

Mur, J.M., Moulin, J.J., Meyer-Bisch, C., Massin, N., Coulon, J.P. & Loulergue, J. (1987) Mortality of aluminium reduction plant workers in France. *Int. J. Epidemiol*., **16**, 257–264

Nadon, L., Siemiatycki, J., Dewar, R., Krewski, D. & Gérin, M. (1995) Cancer risk due to occupational exposure to polycyclic aromatic hydrocarbons. *Am. J. ind Med*., **28**, 303–324

O'Donovan, W.J. (1920) Epitheliomatous ulceration among tar workers. *Br. J. Dermatol. Syphilis*, **32**, 215–228

Ojajärvi, I.A., Partanen, T.J., Ahlbom, A., Boffetta, P., Hakulinen, T., Jourenkova, N., Kauppinen, T.P., Kogevinas, M., Porta, M., Vainio, H.U., Weiderpass, E. & Wesseling, C.H. (2000) Occupational exposures and pancreatic cancer: A meta-analysis. *Occup. environ. Med.*, **57**, 316–324

Parent, M.E., Siemiatycki, J. & Fritschi, L. (2000) Workplace exposures and oesophageal cancer. *Occup. environ. Med.*, **57**, 325–334

Partanen, T. & Boffetta, P. (1994) Cancer risk in asphalt workers and roofers: Review and meta-analysis of epidemiologic studies. *Am. J. Ind. Med.*, **26**, 721–740

Partanen, T., Heikkila, P., Hernberg, S., Kauppinen, T., Moneta, G. & Ojajärvi, A. (1991) Renal cell cancer and occupational exposure to chemical agents. *Scand. J. Work Environ. Health*, **17**, 231–239

Pesch, B., Haerting, J., Ranft, U., Klimpel, A., Oelschlagel, B. & Schill, W. (2000) Occupational risk factors for renal cell carcinoma: Agent-specific results from a case–control study in Germany. MURC Study Group. Multicenter urothelial and renal cancer study. *Int. J. Epidemiol*., **29**, 1014–1024

Petrelli, G., Menniti-Ippolito, F., Taroni, F., Raschetti, R. & Magarotto, G. (1989) A retrospective cohort mortality study on workers of two thermoelectric power plants: Fourteen-year follow-up results. *Eur. J. Epidemiol.*, **5**, 87–89

Pott, P. (1775) *Chirurgical Observations Relative to the Cataract, the Polypus of the Nose, the Cancer of the Scrotum, the Different Kinds of Ruptures, and the Mortification of the Toes and Feet*, London, Hawes, Clarke & Collins

Pukkala, E. (1995) *Cancer Risk by Social Class and Occupation*, Vol. 7, *Contributions to Epidemiology and Biostatistics*, Basel, Karger

Redmond, C.K. (1983) Cancer mortality among coke oven workers. *Environ. Health Perspect*., **52**, 67–73

Redmond, C.K., Ciocco, A., Lloyd, J.W. & Rush, H.W. (1972) Long-term mortality study of steelworkers. VI. Mortality from malignant neoplasms among coke oven workers. *J. occup. Med*., **14**, 621–629

Reid, D.D. & Buck, C. (1956) Cancer in coking plant workers. *Br. J. ind. Med*., **13**, 265–269

Risch, H.A., Burch, J.D., Miller, A.B., Hill, G.B., Steele, R. & Howe, G.R. (1988) Occupational factors and the incidence of cancer of the bladder in Canada. *Br. J. ind. Med*., **45**, 361–367

Rockette, H.E. & Arena, V.C. (1983) Mortality studies of aluminum reduction plant workers: Potroom and carbon department. *J. occup. Med*., **25**, 549–557

Romundstad, P., Andersen, A. & Haldorsen, T. (2000a) Cancer incidence among workers in six Norwegian aluminum plants. *Scand. J. Work Environ. Health*, **26**, 461–469

Romundstad, P., Haldorsen, T. & Andersen, A. (2000b) Cancer incidence and cause specific mortality among workers in two Norwegian aluminum reduction plants. *Am. J. ind. Med*., **37**, 175–183

Romundstad, P., Haldorsen, T. & Andersen, A. (2000c) Lung and bladder cancer among workers in a Norwegian aluminium reduction plant. *Occup. environ. Med*., **57**, 495–499

Rønneberg, A. & Andersen, A. (1995) Mortality and cancer morbidity in workers from an aluminium smelter with prebaked carbon anodes — Part II: Cancer morbidity. *Occup. environ. Med.*, **52**, 250–254

Rønneberg, A., Haldorsen, T., Romundstad, P. & Andersen, A. (1999) Occupational exposure and cancer incidence among workers from an aluminum smelter in western Norway. *Scand. J. Work Environ. Health*, **25**, 207–214

Sakabe H., Tsuchiya K., Takekura N., Nomura S., Koshi S., Takemoto K., Matsushita H. & Matuso Y. (1975) Lung cancer among coke oven workers. *Ind. Health*, **13**, 57–68

Schoenberg, J.B., Stemhagen, A., Mason, T.J., Patterson, J., Bill, J. & Altman, R. (1987) Occupation and lung cancer risk among New Jersey white males. *J. natl Cancer Inst.*, **79**, 13–21

Schumacher, M.C., Slattery, M.L. & West, D.W. (1989) Occupation and bladder cancer in Utah. *Am. J. ind. Med*., **16**, 89–102

Schunk, W. (1979) [Zur Frage der Beziehung zwischen der Exposition gegenüber polyzyklischen Kohlenwasserstoffen und der Häufigkeit von Krebserkrankungen in zwei Chemiebetrieben Thüringens]. *Z. ärztl. Fortbild.*, **73**, 84–88 (in German)

Sharpe, C.R., Rochon, J.E., Adam, J.M. & Suissa, S. (1989) Case–control study of hydrocarbon exposures in patients with renal cell carcinoma. *Can. med. Assoc. J.*, **140**, 1309–1318

Shimauchi, T., Rikihisa, W., Yasuda, H., Yamamoto, O. & Asahi, M. (2000) [A case of squamous cell carcinoma that developed on chronic tar dermatosis.] *J. UOEH*, **22**, 183–187 (in Japanese)

Siemiatycki, J. (1991) *Risk Factors for Cancer in the Workplace*, Boca Raton, FL, CRC Press

Simonato, L. (1981) Carcinogenic risk in the aluminium production industry: An epidemiological overview. *Med. Lav.*, **72**, 266–276

Sinha, R., Cross, A., Curtin, J., Zimmerman, T., McNutt, S., Risch, A. & Holden, J. (2005a) Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. *Mol. Nutr. Food Res.*, **49**, 648–655

Sinha, R., Kulldorff, M., Gunter, M.J., Strickland, P. & Rothman, N. (2005b) Dietary benzo[a]pyrene intake and risk of colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2030–2034

Sinha, R., Peters, U., Cross, A.J., Kulldorff, M., Weissfeld, J.L., Pinsky, P.F., Rothman, N. & Hayes, R.B. (2005c) Meat, meat cooking methods and preservation, and risk for colorectal adenoma. *Cancer Res.*, **65**, 8034–8041

Spinelli, J.J., Band, P.R., Svirchev, L.M. & Gallagher, R.P. (1991) Mortality and cancer incidence in aluminum reduction plant workers. *J. occup. Med.*, **33**, 1150–1155

Steineck, G., Plato, N., Alfredsson, L. & Norell, S.E. (1989) Industry-related urothelial carcinogens: Application of a job–exposure matrix to census data. *Am. J. ind. Med.*, **16**, 209–224

Stern, F.B., Ruder, A.M. & Chen, G. (2000) Proportionate mortality among unionized roofers and waterproofers. *Am. J. ind. Med.*, **37**, 478–492

Swaen, G.M.H. & Slangen, J.M.M. (1997) Mortality in a group of tar distillery workers and roofers. *Int. Arch. occup. environ. Health*, **70**, 133–137

Swaen, G.M.H., Slangen, J.J.M., Volovics, A., Hayes, R.B., Scheffers, T. & Sturmans, F. (1991) Mortality of coke plant workers in the Netherlands. *Br. J. ind. Med.*, **48**, 130–135

Teta, M.J., Ott, M.G. & Schnatter, A.R. (1987) Population based mortality surveillance in carbon products manufacturing plants. *Br. J. ind. Med.*, **44**, 344–350

Thériault, G., De Guire, L. & Cordier, S. (1981) Reducing aluminum: An occupation possibly associated with bladder cancer. *Can. med. Assoc. J.*, **124**, 419–422, 425

Thériault, G., Tremblay, C., Cordier, S. & Gingras, S. (1984) Bladder cancer in the aluminium industry. *Lancet*, **323**, 947–950

Tornqvist, S., Norell, S., Ahlbom, A. & Knave, B. (1986) Cancer in the electric power industry. *Br. J. ind. Med.*, **43**, 212–213

Tremblay, C., Armstrong, B., Thériault, G. & Brodeur, J. (1995) Estimation of risk of developing bladder cancer among workers exposed to coal tar pitch volatiles in the primary aluminum industry. *Am. J. ind. Med.*, **27**, 335–348

Watkins, D.K., Chiazze, L., Jr, Fryar, C.D. & Fayerweather, W. (2002) A case control study of lung cancer and non-malignant respiratory disease among employees in asphalt roofing manufacturing and asphalt production. *J. occup. environ. Med*, **44**, 551–558

Wigle, D.T. (1977) Bladder cancer: Possible new high-risk occupation. *Lancet*, **ii**, 83–84

Wong, O. & Harris, F. (2005) Retrospective cohort mortality study and nested case–control study of workers exposed to creosote at 11 wood-treating plants in the United States. *J. occup. environ. Med.*, **47**, 683–697

Wu W. (1988) Occupational cancer epidemiology in the People's Republic of China. *J. occup Med.*, **30**, 968–974

Wu-Williams, A.H., Xu, Z.Y., Blot, W.J., Dai, X.D., Louie, R., Xiao, H.P., Stone, B.J., Sun, X.W., Yu, S.F., Feng, Y.P. *et al*. (1993) Occupation and lung cancer risk among women in northern China. *Am. J. ind. Med*., **24**, 67–79

Zahm, S.H., Brownson, R.C., Chang, J.C. & Davis, J.R. (1989) Study of lung cancer histologic types, occupation, and smoking in Missouri. *Am. J. ind. Med.*, **15**, 565–578

3. Studies of Cancer in Experimental Animals

Acenaphthene

Dermal application (see also Table 3.1)

Mouse

A solution of acenaphthene in 90% benzene was tested in a group of 100 mice [strain, sex and age unspecified] by dermal application for a period of 9 months. No tumours were observed (Kennaway, 1924a,b). [The Working Group noted that no controls were available.]

Dermal initiation-promotion (see also Table 3.2)

Mouse

A group of 85 male white mice was treated weekly for 1 year with three drops of a 5% croton oil solution that contained approximately 3% 'pure' acenaphthene. A group of 160 control mice received croton oil solution only. After 12 months, 5/85 acenaphthene-treated mice were still alive, two of which had skin tumours, and 13/160 croton oil control mice were still alive, one of which had a skin tumour (Graffi *et al*., 1953). [The Working Group noted the poor survival.]

Acepyrene

Dermal application (see also Table 3.1)

Mouse

Groups of 30 female Swiss mice, 9 weeks of age, received twice weekly applications of the same doses of acepyrene for 30 weeks. Repeated applications resulted in tumour incidences of 0/30 (0%), 0/28 (0%), 1/30 (3%) and 1/30 in the control, low-, mid- and high-dose groups, respectively (Cavalieri *et al.*, 1981).

Dermal initiation–promotion (see also Table 3.2)

Mouse

In a mouse skin initiation–promotion study, groups of 30 female CD-1 mice, 9 weeks of age, received dermal applications of a total dose of 0.02, 0.06 or 0.18 μmol acepyrene in

16.7 μL acetone as 10 single subdoses administered on alternate days. The control group received acetone alone. One week later, all mice received dermal applications of 0.017 μmol 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 33.3 μL acetone twice a week for 40 weeks. After 40 weeks of promotion, the incidences of skin papillomas were 3/29 (10%), 0/30 (0%), 1/30 (3%) and 4/30 (13%) in the acetone controls, low-, mid- and high-dose groups, respectively, with an average number of skin tumours/mouse of 0.14, 0.0, 0.03 and 0.13, respectively (Cavalieri *et al.*, 1981).

Anthanthrene

Previous evaluation

Anthanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated four bioassays in which anthanthrene was administered dermally to mice, one of which produce skin tumours. Three initiation–promotion studies in mice were also evaluated, one of which gave positive results. A study in mice by subcutaneous administration was judged to be inadequate, whereas intrapulmonary administration to rats was considered to give positive results. These studies are summarized in Tables 3.1–3.4. On the basis of the available data, the Working Group concluded that there was *limited evidence* that anthanthrene was carcinogenic to experimental animals. Additional studies that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 27 female SENCAR mice [weight unspecified], 8 weeks of age, received a single dermal application of 800 nmol [221 μg] anthanthrene (purity >99% by high-performance liquid chromatography (HPLC)) in 100 μL dioxane:dimethylsulfoxide (DMSO) (75:25). A vehicle-control group of 23 mice was treated with 100 μL dioxane:DMSO alone. Starting 1 week later, all mice were treated topically with 4.26 nmol (2.6 μg) TPA in 100 μL acetone twice weekly for 25 weeks. The mice were killed after the 25th week of promotion and complete necropsies were performed. At the end of the experiment, 3/27 mice in the anthanthrene-treated group and 2/23 mice in the control group had developed skin papillomas. The first skin papillomas appeared after 15 weeks in the anthanthrene-treated group compared with 20 weeks in the control group (Cavalieri *et al*., 1989).

Table 3.1. Carcinogenicity studies of dermal application of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Acenaphthene									
Mouse, NS	NS	100	NS (benzene)	NS	9 mo	0/100	–	No control or histopathology	Kennaway (1924a,b)
Acepyrene									
Mouse, Swiss	F	30	>99% (acetone)	0, 0.2, 0.6, 1.8 μmol 2×/wk, 30 wk	30 wk	Skin: 0/30, 0/30, 1 SGA/30 (3%) and 1 SCC/30 (3%)	–		Cavalieri *et al.* (1981)
Anthanthrene									
Mouse, NS	NS	30	NS (benzene)	0.3% solution; 1×/wk every 3–5 wk	Life; last mouse died on day 712	1/30 (3%) (lung A); no statistics	–	No control	Badger *et al.* (1940)
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05 or 0.1% solution, 3×/wk, 12 mo	15 mo	0/20 (0.05%), 0.20 (0.1%) vs 0/20 solvent controls	–		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, Swiss	F	30	Recrystallized (toluene)	43 μg in 20 μL, 2×/wk, 75 wk (72 wk in the vehicle-control groups)	Up to 100 wk	1/30 (3%) skin C vs 2/30 skin P and C toluene controls, 0/30 acetone controls	–	No statistics	Lijinsky & Garcia (1972)
Mouse, Swiss	F	40	98.65% (acetone)	119 μg in 16.7 μL, 2×/wk, 30 wk	70 wk	Skin: 18/38 (47%) (7 P, 2 K, 14 C, 1 SGA) vs 0/29 solvent controls	+	Purity questionable; no statistics	Cavalieri *et al.* (1977)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Mouse, MS	NS	100	NS (lanolin or ether solution)	40% suspension or solution, NS	5 mo	Skin P: lanolin suspension, 0/100; ether solution, 1/100	–	No control; limited reporting	Kennaway (1924a,b)
Mouse, albino	NS	41	NS (water or sesame oil)	5 mg in 5 ml, 1×/wk	10 mo	0/41	–	No control	Pollia (1939)
Mouse, Swiss	F	5	NS (acetone)	10% solution, 3×/wk, 20 mo	20 mo	0/5	–	Small numbers; no control	Wynder & Hoffmann (1959)
Mouse, C3H/Hej	M	20; 50 controls	99.5% (toluene)	50 µg in 50 µL, 2×/wk, 104 wk	104 wk	0/14 vs 0/39 solvent controls	–		Warshawsky *et al.* (1993)
Benz[*a*]anthracene									
Mouse, NS	NS	50	NS (benzene)	NS	NS	1/50 (2%) (skin P, regressed)	–	No control	Kennaway (1930)
Mouse, NS	NS	30	Purified (benzene)	200 µg in 100 µL, NS	>1 year	1/30 (3%) (skin E)	–	No control	Barry *et al.* (1935)
Mouse, CAF1	M, F	20	NS (mineral oil)	0.4%, 1 drop 2×/wk, 68 wk	68 wk	1/20 (5%) (skin P)	–	No control	Hill *et al.* (1951)
Mouse, C3H	NS	20	NS (acetone)	0.5%, 2×/wk	638 days	0% vs 0% in solvent-treated controls	–		Stevenson & von Haam (1965)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C3H/He	NS	30–40	Pure (toluene or *n*-dodecane)	0.002%, 0.02%, 0.2%, 1% in toluene or 0.0002%, 0.002%, 0.02%, 0.2%, 1% in *n*-dodecane, 3×/wk; total dose, 150 mg	Up to 88 wk	Toluene: 0/32, 1/18 (6%), 3/32 (9%), 5/29 (17%) (malignant skin T); 0/32, 0/18, 0/32, 3/29 (10%) (benign skin T) with increasing dose level *n*-Dodecane: 2/31 (6%), 4/21 (19%), 4/20 (20%), 7/21 (33%), 16/22 (73%) (malignant skin T); 2/31 (6%), 4/21 (19%), 0/20, 4/21 (19%), 1/22 (5%) (benign skin T)	+	No control	Bingham & Falk (1969)
Mouse, Swiss	F	40	Recrystallized (acetone)	0.396 µmol [90.4 µg] in 16. µL, 2×/wk, 30 wk	70 wk	1/39 (3%) (skin P) vs 0/29 solvent-treated controls; no statistics	–		Cavalieri *et al.* (1977)
Rat, Donryu	M	25	NS (acetone)	Saturated solution, 1×daily, 5 mo	18 mo	0/9	–	No control	Tawfic (1965)
Hamster, Syrian golden	M, F	10	NS (mineral oil)	0.5%, 2×/wk, 10 wk	85 wk	0/10	–	No control	Shubik *et al.* (1960)
Benzo[*a*]pyrene									
Mouse, Swiss ICR/Ha	F	50	NS (acetone)	0 (untreated), 0 (vehicle control), 5 µg/animal, 3×/wk, 52 wk	52 wk	Skin T: 0/50, 0/50, 23/50 (46%; 13 P; 10 C)	+		Van Duuren *et al.* (1973)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss	F	40	99% (acetone)	0, 0.396 μmol [0.1 mg]/animal, 2×/wk, 30 wk	38–65 wk	Skin T: 0% [0/29], 78.9% [30/38] (7 P, 7 K, 36 C, 1 malignant Schwannoma)	+		Cavalieri *et al.* (1977)
Mouse, C57BL/6J	F	30	NS (DMSO/ acetone (1:3) or acetone/ NH_4OH (1000:1))	Experiment 1 and 2: 0 (DMSO/ acetone), 0.02 [5.28 μg], 0.1 [26.43 μg], 0.4 [105.75 μg] μmol/ animal, 1×/2 wk, 60 wk (high dose given in two paintings, 30 min. apart) Experiment 3: 0 (acetone/NH_4OH), 0.025 [6.6 μg], 0.05 [13.21 μg], 0.1 [26.43 μg] μmol/animal, 1×/ 2 wk, 60 wk	60 wk	Skin T (mainly SCC based on past experience)*: Experiment 1: 0%, 0%, 38% (13 T), 100% (44 T) Experiment 2: 0%, 4% (1 T), 50% (15 T), 100% (40 T) Experiment 3 : 0%, 7% (2 T), 59% (20 T), 91% (24 T)	+	Effective no. of animals not clearly specified *At most, 7 animals/group died prema- turely without a skin tumour.	Levin *et al.* (1977)
Mouse, NMRI	F	40	>96% (acetone)	0, 1.7, 2.8, 4.6 μg/animal, 2×/wk for life	~88–~130 wk	Local T: 0/35, 8/34 (23.5%; age-standardized, 24.8%), 24/35 (68.6%; age-standardized, 89.3%), 22/36 (61.1%; age-standardized, 91.7%)	+	Type of local tumours NS	Habs *et al.* (1980)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, NMRI	F	20	>96% (acetone)	0, 2, 4 µg/animal, 2×/wk for life	63–109 wk	Skin T: 0/20, 9/20 (45%; 2 P, 7 C); 17/20 (85%; 17 C)	+		Habs *et al.* (1984)
Mouse C3H/HeJ	M	50	99.5% (acetone)	0 (untreated), 0 (vehicle control) or 12.5 µg/animal, 2×/wk, 99 wk	99 wk	Skin T: 0/50, 0/50, 48/50 (96%; 47 C, 1 P)	+		Warshawsky & Barkley (1987)
Mouse, Swiss	F	30	Purified [NS] (acetone)	0, 0.1 [26.4 µg], 0.4 [105.7 µg] µmol/animal, 2×/wk, 20 wk	42 wk	Skin T incidence: 0/30, 26/29 (90%; SGA, 3 P, 23 SCC), 26/30 (90%; 2 P, 26 SCC) Multiplicity: low dose, SGA 1/1 [1], P 9/3 [3], SCC 111/23 [4.8]; high dose, P 3/2 [1.5], SCC 153/26 [5.9]	+		Cavalieri *et al.* (1988b)
Mouse, ICR/Harlan	F	43–50	NS (acetone)	0 (untreated), 0 (vehicle control), 16, 32, 64 µg/ animal, 1×/wk, 29 wk	34 wk	No skin T reported in 86 untreated and vehicle controls combined; 127 skin T in the high-dose group (mainly P) [type of skin T and incidences in the mid- and low-dose groups NS]. Skin tumours/animal: 0, ~1.1, ~1.4, ~8.0 [derived from dose–response curves]	+	Limited reporting of tumour data	Albert *et al.* (1991)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BALB/c	M	6	NS (acetone)	0 (untreated), 0 (vehicle control), 100 µg/ animal, 2×/wk for life (3 wk–6 mo)	3 wk–6 mo	No skin T reported in the control groups; mean time (wk) to skin T onset, 19.8 (range 15–27); mean skin T weight, 0.57 ± 0.17 g; type of skin T, mainly SCC, 1 pleiomorphic SCC, 1 K	+	Small no. of animals; limited data on tumours	Andrews *et al.* (1991)
Mouse, SENCAR	F	24	>99% (acetone)	0, 100 nmol [26.4 µg]/animal, 1×	27 wk	0/24, 1/24 (4%) (1 skin P; multiplicity, 1/1)	–		Cavalieri *et al.* (1991)
Mouse, Swiss	F	23–27	>99% (acetone)	0, 1, 4, 8 nmol [0, 0.264,1.057, 2.11 µg]/animal, 2×/wk, 40 wk	48 wk	No skin T found (effective no. of animals, 23–27); tumours at other sites: 1/27 (1 lung A), 3/24 (4%; 2 lung A, 1 splenic malignant lymphoma), 3/23 (13%; 1 lung A, 1 splenic malignant lymphoma, 1 liver haemangioma), 3/23 (13%; 1 lung A, 1 splenic malignant lymphoma, 1 malignant lymphoma in multiple organs)	–	Low doses	Higginbotham *et al.* (1993)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C3H/HeJ	M	Experiment 1, 20; experiment 2, 20 or 50; experiment 3, 24–100	99.5% (toluene)	Experiment 1: 0, 0.2% [presumably 100 µg/animal], 2×/wk, 6 mo	Experiment 1: 6 mo	Experiment 1: 0/20, 20/20 (100%) (1 benign and 19 malignant T)	Experiment 1: +	Site and type of T NS	Warshawsky *et al*. (1993)
				Experiment 2: 0 (untreated), 0 (toluene control), 0.001% [0.5 µg/animal], 2×/wk, 104 wk [not clearly specified]	Experiment 2: 104 wk	Experiment 2: 0/32, 0/39, 0/14	Experiment 2 and 3: –		
				Experiment 3: 0, 0.0006% [0.3 µg]/animal, 2×/wk, 66 wk	Experiment 3: 66 wk	Experiment 3: 0/23, 0/66			
Mouse, C3H/Hej	M	20, 50 controls	95% (toluene)	50 µg/in 50 µl 2×/wk, 104 wk	104 wk	1/15 vs 0/39 solvent controls	–		Warshawsky *et al* 1993
Mouse, $AhR^{-/-}$, $AhR^{+/-}$, $AhR^{+/+}$	F	NS	NS (acetone)	200 µg/animal, 1×/wk, 25 wk	28 wk	Skin T: $AhR^{-/-}$, 0/10 (0%); $AhR^{+/-}$, 13/14 (92.4%; 4.6 ± 2.4 T/animal; 12 SCC, 1 P; p <0.01); $AhR^{+/+}$, 15/16 (93.8%; 4.2 ± 1.9 T/animal; 13 SCC, 1 P, 1 K; p <0.01)	+		Shimizu *et al.* (2000)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Cyclopenta[*cd*]pyrene									
Mouse, Swiss	F	30	>99% (acetone)	0, 22.2, 66.6, 200 nmol, 2×/wk, 48 wk	48 wk	Skin SCC, SGA, P: (0/29; 0%), (2/29; 7%), (2/29; 7%), (24/29; 83%)	+		Cavalieri *et al.* (1983)
5,6-Cyclopenteno-1,2-benzanthracene									
Mouse, NS	NS	10	Pure (melting-point [mp] or picrate) (benzene)	0.1%, 0.3% [volume, no. and frequency of treatment NS]	Up to 339 days	0.1% (mp): 0/10 (skin P), 4/10 (40%; skin E); 0.3% (mp): 2/10 (20%), 1/10 (10%) (group 1), 1/10 (10%), 4/10 (40%) (group 2); 0.3% (picrate): 0/10, 8/10 (80%)	+	No control; limited reporting	Cook (1932)
Mouse, NS	NS	10–40	NS (benzene)	0.03, 0.1, 0.3% [volume, no. and frequency of treatments NS]	Up to 795 days (low-dose); 358 days (high-dose)	0.03%: 1/20 (5%; skin P), 1/20 (5%; skin E); 0.1%: 0/10, 5/10 (50%); 0.3%: 5/40 (12%), 14/40 (35%)	+	No control; limited reporting	Barry *et al.* (1935)
Dibenz[*a*,*c*]anthracene									
Mouse, Swiss	F	30, 20 controls	>99% (acetone)	85 µg in 16–20 µL, 2×/wk, 65 wk	Lifetime (>100 wk)	9/16 (56%; 1/16 skin P, 8/16 skin C) vs 0/14 acetone controls.	+	No statistics	Lijinsky *et al.* (1970)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Mouse, Swiss (Millerton)	F	20	NS (acetone)	0.001, 0.01, 0.1% [volume NS], 3×/wk, life	≤6, 13 or 21 mo	0.001%: 6/20 (30%) skin P, 6/20 (30%) skin C; 0.01%: 19/20 (95%) skin P, 18/20 (90%) skin C; 0.1%: 18/20 (90%) skin P, 15/20 (75%) skin C	+	No control; no statistics	Wynder & Hoffmann (1959)
Mouse, C3H (Jax), DBA	F	9–11	NS (benzene)	0.25% solution [volume NS], 2×/wk	NS	C3H: 10/11 (91%) mammary T vs 5/10 (50%) controls; DBA: 9/10 (90%) mammary T vs 0/9 controls	+ for DBA	Small numbers; no statistics	Ranadive & Karande (1963)
Mouse, Swiss	F	20, 50 controls	Purified (acetone: benzene (9:1))	38 µg, 2×/wk, 44 wk	≤60 wk	Skin P: 16/20 (80%) vs 2/50 (4%) solvent controls	+	No statistics	Lijinsky *et al.* (1965)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss (ICR/Ha)	F	30–50	NS (acetone or DMSO)	1, 10, 100 µg in 100 µL, 3×/wk	374–663 days	1 µg (in acetone): 1/30 (3%) skin P, 1/30 (3%) skin C vs 0/30 acetone controls; 1 µg (in DMSO): 1/30 (3%) skin P vs 0/30 DMSO controls; 10 µg (in acetone): 43/50 (86%) skin P, 39/50 (78%) skin C vs 0/50 acetone controls; 100 µg (in acetone): 39/40 (97%) skin P, 32/40 (80%) skin C vs 0/40 acetone controls	+	No statistics	Van Duuren *et al.* (1967)
Mouse, IF/Bcr	M	48, 30	NS (acetone)	1.5 mg, 1×/wk, 18 wk	≤29 wk	Thymectomized, 10/10 (100%) skin P; intact, 27/27 (100%) skin P	+	No control	Johnson (1968)
Mouse, NMRI	F	50	>99% (acetone)	0.1, 0.4, 1.1 µg in 17 µL, 3×/wk, 112 wk; total doses, 37.8, 125, 378 µg	112 wk	0.1 µg, 3/50 (6%); 0.4 µg, 4/50 (8%); 1.1 µg, 16/50 (32%) skin P vs 2/48 (4%) solvent controls	+	No histology; no statistics	Platt *et al.* (1990)
Hamster, Syrian golden	M, F	5	NS (mineral oil)	~320 µg in ~160 µL, 2×/wk, 10 wk	≤75 wk	0/5 M and 0/5 F	–	No control; small numbers; only 1 M and 1 F survived 75 wk; no statistics	Shubik *et al.* (1960)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*j*]anthracene									
Mouse, Swiss	F	30, 20 controls	>99% (acetone)	39, 78 µg in 16–20 µL, 2×/wk, 81 wk (39 µg) or 60 wk (78 µg)	Lifetime (>100 wk)	Skin T: 39 µg, 2/9 (22%; P), 2/9 (22%; C); 78 µg, 2/20 (10%; P), 6/20 (30%; C) vs 0/14 acetone controls	+	No statistics	Lijinsky *et al*. (1970)
Dibenzo[*a*,*g*]fluorene									
Mouse, CF1	M, F	20	NS (acetone)	One drop (0.02 mL) of 0.3% in acetone (~60 µg)/animal, 2×/wk, 31 wk	48 wk	Skin T: experiment 1, 6/20 (30%; 6 SCC, 1 S); experiment 2, 9/20 (45%; 9 SCC, 2 S)	+	No control, limited histology	Riegel *et al.* (1951)
Dibenzo[*a*,*e*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	40 or 20, 20 controls	Recrystallized (dioxane)	0.05, 0.1% solution (volume NS), 3×/wk, 12 mo	15 mo	Skin T: 0.05%, 16/40 (40%; P), 9/40 (22%; E); 0.1%, 9/20 (45%; P), 6/20 (30%; E) vs 0/20 solvent controls	+		Hoffmann & Wynder (1966); LaVoie *et al*. (1979)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*h*]pyrene									
Mouse, NS	M, F	74	NS (benzene)	50 M: 1 drop of a 0.15–0.18% solution, every other day, 86×; 14 M, 10 F: 1 drop of a 0.15–0.18% solution, every 3rd day, 55×	4.5 mo	86×, 32/49 (65%; skin E); 55×, 11/23 (48%; skin E)	+	No control; no statistics	Kleinenberg (1939)
Mouse, NS	NS	30	NS (benzene)	0.3% solution (volume NS), 2×/wk	350 days	10/30 (33%; skin E)	+	No control; no statistics	Badger *et al.* (1940)
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05, 0.1% solution (volume NS), 3×/wk, 12 mo	11 (0.05%), 15 (0.1%) mo	Skin: 0.05%, 16/20 (80%; P), 13/20 (65%; E); 0.1%, 15/20 (75%; P), 15/20 (75%; E) vs 0/20 solvent controls (p <0.01)	+		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, Swiss	F	40	99.6% (acetone)	119 µg in 16.7 µL, 2×/wk, 30 wk	45 wk; control, 70 wk	35/39 (88%; skin P and C) vs 0/29 solvent controls	+	No statistics	Cavalieri *et al.* (1977)
Dibenzo[*a*,*i*]pyrene									
Mouse, XVII	M	23, NS	NS (*ortho*-dichloro-benzene)	1 drop of saturated solution (concentration, volume NS), 2×/wk	14 mo	21/23 (91%; skin P, 8 skin E) vs 0 solvent control	+	Control group not treated simultaneously; no statistics	Lacassagne *et al.* (1958)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, albino	M	12	Recrystallized (benzene or DMF)	Saturated solution (~0.04% in benzene; concentration in DMF, volume NS), 2×/wk, 3.5 mo (no. of applications NS), followed by a single application as a solid (dose NS) covered with resin	>8 mo	0/12 after solution, 3/12 (25%; skin P) after application as a solid	±	No control; limited duration; no statistics	Schoental (1959)
Mouse, Swiss (Millerton)	F	10	NS (acetone)	0.01, 0.1% solution (volume NS), 3×/wk	Life (up to 17 wk)	0.01%, 1/10 (10%; skin P), 0/10 (skin C); 0.1%, 5/10 (50%; skin P), 1/10 (10%; skin C)	+	No control; no statistics	Wynder & Hoffmann (1959)
Mouse, Swiss	F	12	NS (benzene)	25 µg in 25 µL, 3×/wk, 18–22 mo	Up to 22 mo	4/12 epidermoid C	±	No control; no statistics	Pai & Ranadive (1965)
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05, 0.1% solution (volume NS); 3×/wk, 12 mo	15 mo	0.05%, 16/20 (80%; skin P), 13/20 (65%; skin E); 0.1%, 16/20 (80%; skin P), 15/20 (75%; skin E) vs 0/20 solvent controls	+	No statistics	Hoffmann & Wynder (1966); LaVoie *et al*. (1979)
Rabbit	NS	3	Recrystallized (benzene)	~0.04% (volume NS), 2×/wk, 6 mo	6 mo	0/3	–	No control; small number	Schoental (1959)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Mouse, ICR Swiss albino	F	19–21	Pure (NS)	1 µg [55×], 5 µg [40×], 10 µg [24×], 50 µg [7×], 100 µg [7×] in 100 µL, 3×/wk	7 mo	Skin T: 1 µg, 20/20 (100%); 5 µg, 19/19 (100%); 10 µg, 21/21 (100%); 50 µg, 19/20 (95%); 100 µg, 16/20 (80%)	+	No control; no histology; limited reporting; no statistics	Masuda & Kagawa (1972)
Mouse, SENCAR	F	22–24, 27 controls	>99% (acetone)	0.3, 1.2, 2.4 µg in 100 µL, 2×/wk, 40 wk	48 wk	0.3 µg, 1/24 (4%) skin squamous P (3 T/TBA), 5/24 (21%) lung A; 1.2 µg, 16/23 (70%) skin C (1.8 T/TBA), 9/23 (39%) skin squamous P (1.9 T/TBA), 2/23 (9%) SGA (1.5 T/TBA), 9/23 (39%) T at other sites; 2.4 µg, 20/22 (91%) skin C (2.6 T/TBA), 16/22 (73%) skin squamous P (1.9 T/TBA), 3/22 (14%) SGA (1.7 T/TBA), 14/22 (64%) T at other sites vs 0/27 skin T, 1/27 (4%) lung A solvent controls	+		Higginbotham *et al*. (1993)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C57BL/6J, *AhR*$^{+/+}$, *AhR*$^{-/-}$	NS	*AhR*$^{+/+}$, 17; *AhR*$^{-/-}$, 15	NS (NS)	30 µg (volume NS), followed (elapsed time NS) by 6 µg (volume NS), 1×/wk, 20 wk	Up to 2 years	*AhR*$^{+/+}$, 17/17 (76% skin P, 24% skin C; 2.7 ± 1.4 T/mouse); *AhR*$^{-/-}$, 5/15 (100% skin P; 0.46 ± 0.83 T/mouse)	+	No control; limited reporting	Nakatsuru *et al*. (2004)
Dibenzo[*e*,*l*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05, 0.07% solution (volume NS), 3×/wk, 12 mo	15 mo	Skin T: 0.05%, 0/20; 0.07%, 0/20 vs 0/20 solvent controls	–		Hoffmann & Wynder (1966)
Fluoranthene									
Mouse C3H/HeJ	M	20	NS (toluene)	0.1% fluoranthene, 0.001% benzo[*a*]pyrene 2×/wk	104 wk	1/12 skin (8%) vs 0% fluoranthene alone, benzo[*a*]pyrene alone or solvent controls	±		Warshawsky *et al*. (1993)
1-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	No skin T observed; 7 animals survived	–		Hecht *et al.* (1974); LaVoie *et al.* (1979)
2-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 11/11 (100%; 7 C; 1.0 T/mouse); 10/20 (50%) treated animals survived to study termination	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
3-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 5/8 (63%; 4 C in 4 animals; 0.8 T/mouse); 8/20 treated animals survived to study termination	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
4-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 3/10 (30%; 2 C in 2 animals; 0.5 T/mouse); 10/20 treated animals survived to study termination	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
5-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 20/20 (100%) (37 C; in 12 animals; 5 T/mouse); no animals alive when the study was terminated at 35 wk	+		Hecht *et al.* (1974); LaVoie *et al*. (1979)
6-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 3/12 (25%; 1 C in 1 animal; 1 T/mouse)	–		Hecht *et al.* (1974); LaVoie *et al*. (1979)
2-Methylfluoranthene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Pure (acetone)	0.2% solution, 3×/wk, 12 mo	15 mo	Skin C: 4/10 (40%; 2 T/mouse)	±	No control	Hoffman *et al.* (1972)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Naphtho[2,3-*e*]pyrene									
Mouse, Swiss albino Ha/ICR	F	20	NS (dioxane)	100 µg of 0.1% solution, 3×/wk	15 mo	Skin P: 0/20 (0.05%); 1/20 (0.1%) vs 0/20 solvent controls	–		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Perylene									
Mouse, Swiss	M	20	NS (benzene)	2 drops of a 0.3% solution every 4th day, 22 wk	22 wk	0%	–	No control	Finzi *et al.* (1968)
Mouse, Swiss albino Ha/ICR	F	20	'Rigorously purified' (benzene)	0, 800 µg in 200 µL, 1×, alone or followed by (2.5 µg TPA in 100 µL), 3×/wk, 58–60 wk	58–60 wk	0/20 skin P vs 0/20 solvent controls; 3/20 (15%) skin P vs 1/20 TPA controls	–	No statistics	Van Duuren *et al.* (1970)
Mouse, C3H	M	20	Pure (decalin or decalin + *n*-dodecane (1:1))	0, 50 mg in 60 µL, 2×/wk, 82 wk	82 wk	50 mg (decalin), 0/16 skin P, 0/16 skin C; 50 mg (decalin + *n*-dodecane), 1/15 (7%) skin P, regressed, 0/15 skin C vs 2/13 (15%) skin P, 0/13 skin C decalin + *n*-dodecane controls	–	Small number; no control in decalin experiment; no statistics	Horton & Christian (1974)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Phenanthrene									
Mouse, NS	NS	100	NS (90% benzene)	NS	9 mo	0/100 skin	–	Limited reporting; no control	Kennaway (1924a,b)
Mouse, white	NS	100	'Pure' (5% croton oil)	3 drops of a 3% solution, 1×wk	1 year	1/6 (17%)	±	Low survival; no statistics	Graffi *et al.* (1953)
Mouse, C3H/HeJ	M	20, 50 controls	>99% (toluene)	0, 50 μg in 50 μL, 2×/wk, 104 wk	104 wk	1/12 (8%) benign skin T vs 0/39 benign or malignant skin T solvent controls	–	No statistics	Warshawsky *et al.* (1993)
Picene									
Mouse, NMRI	F	50	>99% (acetone)	0, 0.1, 0.4, 1.1 μg in 17 μL, 3×/wk, 112 wk (total doses, 38, 125, 378 μg)	112 wk	Skin T: 0.1 μg, 3/49 (6%); 0.4 μg, 11/48 (23%); 1.1 μg, 11/50 (22%) vs 2/48 (4%) solvent controls	+	No histology; no statistics	Platt *et al.* (1990)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Triphenylene									
Mouse	NS	10	NS (benzene)	0.3% solution in 100 µL, 2×/wk, 548 days	548 days	0/10 skin T	–	Small number; no control	Barry *et al*. (1935)
Mouse, C3H	M	15 or 20	Pure (decalin or decalin + *n*-dodecane (1:1))	0, 300 µg in 60 µL, 2×/wk, 82 wk	82 wk	300 µg (decalin), 0/14 skin P, 0/14 skin C; 300 µg (decalin + *n*-dodecane): 4/11 (36%) skin P, 1/11 (9%) skin C vs 2/13 (15%) skin P, 0/13 skin C decalin-*n*-dodecane controls	±	Small number; no control for decalin alone; no statistics	Horton & Christian (1974)

A, adenoma; C, carcinoma; DMF, *N,N*- dimethylformamide; DMSO, dimethylsulfoxide; E, epithelioma; F, female; K, keratocanthoma; M, male; mo, month; NH_4OH, ammonium hydroxide; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; SGA, sebaceous gland adenoma; T, tumour; TBA, tumour-bearing animal; TPA, 12-*O*-tetradecanoyl-13-acetate; vs, versus; wk, week

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

Table 3.2. Dermal initiation–promotion studies of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Acenaphthene									
Mouse, NS	M	85; 160 controls	'Pure' (acetone)	3 drops of a ~3% solution, 1×/wk, co-administered with 1 drop of 5% croton oil; 1 year	1 year	Skin: 2/5 (40%) vs 1/13 (8%) croton oil controls	±	Low survival	Graffi *et al.* (1953)
Acepyrene									
Mouse, CD-1	F	30	>99% (acetone)	0, 0.02, 0.06, 0.18 μmol, ×10, followed by 2×/wk 0.017 μmol TPA, 40 wk	44 wk	Skin P: 3/29 (10%), 0/30 (0%), 1/30 (3%), 4/30 (13%); 0.14, 0.03, 0.31, 0.31 T/mouse	–		Cavalieri *et al.* (1981)
Anthanthrene									
Mouse, Swiss albino Ha/ICR/Mil	NS	30	Recrystallized (dioxane)	25 μg in 25 μL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone	6 mo	Skin P: 2/25 (8%) vs 2/26 (8%) promoter controls	–	Short duration of study	Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, Swiss ICR/Ha	F	13	Pure (benzene)	4 × 250 μg in 0.1 mL, followed 2 wk later by 25 μg croton oil in 0.1 mL acetone (3×/wk)	54–66 wk	Skin P: 2/13 (15%) vs 0/13 non-promoted group, 0/20 untreated controls, 0/20 vehicle controls, 6/40 (15%) promoter controls Skin C: 1/40 (2%) promoter controls	–	No statistics	Van Duuren *et al.* (1968)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	30	Pure (benzene)	690 µg 1×, followed 1 wk later by 5 µmol (3.1 mg) TPA 2×/wk (10 µmol TPA in promoter controls), 34 wk	35 wk	Skin P: 5/28 (18%) vs 0/30 promoter controls	+	No statistics	Scribner (1973)
Mouse, SENCAR	F	27, 23 controls	>99% (dioxane: DMSO (75:25))	221 µg in 100 µL, 1×, followed 1 wk later by 2.6 µg TPA in 100 µL acetone, 2×/wk, 25 wk	26 wk	Skin P: 3/27 (11%) vs 2/23 (9%) promoter controls	–		Cavalieri *et al.* (1989)
Anthracene									
Mouse, NS	NS	44; 44; 100	NS (petroleum jelly–olive oil)	5% solution on ears, 3×/wk; 5% solution, 3×/wk, then 40 or 60 min UV; 5% solution, 3×/wk, then 90 min UV	11 mo; 9 mo; 9 mo	0/44 (only 1 alive); 0/44 (none alive after 9 mo); 0/100 (none alive after 9 mo)	–	No controls	Miescher (1942)
Mouse, 'S'	NS	20; 20 controls	NS (acetone)	1.5 mg in 300 µL, 2×/day, 3 days/wk, 20×; followed by 300 µL 0.17% croton oil 1×/wk, 16 wk; 300 µL 0.085% 1×/wk, 2 wk	21 wk	Skin P: 3/17 (18%) vs 4/19 (21%) acetone, croton oil control	–	No statistics	Salaman & Roe (1956)
Mouse, CD-1	F	30; 30 controls	Purified (acetone)	1.78 mg 1×; followed by 5 µg TPA, 2×/wk, 34 wk	35 wk	Skin P: 4/28 (14%) vs 1/30 (3%) acetone, TPA control	–	No histo-pathology; no statistics	Scribner (1973)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss albino Crl:CD/1 (ICR)BR	F	20; 20 controls	>99% (acetone)	100 μg in 100 μL every other day, 10×; followed by 2.5 μg TPA in 100 μL, 3×/wk, 20 wk	24 wk	Skin P: 3/20 (15%) vs 2/20 (10%) acetone, TPA control	–		LaVoie *et al.* (1983, 1985)
11*H*-Benz[*b*,*c*]aceanthrylene									
Mouse, CD-1	F	20	>99% (acetone)	0.05, 0.2, 0.4 μmol [12, 48, 96 μg] in 100 μL; 10× on alternate days; followed 10 days later by 2.5 μg TPA in 100 μL acetone, 3×/wk, 20 wk	24 wk	0.05 μmol, 15/20 (75%); [type NS]; 0.2 μmol, 18/20 (90%); 0.4 μmol, (90%) 18/20 vs 1/20 (5%) solvent-treated controls (p <0.005 at all doses); 1.60, 4.90, 7.60 T/ mouse vs 0.05 solvent controls	+		Rice *et al.* (1988)
Benz[*j*]aceanthrylene									
Mouse, SENCAR	F	20	NS (acetone)	40, 200, 400 μg in 200 μL, 1×; followed by 2 μg TPA, 2×/wk, 21 wk	22 wk	Skin P: 40 μg, 20/20 (100%) (8.7 P/mouse); 200 μg, 20/20 (100%) (10.8 P/mouse); 400 μg, 20/20 (100%) (7.7 P/mouse) vs ~1/20 (5%) acetone, TPA controls	+	Limited histo-pathology; no statistics	Nesnow *et al.* (1993)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*l*]aceanthrylene									
Mouse, SENCAR	M, F	20–22	NS (acetone)	12.6, 25.2, 63.1, 126, 252 µg in 200 µL, 1×; followed by 2 µg TPA in 200 µL acetone, 2×/wk, 30 wk	31 wk	Skin P: males – 12.6 µg, 12/20 (60%; 1.4 P/mouse); 25.2 µg, 16/17 (94%; 2.3 P/mouse); 63.1 µg, 21/21 (100%; 8.4 P/mouse); 126 µg, 16/16 (100%; 10.8 P/mouse); 252 µg, 19/20 (95%; 8.7 P/mouse) vs 0/20 acetone, TPA-treated controls; females – 12.6 µg, 13/20 (65%; 1.1 P/mouse); 25.2 µg, 18/19 (95%; 3.1 P/mouse); 63.1 µg, 19/21 (90%; 4.7 P/mouse); 126 µg, 20/21 (95%; 6.6 P/mouse); 252 µg, 20/20 (100%; 10.8 P/mouse) vs 1/19 (5%; 0.05 P/mouse) acetone, TPA-treated controls	+	No histopathology; no statistics	Nesnow *et al.* (1984a)
Benz[*a*]anthracene									
Mouse, NS	NS	75	NS (acetone)	0.05%, alternating applications (1×/wk) with 5% croton oil in mineral oil; NS	12 mo	Skin P: 9/18 (50%) vs 1/13 (8%) croton oil controls	+		Graffi *et al.* (1953)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, albino S	M	20	NS (acetone)	1% solution (300 µL), 2×/wk (total dose, 6 mg), followed 3 wk later by 300 µL of 0.5% solution of croton oil in acetone, 1×/wk	21 wk	Skin: 7/18 (39%; 1.17 T/mouse) vs 0/8 without promotion, 1/20 (5%) croton oil controls	+		Roe & Salaman (1955)
Mouse, Swiss	F	20	'Purified' (acetone)	0.9 µg in 50 µL, 1×; followed by 0.5% croton oil in 200 µL acetone, 2×/wk, 60 wk	61 wk	8/20 (40%; mostly skin P) vs 0/20 without promotion	+	No control	Hadler *et al.* (1959)
Mouse, Swiss (ICR/Ha)	F	20	'Rigorously purified' (benzene)	1.0 mg in 100 µL, 1×; 1.0 mg in 100 µL, 1×; followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 56–58 wk	58–60 wk	Skin P: 1.0 mg, 0/20 vs 0/20 solvent controls; 1.0 mg + TPA, 10/20 (50%) vs 1/20 (5%) TPA controls	+	No statistics	Van Duuren *et al.* (1970)
Mouse, CD-1	F	30	Pure (acetone)	2.2 µmol [502 µg], 1×, followed 1 wk later by 10 µmol (6.2 mg) TPA, 2×/wk, 34 wk	35 wk	18/29 (62%; skin P) vs 0/30 TPA controls	+	TPA dose probably 10 µg; no statistics	Scribner (1973)
Mouse, CD-1	F	30	>99% (acetone)	2 µmol [457 µg], 1×, followed 1 wk later by 10 µg TPA, 2×/wk, 26 wk	27 wk	17/30 (57%; skin P; 1.2 P/mouse) vs 2/29 (6%) in TPA controls	+	No statistics	Slaga *et al.* (1978)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	30	NS (acetone)	1.0, 2.5 μmol [2.28, 571 μg] in 200 μL, 1×, followed 1 wk later by 16 nmol (9.9 μg) TPA in 200 μL acetone, 2×/wk, 26 wk	27 wk	Skin: 1.0 μmol, 5/30 (17%; 0.17 ± 0.07 T/mouse); 2.5 μmol, 11/29 (38%; 0.67 ± 0.17) vs 1/25 (4%; 0.04 ± 0.04) (p <0.05 at 2.5 μmol)	+	No statistics	Wood *et al.* (1980)
Mouse, CD-1	F	30	>99% (acetone)	0.4, 2.5 μmol [91, 571 μg] in 200 μL, 1×, followed 2 wk later by 16 nmol [9.9 μg] TPA in 200 μL acetone, 2×/wk, 25 wk	27 wk	Skin: 0.4 μmol, 4/28 (14%;0.14 ± 0.07 T/mouse); 2.5 μmol, 10/27 (36%; 0.64 ± 0.20) vs 2/29 (7%; 0.07 ± 0.05) in solvent controls (p <0.05 for T incidence and T/mouse at 2.5 μmol)	+		Levin *et al.* (1984)
Benzo[*b*]chrysene									
Mouse, CD-1	F	30	Pure (benzene)	2.5 mmol [695 μg], 1×, followed 1 wk later by 5 μmol (3.1 mg) TPA 2×/wk (10 μmol TPA in promoter controls), 34 wk	35 wk	14/29 (48%) (skin P) vs 0/30 in promoter controls	+	No statistics	Scribner (1973)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]fluoranthene									
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 20 wk	24 wk	Skin P: 2/20 (10%), 19/20 (95%; p <0.001), 18/20 (90%; p <0.001); 0, 1, 3.3, 4.3 T/animal in TPA control, low- and high-dose groups	+		Weyand *et al.* (1990)
Benzo[*b*]fluoranthene									
Mouse, Crl:CD-1 (ICR)BR	F	20	>99% (acetone)	0, 10, 30, 100 µg; 10 subdoses, 1× every other day followed by TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 45%, 60%, 80%; 0, 0.9, 2.3, 7.1 T/animal	+		LaVoie *et al.* (1982a)
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	20	>99% (acetone)	0, 40, 100 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA in 100 µL acetone, 3×/wk	34 wk	Skin P: 10%, 45%, 95%; 0.2, 0.9, 3.3 T/animal	+		Amin *et al.* (1985a)
	F	20	>99% (acetone)	0, 40, 100 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA in 100 µL acetone, 3×/wk	34 wk	Skin P: 5%, 42%, 53%; 0.1, 0.5, 0.9 T/animal	+		
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	25	>99% (acetone)	400 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 92%; 6.0 T/animal	+		Geddie *et al.* (1987)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
	F	20	>99% (acetone)	100 nmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	24 wk	Skin P: 75%; 2.8 T/animal	+		Geddie *et al.* (1987) (contd)
	F	20	>99% (acetone)	100 nmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	24 wk	Skin P: 70%; 1.8 T/animal	+		
	F	20	>99% (acetone)	100 nmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	34 wk	Skin P: 53%; 0.9 T/animal	+		
Mouse, CD-1	F	20	NS (acetone)	0, 30, 100 μg; 10 subdoses, 1× every other day followed by 2.5 μg TPA in 100 μL acetone, 3×/wk, 20 wk	24 wk	Skin P: 15%, 65%, 100%; 0.1, 1.4, 5.4 T/animal	+		Weyand *et al.* (1989)
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 μmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	24 wk	Skin P: 10%, 100% (p <0.001), 100% (p <0.001), 0.1, 8.5, 11.0 T/animal	+		Weyand *et al.* (1990)
Mouse, Crl:CD-1 (ICR) BR outbred albino	F	20	NS (acetone)	0, 30, 100 μg; 10 subdoses, 1× every other day followed by 2.5 μg TPA in 100 μL acetone, 3×/wk, 30 wk	34 wk	Skin P: 15%, 65%, 100%; 0.1, 1.4, 5.4 T/animal	+		Amin *et al.* (1991)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Crl:CD-1 outbred albino	F	20	>99% (acetone)	0, 120, 400 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 15%, 70% (p <0.001), 95%; 0.15, 1.40, 7.15 T/animal	+		LaVoie *et al.* (1993)
	F	20	>99% (acetone)	0, 40, 120, 400 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 5%, 35% (p <0.05), 90% (p <0.001), 95% (p <0.001); 0.05, 0.45, 3.70, 8.65 T/animal	+		
Benzo[*j*]fluoranthene									
Mouse, Crl:CD-1 (ICR)BR	F	20	>99% (acetone)	0, 30, 100, 1000 µg; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 30%, 55%, 95%; 0, 0.6, 1.9, 7.2 T/animal	+		LaVoie *et al.* (1982a)
Mouse, CD-1	F	20	NS (acetone)	0, 1, 3 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 10% (p <0.005), 70% (p <0.005), 90% (p <0.005); 0.1, 3.4, 7.8 T/animal	+		Rice *et al.* (1987)
Mouse, Crl:CD-1 outbred albino	F	20–24	>99% (acetone)	0, 0.3, 1.0, 2.0 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 5% (p <0.01), 55% (p <0.01), 88% (p <0.01), 100% (p <0.01); 0.05, 1.75, 4.08, 7.17 T/animal	+		Weyand *et al.* (1992)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	20	NS (acetone)	0, 25, 50, 100, 1000 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA. 3×/wk, 20 wk	24 wk	Skin P: 0%, 5% (p <0.05), 10% (p <0.05), 45% (p <0.001), 95% (p <0.001); 0, 0.05, 0.40, 0.65, 8.75 T/animal	+		LaVoie *et al.* (1993)
Benzo[*k*]fluoranthene									
Mouse, Crl:CD-1 (ICR)BR	F	20	>99% (acetone)	0, 30, 100, 1000 µg; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 5%, 25%, 75%; 0, 0.1, 0.4, 2.8 T/animal	+		LaVoie *et al.* (1982a)
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	20	>99% (acetone)	0, 4 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 30 wk	30 wk	Skin P: 0%, 37%; 0.7 T/animal	+		Amin *et al.* (1985b)
Benzo[*a*]pyrene									
Mouse, SENCAR	F	Experiment 1, 30; experiment 2, 60	98.5% (acetone or acetone + DMSO)	Experiment 1: 50, 100, 200 nmol [13.21, 26.43, 52.87 µg]/animal, 1× followed after 1 wk by 8.5 nmol TPA, 2×/wk, 19–28 wk	28/50 wk	Experiment 1: Skin P (after 28 wk): 19/30 (63%; 1.7 P/animal), 27/30 (89%; 3.8 P/animal), 29/30 (97%; 7.8 P/animal) Skin C (after 50 wk): 5/30 (18%), 9/30 (30%), 17/30 (55%)	+	No control in experiment 1	DiGiovanni *et al.* (1980)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
				Experiment 2: 0, 10, 50, 100, 200 nmol [0, 2.64, 13.21, 26.43, 52.87 µg]/animal, 1× followed after 1 wk by 8.5 nmol TPA, 2×/wk, 25 wk	25 wk	Experiment 2: Skin P: 6/60 (10 %; 0.1 P/animal), 25/60 (42%; 0.9 P/animal), 36/60 (60%; 1.6 P/animal), 48/60 (80%; 3.8 P/animal), 60/60 (100%; 8.2 P/animal)			DiGiovanni *et al.* (1980) (contd)
Mouse, CD-1	F	30	98.5% (acetone or acetone + DMSO)	0, 10, 50, 100, 200 nmol [0, 2.64, 13.21, 26.43, 52.87 µg]/animal, 1× followed after 1 wk by 8.5 nmol croton oil, 2×/wk, 25 wk	25 wk	Skin P: 6/60 (10%; 0.1 P/animal), 6/60 (10%; 0.1 P/animal), 24/60 (40%; 0.7 P/animal), 35/60 (58%; 1.8 P/animal), 43/60 (72%; 3.8 P/animal)	+		DiGiovanni *et al.* (1980)
Mouse, Crl/CD-1(ICR)BR	F	20	>99% (acetone)	0, 5 µg animal, 1×/2 days, 20 days (total dose, 50 µg/animal) followed after 10 days by 2.5 µg TPA/animal, 3×/wk, 25 wk	25 wk	Skin T (predominantly P): 1/20 (5%; 0.1 T/animal), 18/20 (90%; 7.1 T/animal) (p <0.01)	+		El-Bayoumy *et al.* (1982)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	30	NS (acetone)	0, 10, 25, 50, 100, 200 µg/animal, 1× followed 1 wk later by 2 µg TPA/animal, 2×/wk, 25 wk	25 wk	Skin P: 3/29 (10%; 0.2 T/animal), 17/29 (58%; 1.3 T/animal), 21/28 (76%; 3.8 T/animal), 24/28 (87%; 6.2 T/animal), 27/27 (100%; 8.8 T/animal), 26/26 (100%; 9.0 T/animal)	+		Raveh *et al.* (1982)
Mouse, SENCAR	M, F	22–40	NS (acetone or acetone/DMSO mixture (1:1))	Experiment 1: 0, 51 µg/animal in acetone, 1× followed after 1 wk by 2.0 µg TPA/animal, 2×/wk, 30 wk	30 wk	Skin P Experiment 1: M: 37/37 (100%; 7.2 P/animal), 39/40 (97%; 5.3 P/animal) F: 35/35 (100%; 7.7 P/animal), 36/40 (90%; 4.5 P/animal)	+		Nesnow *et al.* (1984b)
				Experiment 2: 0 (acetone/DMSO mixture), 51 µg/animal (in acetone) followed after 1 wk by 2.0 µg TPA/animal, 2×/wk, 30 wk		Experiment 2:M: (0/21; 0%), 11/18 (61%; 1.5 P/animal); F: (0/20; 0%), 9/19 (47%; 0.8 P/animal)			
Mouse, SENCAR	F	20	NS (acetone)	0, 396 nmol [0.1mg]/animal, 1× followed after 7 days by 3.24 nmol TPA, 2×/wk, 11 wk	11 wk	Skin P: 0/20, 20/20 (100%; 6.6 P/animal)	+		Mukhtar *et al.* (1986)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24, 29 controls	NS (acetone)	0, 0.8 μmol [0, 0.2 mg]/ animal, 1× followed after 1 wk by 4.26 nmol TPA, 2×/wk, 23 wk	23 wk	Skin P: 1/29 (3%; multiplicity, 2), 22/24 (92%; multiplicity, 6.6; p <0.001)	+		Cavalieri *et al.* (1988b)
Mouse, CD-1	F	20	>99% (acetone)	0, 0.01 μmol [2.64 μg]/animal, 1×/2 days, 20 days, followed 10 days after the final dose by 2.5 μg TPA, 3×/wk, 20 wk	20 wk	Skin T: 1/20 (5%; 0.05 T/animal), 17/19 (89%; 5.53 T/animal; (p <0.005)	+	Type of skin T NS	Rice *et al.* (1988)
Mouse, CD-1	F	30	>99% (acetone)	0, 10 nmol [2.64 μg]/animal, 1×/2 days, 20 days, followed 10 days after the last dose by 2.5 μg TPA, 3×/wk, 20 wk	20 wk	Skin P: 24/25 (0, 96%; 3.4 T/animal; p <0.005)	+		Rice *et al.* (1990)
Mouse, SENCAR	F	24	>99% (acetone)	0, 33.3, 100, 300 nmol [0, 8.8, 26.4, 79 μg]/ animal, 1× followed by 3.24 nmol TPA, 2×/wk, 1 wk, then stopped for 2 wk then resumed and continued with 2 ×/wk for 11 wk	15 or 16 wk	Skin T (predominantly P): 0/24, 10/23 (43%; 15 T, 0.65 T/animal), 17/24 (71%; 66 T, 2.75 T/animal), 21/23 (91%; 120 T, 5.22 T/animal)	+		Cavalieri *et al.* (1991)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24	>99% (acetone)	0, 4, 20, 100 nmol [0, 1, 5.3, 26.4 μg]/animal, 1×, followed after 1 wk with 3.24 nmol TPA, 2×/wk, 24 wk	27 wk	Skin T (predominantly P): 0/24, 1/24, (4%; 1 T, 0.04 T/animal), 10/24 (42%; 18 T, 0.75 T/animal), 22/24 (92%; 82 T, 3, 42 T/ animal)	+		Cavalieri *et al.* (1991)
Mouse, SENCAR	F	24	>99% (acetone)	0, 1 nmol [0.26 μg]/animal, 1× followed after 1 wk by 2.16 nmol TPA, 2×/wk, 27 wk	27 wk	No skin T found	–	Very low dose	Higginbotham *et al.* (1993)
Mouse, CD-1	F	20	NS (acetone)	0, 4, 10, 25 nmol [1, 2.5, 6.6 μg]/animal, 10]×, 1×/2 days, 20 days followed after 10 days by 2.5 μg TPA/animal, 3×/wk, 20 wk	25 wk	Skin T: 3/20 (15%; 0.15 T/animal), 3/20 (15%; 0.15 T/animal), 2/20 (10%; 0.10 T/ animal), 5/20 (25%; 0.4 T/animal; p >0.05)	–	Type of skin T NS	LaVoie *et al.* (1993)
Benzo[*e*]pyrene									
Mouse, CD-1	F	30	>99% (acetone/DMSO)	0, 1.0, 2.5, 6.0 μmol, 1×; followed by TPA (16 nmol/200 μL acetone), 2×/wk, 25 wk	36 wk	Skin P: 7% 15%, 11%, 14%; 0.07, 0.15, 0.11, 0.14 T/animal	±		Buening *et al.* (1980)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Chrysene									
Mouse CD-1	F	30	NS (acetone/DMSO)	0, 0.4, 1.2 µmol, 1×, followed by 16 nmol TPA, 2×/wk, 25 wk	26 wk	Skin P: 10%, 43% (p <0.05), 43% (p <0.05); 0.10 ± 0.06, 0.77 ± 0.25, 1.07 ± 0.34 P/animal	+		Chang *et al.* (1983)
				0, 1.2 µmol, 1×, followed by 16nmol TPA, 2×/wk, 25 wk	26 wk	Skin P: 0%, 30% (p <0.05);0, 0.93 ± 0.27 P/animal	+		
Mouse, CD-1	F	25	>99% (acetone)	0,1.0 mg, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 2/25 (8%), 23/25 (92%)	+		Rice *et al.* (1985)
Mouse, CD-1	F	20	>99% (acetone)	0, 0.15, 0.5, 1.5 µmol, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	20 wk	Skin P: 20%, 25% (p <0.05), 90% (p <0.005), 95% (p <0.005); 0.05, 0.45, 2.95, 4.45 P/animal	+		Rice *et al.* (1988)
Mouse, CD-1	F	20	>99% (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 10%, 10%; 0.6, 0.1 T/animal	–		Amin *et al.* (1990)
Mouse, SENCAR	M, F	16	NS (toluene)	0, 1600 nmol, 1×, followed by croton oil/toluene (1:99, v/v), 2×/wk, 100 wk	101 wk	No skin T in treated or control mice	–		Bhatt & Coombs (1990)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
4*H*-Cyclopenta[*def*]chrysene									
Mouse, CD-1	F	25	>99% (acetone)	0, 0.1 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 2/25 (8%), 25/25 (100%)	+		Rice *et al*. (1985)
Mouse, CD-1	F	20	>99% (acetone)	0, 0.15, 0.5, 1.5 µmol, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 1/20 (5%; 0.05), 13/20 (65%; 1.0), 19/19 (100%; 6.85), 19/19 (100%; 8.47) (p <0.05)	+		Rice *et al*. (1988)
Dibenz[*a*,*c*]anthracene									
Mouse, Swiss (ICR/Ha)	F	20, 40 controls	Purified (benzene)	1 mg in 100 µL, 1×; 1 mg in 100 µL, 1×, followed 2 wk later by 25 µg croton oil in 100 µL acetone, 3×/wk	54–66 wk	1.0 mg, 0/20 vs 0/20 solvent controls; 1.0 mg + croton oil, 5/20 (25%) skin P, 2/20 (10%) skin C vs 6/40 (15%) skin P, 1/40 (2%) skin C in croton oil controls	±	No statistics	Van Duuren *et al*. (1968)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss (ICR/Ha)	F	20 or 50, 20 controls	'Rigorously purified' (benzene)	1.0 mg in 100 µL, 1×; 1.0 mg in 100 µL, 1×, followed 2 wk later by 2.5 µg TPA in 100 µL acetone, 3×/wk	58–60 wk	1.0 mg, 0/50 vs 0/20 solvent controls; 1.0 mg + TPA, 19/20 (95%; skin P), 4/20 (20%; skin C) vs 1/20 (5%; skin P) TPA controls	+	No statistics	Van Duuren *et al.* (1970)
Mouse, CD-1	F	30	Pure (benzene)	696 µg, 1×, followed 1 wk later by TPA [5 µmol (3.1 mg); solvent and volume NS], 2×/wk (10 µmol TPA in promoter controls), 34 wk	35 wk	63% skin P vs 0% in TPA controls	+	No statistics; incidence NS	Scribner (1973)
Mouse, SENCAR	F	30	>5% (acetone)	2 µmol [557 µg], 1×; followed 1 wk later by TPA [2 µg, solvent and volume NS], 2×/wk [duration NS]	At least 15 wk	27% skin P vs 10% in TPA controls	±	Limited reporting and histo-pathology; no statis-tics	Slaga *et al.* (1980)
Mouse, CD-1	F	39–40	Pure (acetone)	25, 50 µg in 100 µL, followed 1 wk later by 0.64 µg TPA in 100 µL acetone, 2×/wk; 29 wk, then 1 µg, 38 wk	68 wk	25 µg, 5/39 (13%; skin P); 50 µg, 8/40 (20%) vs 3/40 (8%) TPA controls	±		Chourou-linkov *et al.* (1983)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Mouse, Swiss albino	M	97	NS (acetone)	20, 160 ng in 200 µL, 1×, followed by croton oil [1%, volume NS], 1×/wk, 25 wk	210–218 days	20 ng, 10/30 (33%) skin T (0.5 T/mouse; p <0.05); 160 ng, 14/37 (38%; 0.9 T/mouse; p <0.05) vs 4/32 (13%; 0.1 T/mouse) acetone controls	+		Klein (1960)
Mouse, Swiss (ICR/Ha)	F	20, 50 controls	NS (benzene)	0, 150 µg, 1×, followed by croton oil [100 µL, 0.025%], 3×/wk, 45 wk	331 days	18/20 (90%; skin P), 10/20 (50%; skin C) vs 8/50 (16%; skin P), 0/50 (skin C) croton oil controls	+	No statistics	Van Duuren *et al.* (1967)
Mouse, NMRI	F	16, 30 controls	>99% (acetone or THF)	0, 83.5 µg in 100 µL acetone, 167 µg in 100 µL tetrahydrofuran, 100 µL acetone, 1×, followed by 10 nmol TPA, 2×/wk, 23 wk	24 wk	83.5 µg, 6/16 (38%; skin P); 167 µg, 15/16 (93%; skin P) vs 0/30 acetone controls	+	No histology; no statistics	Platt *et al.* (1990)
Dibenz[*a*,*j*]anthracene									
Mouse, SENCAR	F	30	NS (acetone)	0, 111, 223 µg [400, 800 nmol] in 200 µL, 1×, followed by 2.1 µg TPA, 2×/wk, 20 wk	22 wk	Skin P: 111 µg, 70% (1.3 P/mouse); 223 µg, 97% (3.0 P/mouse) vs 19% (0.19 P/mouse) acetone controls	+	No histology	Sawyer *et al*. (1987, 1988)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24	NS (peroxide-free THF)	0, 111 μg (400 nmol) in 200 μL, 1×, followed by 2.1 μg TPA, 2×/wk, 14 wk	16 wk	Skin P: 111 μg, 29% (0.58 P/mouse) vs 5% (0.05 P/mouse) THF controls (p <0.05)	+	Limited histology	Harvey *et al.* (1988)
Mouse, SENCAR	F	24	NS (peroxide-free THF)	0, 111, 223 μg [400, 800 nmol] in 200 μL, 1×; followed by 2.1 μg TPA, 2×/wk, 20 wk	22 wk	Skin P: 111 μg, 39% (0.86 P/mouse); 223 μg, 65% (1.83 P/mouse) vs 5% (0.05 P/mouse) THF controls	+	No histology	Sawyer *et al*. (1988)
Dibenzo[*a*,*e*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Recrystallized (dioxane)	0, 25 μg in 25 μL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone (frequency, duration NS)	6 mo	10/28 (36%; skin P) vs 2/30 (7%) croton oil controls	+		Hoffmann & Wynder (1966); LaVoie *et al*. (1979)
Mouse, SENCAR	F	21, 23 controls	>99% (dioxane: DMSO (75:25))	0, 242 μg [800 nmol] in 100 μL, 1×, followed 1 wk later by 2.6 μg TPA/100 μL acetone, 2×/wk, 25 wk	26 wk	5/21 (24%; skin P) vs 2/23 (9%) TPA controls	–		Cavalieri *et al*. (1989)
Dibenzo[*a*,*h*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Recrystallized (dioxane)	0, 25 μg, 10×/20 days, followed by 2.5% [2.3 mg] croton oil (volume NS), 3×/wk)	6 mo	21/29 (74%; skin P) vs 2/30 (7%; skin P) croton oil control (p <0.01)	+		Hoffmann & Wynder (1966); LaVoie *et al*. (1979)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	31, 32 controls	NS (acetone)	0, 200 µg, 1×, followed by 10 µg TPA, 2×/wk, 26 wk	27 wk	26/28 (93%; skin T) vs 2/32 (6%) TPA controls	+	No statistics	Sardella *et al.* (1981)
Mouse, CD-1	F	30	'Essentially pure' (10% DMSO in THF)	0, 15, 60, 180 µg in 200 µL, 1×, followed by 16 nmol TPA, 2×/wk, 16 or 24 wk	15 µg, 25 wk; 60 and 180 µg, 17 wk	Skin P: 15 µg, 72% (3.97 P/mouse) vs 0% in TPA control; 60 µg, 79% (4.72 P/mouse); 180 µg, 72% (5.52 P/mouse) vs 10% (0.10 P/mouse) in TPA control	+	No histology	Chang *et al.* (1982)
Mouse, SENCAR	F	24, 23 controls	>99% (dioxane: DMSO (75:25))	0, 240 µg [800 nmol] in 100 µL, 1×, followed by 4.26 nmol TPA, 2×/wk, 25 wk	26 wk	18/24 (75%) skin P vs 2/23 (9%) TPA control	+		Cavalieri *et al.* (1989)
Dibenzo[*a*,*i*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	NS	30	Recrystallized (dioxane)	0, 25 µg in 25 µL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone (frequency, duration NS)	6 mo	12/30 (40%; skin P) vs 2/30 (7%; skin P) croton oil controls	+		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, 20Ha/ICR Swiss	F	20	95% (acetone)	0, 10, 50 µg in 100 µL (total dose, 100, 500 µg), 10×/20 days, followed 10 days later by 2.5 µg TPA in 100 µL acetone 3×/wk, 20 wk	NS	Skin T: 100 µg, 40% (0.5 T/mouse, type NS); 500 µg, 85% (5.8 T/mouse, type NS) vs 0% solvent controls	+		Hecht *et al.* (1981)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	30	'Essentially pure' (10% DMSO in THF)	0, 15, 60, 180 µg in 200 µL, 1×, followed 1 wk later by 10 µg TPA in 200 µL, 2×/wk, 16 wk (all dose groups) and 24 wk (15 µg group)	NS	Skin P: 15 µg (16 wk), 28% (0.52 P/mouse) vs 0% in TPA controls; 60 µg, 67% (5.33 P/mouse); 180 µg, 79% (5.25 P/mouse) vs 10% (0.10 P/mouse) in TPA controls; 15 µg (24 wk), 69% (2.07 ± 0.44 (mean ± SE) P/mouse) vs 0% in TPA controls	+	No histology	Chang *et al*. (1982)
Mouse, SENCAR	F	24, 23 controls	>99% (dioxane: DMSO (75:25))	0, 242 µg (800 nmol) in 100 µL, 1×, followed 1 wk later by 2.6 µg TPA in 100 µL acetone, 2×/wk, 25 wk	26 wk	15/24 (62%; skin P) vs 2/23 (9%) TPA controls	+		Cavalieri *et al*. (1989)
Dibenzo[*a*,*l*]pyrene									
Mouse, SENCAR	F	24, 23 controls	>99% (dioxane: DMSO (75:25))	0, 242 µg (800 nmol) in 100 µL, 1×, followed 3 wk later by 2.6 µg TPA in 100 µL acetone, 2×/wk, 22 wk	26 wk	22/24 (92%; skin P) vs 2/23 (9%) TPA controls	+		Cavalieri *et al*. (1989)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24	>99% (acetone)	0, 10.1, 30.2, 90.7 μg in 100 μL, 1×, followed 1 wk later by 2.0 μg TPA in 100 μL acetone; promotion suspended after first treatment and resumed by 4 wk, 2×/wk, 12 wk	15 wk	Skin P: 10.1 μg, 23/24 (96%; 6.75 T/mouse); 30.2 μg, 22/24 (92%; 7.92 T/mouse); 90.7 μg, 24/24 (100%; 8.50 T/mouse) vs 0/24 TPA controls	+		Cavalieri *et al*. (1991)
Mouse, SENCAR	F	24	>99% (acetone)	1.2, 6.0, 30.2 μg in 100 μL, 1×, followed 2 wk later by 2.0 μg TPA in 100 μL acetone, 2×/wk, 24 wk	27 wk	Skin P: 1.2 μg, 22/24 (92%; 6.96 T/mouse); 6.0 μg, 20/24 (83%; 5.29 T/mouse); 30.2 μg, 20/24 (83%; 3.29 T/mouse) vs 0/24 TPA controls	+		Cavalieri *et al*. (1991)
Mouse, SENCAR	F	24	>99% (acetone)	75.5, 302 ng in 100 μL, 1×, followed 1 wk later by 1.3 μg TPA in 100 μL acetone, 2×/wk, 27 wk	27 wk	Skin P: 75.5 ng, ~30%; 302 ng, ~80% vs 0% in TPA controls	+		Higginbotham *et al*. (1993)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	20	NS (acetone)	0.3, 1.2, 3.0, 7.6 μg (total doses); 10 subdoses in 100 μL, every other day, followed 10 days later by 2.5 μg TPA in 100 μL, 3×/wk, 20 wk	>22 wk	Unspecified skin T: 0.3 μg, 18/19 (95%; 5.0 T/mouse); 1.2 μg, 20/20 (100%; 17.8 T/mouse); 3.0 μg, 18/20 (90%; 11.3 T/mouse); 7.6 μg, 20/20 (100%; 15.0 T/mouse) vs 3/20 (15%; 0.15 T/mouse) solvent controls (p <0.001 for all dose groups)	+	No histology	LaVoie *et al*. (1993)
Mouse, SENCAR	F	23–25	Pure (acetone)	0.4, 1.2, 3.6 μg in 100 μL, 1×, followed 1 wk later by 1 μg TPA in 100 μL, 2×/wk, 28 wk	29–30 wk	Skin T: 0.4 μg, 16/23 (70%; 5.22 T/mouse; 2 SCC in 2 mice); 1.2 μg, 19/23 (83%; 7.09 T/mouse); 3.6 μg, 23/25 (92%; 9.28 T/mouse; 7 SCC in 5 mice)	+	No control	Gill *et al*. (1994)
Mouse, NMRI	F	16	>99.7% (acetone)	0, 12 μg in 100 μL, 1×, followed 1 wk later by TPA (6.2 μg in 100 μL acetone, 2 ×/wk, 30 wk)	31 wk	15/16 (94%; skin T; 6.5 T/mouse) vs 0/16 acetone controls	+	No histology	Luch *et al*. (1999)
Mouse, SENCAR	F	35, 10 controls	NS (toluene)	0, 0.6 μg in 200 μL, 1×, followed 2 wk later by 1 μg TPA in 200 μL acetone, 2×/wk, 25 wk	26 wk	30/30 (100%; skin P; 7.97 T/mouse) vs 1/9 (11%; 0.25 T/mouse) TPA controls	+		Marston *et al*. (2001)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*e*,*l*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	NS	30	Recrystallized (dioxane)	0, 25 µg in 25 µL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone (frequency, duration NS)	6 mo	0/30 skin P vs 2/30 (7%) promoter controls	–		Hoffmann & Wynder (1966)
1,4-Dimethylphenanthrene									
Mouse, Swiss albino (Ha/ICR)	F	20	>99.5% (acetone)	0, 100 µg in 100 µL, 10×, every other day, followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 20 wk	24 wk	95% skin P (8.2 T/ mouse) vs 0% in TPA controls	+	No histology; no statistics	LaVoie *et al*. (1981)
Mouse, outbred albino Crl:CD-1 (ICR) BR	F	20	>99.5% (acetone)	0, 30, 100 µg in 100 µL, 10×, every other day, followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 20 wk	24 wk	30 µg, 80% skin T (3.25 T/mouse); 100 µg, 100% (5.30 T/mouse) vs 0% in TPA control; (p <0.01)	+		LaVoie *et al*. (1982b)
Indeno[1,2,3-*cd*]pyrene									
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	25	NS (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 90% (2.83 T/mouse) vs 5% in controls	+		Rice *et al.* (1986)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	25	>99% (acetone)	0, 4.0 µmol [1105 µg], 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 72% (2.1 T/mouse) (p <0.005)	+		Rice *et al.* (1990)
1-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 6/19 (32%; 0.32 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
2-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 8/19 (42%; 0.68 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
3-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1, 0.3 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T (T/mouse): 0/20, 3/17 (18%; 0.18), 4/16 (25%; 0.50)	+		Hecht *et al.* (1974)
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 14/20 (70%; 1.3 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
4-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 7/20 (35%; 0.45 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
5-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0.1, 0.3 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 20/20 (100%; 5.5 T/mouse), 20/20 (100%; 8.0 T/mouse)	+		Hecht *et al.* (1974)
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 17/18 (94%; 5.3 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
Mouse, CD-1	F	20	>99.9% (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P (T/mouse): 5% (0.05), 65% (1.7; p <0.01)	+		Amin *et al.* (1985c)
Mouse, CD-1	F	20	100% (NS)	0, 33, 100 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 25 wk	26 wk	Skin P (T/mouse): 10% (0.1), 80% (3.9), 90% (5.2)	+		Hecht *et al.* (1985)
Mouse, CD-1	F	20	>99.9% (acetone)	0, 100 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 25 wk	25 wk	Skin P (T/mouse): 10% (0.1), 90% (5.2)	+		El-Bayoumy *et al.* (1986)
Mouse, CD-1	F	20	>99% (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P: 0%, 84% (4.8 T/mouse)	+		Hecht *et al.* (1987)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	20	>99% (acetone)	0, 0.15, 0.5, 1.5 µmol, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P (T/mouse): 20% (0.3), 100% (9.2; p <0.005), 100% (10.68; p <0.005), 100% (9.42; p <0.005)	+		Rice *et al.* (1988)
Mouse, CD-1	F	22	NS (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P (T/mouse): 10% (0.6), 85% (4.3; p <0.05)	+		Amin *et al.* (1990)
Mouse, CD-1	F	20	NS (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 25 wk	25 wk	Skin P (T/mouse): 10% (0.01), 65% (2.80; p <0.01)	+		Amin *et al.* (1992)
6-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 7/19 (37%; 1.5 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al*. (1979)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
2-Methylfluoranthene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Pure (acetone)	0, 1.0 mg (2 studies), 10 subdoses in 50 µL, 1× every other day, followed by 2.5% croton oil, 3×/wk, 20 wk	4 mo	**Skin T (T/mouse)**: Study 1: 1/30 (3%; 0.03), 9/30 (30%; 0.47) Study 2: 1/30 (3%; 0.03), 10/29 (34%; 0.55; p <0.01)	+		Hoffman *et al.* (1972); LaVoie *et al.* (1979)
3-Methylfluoranthene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Pure (acetone)	0, 1.0 mg (2 studies), 10 subdoses of 50 µL, 1× every other day, followed by 2.5% croton oil, 3×/wk, 20 wk	4 mo	Skin T (T/mouse): 1/30 (3%; 0.03), 9/28 (32%; 0.5; p <0.01)	+		Hoffman *et al.* (1972); LaVoie *et al.* (1979)
1-Methylphenanthrene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.5% (acetone)	0, 100 µg in 100 µL, 10× every other day, followed by 2.5 µg TPA in 100 µL, 3×/wk, 20 wk)	24 wk	0% skin P vs 0% in TPA control	–	No histology	LaVoie *et al.* (1981)
Naphtho[1,2-*b*]fluoranthene									
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 µmol, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 10% (0.1), 65% (2.5), 100% (6.6; p <0.001)	+		Weyand *et al.* (1990)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Naphtho[2,1-*a*]fluoranthene									
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 µmol, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 10% (0.1), 90% (5.9), 100% (7.3; p <0.001)	+		Weyand *et al.* (1990)
Naphtho[2,3-*e*]pyrene									
Mouse, Swiss albino Ha/ICR	F	30	NS (dioxane)	0, 250 µg, 10 subdoses, 1× every other day followed by 2.5% croton oil, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 7.6% (0.1), 33% (0.5; p <0.01)	+		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Perylene									
Mouse, Swiss albino Ha/ICR	F	20	'Rigorously purified' (benzene)	0, 800 µg in 200 µL, 1×, alone or followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 56–58 wk	58–60 wk	0/20 skin P vs 0/20 solvent controls; 3/20 (15%) skin P vs 1/20 TPA controls	±	No statistics	Van Duuren *et al.* (1970)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Crl:CD-1 (ICR)Br	F	20	>99% (acetone)	0, 100 µg in 100 µL, 10× every other day followed by 2.5 µg TPA in 100 µL, 3×/wk, 25 wk	29 wk	5% skin T vs 5% in TPA controls	–		El-Bayoumy *et al.* (1982)
Phenanthrene									
Mouse, 'S'	NS	20	NS (acetone)	0, 54 mg, 10×, 3×/wk, followed by (0.17%) croton oil, 300 µL, 1×/wk, 16 wk; 300 µL (0.085%) 1×/wk, 2 wk	24 wk	5/20 (25%) skin P (12 T) vs 4/19 (21%) skin P (4 T) croton oil controls	±		Salaman & Roe (1956)
Mouse, 'stock albino'	M, F	10	'High purity' (acetone)	0, 300 µg on days 0, 2, 6, 8, followed by 250 µl (0.1%) croton oil, 1×/wk, 20 wk	24 wk	4/19 (21%) skin P vs 2/20 (10%) skin P croton oil controls	±		Roe (1962)
Mouse, CD-1	F	30	Purified (acetone)	0, 1.78 mg, 1×, followed by 5 µmol TPA, 2×/wk, 34 wk	35 wk	12/30 (40%) skin P vs 1/30 (3%) skin P TPA controls	+	No histology; no statistics	Scribner (1973)
Mouse, CD-1	F	30	>98% (acetone: ammonium hydroxide (1000:1))	0, 1.78 mg, 1×, followed by 16 nmol TPA, 2×/wk, 35 wk	36 wk	4/30 (13%) skin P vs 2/30 (7%) skin P TPA controls	±	No histology	Wood *et al.* (1979)
Mouse, Swiss albino Ha/ICR	F	20	>99.5% (acetone)	0, 100 µg in 100 µL, 10× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	0/20 skin P vs 0/20 skin P TPA controls	–	No histology	LaVoie *et al.* (1981)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Picene									
Mouse, CD-1	F	30	Purified (benzene)	0, 2.8 mg (volume NS), 1×, followed 1 wk later by 10 μmol TPA, 2×/wk, 34 wk	35 wk	8/30 (27%) skin P vs 0/30 TPA controls	+	No histology; the TPA dose was probably 10 μg; no statistics	Scribner (1973)
Mouse, NMRI	F	16 or 30	>99% (acetone, THF, or benzene)	83.5 μg in 100 μL acetone, 167 μg in 100 μL tetrahydrofuran, 2800 μg in 100 μL benzene, 1×, followed by 6.2 μg TPA in 100 μL, 2×/wk, 23 wk	24 wk	83.5 μg in acetone, 0% skin T; 167 μg in THF, 0%; 2800 μg in benzene, 19% vs 0% in acetone controls	+	No histology; no statistics	Platt *et al*. (1990)

C, carcinoma; DMSO, dimethylsulfoxide; F, female; M, male; mo, month; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; SCC, squamous-cell carcinoma; SE, standard error; SGA, sebaceous gland adenoma; T, tumour; THF, tetrahydrofuran, TPA, 12-*O*-tetradecanoylphorbol-19-acetate; UV, ultraviolet; vs, versus; wk, week

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

Table 3.3. Carcinogenicity studies of subcutaneous administration of various PAHs in experimental animals

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthanthrene									
Mouse, XVII	M, F	7 M, 7 F	NS (olive oil)	600 μg in 200 μL, 1×/mo, 3 mo	NS	M: 0/7; F: 0/7	–	No control; small no. of animals; short duration of treatment; no statistics	Lacassagne *et al.* (1958)
Anthracene									
Mouse, C57BL	M, F	40–50	NS (tricaprylin)	5 mg 1×	22–28 mo	0/26 mice surviving >4 mo	–	No control	Steiner (1955)
Mouse, NMRI	M, F	40; 40 controls	99.9% (aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20))	71.3 μg in 50 μL (400 nmol), 1× on PND 2	40 wk	Pulmonary T: F, 1/12 (8%) vs 1/19 (5%) solvent control; M, 2/17 (12%) vs 1/14 (7%) solvent control	–	No statistics	Platt *et al.* (1990)
Rat, NS	NS	10	NS; aqueous suspension	1 mg in 2 mL, 1×/wk, 103 wk	103 wk	0/10 (only 2 survived 18 mo)	–	Small no. of animals; limited reporting; no control	Boyland & Burrows (1935)
Rat, Wistar	NS	5	NS (sesame oil)	5 mg, 1×/wk, 6–7 wk	10 mo	0/5 (only 4 survived 10 mo)	–	Small no. of animals; short duration; no control; no statistics	Pollia (1941)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, BDI and BDIII	NS	10	Pure (oil)	20 mg, 1×/wk, 33 wk	Lifetime	5/9 (55%) (1 myosarcoma, 4 fibroma, some of which had sarcomatous proliferations)		No control	Druckrey & Schmähl (1955); Schmähl (1955)
11*H*-Benz[*b*,*c*]aceanthrylene									
Mouse, C3H	M	15	NS (tricaprylin)	1 mg in 500 µL, 1×	>539 days	1/15 (7%) (3 T/TBA, pulmonary A, hepatic A, benign haemangioma of rump)	–		Dunlap & Warren (1946)
Benz[*a*]anthracene									
Mouse, A	M, F	10; 20 controls	NS (tricaprylin)	500 µg in 100 µL, 1×	14 wk	0/10 vs 1/20 control	–		Andervont & Shimkin (1940)
Mouse, C57BL	M, F	50	NS (tricaprylin)	5.0 mg in 500 µL, 1×	22 mo	S: 8/50 (16%; S) vs 3/304 (1%) tricaprylin controls	+		Steiner & Falk (1951)
Mouse, C57BL	M, F	16 (10 mg); 36–45 (≤5 mg)	Purified (tricaprylin)	0.05, 0.2, 1, 5, 10 mg in 500 µL, 1×	22–28 mo	S: 0.05 mg, 5/44 (2%); 0.2 mg, 11/45 (24%); 1 mg, 15/44 (34%); 5 mg, 20/36 (56%); 10 mg, 5/16 (31%)	+	No control	Steiner & Edgcomb (1952); Steiner (1955)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BALB/c	M, F	60	NS (1% aqueous gelatine)	50 µg in 20 µL, 1× on PND1, 2, 4 or 8	36–43 wk	Pulmonary A + AdC: PND1, 24/52 (46%); PND2, 11/39 (28%); PND4, 10/33 (30%); PND8, 10/41 (24%) vs 2/21 (10%) solvent controls	+		Roe *et al.* (1963)
Mouse, C3H	NS	20	NS (tricaprylin)	5 mg, 1×	638 days	0% vs 0% in solvent controls	–		Stevenson & von Haam (1965)
Mouse, C57BL	M, F	10	NS (arachis oil)	1 mg in 100 µL, 1×/wk, 10 wk	80 wk	S: M, 8/10 (80%); F, 6/10 (60%) vs 0/10 solvent controls	+		Boyland & Sims (1967)
Mouse, C57BL/6J	M	40–50	NS (tricaprylin)	500 µg in 100 µL, 1×; 500 µg in 100 µL, 1×, followed by transplantation of injection sites to secondary hosts 8–24 wk later	Up to 52 wk	FibroS: no transplant, 4.1%; transplant, 67% (8 wk), 80% (12 wk), 20% (16 wk), 44% (24 wk) vs 0% in transplanted vehicle controls	+		Homburger & Treger (1970)
Rat, albino	M	20	NS (tricapry lin)	2 mg in 250 µL, 1×	14.5 mo	0/20 vs 0/19 solvent controls	–		Miller & Miller (1963)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene									
Mouse, NMRI	F	60–90	NS (tricaprylin, 0.9% saline solution or lutrol 9 (polyethylene-oxide))	Experiment 1: 0, 25, 50, 100, 200, 400 µg/animal (in tricaprylin), 1×	420–500 days	T (mainly fibroS but also myofibroS, carcinoS, SCC, AdC and reticuloS) at injection site [incidence derived from dose–response curves] Experiment 1: [0/90] (0%), [24/90] (~ 27%), [49/90] (~54%), [51/90] (~57%), [66/90] (~73%), [56/90] (~62%)	+		Pott *et al*. (1973a)
				Experiment 2: 0, 25, 50, 100, 200 µg/animal (in tricaprylin), 1×	~420 days	Experiment 2: [1/80] (~1%), [40/80] (~50%), [50/80] (~62%), [60/80] (~74%), [61/80] (~76%)			
				Experiment 3: 0, 200, 400, 800 µg/animal (in 0.9% saline solution), 1×	~270 days	Experiment 3: [0/ 60] (0%), [2/60] (~3%), [18/60] (~30%), [26/60] (~44%)			
				Experiment 4: 100 µg/animal in lutrol or tricaprylin, 1×	~410 days	Experiment 4: [2/60] (~3%), [30/60] (~50%)			
				Experiment 5: 50 µg in 1 mL or 0.1 mL tricaprylin, 1×	~310 days	Experiment 5: [11/60] (~18%), [37/60] (~62%)			

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, NMRI	F	80	NS (tricaprylin, 0.9% saline solution, Tween 60 or Lutrol 9 (polyethylene oxide))	Experiment 1: 0, 25, 50, 100, 200 µg/animal in tricaprylin, 1×	19 mo	**Subcutaneous T at injection site** Experiment 1: 1/80 (1%), 44/80 (~55%), 52/80 (~65%), 60/80 (~75%), 66/80 (~83%)	+		Pott *et al.* (1973b)
		60		Experiment 2: 0, 100, 200, 400, 800 µg /animal in 0.9% saline/Tween 60 solution, 1×	17 mo	Experiment 2: 1/60 (2%), 1/60 (2%), 5/60 (~8%), 22/60 (~37%), 32/60 (~54%)			
		120		Experiment 3: 100 µg/animal in Lutrol 9, 1×	14 mo	Experiment 3: 2/97 (2%)			

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C3H/fCum	M	20 or 40	Pure (trioctanoin, DMSO)	Experiment 1: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or DMSO, 1×	15 mo	**FibroS at injection site** Experiment 1: 0/16, 0/20, 15/18 (83%), 12/19 (63%)	+		Kouri *et al.* (1980)
				Experiment 2: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or in DMSO, 1×	18 mo	Experiment 2: 0/20, 0/18, 14/18 (78%), 7/19 (37%)			
				Experiment 3: 0 (trioctanoin/ DMSO; 100:1), 0.9 µmol [0.23 mg] in trioctanoin/ DMSO (100:1), 1×	18 mo	Experiment 3: 0/20, 36/40 (90%)			
Mouse, NS, newborn	M, F	NS	NS saline (solution + 1% gelatine + 0.4% Tween 20)	0, 10, 100 µg/ animal, 1×	30 wk	Lung T: 5/38 (13%; 0.13 T/ animal), 5/31 (16%; 0.23 T/animal), 21/33 (64%; 2.52 T/animal)	+	Type of lung tumours NS; statistics NS	Rippe & Pott (1989)
Mouse, NS	F	NS	NS (tricaprylin)	0, 10,100 µg/ animal, 1×	78 wk	S at injection site: 1/30 (3%), 13/30 (43%), 20/30 (67%)	+		Rippe & Pott (1989)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, $AhR^{-/-}$, $AhR^{+/-}$, $AhR^{+/+}$	M	17, 16 controls	NS (olive oil)	2 mg/animal, on day 1, day 8	18 wk	$AhR^{-/-}$: 0/16 (0%); $AhR^{+/-}$: 17/17 (100%; 15 fibroS, 1 rhabdomyoS, 1 SCC; p <0.01); $AhR^{+/+}$: 17/17 (100%; 13 fibroS, 2 rhabdomyoS, 2 SCC; p <0.01)	+		Shimizu *et al.* (2000)
Rat, Wistar	F	50	NS (tricaprylin)	0, 33, 100, 300, 900, 2700 µg/ animal, 1×	~530 days	T (mainly fibroS) at injection site [incidence derived from dose–response curves]: 2/50 (~4%), 4/50 (~8%), 7/50 (~14%), 23/50 (~46%), 35/50 (~70%), 38/50 (~76%)	+		Pott *et al.* (1973a)
Rat, NS	F	NS	NS (tricaprylin)	0, 1 mg, 1×	132 wk	S at injection site: 0/24 (0%), 20/24 (83%)	+		Rippe & Pott (1989)
Rat, NS	F	NS	NS (DMSO)	0, 15 mg, 1×	132 wk	S at injection site: 1/24 (4%), 19/24 (79%)	+		Rippe & Pott (1989)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian, RB (randomly bred), BIO inbred strains designated as: 1.5, 4.22, 4.24, 7.88, 12.14, 15.16, 45.5, 54.7, 82.73, 86.93, 87.20	M	25 M, 25 F	NS (tricaprylin)	500 µg/animal, 1×	53 wk	**FibroS at injection site** RB: M, 4/25 (16%); F, 6/23 (26%) 1.5: M, 5/25 (20%); F, 4/23 (17%) 4.22: M, 3/25 (12%); F, 8/25 (32%) 4.24: M, not tested, F, 9/25 (36%) 7.88: M, 13/25 (52%); F, 5/23 (23%) 12.14: M, 3/25 (12%); F, 9/22 (41%) 15.16: M, 9/25 (36%); F, 16/25 (64%) 45.5: M, 12/25 (48%); F, 7/15 (47%) 54.7: M, 5/25 (20%); F, 5/25 (20%) 82.73: M, 4/21 (19%); F, 4/24 (17%) 86.93: M, 9/25 (36%); F, 8/25 (32%) 87.20: M, 16/25 (64%); F, 11/25 (42%)	+	No sub-cutaneous T observed in historical controls	Homburger *et al*. (1972)
Monkey, Old World	NS	17	NS (NS)	30–90 mg/kg; multiple doses [no. of injections and duration of treatment NS]	Under observation up to 18 years	No T found	–		Adamson & Sieber (1983)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*c*]anthracene									
Mouse, C57BL6/J	M, F	30, 10 controls	NS (trioctanoin)	150, 300 µg in 50 µL, 1×	12 mo	150 µg, 0/30; 300 µg, 1/30 (3%) (fibroS)	–	No incidence reported for the solvent control group; no statistics	Kouri *et al*. (1983)
Mouse, DBA2/J	M, F	30, 10 controls	NS (trioctanoin)	150, 300 µg in 50 µL, 1×	12 mo	150 µg, 0/30; 300 µg, 0/30	–	No incidence reported for the solvent control group; no statistics	Kouri *et al*. (1983)
Mouse, B6D2F$_1$	M, F	30, 10 controls	NS (trioctanoin)	150, 300 µg in 50 µL, 1×	12 mo	150 µg, 0/30; 300 µg, 1/30 (3%) (fibroS)	–	No incidence reported for the solvent control group; no statistics	Kouri *et al*. (1983)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Mouse, CH3	M	19–79	Melting-point (tricaprylin)	1.95, 7.8, 15.6, 31, 62, 125, 250, 500 µg, 1, 2, 4 mg in 250 µL, 8 mg in 500 µL, 1×	NS	S: 1.95 µg, 2/79 (3%); 7.8 µg, 6/40 (15%) 0; 15.6 µg, 6/19 (31%); 31 µg, 16/21 (76%); 62 µg, 20/20 (100%); 125 µg, 21/23 (91%); 250 µg, 19/21 (90%); 500 µg, 20/21 (96%); 1 mg, 22/22 (100%); 2 mg, 19/19 (100%); 4 mg, 17/20 (85%); 8 mg, 16/21 (76%)	+	No control	Bryan & Shimkin (1943)
Mouse, C57BL	M, F	50, 304 controls	NS (tricaprylin)	20 µg, 1×	≤22 mo	S: 28/48 (58%) vs 3/280 (1%) solvent-treated controls	+	No statistics	Steiner & Falk (1951)
Mouse, C57BL	M, F	40–50	NS (tricaprylin)	20, 40 µg in 500 µL, 1×	22–28 mo	S: 20 µg, 7/21 (33%); 40 µg, 6/18 (33%)	+	No control; no statistics	Steiner (1955)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, General Purpose/NTH	M, F	592, 79 controls	NS (olive oil)	0, 0.003, 0.01, 0.03, 0.08, 0.2, 0.7, 2.2, 6.7 µg in 50 µL, 1× as newborn	M, 54–55 wk; F, 78–79 wk	0.003 µg, 1/61 (2%; fibroS), 23/60 (38%; pulmonary T); 0.01 µg, 1/45 (2%; fibroS), 10/45 (22%; pulmonary T); 0.03 µg, 0/54 fibroS, 15/53 (28%; pulmonary T); 0.08 µg, 5/42 (12%; fibroS), 10/42 (24%; pulmonary T); 0.2 µg, 5/48 (10%; fibroS), 18/48 (37%; pulmonary T); 0.7 µg, 11/45 (24%; fibroS), 15/45 (33%; pulmonary T); 2.2 µg, 13/38 (34%; fibroS), 14/38 (37%; pulmonary T); 6.7 µg, 29/50 (58%; fibroS), 22/50 (44%; pulmonary T) vs 0/79 fibroS, 26/79 (33%; pulmonary T) solvent controls	+	No dose–response for pulmonary T; no statistics	O'Gara *et al.* (1965)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C57BL	M, F	20	NS (arachis oil)	0, 1 mg, 1×/wk, 10 wk	60–80 wk	S: M, 20/20 (100%); F, 17/19 (90%) vs 0/40 solvent controls	+	No statistics	Boyland & Sims (1967)
Mouse, NMRI	F	60	NS (tricaprylin)	10, 30, 90, 270, 810 µg, 1×	≤16 mo	Mainly fibroS: 10 µg, 24/60 (40%); 30 µg, 21/60 (35%); 90 µg, 39/60 (65%); 270 µg, 45/60 (75%); 810 µg, 54/60 (90%)	+	No control; no statistics	Pott *et al.* (1973a)
Mouse, NMRI	F	100	NS (tricaprylin)	0, 2.35, 4.7, 9.3, 18.7, 37.5, 75.0 µg in 500 µL, 1×	114 wk	S: 2.35 µg, 37/100; 4.7 µg, 39/100; 9.3 µg, 44/100; 18.7 µg, 56/100; 37.5 µg, 65/100; 75.0 µg, 69/100	+	Control data not reported; no statistics	Pfeiffer (1977)
Mouse, B6, D2, $B6D2F_1$	M	30 B6, 30 D2, 57 or 60 $B6D2F_1$; 10 controls of each	NS (tricaprylin)	0, 150, 300 µg in 50 µL, 1×	12 mo	FibroS: B6: 150 µg, 16/30 (53%); 300 µg, 14/30 (46%); D2: 150 µg, 1/30 (3%); 300 µg, 0/30; $B6D2F_1$: 150 µg, 8/57 (14%); 300 µg, 33/60 (55%)	+	No histology; control data not reported; no statistics	Kouri *et al.* (1983)
Mouse, C3H/HeJ, C57BL/6J, AKR/J, DBA/J2	M	30, 10 controls	NS (trioctanoin)	0, 150 µg in 50 µL, 1×	9 mo	FibroS: C3H/HeJ, 24/30 (80%) vs 0/10 controls; C57BL/6J, 16/30 (53%) vs 0/10 controls; AKR/J, 0/30 vs 0/10 controls; DBA/J2, 1/30 (3%) vs 0/10 controls	+	No statistics	Lubet *et al.* (1983)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mice, NMRI	F	50	>99% (tricaprylin)	0, 10, 11, 30, 86, 112 µg in 500 µL, 1×	112 wk	FibroS: 10 µg, 25/48 (52%); 11 µg, 16/47 (34%); 30 µg, 25/50 (50%); 86 µg, 31/49 (63%); 112 µg, 38/48 (79%) vs 3/49 (6%) and 1/50 (2%) solvent controls	+	No histology; no statistics	Platt *et al.* (1990)
Mouse, NMRI		40, 44 and 49 controls	>99% (aqueous solution [1% gelatine, 0.9% saline, 0.4% Tween 20])	0, 11, 111 µg in 50 µL, 1× on PND 2	40 wk	Pulmonary T: F: 11 µg, 6/13 (46%; 3.3 T/mouse); 111 µg, 16/17 (94%; 30.6 T/mouse) vs 1/19 (5%; 1.0 T/mouse) solvent controls; M: 11 µg, 6/22 (27%; 3.8 T/mouse); 111 µg, 19/21 (90%; 27.3 T/mouse) vs 1/14 (7%; 2.0 T/mouse) solvent control	+	No statistics	Platt *et al.* (1990)
Rat, NS	NS	2 series of 10, 20 controls	NS (lard)	2 mg in 1 mL, 1×/wk, then 6 mg in 3 mL at unspecified intervals	161–217 days	S: 1/10 (10%), 7/10 (70%) vs 0/20 solvent controls		Limited survival and reporting	Barry & Cook (1934)
Rat, albino	NS	40	Melting-point (lard)	8 mg, 1×/mo; 4×	1 year	11/18 (61%) (T)	+	No control or histology	Shear (1936)
Rat, Wistar	NS	5	NS (sesame oil)	5 mg in 500 µL, 1×/wk, 4, 5 or 8×	10 mo	2/5		Small numbers; no histology	Pollia (1941)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, NS	M, F	10	NS (olive oil)	0.1, 1 mg in 1 mL, 1×	0.1 mg, 21 mo; 1 mg, 15 mo	S: 0.1 mg, 3/9 (33%); 1 mg, 6/10 (60%)	+	No control	Roussy & Guérin (1942)
Rat, Sprague-Dawley	F	12	Pure (sesame oil:DMSO (9:1))	300 µg in 100 µL, 3×/wk, 20×	37 wk	S: 12/12 (100%) vs 0/12 solvent controls	+	No statistics	Flesher *et al.* (2002)
Dibenz[*a,j*]anthracene									
Mouse, Swiss	F	25	>99% (olive oil)	400 µg in 200 µL, 1×	Life	S: 3/21 (14%); vs 0/16	±	No statistics	Lijinsky *et al*. (1970)
Dibenzo[*h,rst*]pentaphene									
Mouse XVII/nc/Z	M, F	8 M, 19 F	NS (olive oil)	600 µg in 200 µL, 1×/mo, 3 mo	320 days	S: M, 3/8 (37%; S); F, 6/19 (32%)	+	No control; no statistics	Lacassagne *et al*. (1964)
Dibenzo[*a,e*]pyrene									
Mouse, XVII	M, F	12 or 21 M, 15 or 14 F	NS (olive oil)	600 µg in 200 µL, 1×; or 1×/mo, 3 mo	NS	600 µg: M, 10/12 (83%; S); F, 10/15 (67%; S); 1.8 mg: M, 18/21 (86%; S); F, 14/14 (100%)	+	No control; no statistics	Lacassagne *et al*. (1963)
Dibenzo[*a,h*]pyrene									
Mouse, white	NS	20	NS (olive oil)	6 mg, 1×	9.5 mo	17/20 (85%) papillomas at 3.5 mo; none survived 9.5 mo		No control; no statistics	Kleinenberg (1938)
Mouse, XVII	M, F	35 M, 10 F	NS (olive oil)	600 µg in 200 µL; 1×/mo, 3 mo	NS	M, 34/35 (97%; S); F, 1/10 (10%; S)		No control; no statistics	Lacassagne *et al.* (1958)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*i*]pyrene									
Mouse, XVII	NS	11	NS (peanut oil)	600 µg (volume NS), 1×/mo, 3 mo	Up to 135 days	11/11 (100%; S); none survived 135 days	+	No control; low survival; no statistics	Lacassagne *et al*. (1957)
Mouse, XVII	M, F	17 M, 18 F	NS (olive oil)	600 µg in 200 µL, 1×/mo, 3 mo	NS	M, 17/17 (100%; S); F, 16/18 (89%; S)	+	No control; limited duration; no statistics	Lacassagne *et al*. (1958)
Mouse, hybrid XVII × C57BL	M, F	8 M, 8 F	NS (propylene glycol)	2 mg in 200 µL, 1×	NS	M, 8/8 (100%; S); F, 8/8 (100%; S)	+	No control; limited duration; no statistics	Waravdekar & Ranadive (1958)
Mouse, albino	M	12	Recrystallized (paraffin oil)	2 mg in 100 µL, 4 mg in 200 µL, 1×	Up to 4.5 mo	2 mg, 6/6 (100%; S); 4 mg, 6/6 (100%; S)	+	No control; limited duration; no statistics	Schoental (1959)
Mouse, C57Br/cd	M	10–12	NS (peanut oil, tricaprylin or tricaprylin/ cholesterol (% NS)	0.01–600 µg (volume NS), 1×	66 wk	<1 µg, 0%; 1 µg, 9%; 2 µg, 33%; 6.25 µg, 50%; 12.5 µg, 64%; 25 µg, 92%; ≥50 µg, 100%	+	No control; T were fibroS; leiyomioS; no statistics	Homburger & Tregier (1960)
Mouse, C57Br/cd	M	8850	NS (peanut oil)	500 µg (volume NS), 1×	>22 wk	'Nearly' 100% (fibroS or leiyomioS)	+	No control; no statistics	Homburger & Tregier (1960)
Mouse, C57BL/6	M	438	NS (peanut oil)	500 µg (volume NS), 1×	>22 wk	100% fibroS	+	No control; no statistics	Homburger *et al*. (1962)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BALB/c	M, F	8 M, 22 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 8/8 (100%; fibroS); F, 22/22 (100%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, C3H/An	M, F	9 M, 22 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 9/9 (100%; fibroS); F, 20/22 (91%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, I strain	M, F	10 M, 20 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 5/10 (50%; fibroS); F, 17/20 (85%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, (BALB/c × C3H)F_1	M, F	10 M, 25 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 10/10 (100%; fibroS); F, 25/25 (100%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, (C3H × I)F_1	M, F	9 M, 18 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 6/9 (67%; fibroS); F, 15/18 (83%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, Swiss	M, F	8 M, 5 F	NS (tricaprylin)	2 mg in 200 µL, 1×	8 mo	M, 8/8 (100%; fibroS); F, 5/5 (100%; fibroS)	+	No control; no statistics	Pai & Ranadive (1965)
Mouse, XVII × C57BL	M, F	8 M, 8 F,	NS (tricaprylin)	2 mg in 200 µL, 1×	10 mo	M, 8/8 (100%; fibroS); F, 8/8 (100%; fibroS)	+	No control; no statistics	Pai & Ranadive (1965)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss ICR/Ha	F	138	NS (tricaprylin)	100 mg in 200 µL, 1×	38 wk	125/138 (91%; fibroS); 2/138 (1%; local C); 1/138 (1%; lymphoma); 6/138 (4%; solitary pulmonary A); 2/138 (1%; multiple pulmonary A)	+	No control; the dose was probably 100 µg; no statistics	Epstein *et al*. (1967)
Mouse, C57BL/6	M	20–80	NS (peanut oil)	500 µg in 100 µL, 1×, followed by transplantation of injection sites to secondary hosts 1–8 wk later	26 wk	No transplant: M, 95% (fibroS); F, 90% (fibroS); with transplant: decreased latency times for the same incidences in both M and F	+	No control; no statistics	Homburger & Treger (1967)
Mouse, C57BL/6J	M	40	NS (tricaprylin)	25 µg (volume NS), 1×, followed by transplantation of injection sites to secondary hosts 7 wk later	30 wk	No transplant, 90% fibroS; with transplant, 90% fibroS at 18 wk	+	No control; no statistics	Homburger & Baker (1969)
Mouse, C57BL/6J	M	40–50	NS (tricaprylin)	25 µg in 100 µL, 1×; 25 µg in 100 µL, 1×, followed by transplantation of injection sites to secondary hosts 6 wk later	34 wk	No transplant: 50% fibroS at 19.5 wk; 92% fibroS at 34 wk; with transplant: 50% fibroS at 14.5 wk; 100% fibroS at 31 wk vs 0% in transplanted vehicle controls	+	No statistics	Homburger & Treger (1970)
Mouse, NS	NS	50, 25 controls	NS (tricaprylin)	100 µg (volume NS), 1×	75 wk	40/50 (80%; local S) vs 0/25 solvent controls	+	No histology; no statistics	Sardella *et al*. (1981)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian	M	6–10	NS (tricaprylin)	250–2000 µg in 400 µL, 1×	>17 wk	250 µg, 5/9 (56%; fibroS); 500 µg, 9/10 (90%; fibroS); 1000 µg, 10/10 (100%; fibro S); 2000 µg, 6/6 (100%; fibroS) vs 0 solvent controls	+	Limited reporting; no statistics	Wodinsky *et al.* (1964)
Hamster, Syrian	F	8–20	NS (tricaprylin)	250–2000 µg in 200 µL, 2500 µg in 400 µL, 1×	40 wk	250 µg, 85% fibroS; 500 µg, 87% fibroS; 1000 µg; 100% fibroS; 1500 µg, 100% fibroS; 2000 µg, 100% fibroS; 2500 µg, 95% fibroS	+	No control; no statistics	Wodinsky *et al.* (1965)
Rabbit, NS	NS	3	Recrystallized [arachis or paraffin oil]	100–300 µg in 100–300 µL, 5× [periodicity NS], 3 mo	7 mo	1/3 (33%) C (developed after biopsy)	±	No control; limited reporting; small number; no statistics	Schoental (1959)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Mouse, XVII nc/ZE	M, F	19 M, 17 F, 500 controls	NS (olive oil)	600 µg in 200 µL, 1×/mo, 2×; 1 additional 600 µg injection 2 mo later [only to mice without strong fibrous reaction at injection site; sex, no. NS]	M, 195 days; F, 217 days	M, 12/12 (100%; S; av. latency, 130 days); F, 12/12 (100%; S av. latency, 113 days) vs 'inactive' solvent	+	No histology; no statistics	Lacassagne *et al*. (1968)
1,2-Dihydroacenanthrylene									
Mouse, NS	F	9	Crystalline (NS)	5 mg (volume NS), 1×	20 mo	0/9	–	No control; limited duration; limited reporting; no statistics	Shear (1938)
Phenanthrene									
Mouse, C57BL	M, F	40–50	NS (tricaprylin)	5 mg, 1×	22–28 mo	0/27 surviving >4 mo	–	No control	Steiner (1955)
Mouse, 'stock albino'	M, F	10	NS (3% aqueous gelatine)	0, 300 µg on days 0, 2, 6, 8, followed by 250 µL croton oil, 1×/wk, 20 wk	24 wk	3/17 (18%) skin P vs 2/20 (10%) skin P croton oil control	–		Roe (1962)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, 'stock albino'	M, F	57, 45 controls	'Highest purity' (1% aqueous gelatine)	0, 40 µg in 20 µL, 1× neonatal treatment	62 wk	3/49 (6%) lung A vs 8/34 (24%) lung A solvent control	–	No histology	Grant & Roe (1963); Roe & Waters (1967)
Picene									
Mouse, NMRI	F	50	>99% (tricaprylin)	10, 11, 30, 86, 111 µg in 500 µL, 1×	112 wk	10 µg, 9/50 (18%) fibroS; 11 µg, 12/46 (26%); 30 µg, 17/49 (35%); 86 µg, 31/49 (63%); 111 µg, 29/50 (58%) vs 3/49 (6%), 1/50 (2%) solvent control	+	No histology; no statistics	Platt *et al*. (1990)
Mouse, NMRI	M, F	45 M, 50 F, 49 controls	>99% (aqueous solution (1% gelatine, 0.9% saline, 0.4% Tween 20))	0, 11, 111 µg [40, 400 nmol] in 50 µL, 1× on PND 2	40 wk	**Pulmonary T (T/mouse)**: F: 11 µg, 4/16 (25%; 2.8); 111 µg, 8/23 (35%; 2.1) vs 1/19 (5%; 1.0) solvent controls; M: 11 µg, 1/22 (5%; 1.0); 111 µg, 2/13 (15%; 1.5) vs 2/14 (14%; 2.0) solvent controls	+	No statistics	Platt *et al*. (1990)
Rat, Sprague-Dawley	F	12	Pure (sesame oil:DMSO (9:1))	0, 300 µg in 100 µL, 3×/wk, 20 ×	37 wk	No skin T found	–		Flesher *et al*. (2002)

A, adenoma; AdC, adenocarcinoma; av., average; C, carcinoma; DMSO, dimethylsulfoxide; F, female; M, male; mo, months; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; TBA, tumour-bearing animal; vs, versus; wk, week

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

Table 3.4. Carcinogenicity studies of intrapulmonary administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthanthrene									
Rat, Osborne-Mendel	F	35	99.4% (beeswax:tricaprylin (1:1))	160 of 830 µg in 50 µL, 1×	Life; av. 88 wk for high-dose group, 118 wk for untreated group	SCC: 1/35 (3%) low-dose, 19/35 (54%) high-dose vs 0/35 untreated, 0/35 vehicle controls	+		Deutsch-Wenzel *et al.* (1983)
Anthracene									
Rat, Osborne-Mendel	F	60, 108 controls	'Recrystallized' (beeswax:tricaprylin (1:1))	500 µg in 50 µL, 1×	>2 years	0/60 (approx. 50% killed after 1 year) vs 0/108 solvent control	–	No statistics	Stanton *et al.* (1972)
Benzo[*b*]fluoranthene									
Rat, Osborne-Mendel	F	35	>99.5% (beeswax/trioctanoin)	0, 0.1, 0.3, 1.0 mg, 1×	Lifetime	Lung SCC/S: 0%, 2.9%, 8.6%, 37.1%	+		Deutsch-Wenzel *et al.* (1983)
Benzo[*j*]fluoranthene									
Rat, Osborne-Mendel	F	35	>99.9% (beeswax/trioctanoin)	0, 0.2, 1.0, 5.0 mg, 1×	Lifetime	Lung SCC: 0%, 2.9%, 8.6%, 51.4%	+		Deutsch-Wenzel *et al.* (1983)
Benzo[*k*]fluoranthene									
Rat, Osborne-Mendel	F	35	>99.5% (beeswax/trioctanoin)	0, 0.16, 0.83, 4.15 mg	Lifetime	Lung SCC: 0%, 0%, 9.7%, 44.4%	+		Deutsch-Wenzel *et al.* (1983)

Table 3.4 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*ghi*]perylene									
Rat, Osborne-Mendel	F	35	>98.5% (beeswax/trioctanoin)	0, 0.16, 0.83, 4.15 mg	Lifetime	Lung SCC/S: 0%, 0%, 2.9%, 11.8%	+		Deutsch-Wenzel *et al.* (1983)
Benzo[*a*]pyrene									
Rat, OM	F	35	99.1% (1:1 mixture of beeswax and trioctanoin)	0 (untreated), 0 (vehicle control), 0.1, 0.3, 1.0 mg/animal, 1×	64 (high-dose group)–133 wk (untreated controls)	Lung T: [0/35] (0%), [0/35] (0%), [10/35] (28.6%) (4 epidermoid C; 6 pleomorphic S), [23/35] (65.7%) (21 epidermoid C; 2 pleomorphic S), [33/35] (94.3%) (33 epidermoid C)	+		Deutsch-Wenzel *et al.* (1983)
Rats, F344/NSlc	M	NS	NS (beeswax/tricaprylin mixture (1:1))	0, 0.03, 0.1, 0.3, 1.0 mg/animal, 1×	104 wk	Lung T:0/40, 1/29 (3%; 1 undifferentiated T), 7/30 (23%; 6 SCC, 1 undifferentiated T), 22/29 (76%; 20 SCC, 2 undifferentiated T), 9/13 (69%; 9 SCC)	+		Iwagawa *et al.* (1989)

Table 3.4 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Osborne-Mendel	F	35	99.6% (beeswax/tri-octanoin mixture of varying composition)	0 (untreated), 0 (vehicle control), 30, 100, 300 µg/animal, 1×	134 wk (low-dose group)–140 wk (vehicle controls)	Lung T: [0/35] (0%), [0/35] (0%), [3/35] (8.6%; 3 SCC), [11/35] (31.4%; 11 SCC), [77.1%; 27/35] (27 SCC).	+	SCC predominantly keratinized	Wenzel-Hartung *et al.* (1990)
Rat, F344/DuCrj	M	9–10	NS (beeswax/trica-prylin mixture (1:1))	0, 50, 100, 200 µg/animal, 1×	100 wk	Lung T: 0/19, 0/10, 3/10 (30%; 2 SCC, 1 AdSC), 4/9 (44.4%; 3 SCC, 1 undifferen-tiated T)	+		Horikawa *et al.* (1991)
Benzo[*e*]pyrene									
Rat, Osborne-Mendel	F	35	>99.7% (beeswax/trioctanoin)	0, 0.2, 1.0, 5.0 mg	Lifetime	Lung SCCS: 0%, 0%, 3.3%, 2.9%	±		Deutsch-Wenzel *et al.* (1983)
Chrysene									
Rat, Osborne-Mendel	F	35	>99.6% (beeswax/trioctanoin)	0, 1, 3 mg	Lifetime	Lung C: 0/35 (0%), 5/35 (14.3%), 10/35 (28.6%)	+		Wenzel-Hartung *et al.* (1990)
Dibenz[*a,h*]anthracene									
Mice, Street	M, F	80	NS (paraffin)	20 µg in 10 µL, 1×	<27 mo	4/16 (25%) pulmonary T vs 1/41 (2%) controls	+	Controls not solvent-treated; no statistics	Rask-Nielsen (1950)

Table 3.4 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Osborne/Mendel	F	35	99.3% (beeswax, tricaprylin)	100 μg, 1×	123 wk	20/35 (57%) vs 0/35 solvent control	+	No statistics	Wenzel-Hartung *et al.* (1990)
Indeno[1,2,3-*cd*]pyrene									
Rat, Osborne-Mendel	F	35	>99.4% (beeswax/trioctanoin)	0, 0.16, 0.83, 4.15 mg	Lifetime	Lung SCC S: 0%, 11.4% (4/35), 22.9% (8/35), 60.0% (21/35)	+		Deutsch-Wenzel *et al.* (1983)
Phenanthrene									
Rat, Osborne/Mendel	F	35	99.9% (beeswax:tricaprylin)	0, 1, 3, 10 mg, 1×	135 wk	1 mg, 0/35 lung C; 3 mg, 0/35; 10 mg, 1/35(3%) vs 0/35 solvent control	–	No statistics	Wenzel-Hartung *et al.* (1990)

AdSC, adenosquamous carcinoma; C, carcinoma; F, female; M, male; mo, month; NS, not specified; P, papilloma; PAH, polycylic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; vs, versus; wk, week

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

Intramamillary administration (see also Table 3.5)

Rat

A group of 20 female Sprague-Dawley rats [weight unspecified], 8 weeks of age, received a single intramamillary injection of 4 μmol [1.1 mg]/gland anthanthrene (purity >99% by HPLC; total dose, 32 μmol [8.8 mg]) dissolved in 100 μL trioctanoin. One control group of 21 rats was treated with 100 μL solvent and another control group of 20 rats remained untreated. The animals were monitored weekly by palpation for tumour development and were killed when tumours were ≥2 cm in diameter; all the remaining animals were killed and necropsied at 40 weeks. Survival rates (mean ± standard deviation (SD)) were 38 ± 0, 37 ± 4 and 40 ± 0 weeks in the anthanthrene-treated, untreated and vehicle control groups, respectively. Tumour latencies were 36 ± 0 and 25 ± 13 weeks in the anthanthrene-treated and untreated groups, respectively. At the end of the study, 1/20 rats in the anthanthrene-treated group and 2/20 rats in the untreated group had developed mammary epithelial tumours (one adenofibroma in the anthanthrene-treated rat; one adenofibroma and one adenocarcinoma in the untreated rats). No tumours were observed in the vehicle control group (Cavalieri *et al.*, 1989).

Anthracene

Previous evaluation

Anthracene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which anthracene was fed to rats, administered dermally to mice, administered by subcutaneous, intraperitoneal or pulmonary injection to rats and implanted into the brain or eyes of rabbits. These studies are summarized in Tables 3.1–3.4 and 3.6–3.8. On the basis of the available data, the Working Group concluded that there was *inadequate evidence* that anthracene was carcinogenic to experimental animals (IARC, 1987). Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

A group of 20 male C3H/HeJ mice, 6–8 weeks of age, was treated twice weekly with dermal applications of 50 μL of a 0.1% toluene solution of anthracene (99.5% pure by HPLC; 50 μg per treatment) for 104 weeks. A control group of 50 male mice was treated with toluene alone. Lesions (≥1 mm^3) that persisted for at least 1 week were diagnosed as papillomas. After 104 weeks, no benign or malignant skin tumours had developed in the surviving 14 experimental or 39 control mice. Gross examination of internal organs indicated no tumours in either the experimental or control groups (Warshawsky *et al.*, 1993).

Table 3.5. Carcinogenicity studies of intramammary or intramamillary administration of various PAHs in female experimental animals

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthanthrene								
Rat, Sprague-Dawley	20	>99% (trioctanoin)	1.1 mg/gland in 100 µL, 1×; 8 glands total	40 wk	1/20 (5%) mammary epithelial T (AdF) vs 0/21 vehicle controls, 2/20 (10%) (1 AdC, 1 AdF) untreated controls	–		Cavalieri *et al.* (1989)
Benz[*a*]anthracene								
Rat, Sprague-Dawley	20	>99% (none)	4 [913 µg] or 16 µmol [3.65 mg] applied as powder to 1 gland, 1×; untreated contralateral gland used as negative control	20 wk	0/20 at both doses	–		Cavalieri *et al.* (1988a)
Benzo[*a*]pyrene								
Rat, Sprague-Dawley	20	>99% (no vehicle)	0 (untreated contralateral mammary glands), 4 [1 mg], 16 µmol [4.2 mg], 1×	20 wk	Mammary gland T: [0/20] (0%), [10/20] (50%; 6 AdC, 4 fibroS; multiplicity: AdC, 6/6; fibroS 4/4), [16/20] (80%; 8 AdC, 2 fibroA, 10 fibroS; multiplicity: AdC, 8/8; fibroA, 2/2; fibroS, 10/10)	+		Cavalieri *et al.* (1988a)

Table 3.5 (contd)

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Sprague-Dawley	20	>99% (trioctanoin)	0, 4 μmol [1 mg]/mammary gland (2nd, 3rd, 4th and 5th mammary gland on both sides injected), 1×	45 wk	Epithelial mammary T: [3/20] (15%; 3 fibroA), [14/20] (70%; 13 AdC, 3 fibroA) Multiplicity: controls, 3/3 [1]; dosed rats: AdC, 18/13 [1.4]; fibroA, 4/3 [1.3] Mesenchymal (mammary) T: [0/20] (0%), [11/20] (55%; 11 fibroS; multiplicity, 20/11 [1.8]) Skin T: [0/20] (0%), [9/20] (45%; 9 SCC; multiplicity, 11/9 [1.2])	+		Cavalieri *et al.* (1988a,b)
Rat, Sprague-Dawley	20	>99% (trioctanoin)	0, 0.25, 1 μmol [66, 264 μg]/mammary gland (the 2nd, 3rd, 4th and 5th on both sides), 1×	24 wk	Epithelial mammary gland T: 1/18 (6%; 1 fibroA; multiplicity, 1/1), 1/20 (5%; 1 AdC; multiplicity 1/1), 0/20 (0%) Mesenchymal (mammary) T: 0/18 (0%), 6/20 (30%; 6 fibroS; multiplicity, 7/6 (1.2)), 8/20 (40%; 8 fibroS; multiplicity, 10/8 (1.3)) Skin T: [0/18] (0%), (0/20, 0%), (1/20, 5%); 1 SCC; multiplicity 1/1	+	Statistics not specified	Cavalieri *et al.* (1991)
Dibenz[*a*,*h*]anthracene								
Rat, Sprague-Dawley	20	>99% (fine powder)	1.1, 4.5 mg at 50 day of age, 1×	20 wk	0/20 vs 0/20 control	–	Control was untreated contralateral breast; no statistics	Cavalieri *et al.* (1988a)

Table 3.5 (contd)

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*e*]pyrene								
Rat, Sprague-Dawley	19, 21 controls	>99% (tricaprylin)	1.2 mg/gland in 100 µL, 1×; 8 glands total	40 wk	1/19 (5%; mammary epithelial T (AdF) vs 0/21 solvent controls	–		Cavalieri *et al.* (1989)
Dibenzo[*a*,*h*]pyrene								
Rat, Sprague-Dawley	20, 21 controls	>99% (tricaprylin)	1.2 µg/gland in 100 µL, 1×; 8 glands total	40 wk	19/20 (95%; fibroS) vs 0/20 solvent controls; 4/20 (20%; mammary gland AdF, AdC) vs 0/21 solvent controls	+		Cavalieri *et al.* (1989)
Dibenzo[*a*,*i*]pyrene								
Rat, Sprague-Dawley	20, 21 controls	>99% (tricaprylin)	1.2 mg/gland in 100 µL, 1×; 8 glands total	40 wk	18/19 (95%; fibroS) vs 0/21 solvent controls; 11/19 (58%; mammary AdC), 1/19 (5%; mammary AdF) vs 0/21 solvent controls	+		Cavalieri *et al*. (1989)

Table 3.5 (contd)

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene								
Rat, Sprague-Dawley	9 [from 19; 10 died within 9 wk], 21 controls	>99% (trioctanoin)	1.2 mg/gland in 100 µL, 1×; 8 glands total	15 wk; controls, 40 wk	100% [8/9 mammary AdC, 0/9 mammary AdF vs 2/20 solvent controls; 7/9 fibroS vs 0/20 solvent controls; 8/9 skin SCC vs 0/20 solvent controls]	+		Cavalieri *et al.* (1989)
Rat, Sprague-Dawley	20	>99% (trioctanoin)	75.6, 302 µg/gland in 50 µL, 1×; 8 glands total	24 wk	75.6 µg, 20/20 (100%) mammary AdC, 0/20 mammary AdF, 4/20 (20%) fibroS, 1/20 (5%) SCC; 302 µg, 19/19 (100%), 0/19, 14/19 (74%), 1/19 (5%) vs 0/18, 1/18 (6%), 0/18, 0/18 solvent controls	+		Cavalieri *et al.* (1991)

A, adenoma; AdC, adenocarcinoma; AdF, adenofibroma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; vs, versus, wk, week

[a] –, negative; +, positive

Table 3.6. Carcinogenicity studies of oral administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Rat, BDI and BDIII	NS	28	Pure (oil)	5 mg/day, then 15 mg/day, 6 days/wk, 91 wk; total dose, 4.5 g/animal	Lifespan; mean survival time, 700 days	2/28 (7%) (1 liver S, 1 uterine AdC)	–	No controls; both tumours considered to be unrelated to treatment	Druckrey & Schmähl (1955); Schmähl (1955)
Benz[*a*]anthracene									
Mouse, C57BL	NS	8–19; 6–16 controls	NS (heavy mineral oil)	500 µg in 10 µL, 1, 8 or 16× at 3–7-day intervals	16 months	Forestomach P: 1×, 0/13; 8×, 1/19 (5%); 16×, 1/8 (12%) vs 0/12, 0/16, 0/6 solvent controls	±		Bock & King (1959)
Mouse, B6AF1/J	M	20 or 40	99% (methocel-aerosol)	3% suspension in 50 µL, 3×/wk, 15×	340–444 or 547–600 days	340–444 days: 18/39 (46%) H, 37/39 (95%) pulmonary A, 1/39 (3%) reticulum-cell neoplasm, 2/39 (5%) forestomach P vs 0/39, 4/39 (10%), 0/39, 0/39 solvent controls 547–500 days: 20/20 (100%), 19/20 (95%), 0/20, 0/20 vs 2/20 (10%), 7/20 (35%), 0/0, 0/0, 2/20 (10%) lymphocytic neoplasms in solvent controls	+		Klein (1963)
Rat, Sprague-Dawley	F	18	NS (sesame oil)	200 mg	NS	0/18	–		Huggins & Yang (1962)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*c*]fluorene									
Mouse, A/J	F	30	≥98% (mixed with diet)	27, 397 µmol/kg diet; 260 days	260 days	Lung A: 27 µmol/kg, [13/28] (46%; 0.57 ± 0.13 T/mouse; mean ± SE); 397 µmol/kg, [29/29] (100%; 46.0 ± 2.8) (p <0.001) vs [7/29] (24%; 0.31 ± 0.11) in basal diet controls	+		Weyand *et al.* (2004)
Benzo[*a*]pyrene									
Mouse, A/J	F	15	NS (cottonseed oil or corn oil [not clearly specified])	2 mg/animal or 2 mg/animal preceded by oral intubation of 0.2 mL cottonseed oil (96 and 48 h before) 3× with 18-day intervals	26 wk	Forestomach T: 15/15 (100%; 12 P, 3 C; 2.9 ± 0.5 T/animal), 14/14 (100%; 14 P; 3.7 ± 0.4 P/animal) Pulmonary A: 15/15 (100%; 24 ± 1.8 A/animal), 14/14 (100%; 18 ± 2.0 A/animal)	+	No control	Sparnins *et al.* (1986)
Mouse, A/J	F	15–16	>98% (cottonseed oil)	0, 2, 3 mg/animal on day 1, 4, 7 of 1-wk period at age 9 wk	22 wk	Forestomach P: 4.1 ± 0.8, 8.7 ± 1.1 and 9.9 ± 0.9 P/animal Pulmonary A: 28.3 ± 3.0, 64.1 ± 5.8 and 52.3 ± 4.3 A/animal	+	No control; limited data on tumour incidence	Estensen & Wattenberg (1993)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, A/J	F	30	NS (gel diet)	0, 16, 98 ppm (total dose; 0, 11, 67 mg/animal)	260 days	Lung T: 4/21 (19%; A; 0.19 ± 0.09 A/animal), 9/25 (36%*; 7 A, 2 AdC; 0.48 ± 0.14* T/animal), 14/27 (52%*; 14 A; 0.59 ± 0.12* A/animal) Forestomach T: (0%) 0/21, (5/25) (20%; 3 P, 2 C; 0.24 ± 0.11** T/animal), 27/27 (100%**; 13 P, 14 C; 4.22 ± 0.41**) * (p <0.05); ** (p <0.001)	+		Weyand *et al.* (1995)
Mouse B6C3F1	F	48	98.5% (acetone)	0 (untreated), 0 (acetone control diet), 0.0005 % (5 ppm), 0.0025% (25 ppm), 0.01% (100 ppm) in the diet	2 years	Liver (A): 4/21 (4%), 7/48 (15%), 5/47 (11%), 0/45 (0%) Lung (A and/or C): 5/48 (10%), 0/48 (0%), 4/45 (9%), [0/48] (0%) Forestomach (P and/or C): 1/48 (2%), 3/47 (6%), 36/46 (78%****), 46/47 (98%****) Oesophagus (P and/or C): 0/48 (0%), 0/48 (0%), 2/45 (4%), 27/46 (59%**) Tongue (P and/or C): 0/48 (0%), 0/48 (0%), 2/46 (4%), 23/48 (48%***) Larynx (P and/or C): 0/35 (0%), 0/35(0%), 3/34 (9%), 5/38 (13%*) *(p <0.014); **(p <0.0014); ***(p <0.0003); ****(p <0.00001)	+		Culp *et al.* (1998)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss albino, inbred	F	10	Highest purity grade (corn oil) (drinking-water contained 0.005% ethanol)	0, 1 mg/animal, 2×/wk, 4 wk	27 wk	0, 10/10 (100%) (forestomach P; multiplicity, 7.11 ± 1.05)	+		Badary *et al.* (1999)
Mouse, A/J	F	11 or 18	>99% (cottonseed oil)	100, 125, 150 [dose not clearly specified] mg/kg bw on day 1, 3, 7 of a 1-wk period at age 8–14 wk	34 or 36 wk	Lung T/cm^2 lung tissue: 100 mg/kg bw, 4.2 ± 2.2 A, 2.9 ± 2.7 A with progression* combined with C; 100 [or 125 or 150] mg/kg bw, 6.3 ± 5.6 A, 2.2 ± 1.6 A with progression*, 2.1 ± 1.9 C *A with clones of 10 cells or more with hyperchromatic nuclei and nuclear size greater than that of nuclei in A cells	+	No control; lack of data on dose and lung tumour incidence	Estensen *et al.* (2004)
Mouse, Eμ-*pim*-1 transgenic, wild-type counterpart	M	20–31	NS (soya oil)	0, 4.3, 13, 39 mg/kg bw, by gavage 3×/wk, 13 wk (0, 4.3 mg/kg bw only administered to Eμ-*pim*-1 transgenic mice)	287 days	**Eμ-*pim*-1 transgenic mice** Lymphoma: 2/20 (10%), 4/30 (13%), 15/31* (48%), 24/29** (83%) *(p <0.005), **(p <0.0001) Forestomach T (P + C combined): 0/20, 4/30 (13%), 8/31 (26%), 6/29 (21%) **Wild-type mice** Lymphoma: 2/30 (7%), 6/20 (30%) Forestomach T (P + C combined): 5/30 (17%), 12/20 (60%)	+	No control for wild-type mice; statistics NS	Kroese *et al.* (1997)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, *XPA*$^{+/+}$, *XPA*$^{+/-}$, *XPA*$^{-/-}$	NS	17–34	NS (soya oil)	0, 4.3, 13 mg/kg bw/animal by gavage, 3×/wk, 13 wk	273 days	*XPA*$^{+/+}$: 1/26 (4%; 1 lymphoma), 1/14 ([7%] 1 lymphoma), 5/17 (29%; 2 lymphoma, 3 other types of T); *XPA*$^{+/-}$: 0/34, 0/28, 6/28 (21%; 3 lymphoma, 3 other T); *XPA*$^{-/-}$: 2/13 (15%; 2 hepatocellular A), 2/11 (18%; 2 lymphoma), 5/9 (56%; 4 lymphoma, 1 other T)	+	Limited reporting of tumours other than lymphomas; tumour response in high-dose *XPA*$^{-/-}$ mice significantly stronger than that in high-dose *XPA*$^{+/-}$ or *XPA*$^{+/+}$ mice (p <0.005)	de Vries *et al.* (1997)
Mouse, Muta^{TM} (λ*lacZ* transgenic)	M	8–12	NS (corn oil)	0, 75, 125 mg/kg bw/day, 5 consecutive days	41 wk	**Forestomach** SCC: 0/8, 2/11 (18%), 2/11 (18%) P: 0/8, 4/11 (36%), 5/11 (45%) (p ≤0.05) Splenic malignant lymphoma: 0/8, 0/11, 2/11 (18%)	+		Hakura *et al.* (1998)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, wild-type (C57BL/6), *XPA*$^{-/-}$, *p53*$^{+/-}$, *XPA*$^{-/-}$/*p53*$^{+/-}$	M, F	5–31	NS (soya oil)	0, 13 mg/kg bw/animal, by gavage, 3×/wk, 13 wk	52 wk	Wild type: 0/11, 6/17 (35%; total no. of T, 7; 3 lymphomas, 1 bronchiolo-alveolar A, 1 histiocytic S, 1 hepatocellular C, 1 intestinal A) *XPA*$^{-/-}$: 0/5, 5/7 (71%; total no. of T, 9; 3 lymphoma, 2 fore-stomach P, 1 bronchiolo-alveolar A, 1 histiocytic S, 1 ovary T, 1 intestinal A) *p53*$^{+/-}$: 3/18 (17%; 1 lymphoma, 1 osteosarcoma, 1 mammary AdC), 22/31 (71%; total no. of T, 33; 6 lymphoma, 9 forestomach P, 4 bronchiolo-alveolar A, 4 fibroS, 3 histiocytic S, 2 osteoS, 2 mammary AdC, 1 skin SCC, 1 hepatocellular C, 1 intestinal A) *XPA*$^{-/-}$/*p53*$^{+/-}$: 0/8, 15/15 (100%; total no. of T, 25; 8 lymphoma, 8 forestomach tumours, 6 histiocytic S, 1 rhabdomyoS, 1 haemangioma, 1 leukaemia)	+	Treated double transgenic *XPA*$^{-/-}$/*p53*$^{+/-}$ mice developed tumours much earlier and in higher incidences than their similarly treated single transgenic *XPA*$^{-/-}$ or *p53*$^{+/-}$ counterparts; statistics NS	van Oostrom *et al.* (1999)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, $CSB^{-/-}$ or wild-type ($CSP^{+/-}$ /$CSB^{+/+}$)	M, F	6–18 M; 6–13 F	NS (soya oil)	0, 13 mg/kg bw, 3×/wk, 13 wk	52 wk	Wild-type: 5/27 (14 M, 13 F; 19%; 4 bronchiolo-alveolar A, 2 lymphoma), 17/29* (18 M, 11 F; 59%; 6 bronchiolo-alveolar A, 10 forestomach P, 2 forestomach SCC, 2 histiocytic S, 2 hepatocellular A, 1 intestinal AdC, 1 skin P) $CSB^{-/-}$: 0/13 (6 M, 7 F), 7/12** (6 M, 6 F; 58%; 2 bronchiolo-alveolar A, 2 uterine S, 1 forestomach SCC, 1 intestinal AdC, 1 skin histiocytic S) *($p = 0.0023$) **($p = 0.0017$)	+		Wijnhoven *et al.* (2000)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, wild-type (C57BL/6), *Xpa*$^{-/-}$, *Xpa*$^{-/-}$/*p53*$^{+/-}$	M, F	5 or 10 M, 5 or 10 F of each genotype	NS	0, 75 ppm in the diet, 13 wk	9 months	Wild-type (M + F combined): 1/10 (10%; 1 intestinal A), 2/20 (10%; 2 forestomach P) *Xpa*$^{-/-}$ (M + F combined): 1/10 (10%; 1 bronchiolo-alveolar A), 16/20 (80%; 15 forestomach P, 1 SCC) *Xpa*$^{-/-}$/*p53*$^{+/-}$ (M + F combined): 2/10 (20%; 1 forestomach P, 1 mammary AdC), 13/15 (87%; total no. of T, 21; 3 oesophageal P, 11 forestomach P, 1 SCC, 2 haemangioS, 1 intestinal A, 1 abdominal osteoS, 1 widespread C, 1 abdominal S)	+	Treated cancer-prone nucleotide excision repair-deficient mice (*Xpa*$^{-/-}$ or *Xpa*$^{-/-}$/*p53*$^{+/-}$ mice) showed a much stronger tumour response than their treated wild-type counterparts (C57Bl/6 mice); no statistics	Hoogervorst *et al.* (2003)
Rat, Sprague-Dawley	M, F	32 M, 32 F	Highly pure (caffeine solution)	0, 0.15 mg/kg bw, 1×/9 days (6 mg/kg bw/year), 0.15 mg/kg bw, 5×/wk (39 mg/kg bw/year) for life in diet	Lifespan median survival times, 128–131 wk	T incidence (M + F combined): 3/64 (47%; 2 forestomach P, 1 oesophageal P), 3/64 (47%; 1 forestomach P, 1 oesophageal P, 1 laryngeal P), 10/64 (16%; 9 forestomach P (p <0.1), 1 oesophageal P)	+		Brune *et al.* (1981)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Crl:CD(SD)BR	F	30	99% (trioctanoin)	0, 50 µmol/ animal, 1×/wk, 8 wk by gavage	49 wk	Mammary T incidence: 11/30 [37%] [incidence not clearly specified] (8 desmoplastic A, 2 A, 1 AdC), 29/30 (96.7%; 8 fibroA**, 17 desmoplastic A*, 7 A, 22 AdC**) No. of mammary T: Controls, 14 desmoplastic A, 2 A, 1 AdC; treated animals, 14 fibroA*, 35 desmoplastic A, 11 A, 56 AdC** *(p <0. 05), **(p <0.01)	+		El-Bayoumy *et al.* (1995)
Dibenz[*a*,*h*]anthracene									
Mouse, C57 strain A BL, C3H, DBA,	M, F	10 or 20	Melting-point (olive oil emulsion in drinking-water)	500–800 µg/day, 156–303 days	156–303 days	C57 BL, 2/20 (10%; small intestine AdC); C3H, 6/20 (30%; small intestine AdC), 15/20 (75%; pulmonary T); DBA, 3/20 (15%; small intestine AdC), 15/20 (75%; pulmonary T); A: 7/30 (23%; small intestine AdC), 22/30 (73%; pulmonary T)	+	No. of controls NS; only observation in controls: pulmonary T in strain A mice; no statistics	Lorenz & Stewart (1947)
Mouse, A back-cross	M	10	Melting-point (mineral oil/aerosol emulsion)	400 µg/day, 406 days	406 days	2 forestomach SCC, 11 forestomach P		Small no. of animals; controls not treated with identical vehicle; no statistics	Lorenz & Stewart (1948)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss	M	42	NS (polyethylene glycol-400)	1.5 mg, 1× by gavage	30 wk	2/42 (5%) forestomach P	±	No control	Berenblum & Haran (1955)
Mouse, BALB/c	F	44, 30 controls	NS (almond oil)	500 µg, 2×/wk, 15 wk	60 wk	Virgin, 1/20 (5%; mammary gland T); pseudo-pregnant, 13/24 (54%; mammary gland T) vs untreated control pseudo-pregnant, 2/30 (7%; mammary gland T)	+	No solvent-treated control; no statistics	Biancifiori & Caschera (1962)
Mouse, DBA/2	M, F	21; 25 M, 10 F controls	Melting-point (olive oil emulsion in drinking-water)	800 µg/day, 8–9 months	8–9 months	M: 14/14 (100%; lung A), 14/14 (100%; alveologenic C), 10/14 (71%; haemangio-endotheliomas) versus 1/25 (4%; lung A), 0/25 (alveologenic C), 0/25 (haemangio-endothelioma) solvent controls; F: 13/13 (100%; lung A), 10/13 (77%; alveologenic C), 6/13 (46%; haemangio-endotheliomas), 12/13 (92%; mammary C) 0/10 (lung A), 0/10 (alveologenic C), 0/10 (haemangio-endotheliomas), 0/10 (mammary C) solvent controls	+	No statistics	Snell & Stewart (1962)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Fish, Japanese medaka (*Oryzias latipes*)	M, F	65, 75 controls	>97% (menhaden oil)	100 ppm in diet, 5×/wk, 28 days	10 months	17/65 (26%) vs 6/75 (8%) diet controls (p <0.05); hepatic neoplasia, 12/65 (18%) vs 0/75 (p <0.001); hepatocellular C, 7/65 vs 0/75 predominated	+		Reddy *et al*. (1999a)
Fish, Shasta rainbow trout (*Oncorhynchus mykiss*)	M, F	260	NS	200 ppm in diet, 4 wk, 500 ppm, 2 wk	200 ppm, 9 months; 500 ppm, 11 months	200 ppm, 36% liver T (hepatocellular C, 43% relative incidence; hepatocellular A, 44% relative incidence; 2.00 T/TBA), 48% stomach papillary A (3.80 T/TBA), 30% swimbladder papillary A (2.40 T/TBA); 500 ppm, 61% liver T (hepatocellular C, 64% relative incidence; hepatocellular A, 14% relative incidence; 2.58 T/TBA), 91% stomach papillary A (5.67 T/TBA), 53% swimbladder papillary A (2.25 T/TBA) vs 0% liver, stomach and swimbladder T in the diet control group	+		Reddy *et al*. (1999b)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Phenanthrene									
Rat, Sprague-Dawley	F	10	NS (sesame oil)	200 mg, 1×	60 days	0/10 vs 8/164 (5%) mammary gland T non-concurrent controls after 310 days	–	Small numbers; no statistics	Huggins & Yang (1962)

A, adenoma; AdC, adenocarcinoma; C, carcinoma; F, female; H, hepatoma; M, male; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; SE, standard error; T, tumour, TBA, tumour-bearing animal; vs, versus; wk, week

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

Table 3.7. Carcinogenicity studies of intraperitoneal administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Mouse, Swiss	M	5	NS (olive oil)	25 mg in ~750 µL, 1×	5 months	0/4 remaining mice vs 0/4 remaining solvent controls	–	Small no. of animals; limited reporting; no statistics	Shubik & Della Porta (1957)
Rat, BDI and BDIII	NS	10	Pure (oil)	20 mg, 1×/wk, 33 wk	Lifetime (>2 years)	1/10 (10%; spindle-cell S)	±	No control; no statistics	Schmähl (1955)
Benz[*j*]aceanthrylene									
Mouse, A/J	M	27	NS (tricaprylin)	20, 50, 100 mg/kg bw, 1×	8 months	Lung A: 20 mg/kg, 12/12 (100%; 60.3 ± 14.6 A/mouse); 50 mg/kg, 13/13 (100%; 140.6 ± 21.5 A/mouse); 100 mg/kg, 14/14 (100%; 97.6 ± 28.2 A/mouse) vs 19/34 (56%; 0.85 ± 0.9 A/mouse) solvent controls	+	Limited histopathology; no statistics	Mass *et al.* (1993); Nesnow *et al.* (1998a)
Benz[*a*]anthracene									
Mouse, BLU:Ha (ICR)	M, F	140, 100 controls	NS (DMSO)	2800 nmol [63.9 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	Pulmonary T: M, 10/47 (21%; 0.26 T/mouse) vs 7/43 (16%; 0.19 T/mouse) solvent controls; F, 4/38 (11%; 0.08 T/mouse) vs 2/24 (8%; 0.08 T/mouse) solvent controls	±		Wislocki *et al.* (1979)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BLU:Ha(ICR)	M, F	90, 80 controls	≥99% (DMSO)	2.8 μmol [639 μg] in 35 μL (total dose, given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26–32 wk	M: 17/27(63%; pulmonary A; 1.44 T/mouse), 2/27 (8%; hepatic T, mostly A or neoplastic nodules; 0.08 T/mouse) vs 1/28 (4%; 0.04 T/mouse), 4% (1/28; 0.04 T/mouse) in solvent controls; F: 14/22 (64%; pulmonary A; 2.00 T/ mouse), 0/22 (0%; hepatic T) vs 4/37 (11%; 0.11 T/ mouse 0/37 (0%) in solvent controls (p <0.05 for lung T incidence and number of T/mouse (M + F combined))	+		Levin *et al.* (1984)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	39 M; 32 F; 28 M controls, 31 F controls	>99% (DMSO)	2.8 μmol [639 μg] in 70 μL (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	1 year	M: 31/39 (79%; liver T; 6/39 A, 25/39 C; 3.0 nodules/TBA) vs 2/28 (7%; 2/28 A, 0/28 C; 1.0/TBA) solvent controls (p <0.05 for C and combined liver T); 6/39 (15%; lung T; 5/39 A, 1/39 C) vs 1/28 (4%; 1/28 A, 0/28 C) in solvent controls; 3% (1/39, malignant lymphoma) vs 4% (1/28) solvent controls F: 0/32 (0%; liver T) vs 0/31 (0%) solvent controls; 6/32 (19%; lung T; 6/32 A, 0/32 C) vs 0/32(0%) solvent controls (p <0.025); 3/32 (9%; malignant lymphoma) vs 1/31 (3%) solvent controls	+		Wislocki *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*b*]fluoranthene									
Mouse, CD-1	M	15	>99% DMSO	0, 0.5 µmol (total dose; given as on 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 0/17 (0%), 2/15 (13.3%); liver A + H: 1/17 (5.6%), 8/15 (53.3%)	+		LaVoie *et al.* (1987)
	F	17		0, 0.5 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 0/18 (0%), 3/17 (17.6%); liver A: 0%, 0%	+		
Mouse, strain A/J	M	20	99% (tricaprylin)	0, 10, 50, 100, 200 mg/kg, 1×, tricaprylin, negative control; 1000 mg/kg urethane, positive control	8 months	Lung A: 55%, 50%, 80% (p <0.05), 100% (p <0.05), 100% (p <0.05); 0.60 ± 0.58, 0.67 ± 0.75, 2.00 ± 1.82, 5.30 ± 3.21, 6.95 ± 03.52 T/animal	+		Nesnow *et al.* (1995); Ross *et al.* (1995); Mass *et al.* (1996); Nesnow *et al.* (1998a)
Benzo[*j*]fluoranthene									
Mouse, CD-1	M	40	>99% (DMSO)	0, 1.1 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 0/17 (0%), 11/21 (52.3%) (p <0.005); liver H: 1/17 (5.6%), 11/21 (52.3%) (p <0.005)	+		LaVoie *et al.* (1987)
	F	40				Lung A: 0/17 (0%), 4/18 (22.2%) (p <0.05) [p = 0.058, Fisher's exact test, one-tailed]; liver H: 0%, 0%	±		

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M	80	>99% (DMSO)	0, 1.10, 0.275, 1.10 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 18.2% (p >0.05), 21.6% (p >0.05), 50.0% (p <0.01), 100% (p <0.001); liver A: 9.1%, 27.0% (p >0.05), 38.2% (p <0.005), 56.0% (p <0.001); 0.18, 0.30, 0.65, 2.96 lung T/animal; 0.18, 0.35, 0.47, 2.20 liver T/animal	+		LaVoie *et al.* (1994a)
	F	80				Lung A: 21.2% (p >0.05), 20.7% (p >0.05), 43.8% (p >0.05), 92.1% (p <0.001); liver A: 0%, 6.8%, 0%, 2.6%; 0.24, 0.28, 0.53, 2.63 lung T/animal; 0, 0.07, 0, 0.03 liver T/animal	+		
Benzo[*k*]fluoranthene									
Mouse, CD-1	M, F	16 M, 18 F	>99% (DMSO)	0, 2.1 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	M : liver T, 3/16 (18.8%; 2 A, 1 H; p <0.05); lung T, 1/16 (6.3%; 1 A; p <0.05) vs 1/17 (6%) solvent controls F : liver T, 0/18; lung T, 3/18 (16.7%; 3 A; p <0.05) vs 0/18 solvent controls	±		LaVoie *et al.* (1987)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*c*]fluorene									
Mouse, A/J	F	30	≥98% (tricaprylin)	1.75 mg (100 mg/kg bw) in 250 µL, 1×	260 days	Lung A: [26/28] (92%; 4.0 ± 0.53 T/mouse; p <0.01) vs 14/29 (48%; 0.6 ± 0.14) solvent controls	+		Weyand *et al.* (2004)
Benzo[*c*]phenanthrene									
Mouse, CD-1	M, F	75	>99% (DMSO)	0, 50, 150 nmol, 1×, (total dose; given as as 1/7, 2/7, 4/7 on PND 1, 8, 15)	33–39 wk	Lung T (T/mouse): M: 3% (0.06), 6% (0.06), 57% (1.6) F: 5% (0.07), 9% (0.09), 65% (2.6)	+		Levin *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene									
Mouse, B6C3F1; C3A/JF1	M, F	30–63, 96–100 controls	NS (trioctanoin)	0, 75, 150 µg in 10 µL/g bw, 1× at 1, 15, 42 days of age	90 wk or lifespan	*B6C3F1 mice (all ages combined)* Liver T (A and hepatocelluar C): M, 1/98 (1%), 69/162 (43%), 81/165 (49%); F, 0/96 (0%), 7/147 (5%), 10/126 (8%) Lung T (A and AdC): M, 7/98 (7%), 57/162 (35%), 73/165 (44%); F, 2/90 (2%), 53/147 (36%), 50/126 (40%) Stomach T (P and SCC): M, 0/98 (0%), 39/162 (24%), 64/165 (39%); F, 0/96 (0%), 22/147 (15%), 40/126 (32%) Lymphoreticular T (mainly reticulum-cell): M, 2/98 (2%), 104/314 (33%) (high- and low-dose groups combined); F, 2/96 (2%), 148/281 (53%) (high-and low-dose groups combined)	+		Vesselinovitch *et al.* (1975a,b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						C3A/JF1 mice (all ages combined) Liver T (A and hepatocelluar C): M, 3/97 (3%), 30/148 (20%), 33/137 (24%); F, 0/100 (0%), 1.3% 2/126 (1.3%), 2/153 1.3%) Lung T (A and AdC): M, 49/97 (49%), 1438/148 (93%), 125/137 (91%); F, 26/100 (26%), 115/126 (91%), 141/153 (92%) Stomach T (P and SCC): M, 0/97 (0%), 18/148 (12%), 42/137 (31%); F, 0/100 (0%), 18/126 (14%), 31/153 (20%) Lymphoreticular T (mainly reticulum-cell): M, 0/97 (0%), 26/285 (9%); F, 2/100 (2%), 50/278 (18%) (high- and low-dose groups combined)			Vesselinovitch *et al.* (1975a,b) (contd)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss-Webster BLU:Ha (ICR)	NS	120–184	NS (DMSO)	0, 7, 14 nmol [0, 1.8, 3.7 µg]/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	34–37 wk	Lung T (mainly A + few AdC): 11%, [11/98] (0.12 [should be 0.11] T/animal), 22% [18/82] (0.22 T/animal), 15% [14/92] (0.15 T/animal)	–	Limited reporting of the type of pulmonary tumours; statistics NS; no significant differences in tumour incidence between M and F	Buening *et al.* (1978)
Mouse, CD-1	M, F	37 M, 27 F	>99% (DMSO)	0, 560 nmol [148 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 0, 8, 15)	1 year	Liver T: M, 2/28 (7%; 2 A), 18/37* (49%; 11 A, 7 C*); F, no liver T found Lung T: M, 1/28 (4%; 1 A), 13/37** (35%; 13 A); F, 0/31, 13/27** (48%) (13 A) Malignant lymphoma: M, 1/28 (4%), 2/37 (5%); F, 1/31 (3%), 4/27 (15%) *(p<0.005), **(p<0.05)	+		Wislocki *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1,	M, F	17 M, 14 F	>99% (DMSO)	0, 1.1 μmol [290 μg]/animal (total dose; given as 1/6, 2/6, 4/6 on PND 1, 8, 15)	52 wk	Liver T: M, 1/17 (6%; 1 H), 13/17 (76%; 9 hepatic A, 4 H; p <0.005); F, 0 Lung A: M, 0/17, 14/17 (82%; p <0.005); F, 0/18, 9/14 (64%)	+	Statistics NS	LaVoie *et al.* (1987)
Mouse, Swiss-Webster BLU: Ha(ICR)	M, F	NS	>99% (DMSO)	0, 59.5 μg/animal (total dose; given as 8.5, 17, 34 μg on PND 1, 8, 15)	26 wk	Lung T: M, 12/91 (13%; 12 A, 1 AdC; 0.15 ± 0.04 T/mouse), 13/28 (46%; 13 A; 0.71 ± 0.19 A/mouse); F, 7/101 (7%; 7 A; 0.08 ± 0.03 A/mouse), 18/27 (67%; 18 A, 1 AdC; 1.19 ± 0.21 T/mouse)	+	Statistics NS	Busby *et al.* (1989)
Mouse, NS, newborn	M, F	NS	NS (saline solution + 1% gelatine + 0.4% Tween 20)	0, 10, 100 μg/ animal, 1×	30 wk	Lung T: 13% [5/38] (0.13 T/animal), 16% [5/31] (0.23 T/animal), 64% [21/33] (2.52 T/animal)	+	Type of lung tumour NS; no statistics	Rippe & Pott (1989)
Mouse, A/J	NS	27	NS (tricaprylin)	0, 20, 50, 100 mg/kg bw/animal, 1×	8 months	Lung T: 19/34 [56%] (0.85 ± 0.9 T/animal), 10/16 [63%] (1.0 ± 1.0 T/animal), 15/16 [94%] (3.9 ± 2.9 T/animal), 14/14 [100%] (5.9 ± 3.3 T/animal)	+	Type of lung tumours NS; statistics NS	Mass *et al.* (1993); Ross *et al.* (1995)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, A/J	M	20	NS (tricaprylin, urethane)	0, 10, 50, 100 or 200 mg/kg bw, 1×; positive control, 1000 mg/kg bw urethane	8 months	Lung A/animal (partly derived from dose–response curve): 0.6, ~0.5, ~6, 12.8, ~35: at the 3 highest doses, T incidence was 100% (20/20); positive controls, 27.3 ± 4.7	+		Nesnow *et al.* (1995)
Mouse, A/J	M	55	NS (tricaprylin)	0, 5, 10, 50, 100, 200 mg/kg bw, 1×; positive control, 1000 mg/kg bw urethane, 1×	240 days	Lung A/animal: 0.6 ± 0.6, (~ 0), (~ 0), (~ 3), (~14), (~ 33); positive controls, 25.6 ± 5.7 [figures derived from dose–response curve except for those for both control groups; incidence of lung A NS]	+		Ross *et al.* (1995)
Mouse, A/J	F	29	NS (tricaprylin)	0 (untreated), 0 (vehicle control), 1.79 mg/animal, 1×	260 days	Lung T: 7/30 (23%; 7 A; 0.27 ± 0.12 A/animal), 11/30 (37%; 11 A; 0.43 ± 0.11 A/animal), 29/29 (100%; 27 A, 2 AdC; 15.8 ± 1.28 T/animal; $p < 0.05$); forestomach T: 0/30 (0%), 0/30 (0%), 24/29 (83%; 15 P, 9 C; 1.83 ± 0.25 T/animal; $p < 0.001$)	+		Weyand *et al.* (1995)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, B6C3F1, infant	M, F	>30 M, >30 F	NS (corn oil)	0 (untreated), 0 (vehicle controls), 125, 250, 375 µg/7 g bw, 1×	26 wk, 39 wk, 52 wk	**Liver T in M:** At wk 26: 0/41, 0/58, 0/29, 0/25, 3/34 (9%; multiplicity, 1.0); at wk 39: 0/34, 0/59, 6/26 (23%; multiplicity, 1.0), 13/34 (38%; multiplicity, 1.9), 15/23 (65%; multiplicity, 1.9); at wk 52: 4/64 (6%; multiplicity, 1.0), 3/63 (5%; multiplicity, 1.0), 13/29 (45%; multiplicity, 1.8), 14/27 (52%; multiplicity, 2.2), 19/24 (79%; multiplicity, 2.5) No liver T found in F	+		Rodriguez *et al.* (1997)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M	24	>99% (DMSO)	0, 100, 26, 400 nmol [111 µg]/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15); positive controls, 100, 400 nmol 6-nitro-chrysene/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	12 months	Liver T: 3/20 (15%; 1 A, 2 C; 1.7 T/liver section), 5/21 (24%; 4 A, 1 C; 1.5 T/liver section), 9/20 (45%; 7 A*, 2 C; >2.3 T/liver section) *(p = 0.0234) Lung T: 4/20 (20%; 4 A; 1.0 T/lung section), 1/21 (5%; 1 A; 1.0 T/lung section), 9/20 (45%; 7 A, 2 C; 1.9 T/lung section); positive controls: over 90% liver T (A and/or C) and 100% lung T (A and/or C) in both groups	+		von Tungeln *et al*. (1999a)
Rat, Wistar	F	NS	NS (tricaprylin/ beeswax mixture (3:1))	0, 5 mg/animal, 1×	~112 wk	Abdominal mesothelioma and S: 3/41 (7.3%), 33/37 (89.2%)	+	Limited reporting	Roller *et al.* (1992)
Rat, Wistar	F	NS	NS (saline solution)	5 mg/animal, 1×	~116 wk	19/38 (50%; abdominal mesothelioma and S); historical controls, 11/369 (3%)	+	No control; limited reporting of tumour data	Roller *et al.* (1992)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*e*]pyrene									
Mouse, Swiss-Webster BLU/Ha (ICR)	M, F	80	>99% (DMSO)	0, 2.8 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	62–66 wk	M: lung T, 42%, 41%; liver T, 11%, 21%; 0.53, 0.55 lung T/animal; 0.11, 0.21 liver T/animal F: lung T, 57%, 40%; liver T, 0%, 0%; 0.95, 0.57 lung T/animal	– –		Buening *et al.* (1980)
Mouse, Swiss-Webster BLU/Ha (ICR)	M, F	80	>99% (DMSO)	0, 5.6 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	62–66 wk	M: lung T, 31%, 48%; liver T, 0%, 12%; 0.48, 0.56 lung T/animal; 0, 0.12 liver T/animal F: Lung T, 40%, 26%; liver T, 0%, 0%; 0.43, 0.26 lung T/animal	+ –		Buening *et al.* (1980)
Chrysene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	80	NS (DMSO)	0, 1.4 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	37–41 wk	M: lung T, 8% (4/52), 15% (4/27); liver T, 0% (0/52), 22% (6/27); 0.08, 0.19 lung T/animal; 0.41 liver T/animal F: lung T, 15% (6/41), 9% (1/11); liver T, 0% (0/41), 0% (0/11); 0.09 lung T/animal	+ –		Chang *et al.* (1983)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	90 or 100	>99% (DMSO)	0, 700 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	M: lung A or AdC, 9%, 17%; liver A and C, 11% (5/45), 29% (10/35) [p <0.015]; lymphoma, 4%, 9% F: lung A or AdC, 6%, 6%; liver A or C, 0%, 0%; lymphoma, 0%, 3%	+ –		Wislocki *et al.* (1986)
Mouse, CD-1	M, F	90 or 100	>99% (DMSO)	0, 2800 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	M: lung, A or AdC, 4% (1/28), 21% (7/34) [p <0.05]; liver A and C, 7% (2/28), 41% (14/44) [p <0.05]; lymphoma, 4%, 0% F: lung A and AdC, 0%, 4%; liver A and C, 0%, 0%; lymphoma, 3%, 0%	+ –		Wislocki *et al.* (1986)
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	~297	>98% (DMSO)	0, 0.03, 0.92 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	M: lung A and AdC, 13/91 (14%), 2/27 (7%), 3/20 (15%); 0.15 ± 0.08, 0.07 ± 0.05, 0.15 ± 0.08 T/animal F: lung, A and AdC, 7/101 (7%), 3/29 (10%), 0/29 (0%); 0.08 ± 0.03, 0.10 ± 0.06, 0.0.06 ± 0.03 T/animal	– –		Busby *et al.* (1989)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Cyclopenta[*cd*]pyrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	~ 150	>99% (DMSO)	0, 1.55, 3.09, 4.64, 6.19, 7.73 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	M: lung A or AdC, 2/25 (8%), 5/8 (62%), 5/9 (56%), 6/7 (86%), 10/13 (77%), 8/9 (89%); 0.08 ± 0.06, 1.12 ± 0.48, 2.78 ± 1.10, 9.29 ± 4.78, 4.08 ± 0.98, 7.33 ± 1.80 T/animal F: lung A or AdC, 2/24 (8%), 6/10 (60%), 7/10 (70%), 13/14 (93%), 7/7 (100%), 9/9 (100%); 0.08 ± 0.06, 2.20 ± 0.83, 3.20 ± 1.04, 6.71 ± 2.01, 13.57 ± 5.50, 5.33 ± 1.62 T/animal	+ +		Busby *et al.* (1988)
Mouse, Strain A/J	M	20	99% (tricaprylin)	0, 10, 50, 100, 200 mg/kg, 1×	8 months	Lung A: 55%, 40%, 100% (p <0.05), 100% (p <0.05), 100% (p <0.05); 0.60 ± 0.58, 0.58 ± 0.82, 4.63 ± 2.11 (p <0.05, one-way ANOVA), 32.8 ± 15.4 (p <0.05, one-way ANOVA), 97.7 ± 28.7 (p <0.05, one-way ANOVA) T/animal	+		Nesnow *et al.* (1994, 1995); Ross *et al.* (1995); Nesnow *et al.* (1998a,b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,c]anthracene									
Mouse, B6C3F$_1$	M	24	>99% (DMSO)	400 nmol [111 µg] in 35 µL (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	12 months	9/24 (38%; liver A), 0/24 (liver C), 0/24 (lung T) vs 2/24 (8%), 1/24 (4%), 0/24 solvent controls	±		von Tungeln *et al.* (1999b)
Dibenz[*a*,*h*]anthracene									
Mouse, NS	NS	20	NS (aqueous suspension)	200 µg in 400 µL, 2×/wk, ~48 wk	~48 wk	5/20 (25%) peritoneal T	+	No control	Boyland & Burrows (1935)
Mouse, A/J	M	20	97% (tricaprylin)	0, 1.25, 2.5, 5, 10 mg/kg bw in 100 or 200 µL, 1×	8 months	Lung A: >2.5 mg/kg, 100%; 1.25 mg/kg, 1.44 A/mouse; 2.5 mg/kg, 3.05 A/mouse; 5 mg/kg, 13.1 A/mouse; 10 mg/kg, 32.1 A/mouse vs 0.6 A/mouse in solvent control significantly different (p <0.05) at doses >1.25 mg/kg	+	No histology	Nesnow *et al.* (1995); Ross *et al.* (1995); Nesnow *et al.* 1998a,b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*h*]pyrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	25 M, 14 F, 32 M controls, 39 F controls	'Essentially pure' (DMSO)	3.8, 7.6, 15.1 µg in 5, 10, 20 µL on PND 1, 8, 15	49–54 wk	Pulmonary T: F, 13/14 (93%) vs 11/39 (28%) solvent controls; M, 25/25 (100%) vs 7/32 (22%) solvent controls Hepatic T: F, 1/14 (7%) vs 0/39 solvent controls; M 11/25 (44%) vs 0/32 solvent controls	+		Chang *et al.* (1982)
Dibenzo[*a*,*i*]pyrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	39 M, 21 F, 32 M controls, 39 F controls	'Essentially pure' (DMSO)	3.8, 7.6, 15.1 µg in 5, 10, 20 µL on PND 1, 8, 15	49–54 wk	M, 38/39 (97%; pulmonary T; 3.64 T/mouse), 21/39 (54%; hepatic T; 0.82 T/mouse) vs 7/32 (22%; pulmonary T; 0.80 T/mouse), 0/32 (hepatic T) solvent controls; F, 21/21 (100%; pulmonary T; 5.80 T/mouse) vs 11/39 (28%; pulmonary T, 0.44 T/mouse) solvent controls	+	Limited histo-pathology	Chang *et al.* (1982)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Mouse, A/J	M	30–35 or 5	Pure analytical grade (tricaprylin)	0.3, 1.5, 3.0, 6.0 mg/kg bw or 12, 18, 24 mg/kg bw, 1× (volume NS)	8 months	Lung A: 0.3 mg/kg, 14/33 (42%; 0.42 ± 0.56 T/mouse); 1.5 mg/kg, 33/34 (97%; 4.30 ± 2.86); 3.0 mg/kg, 35/35 (100%; 7.50 ± 3.79); 6.0 mg/kg, 30/30 (100%; 16.1 ± 7.26); 12, 18 mg/kg, NS; 24 mg/kg, 5/5 (100%; 36.67 ± 10.64) vs 15/30 (50%; (0.67 ± 0.80) solvent controls	+		Prahalad *et al.* (1997)
Mouse, A/J	M	20	NS (tricaprylin)	0–6 mg/kg, 1× (volume NS)	8 months	Incidence of T, NS; lung A/mouse increased in a dose-dependent manner (p <0.005 for doses ≥1.5 mg/kg compared with solvent controls)	+		Nesnow *et al.* (1998a)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Crl:CD-1 (ICR)BR	M, F	25–40	NS (DMSO)	12.1, 121 µg in 80 µL, 3× (total dose; given as 1/8, 1/4, 5/8 on PND 1, 8, 15)	12.1 µg, 55 ± 1 wk; 121 µg, 17 wk	**Pulmonary T (T/mouse)** M: 12.1 µg, 84.8% (2.85 ± 0.44 [mean ± SEM]); 121 µg, 41.2% (0.65 ± 0.21) vs 25.0% (0.33 ± 0.14) in solvent controls F: 12.1 µg, 89.5% (2.95 ± 0.67); 121 µg, 35.7% (0.57 ± 0.29) vs 10.0% (0.10 ± 0.07) in solvent controls **Hepatic T (T/mouse)** M: 12.1 µg, 84.8% (5.67 ± 0.86); 121 µg, 35.3% (1.00 ± 0.38) vs 0% in solvent controls F: 12.1 µg, 10.5% (0.11 ± 0.07); 121 µg, 14.3% (0.21 ± 0.15) vs 0% in solvent controls **Other T (T/mouse)** M: 12.1 µg, 30.3% (0.58 ± 0.17); 121 µg, 23.5% (0.35 ± 0.19) vs 0% in solvent controls F: 12.1 µg, 47.4% (0.53 ± 0.14); 121 µg, 42.9% (0.50 ± 0.17) vs 0% in solvent controls	+		Platt *et al.* (2004)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
1,2-Dihydroacenanthrylene									
Mouse, CD-1	M, F	17–31 M, 13–23 F, 24 M controls, 34 F controls	Pure (DMSO)	175, 437.5, 875 µg in 35 µL (total dose; given as on 1/7, 2/7, 4/7 on PND 1, 8, 15)	9 months	**Liver T** M: 175 µg, 1/17 (6%); 437.5 µg, 1/25 (4%); 875 µg, 0/31 vs 0/24 solvent controls F: 175 µg, 0/23; 437.5 µg, 0/21; 875 µg, 0/13 vs 0/34 solvent controls **Lung A** M: 175 µg, 2/17 (12%); 437.5 µg, 2/25 (8%); 875 µg, 5/31(16%) vs 0/24 solvent controls F: 175 µg, 0/23; 437.5 µg, 1/21 (5%); 875 µg, 1/13 (8%) vs 1/34 (3%) solvent controls **Lung AdC** M: 175 µg, 2/17 (12%); 437.5 µg, 0/25; 875 µg, 1/31 (3%) vs 1/24 (4%) solvent controls F; 175 µg, 1/23 (4%); 437.5 µg, 0/21; 875 µg, 1/13 (8%) vs 0/34 solvent controls	±		Wang *et al.* (1999)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BLU:Ha	M, F	10 M, 23 F, 22 M controls, 25 F controls	Pure (DMSO)	175 µg in 35 µL (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	6 months	M: 0/10 lung A, 0/10 lung AdC vs 1/22 (5%), 0/22 solvent controls F: 1/23 (4%) lung A, 0/23 lung AdC vs 1/25 (4%), 0/25 solvent controls	–		Wang *et al.* (1999)
Fluoranthene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	58 M, 41 F, 27 M controls, 27 F controls	98% (DMSO)	0, 3.46, 17.3 µmol (total dose; given as 1/7, 2/7, 4/7 of total dose on PND 1, 8, 15)	24 wk	**Lung A/AdC (T/mouse)** M: 1/27 (4%; 0.04 ± 0.04), 7/31 (23%; 0.29 ± 0.15), 20/27 (74%; 1.52 ± 0.32) (p <0.014) F: 4/28 (14%; 0.14 ± 0.07), 3/20 (15%; 0.15 ± 0.11), 8/21 (38%; 0.52 ± 0.18)	+		Busby *et al.* (1984)
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	23 M, 29 F, 91 M controls, 101 F controls,	>99% (DMSO)	0, 1.27 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	**Lung A/AdC (T/mouse)** M: 13/91 (14%; 0.15 ± 0.04), 5/23 (22%; 0.22 ± 0.09) (0.002 <p < 0.004) F: 7/101 (7%; 0.08 ± 0.03), 9/29 (31%; 0.41 ± 0.13) (0.002 <p <0.004)	+		Busby *et al.* (1989)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	57 M, 64 F, 23 M controls, 22 F controls	>99% (DMSO)	0, 3.46, 8.65, 17.30 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	6 months	**Lung A/AdC (T/mouse)** M: 0/23 (0%), 1/19 (5%; 0.05 ± 0.05), 2/18 (11%; 0.17 ± 0.12), 10/20 (50%; 0.70 ± 0.19); F: 0/22 (0%), 0/20 (0%), 2/21 (10%; 0.10 ± 0.07), 9/23 (39%; 0.43 ± 0.12) **Liver A (T/mouse)** M: 0/23 (0%), 1/19 (5%), 0/18 (0%), 2/20 (10%)	+		Wang & Busby (1993)
					9 months	**Lung A AdC (T/mouse)** M: 1/20 (5%; 0.05 ± 0.05), 3/18 (17%; 0.17 ± 0.09), 9/21 (43%; 0.52 ± 0.15), 9/14 (64%; 1.00 ± 0.26) F: 1/18 (6%; 0.06 ± 0.06), 6/19 (32%; 0.37 ± 0.14), 2/23 (9%; 0.09 ± 0.06), 7/24 (29%; 0.50 ± 0.21) **Liver A (T/mouse)** M: 0%, 4/18 (22%; p <0.03), 12/21 (57%; p <0.001), 5/14 (36%; p <0.01)	+		

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	64–79	>99.5% (DMSO)	0, 3.46, 17.3 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung A/AdC (T/mouse)** M: 17% (0.17), 43% (0.64; p <0.05), 65% (1.12; p <0.005) F: 12% (0.15), 35% (0.35; p <0.05), 86% (2.45; p <0.001) **Liver A/C (T/mouse)** M: 3/29 (10%; 0.17), 14/28 (50%; 0.64; p <0.012 χ^2), 14/17 (82%; 1.12; p <0.001 χ^2)	+		LaVoie *et al.* (1994b)
Indeno[1,2,3-*cd*]pyrene									
Mouse, CD-1	M, F	30	>99% (DMSO)	0, 2.1 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung T** M: 0/17 (0%), 1/11 (9.1%); F: 0/18 (0%), 0/9 (0%) **Liver T** M: 0/11 (0%), 0/11 (0%); F: 0/18 (0%), 0/9 (0%)	M, + F, –		LaVoie *et al.* (1987)
5-Methylchrysene									
Mouse, ICR/HA	M, F	100	Pure (DMSO)	0, 56 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	35 wk	**Lung A (T/mouse)** M: 4% (0.04), 20% (0.26) F: 7% (0.07), 21% (0.25) **Liver A** M: 2% (0.02), 23% (0.43) F: 2% (0.02), 12% (0.29)	+		Hecht *et al.* (1985)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, strain A/J	M	20	99% (tricaprylin)	0, 10, 50, 100, 200 mg/kg bw, 1×	8 months	Lung, A (T/mouse): 55% (0.6 ± 0.06), 65% (1.8 ± 1.6), 100% (39.0 ± 13.7; p <0.05), 100% (93.1 ± 19.9; p <0.05), 100% (too numerous to count; p <0.05)	+		You *et al.* (1994); Nesnow *et al.* (1995); Ross *et al.* (1995); Nesnow *et al.* (1998a)
2-Methylfluoranthene									
Mouse, CD-1, weanling	M, F	64–79	>99.5% (DMSO)	0, 3.46, 17.3 µmol, (total dose; given as 1/7, 2/7, 4/7 of total dose on PND 1, 8, 15)	52 wk	**Lung A/AdC (T/mouse)** M: 5/29 (17%; 0.17), 5/31 (16%; 0.25), 23/24 (96%; 3.04; p <0.001) F: 4/34 (12%; 0.15), 6/34 (18%; 0.35; p <0.001), 11/16 (69%; 2.45) **Liver A/C** M: 3/29 (10%), 8/31 (26%), 22/24 (92%; p <0.001) F: 0/34, 0/34, 5/16 (31%; p <0.001)	+		LaVoie *et al.* (1994b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
3-Methylfluoranthene									
Mouse, CD-1, weanling	M, F	64–79	>99.5% (DMSO)	0, 3.46, 17.3 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung A/AdC (T/mouse)** M: 5/29 (17%; 0.17), 6/24 (25%; 0.25), 5/26 (19%; 0.23) F: 4/34 (12%; 0.15), 5/33 (15%; 0.18), 6/28 (21%; 0.39) **Liver A/C** M: 3/29 (10%), 7/24 (30%), 15/26 (55%) F: 0/34, 0/33, 2.28 (7%)	+		LaVoie *et al.* (1994b)
Phenanthrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	100	>98% (DMSO)	0, 35, 70, 140 µg on PND 1, 8, 15	38–42 wk	17/35 lung A vs 15/59 (25%) solvent controls; 0/35 liver T vs 0/59 solvent controls; 6/35 (17%) malignant lymphoma vs 0/59 solvent controls	–		Beuning *et al.* (1979)
Pyrene									
Mouse, CD-1	M, F	90 or 100	>99% (DMSO)	0, 700 nmol (total dose; given as 1/7, 2/7, 4/7 of total dose on PND 1, 8, 15)	52 wk	**Lung A/AdC** M: 9%, 8%; F: 6%, 10% **Liver A/C** M: 11%, 12%; F: 0%, 0% **Lymphoma** F: 0%, 10%	–		Wislocki *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	90 or 100	>99%, (DMSO)	0, 200, 2800 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung A/AdC** M: 4%, 3%, 7%; F: 0%, 3%, 6% **Liver A/C** M: 7%, 0%, 21%; F: 0%, 0%, 0% **Lymphoma** M: 4%, 0%, 0%; F: 3%, 3%, 6%	–		Wislocki *et al.* (1986)
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	NS	>98% (DMSO)	0, 0.43, 9.65 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	**Lung A/AdC (T/mouse)** M: 13/91 (14%; 0.15 ± 0.04), 4/23 (17%; 0.17 ± 0.08), 2/27 (7%; 0.07 ± 0.05) F: 7/101 (7%; 0.08 ± 0.03), 1/28 (4%; 0.04 ± 0.04), 3/26 (12%; 0.12 ± 0.06)	M, –; F, ±		Busby *et al.* (1989)
Mouse, strain A/J	M	20	99.7% (tricaprylin)	0, 10, 50, 100, 200 mg/kg bw, 1 ×	8 months	No induction of lung T at any of the doses administered	–		Ross *et al.* (1995)

A, adenoma; AdC, adenocarcinoma; C, carcinoma; DMSO, dimethylsulfoxide; F, female; H, hepatoma; M, male; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; SEM, standard error of the mean; T, tumour; TBA, tumour-bearing animal; vs versus wk, week

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

Table 3.8. Carcinogenicity studies of implantations of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Rabbit, NS	NS	9	'Pure' (solid pellet)	4, 5, 10, 12, 20 mg, 1×	20–54 months	0/9	–	Cerebral implant; small no. of animals; no control; no statistics	Russell (1947)
Benz[*a*]anthracene									
Mouse, C57×IF F_1	NS	77	NS (paraffin wax)	~2 mg in a 12.5% suspension	40 wk	17/52 (33%) C (p <0.001), 1/52 (6%) P (p <0.001) vs 4/89 (4%), 1/89 (1%) controls	+	Bladder implantation	Clayson *et al.* (1968)

C, carcinoma; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; vs, versus; wk, week
[a] –, negative; +, positive

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 20 female Swiss albino mice (ICR/BR) [age and weight not specified] received dermal applications of 100 μL of a 0.1% solution of anthracene (purity >99%) 10 times on alternate days (total dose, 1 mg). Ten days after the last application, the mice received 2.5 μg TPA in 100 μL acetone thrice weekly for 20 weeks. Three treated mice (15%) developed skin tumours compared with two mice (10%) treated with acetone only and then TPA (LaVoie *et al*., 1983, 1985).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 40 male and female NMRI mice, 2 days of age, received a single subcutaneous injection of 50 μL of an aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20) that contained 71.3 μg anthracene (400 nmol; 99.9% pure). A control group of 49 male and female mice was treated with the solvent alone. After 40 weeks, 1/12 treated female and 2/17 treated male mice had developed pulmonary tumours compared with 1/19 control females and 1/14 control males (Platt *et al*., 1990)

Intraperitoneal administration (see also Table 3.7)

Mouse

A group of five male Swiss albino mice received a single intraperitoneal injection of 25 mg anthracene in 750 μL olive oil. A control group of six males received olive oil only. After 5 months, no tumours had developed in the surviving four experimental mice or four control mice (Shubik & Della Porta. 1957).

11*H*-Benz[*b*,*c*]aceanthrylene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female CD-1 mice [age and body weight unspecified] were treated on alternate days with 10 subdoses of 0.05, 0.2 and 0.4 μmol [12, 48 and 96 μg] (total doses, 0.5, 2.0 and 4.0 μmol] 11*H*-benz[*b*,*c*]aceanthrylene (purity >99% by HPLC) in 100 μL acetone. A control group of 20 mice was treated with acetone alone. Ten days after the last dose, promotion began with applications of 2.5 μg TPA in 100 μL acetone thrice weekly for 20 weeks. At the end of the study, tumour incidences [type not specified] were 75% (15/20), 90% (18/20) and 90% (18/20) in the low-, mid- and high-dose treatment

groups, respectively, compared with 5% (1/20) in the control group (p <0.005 at all dose levels). The corresponding numbers of tumours/mouse were 1.60, 4.90 and 7.90 compared with 0.05 in the control group (Rice *et al*., 1988).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 15 male C3H mice, 3–5 months of age [body weight unspecified], received a single subcutaneous injection of 1 mg 11*H*-benz[*b*,*c*]aceanthrylene [purity unspecified] in 500 μL tricaprylin. The mice were monitored weekly and kept alive as long as possible; upon death, autopsies were performed and all tissues suggestive of neoplasia were examined histologically. No control group was used in the study. At 6 months, the survival rate was 14/15. At 539 days, one mouse had developed three tumours (a pulmonary adenoma, a hepatic adenoma and a benign haemangioma of the rump) (Dunlap & Warren, 1946).

Benz[*j*]aceanthrylene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female SENCAR mice, 7 weeks of age, received a single dermal application of 40, 200 or 400 μg benz[*j*]aceanthrylene [purity not specified] in 200 μL acetone. One week later, the mice were treated twice weekly for 21 weeks with 2 μg TPA in 200 μL acetone. A control group of 20 mice received TPA only. At the end of the study (22 weeks), the incidence of skin papillomas was 100% in each group treated with benz[*j*]aceanthrylene compared with ~5% in the TPA-treated control group (Nesnow *et al*., 1993).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 27 male A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 20, 50 or 100 mg/kg bw benz[*j*]aceanthrylene [purity not specified] in tricaprylin. A control group received tricaprylin alone. At the end of the study (after 8 months), the incidence of lung tumours (primarily adenomas) was 100% in each group treated with benz[*j*]aceanthrylene compared with 56% in the solvent-treated control group. The number of tumours/mouse was 0.85, 60.3, 140.6 and 97.6 in the 0-, 20-, 50- and 100-mg/kg bw benz[*j*]aceanthrylene-treated groups, respectively (Mass *et al*., 1993; Nesnow *et al*., 1998a).

Benz[*l*]aceanthrylene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20–22 male and 20–22 female SENCAR mice, 7 weeks of age, received a single dermal application of 50, 100, 250, 500 or 1000 nmol [12.6, 25.2, 63.1, 126 or 252 μg] benz[*l*]aceanthrylene [purity not specified] in 200 μL acetone or the solvent alone. One week later, all mice were treated twice weekly for 30 weeks with 2 μg TPA in 200 μL acetone. The experiment lasted 31 weeks. At the end of the study, the incidence of skin papillomas [histology not specified] was 12/20 (1.4 papillomas/mouse), 16/17 (2.3 papillomas/mouse), 21/21 (8.4 papillomas/mouse),16/16 (10.8 papillomas/mouse) and 19/20 (8.7 papillomas/mouse) male mice treated with 12.6, 25.2, 63.1, 126 or 252 μg benz[*l*]aceanthrylene compared with 0/20 control male mice. In females, the incidence of skin papillomas was 13/20 (1.1 papillomas/mouse), 18/19 (3.1 papillomas/mouse), 19/21 (4.7 papillomas/mouse), 20/21 (6.6 papillomas/mouse) and 20/20 (10.8 papillomas/mouse) after treatment with 12.6, 25.2, 63.1, 126 or 252 μg benz[*l*]aceanthrylene compared with 1/19 (0.05 papillomas/mouse) controls (Nesnow *et al*., 1984a).

Benz[*a*]anthracene

Previous evaluations

Benz[*a*]anthracene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated multiple bioassays in which benz[*a*]anthracene was administered orally to mice and dermally to mice, rats and hamsters, injected subcutaneously into adult and newborn mice or intramuscularly and intravenously into rats and implanted into the bladders of mice. The transfer of injection sites following subcutaneous injections to mice was also analysed. Among these studies (which are summarized in Tables 3.1–3.3, 3.6, 3.9 and 3.10), some gave negative results, while others were positive. Benz[*a*]anthracene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that benz[*a*]anthracene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

Two groups of 40 female Swiss mice, 7 weeks of age [body weight unspecified], received twice-weekly dermal applications of 0.396 μmol [90.4 μg] benz[*a*]anthracene (recrystallized; purity verified by melting-point) in 16.7 μL acetone or solvent alone for

Table 3.9. Carcinogenicity studies of intravenous administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Mouse, strain A	M, F	10–11	NS (water)	250 µg suspended in 250 µL, 1×	8, 14 or 20 wk	8 wk, 0/10; 14 wk, 2/10 (20%); 20 wk, 2/11 (18%) (lung T, type NS)	±	No control	Andervont & Shimkin (1940)
Rat, Sprague-Dawley	F	28	NS (lipid NS)	0.25% (w/v), 2 mg (~13 mg/kg), 3× (on days 50, 53, 56 of age)	98 days	0/28	–	No control	Pataki & Huggins (1969)
Dibenz[*a*,*h*]anthracene									
Mouse, strain A	M, F	10, 20 controls	Melting-point (aqueous suspension)	250 µg in 250 µL, 1×	20 wk	10/10 (100%) pulmonary T vs 4/19 (21%) solvent controls	+	No statistics	Andervont & Shimkin (1940); Shimkin & Stoner (1975)
Mouse, strain A	M, F	44–55	NS (aqueous colloidal suspension)	100, 200, 300, 400, 500 µg, 1×	6 months	Lung T/mouse: 100 µg, 8.08 ± 0.542; 200 µg, 18.25 ± 1.225; 300 µg, 30.02 ± 1.663; 400 µg, 38.64 ± 1.923; 500 µg, 53.37 ± 2.166 vs 0.29 ± 0.033 in water controls	+	No histology	Heston & Scheidermann (1953)

F, female; M; male; NS, not specified; PAH, polycyclic aromatic hydrocarbon; T, tumour; vs, versus; wk, week; w/v, weight/volume

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

Table 3.10. Carcinogenicity studies of intramuscular administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Rat, Long-Evans	M	16	NS (sesame oil)	2.5 mg in 500 µL, 1×	270 days	0/16	–	No control	Pataki & Huggins (1969)
Dibenz[*a*,*h*]anthracene									
Fowl, NS	NS	31	NS (lard)	4 mg (volume NS), 1 ×	45 months	15/31 (48%; S)	+	No control	Peacock (1935)
Pigeon, NS	M, F	121, 32 controls	NS (benzene)	3 mg in 100 µL, 1×	13 months	14/109 (13%; fibroS) vs 0/32 untreated controls	+	No solvent-treated controls; no statistics	Prichard *et al.* (1964)
Dibenzo[*a*,*h*]pyrene									
Rat, NS	NS	4	NS (sunflower seed oil)	0.5–1.0 mg, 1×	7–8 months	2/4 (50%)		No control; small number; no statistics	Voronjansky *et al.* (1939)
Rat, NS	F	6	NS (rabbit fat)	0.5 mg, 1×	8 months	2/6 (33%)		No control; small number; no statistics	Pisareva *et al.* (1940)

F, female; M, male; NS, not specified; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; vs, versus
[a] –, negative; +, positive

30 weeks. The animals were observed until death or were killed when moribund. Mean survival rates were 65 ± 15 weeks (mean ± SD) in the benz[*a*]anthracene-treated group and 65 ± 11 weeks in the vehicle-control group. At the end of the experiment, 1/39 benz[*a*]anthracene-treated mice had developed a skin papilloma compared with 0/29 acetone-treated controls (Cavalieri *et al*., 1977).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female CD-1 mice, 8 weeks of age, received a single dermal application of 2.2 μmol [502 μg] chromatographically purified benz[*a*]anthracene in benzene [concentration unspecified], followed 1 week later by twice-weekly applications of 10 μmol TPA for 34 weeks. A control group of 30 female mice of the same strain was treated with 10 μmol TPA only [the dose of TPA was probably 10 μg] twice weekly for 34 weeks. At week 35, 29/30 benz[*a*]anthracene-treated animals and all controls were still alive. At that time, 62% [18/29] of benz[*a*]anthracene-treated mice had developed skin papillomas; one skin tumour had occurred in 1/30 (3%) TPA controls by week 25, but this had regressed by the end of the study, at which time no tumours were found in the control group (Scribner, 1973).

A group of 30 female CD-1 mice, 7–9 weeks of age [body weight unspecified], received a single dermal application of 2 μmol benz[*a*]anthracene [457 μg; volume unspecified]. One week later, mice received twice-weekly applications of 10 μg TPA in acetone [volume unspecified] for 26 weeks. A control group was treated with TPA alone. At the end of the study, 57% of the mice in the benz[*a*]anthracene-treated group had developed skin papillomas (1.2 papillomas/mouse) compared with 6% (0.1 papillomas/mouse) of the TPA-treated controls (Slaga *et al*., 1978).

Groups of 30 female CD-1 mice, 8 weeks of age [body weight unspecified], received a single dermal application of 0, 1.0 or 2.5 μmol [228 or 571 μg] benz[*a*]anthracene in 200 μL acetone. Beginning 1 week later, animals received twice-weekly applications of 16 nmol [9.9 μg] TPA in 200 μL acetone for 27 weeks. At the end of the study, 17% of the mice in the low-dose group and 38% of the mice in the high-dose group had developed skin tumours [type not specified] compared with 4% of the TPA-treated controls. The corresponding numbers of tumours/mouse in the benz[*a*]anthracene-treated groups were 0.17 ± 0.07 and 0.67 ± 0.17 (mean ± standard error (SE)) compared with 0.04 ± 0.04 in the TPA-treated controls (Wood *et al*., 1980).

In a similarly designed initiation-promotion study in CD-1 mice that used initiating doses of 0.4 or 2.5 μmol [91 or 571 μg] benz[*a*]anthracene and promotion with TPA for 25 weeks, skin tumours [type not specified] were found in 14% of the mice treated with 0.4 μmol (0.14 ± 0.07 tumours/mouse) and 36% of the mice treated with 2.5 μmol (0.64 ± 0.20 tumours/mouse) compared with 7% (0.07 ± 0.05 tumours/mouse) in the TPA-treated control group. At the highest dose, both the tumour incidence and the number of

tumours/mouse were significantly higher than those in the control group ($p < 0.05$) (Levin *et al*., 1984).

Buccal pouch application (see also Table 3.11)

Hamster

Two groups of 26 male Syrian golden hamsters [age unspecified] received twice-weekly applications of a 20 mM solution of benz[*a*]anthracene in paraffin oil [volume unspecified] to the buccal pouch for either 5 or 20 weeks. A group of 20 control animals was available. The animals were monitored for up to 44 weeks, with no evidence of tumours in any of the groups (Solt *et al.*, 1987).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 140 newborn male and female Swiss Webster BLU:Ha (ICR) mice received a total intraperitoneal dose of 0 (controls) or 2800 nmol [63.9 μg] benz[*a*]anthracene in DMSO [volume not specified] (1/7 of the dose was administered on postnatal day 1, 2/7 on postnatal day 8 and 4/7 on postnatal day 15). A control group of 100 mice was treated with DMSO alone. The incidence of pulmonary tumours was 10/47 treated males (0.26 tumours/mouse), 7/43 control males (0.19 tumours/mouse), 4/38 treated females (0.18 tumours/mouse) and 2/24 control females (0.08 tumours/mouse) (Wislocki *et al.*, 1979).

Groups of 90 newborn male and female BLU:Ha(ICR) mice received intraperitoneal injections of a total dose of 0 (controls) or 2.8 μmol [639 μg] benz[*a*]anthracene [purity >99%] in 35 μL DMSO [1/7, 2/7 and 4/7 of the dose on days 1, 8 and 15 of life, respectively] and were monitored for 26–32 weeks. A control group of 80 mice was treated with DMSO alone. The incidence of pulmonary adenomas was 63% [17/27] in treated males (1.44 tumours/mouse), 4% [1/28] in control males (0.04 tumours/mouse), 64% [14/22] in treated females (2.00 tumours/mouse) and 11% [4/37] in control females (0.11 tumours/mouse). Hepatic tumours (mostly type A and neoplastic nodules) were observed in 8% [2/27] of treated males (0.08 tumours/mouse) and 4% [1/28] of control males (0.04 tumours/mouse) but not in treated or control females. A significant increase in the incidence and number of pulmonary tumours per mouse compared with the controls was found when the results from male and female mice were combined (Levin *et al*., 1984).

A group of newborn male and female CD-1 mice received a total intraperitoneal dose of 2.8 mmol benz[*a*]anthracene in DMSO [1/7 on postnatal day 1, 2/7 on postnatal day 8 and 4/7 on postnatal day 15]. The incidence of liver tumour was 79% (6/39 adenomas and 25/39 carcinomas; 3.0 nodules/tumour-bearing animal) in treated male mice compared

Table 3.11. Carcinogenicity studies of application to the buccal pouch of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Hamster, Syrian golden	M	26, 20 controls	NS (paraffin oil)	20 mM, 2×/wk, 5 or 20 wk	Up to 44 wk	0% vs 0% in solvent-treated controls	–		Solt *et al.* (1987)
Benzo[*a*]pyrene									
Hamster, Syrian golden	M	28, 20 controls	NS (paraffin oil)	0, 20 mM [5046 mg]/animal, 2×/wk, 20 wk	Up to 40–44 wk with interim kills after 5, 20 and 24–32 wk	Forestomach P: only in treated animals killed after 40–44 wk: 80% [8/10] Buccal pouch tumours: only 1 (a SCC) in a treated animal killed in wk 41 Positive (DMBA) controls: 100% [19/19] forestomach (100% P, 68% SCC), 100% [19/19] buccal pouch (a total of 199 well-differentiated SCC) in animals surviving for 20 wk	+		Solt *et al.* (1987)

DMBA, 7,12-dimethylbenz[*a*]antracene; M, male; NS, not specified; P, papilloma; SCC, squamous-cell carcinoma; vs, versus; wk, week

[a] –, negative; +, positive

with 7% (2/28 adenomas and 0/28 carcinomas; 1.0 nodule/tumour-bearing animal) in control males (p <0.05 for liver carcinomas and for combined liver tumours); that of pulmonary tumours in treated males was 15% (5/39 adenomas and 1/39 carcinoma) compared with 4% (1/28 adenoma and no carcinomas) in control males. No liver tumours were observed in treated females which had an incidence of pulmonary tumours of 19% (6/32 adenomas and no carcinomas) compared with 0% in the control group (p <0.025) (Wislocki *et al*, 1986).

Intratratracheal administration (see also Table 3.12)

Hamster

A group of 48 male Syrian golden hamsters, 9–10 weeks of age (average weight, 98 g), was treated intratracheally with 30 weekly doses of 0.5 mg benz[a]anthracene [purity > 99% by thin layer chromagraphy; total dose, 15 mg] that was ground to a finely aggregated dust [1:1, w:w] with haematite [particle size < 1 μm] and then suspended in 200 μL saline [0.9% aqueous]. A second group of 36 male hamsters of the same strain and age was treated in a similar manner with 15 weekly doses of 3.0 mg benz[a]-anthracene [total dose, 45 mg] and an additional group of 90 hamsters remained untreated. No control group received intratracheal instillation of the vehicle alone. The animals were monitored daily, weighed once a week, died spontaneously or were killed when moribund. At 120 weeks, only one animal remained in the 3.0-mg treatment group and all animals in the remaining groups had died. Tumours of the respiratory tract were not observed in any of the groups (Sellakumar & Shubik, 1974).

Intramammary administration (see also Table 3.5)

Rat

A group of 20 female pathogen-free Sprague-Dawley rats, 50 days of age [body weight unspecified], was subjected to a small incision over the right fifth inguinal mammary gland, which was exposed, had finely powdered benz[*a*]anthracene (purity >99% by HPLC; 4 or 16 μmol) [913 μg or 3.65 mg, respectively] dispersed over it and was subsequently closed. The untreated contralateral left gland served as a negative control. All animals were killed at 20 weeks. No mammary tumours were present in the benz[*a*]anthracene-treated animals (Cavalieri *et al.,* 1988a).

Table 3.12. Carcinogenicity studies of intratracheal administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Hamster, Syrian golden	M	36 or 48; 90 controls	>99% (0.9% saline)	Ground with haematite [1:1, <1 μm particles] in 200 μL; 0.5 mg 1×/wk, 30 wk [total dose, 15 mg], 3.0 mg, 1×/wk, 15 wk [total dose, 45 mg]	120 wk	15 mg: 0/47 (respiratory tract T), 6/47 (13%; other T); 45 mg, 0/33, 6/33 (18%) vs 0/82, 11/82 (13%) untreated controls	–	Control group not submitted to tracheal instillation of the vehicle	Sellakumar & Shubik (1974)
Benzo[*a*]pyrene									
Mouse, Iva; NMRI	F	NS	NS (0.9% saline solution)	0 (untreated), 0 (0.9% saline solution), 50 μg/animal, 1×/wk, 20 wk	2 years	Lung T: [9/28] (32%; 0.7 ± 1.7 T/animal), [30/55] (54%; 0.8 ± 1.0 T/animal), [28/50] (56%; 2.2 ± 3.7 T/animal)	+	Numbers not given; no histopathology; statistics NS	Heinrich *et al.* (1986a)
Mouse, *XPA*$^{-/-}$, *XPA*$^{+/-}$, *XPA*$^{+/+}$	F	5 or 30	NS (gelatine/ physiological saline)	0, 0.1 mg/ animal, 1×/wk, 4 wk	16 mo	**Lung T** *XPA*$^{-/-}$: 0/5, 15/21 (71%; 13 A, 2 C; 1.4 ± 0.3 T/animal) *XPA*$^{+/-}$: 0/5, 11/27 (41%; 11 A; 0.8 ± 0.2 A/animal) *XPA*$^{+/+}$: 0/5, 7/20 (35%; 7 A; 0.4 ± 0.1 A/animal)	+		Ide *et al* (2000)
Rat, Wistar-WU/Kisslegg	F	NS	NS (0.9% saline solution)	0, 1 mg/animal, 1×/wk, 20 wk	124–126 wk	Lung T: 0/40, 7/36 (19%; 1 A, 5 SCC, 1 mixed AdC/SCC)	+		Pott *et al.* (1987)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Sprague-Dawley	M, F	20 or 50	NS (physiological saline solution with or without Tween 60)	0, 0 (physiological saline), 7 mg/kg bw/ instillation (physiological saline with Tween 60), 1×/2 wk, 44 wk	Controls, 131 wk; treated animals, 112 wk	M: 0/50, 0/50, 19/20 (95%; 19 malignant lung T) F: 0/50, 0/50, 19/20 (95%; 18 malignant, 1 benign lung T)	+	Limited histology; type of lung T NS	Steinhoff *et al.* (1991)
Hamster, Syrian golden	M, F	35	>99% (0.9% saline solution)	0 (only for M), 1 mg/animal, 1×/wk, 36 wk	78 wk	**Respiratory tract T/adenomatoid lesions** M: 6/27 (22%; 1 tracheal P, 5 pulmonary adenomatoid lesion), 19/29 (66%; 1 tracheal P, 17 SCC, 26 pulmonary adenomatoid lesion, 5 A, 1 AdC, 1 SCC) F: 22/27 (81%; 1 laryngeal SCC, 16 tracheal SCC, 2 bronchial A, 1 AdC, 21 pulmonary adenomatoid lesion, 8 A, 1 AdC)	+	No female controls; no statistics	Feron (1972)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	30	NS (0.9% saline solution)	0, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/animal, 1×/wk, 52 wk	78 wk	**Respiratory tract T** 0/29, 3/30 (10%; 3 tracheal P, 1 pulmonary A), 4/30 (13%; 1 tracheal P, 4 pulmonary A), 9/30 (30%; 5 tracheal P, 7 pulmonary A), 25/29 (86%; 2 tracheal polyp, 9 P, 5 SCC, 1 AdSC, 1 fibroS, 2 bronchial polyp, 1 P, 2 SCC, 1 AdSC), 26/28 (93%; 6 tracheal P, 11 SCC, 1 AdSC, 1 bronchial polyp, 2 P, 4 SCC, 2 AdSC, 4 AdC, 1 anaplastic C, 16 pulmonary A, 4 SCC, 3 AdSC, 1 AdC, 2 anaplastic C)	+		Feron *et al.* (1973)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	50, 25 controls	NS (0.5% gelatine in 0.9% saline solution)	0, 13.3–15.5 mg/animal, 1×/wk, 8 wk	M, 67–88 wk; F, 60–88 wk	**Respiratory tract T** Controls [effective no. of animals NS]: 1 tracheal polyp, 6 pulmonary bronchiolar adenomatoid lesions Treated animals: 26/65 (40%; 1 nasal polyp; 6 laryngeal polyps, 1 P, 1 A, 1 AdC, 7 tracheal polyps, 1 AdC, 1 SCC, 1 fibroS, 2 bronchial AdC, 13 pulmonary bronchiolar adenomatoid lesion, 3 A, 5 AdC, 1 SCC, 2 anaplastic C, 1 mixed C, 1 myelogenous leukaemia, 1 neurofibroS) **T at other sites** Controls: 1 renal A Treated animals: 3 blast-cell leukaemia, 2 adrenocortical A, 1 renal AdC, 1 oesophageal fibroS, 1 haemangioma	+	Tumour data for M and F combined; statistics NS	Henry *et al.* (1973)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	20–32 M, 20–28 F	NS (0.9% saline)	0, 1 mg/animal, 1×/wk, 30 wk	60 wk	**Respiratory tract T** M: 0/20, 11/26 (42.3%; 1 laryngeal polyp, 1 tracheal polyp, 1 P, 1 bronchial SCC, 9 lung A, 7 AdC, 3 SCC, 1 anaplastic C, 2 AdSC) F: 0/20, 14/26 (53.8%; 1 laryngeal P, 2 tracheal polyps, 1 bronchial SCC, 10 lung A, 3 AdC, 1 SCC)	+		Kobayashi (1975)
Hamster, Syrian golden	M, F	17 or 40	>99% (saline solution)	0 (untreated), 0 (vehicle controls), 1 mg/animal, 1×/2 wk, 52 wk	78 wk	**Respiratory tract T** M: 0/40, 0/40, 13/14 (93%; 2 laryngeal P, 1 SCC, 4 tracheal P, 3 SCC, 1 anaplastic C, 1 S, 1 bronchial SCC, 1 AdC, 5 pulmonary A, 1 AdC) F: 0/40, 0/40, 7/12 (58%; 2 tracheal P, 3 SCC, 1 bronchial P, 5 pulmonary A)	+		Kruysse & Feron (1976)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	48	>99% (0.9% saline solution)	0 (untreated) or 3 mg/animal, 1×/wk, 10 wk	100 wk	**Respiratory tract T** 0/48, 7/48 (15%; 2 laryngeal P, 4 tracheal P, 1 lung A) **T at other sites** 6/48 (13%; 3 forestomach P, 2 lymphoma, 1 anaplastic C), 26/48 (54%; 21 forestomach P, 1 skin melanoma, 1 liver haemangioma, 1 adrenocorticoA, 3 adrenocorticoC)	+		Sellakumar *et al.* (1976)
Hamster, Syrian golden	M, F	30	97% (0.9% saline solution or Tris buffer)	Experiment 1: 0, 4, 8, 16 mg in 0.9% saline solution/animal, 1×	Up to 89 wk for M and 70 wk for F	**Respiratory tract T** *Experiment 1*: M: 0/24, 3/30 (10%; 1 laryngeal P, 1 tracheal P, 1 lung S), 5/28 (18%; 1 laryngeal SCC, 1 tracheal P, 4 lung S), 4/27 (15%; 3 tracheal P, 1 lung A, 1 S) F: 0/28, 3/29 (10%; 1 tracheal P, 2 lung A), 1/30 (3%; 1 lung A), 3/28 (13%; 1 laryngeal P, 2 lung A)	+		Kektar *et al.* (1977)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
				Experiment 2: 0, 4, 8, 16 mg in Tris buffer/ animal, 1×	Up to 83 wk for M and 68 wk for F	*Experiment 2*: M: 0/27, 5/24 (21%; 1 tracheal P, 5 lung A), 13/25 (52%; 1 laryngeal P, 7 tracheal P, 4 lung A, 3 AdC), 8/27 (30%; 2 laryngeal P, 1 SCC, 3 tracheal P, 3 lung A) F: 0/27, 3/27 (11%; 2 tracheal P, 1 lung AdC), 2/29 (7%; 2 tracheal P), 8/29 (28%; 1 laryngeal P, 4 tracheal P, 5 lung A)			Ketkar *et al.* (1977) (contd)
Hamsters, Syrian golden	M, F	15 or 30	>99% (0.9% saline solution)	0 (untreated), 0 (vehicle controls), 0.35, 0.7 mg/animal, 1×/wk, 52 wk	81 wk	**Respiratory tract T** M: 0/30 (untreated and vehicle controls combined), 4/29 (14%; 2 tracheal P, 1 bronchial P, 2 pulmonary A), 19/30 (63%; 1 laryngeal P, 5 tracheal P, 1 SCC, 1 anaplastic C, 1 S, 2 bronchial P, 1 AdC, 2 pulmonary A) F: 0/28 (untreated and vehicle controls combined), 3/27 (11%; 1 laryngeal P, 1 bronchial P, 1 pulmonary A), 7/24 (29%; 1 tracheal P, 2 SCC, 1 bronchial AdC, 5 pulmonary A)	+	Statistics NS	Feron & Kruysse (1978)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	30	97% (10% bovine serum albumin)	0, 0.1, 0.33, 1.0 mg/animal, 1×/wk for life	Average survival up to 41 wk for M and 35 wk for F	**Respiratory tract T** M: 0/29, 5/26 (19%; 5 bronchiogenic A), 7/29 (24%; 5 tracheal P, 2 bronchiogenic A), 6/27 (22%; 5 tracheal P, 2 bronchiogenic A) F: 0/30, 12/30 (40%; 1 tracheal P, 1 SCC, 10 bronchiogenic A), 10/28 (36%; 7 tracheal P, 5 bronchiogenic A, 1 SCC), 6/30 (20%; 3 tracheal P, 3 bronchiogenic A, 3 SCC)	+	Average survival time drastically lower in the high-dose group than in the other groups	Ketkar *et al.* (1978)
Hamster, Syrian golden	NS	NS	NS (0.4% Tween 80 in saline solution)	0, 0.3, 0.9 mg/animal, 1×/wk, 20 wk	91 wk	Benign and malignant respiratory tract T: 3, 17, 68%	+	Type of T not further specified	Pott *et al.* (1978)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	48 (M + F)	99.4% (0.9% saline solution); particle size by weight: large: 98% <30 μm, 90% <20 μm, 36% <10 μm, 10% <5 μm; small: 98% <10 μm, 79% <5 μm, 5% <1 μm	0, 3 mg large particles, 3 mg small particles/ animal, 1×/wk, 18 wk	Lifespan, up to 90 wk	Respiratory tract T (M + F combined): 0/46, 31/47 (66%; 5 laryngeal P, 12 tracheal P, 20 SCC, 2 unspecified T, 2 bronchial P, 9 SCC, 3 A, 2 anaplastic C), 5/46 (11%; 1 laryngeal P, 1 SCC, 4 tracheal P)	+		Stenbäck & Rowland (1978)
Hamster, Syrian golden	M, F	70 (M + F)	NS (0.9% saline solution)	0, 0.5 mg/ animal, 1×/2 wk, 52 wk	104 wk	**Respiratory tract T** M: no T in 24 controls and 20 T (1 tracheal polyp, 6 P, 3 SCC, 1 anaplastic C, 1 bronchial polyp, 1 SCC, 7 pulmonary A) in treated animals F: no T in 25 controls and 18 T (8 tracheal P, 3 SCC, 6 pulmonary A, 1 AdC) in treated animals	+	No. of TBA NS	Feron (1979)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	30	97% (Tris buffer + 0.9% saline solution); particle size: majority <10 μm but particles up to 80 μm also present	0 (untreated), 0 (vehicle controls), 0.125, 0.25, 0.5, 1.0 mg/animal, 1×/wk, for life	Average survival up to 88 wk	Respiratory tract T: 0/29, 0/28, 9/29 (31%; 2 laryngeal polyps/P, 1 tracheal P, 1 SCC, 2 lung A, 2 SCC, 5 AdC), 24/29 (83%; 1 nasal SCC, 2 laryngeal polyps/P, 4 tracheal P, 9 SCC, 5 lung A, 5 SCC, 11 AdC), 19/29 (66%; 1 laryngeal P, 2 SCC, 5 tracheal P, 11 SCC, 7 lung SCC, 2 AdC), 9/29 (31%; 1 laryngeal P, 1 SCC, 1 tracheal P, 5 SCC, 1 lung A, 4 SCC) (p <0.001)	+	Average survival in two highest-dose groups very much lower than that in the other groups due to many early deaths from pulmonary lesions other than tumours	Ketkar *et al.* (1979)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	30–35	NS; particles size by weight: fine, 77% <5.2 μm, 60% <3.9 μm; coarse, 77% <42 μm, 3% <16 μm; wide-range, 72% <30 μm, 19% <10 μm (gelatine in 0.9% saline solution)	0 (untreated), 0 (gelatine in 0.9% saline), 0.5, 1.0 mg fine particles, 0.5, 1.0 mg coarse particles, 1.0 mg wide-range particles/animal, 1×/wk, 52 wk	105 wk	**Respiratory tract T** M: 0/29**,** 2/34 (6%; 2 laryngeal P), 7/34 (21%; 1 laryngeal P, 6 tracheal P, 1 lung A), 6/31 (19%; 2 laryngeal P, 1 tracheal P, 1 S,1 pulmonary A), 13/31 (42%; 2 laryngeal P, 3 tracheal P, 9 pulmonary A), 25/34 (74%; 2 laryngeal P, 9 tracheal P, 4 SCC, 2 S, 1 pulmonary A, 1 AdC), 23/34 (68%; 2 laryngeal P, 1 SCC, 6 tracheal P, 2 SCC, 1 bronchial P, 1 SCC, 13 pulmonary A, 2 AdC, 2 anaplastic C) F: 0/28, 2/33 (6%; 1 tracheal P, 1 pulmonary A), 2/34 (6%; 1 bronchial P, 1 A), 5/32 (16%; 1 laryngeal P, 2 tracheal P, 3 pulmonary A), 9/32 (28%; 2 laryngeal P, 5 tracheal P, 6 pulmonary A), 19/32 (31%; 4 tracheal P, 1 SCC, 1 S, 1 bronchial P, 7 pulmonary A, 1 AdC), 11/34 (34%; 1 laryngeal P, 3 tracheal P, 2 bronchial P, 7 pulmonary A, 1 AdC)	+	Statistics NS	Feron *et al.* (1980)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	30	NS (Tris-buffer/saline)	0 (untreated), 0 (vehicle control), 5, 20, 40 µg/animal, 1×/2 wk for life	Mean survival time, 67 (low-dose) to 84 wk (high-dose)	0/29, 0/30, 0/28, 0/27, 2/28 (7%) (1 lung A, 1 respiratory tract mucoepidermoid C)	±	Statistics NS	Künstler (1983)
Hamster, Syrian golden	M	80	>99% (0.5% gelatine in 0.9% saline solution)	0, 5 mg/animal, 1×/wk, 15 wk	129 wk	Malignant T: 4/80 (5%; 1 multicentric undifferentiated lung C, 3 lymphoma), 25/80 (31%; 9 SCC, 2 undifferentiated C of the respiratory tract, 5 lymphoma, 1 SCC, 2 AdC of the gastrointestinal tract, 2 soft-tissue T, 1 hepatoma, 2 mouth SCC, 1 skin C; $p < 0.001$)	+	No. of TBA NS	Godleski *et al*. (1984)
Hamster, Syrian golden	M, F	35	NS (gelatine in saline solution)	0, 1 mg/animal, 1×/2 wk, 52 wk	85 wk	**Respiratory tract T** M: 0/31, 6/ 34 [18%] (3 tracheal P, 1 SCC, 1 S, 1 bronchial P); F: 0/28, 1/29 [3%] (1 laryngeal C *in situ*)	+	Statistics NS	Feron *et al.* (1985)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Hamster, Syrian golden	M	48, 90 controls	>99% (saline)	50, 250 µg (combined with an equal amount of ferric oxide) in 200 µL, 1×/wk, 30 wk	≤120 wk	Respiratory tract T: 50 µg, 0/46; 250 µg, 2/46 (4%) vs 0/82 untreated controls	–		Sellakumar & Shubik (1974)
Hamster, Syrian golden	NS	NS	NS (saline solution containing 0.4% Tween 80)	300, 900 µg in 150 µL, 1×/wk, 20 wk	≤2 years	Respiratory tract T: 300 µg, 55%; 900 µg, 65% vs 3% solvent controls	+	No statistics	Pott *et al.* (1978)
Dibenzo[*a*,*i*]pyrene									
Hamster, Syrian golden	M	36 or 48, 90 controls	>99% (0.9% saline)	Ground with haematite [1:1, <1 µm particles] in 200 µL, 2 mg 1×/wk, 4 wk (total dose, 8 mg); 500 µg, 1×/wk, 24 wk (total dose, 12 mg)	100 wk; controls, 120 wk	8 mg, 16/34 (47%; respiratory tract T, predominantly SCC; 1/34 (3%) larynx, 2/34 (6%) trachea, 13/34 (38%) bronchi, 1/34 (3%) lung); 12 mg, 39/44 (89%; respiratory tract T, predominantly SCC; 6/44 (14%) trachea, 37/44 (84%) bronchi, 1/44 (2%) lung, 2/44 (4%) malignant lymphoma) vs 0/82 (respiratory tract T), 11/82 (13%; T at other sites) untreated controls	+	Control group not submitted to tracheal instillation of the vehicle; no statistics	Sellakumar & Shubik (1974)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	48	>99% (water)	1 mg, 1×/wk, 12 wk (total dose, 12 mg); 500 µg, 1×/wk, 17 wk (total dose, 8.5 mg) [volume NS]	NS	12 mg, 36/48 (respiratory tract T, predominantly SCC; bronchi, 62%; trachea, 19%); 8.5 mg, 39/48 (respiratory tract T, predominantly SCC; bronchi, 82%; trachea, 13%; larynx, lung and pleura T also observed)	+	No control; no statistics	Stenbäck & Sellakumar (1974)

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; C, carcinoma; F, female; M, male; mo, month; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; TBA, tumour-bearing animal; vs, versus; wk, week

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

Benzo[*b*]chrysene

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female CD-1 mice, 8 weeks of age, received a single dermal application of 2.5 μmol [695 μg] benzo[*b*]chrysene (chromatographically purified) in benzene [concentration unspecified], followed 1 week later by twice-weekly applications of 5 μmol TPA (total dose, 10 μmol) for 34 weeks. A control group of 30 female mice of the same strain was treated twice weekly with 10 μmol TPA only for 34 weeks. At week 35, 29/30 benzo[*b*]chrysene-treated mice and all controls were still alive. At that time, 48% (14/29) of benzo[*b*]chrysene-treated mice had developed skin papillomas; only one skin tumour had occurred in 3% (1/30) of TPA controls by week 25, but this had regressed by the end of the study, at which time no tumours were found in the control group (Scribner, 1973).

Benzo[*g*]chrysene

No data were available to the Working Group.

Benzo[*a*]fluoranthene

Dermal initiation–promotion (see also Table 3.2)

Mouse

In a mouse skin initiation–promotion study, groups of 20 female CD-1 mice, 50–55 days of age, received 10 subdoses on alternate days of benzo[*a*]fluoranthene in 100 μL acetone (total initiating doses, 0, 1.0 or 4.0 μmol). Ten days later, 2.5 μg TPA in 100 μL acetone were applied thrice weekly for 20 weeks. The incidence of tumour-bearing animals was 95% (19/20) and 90% (18/20) with averages of 3.3 and 4.3 skin tumours/mouse in the low- and high-dose groups, respectively. Papillomas occurred in 10% (2/20) of TPA controls with an average of 0.1 tumours/mouse (Weyand *et al*., 1990).

Benzo[*b*]fluoranthene

Previous evaluation

Benzo[*b*]fluoranthene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated three bioassays in which the compound was administered dermally or subcutaneously to mice. On the basis of the available data, the Working Group concluded that benzo[*b*]fluoranthene induced skin tumours and local sarcomas. Benzo[*b*]fluoranthene was also considered in February 1983 (IARC, 1983) by a Working

Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that benzo[*b*]fluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since the previous evaluation are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In separate skin initiation–promotion studies with female outbred albino Crl:CD-1 (ICR) BR mice, total initiating doses of 0–100 nmol (Amin *et al.*, 1985a, 1991), 0–100 μg (LaVoie *et al.*, 1982a) and 0–400 nmol (LaVoie *et al.*, 1993) benzo[*b*]fluoranthene resulted in dose-related increases in the numbers of mice with skin papillomas. Similar results were obtained in female outbred albino Crl:CD-1 (ICR) BR mice with total initiating doses of 100 or 400 nmol benzo[*b*]fluoranthene (Geddie *et al.*, 1987), and in other studies in female CD-1 mice with total initiating doses of 0, 30 or 100 μg (Weyand *et al.*, 1989) and 0, 1.0 or 4.0 μmol benzo[*b*]fluoranthene (Weyand *et al.*, 1990).

Intraperitoneal administration (see also Table 3.7)

Mouse

Intraperitoneal injection of 0 or 0.5 μmol benzo[*b*]fluoranthene into newborn CD-1 mice induced lung and liver adenomas in treated males and lung adenomas in treated females (LaVoie *et al.*, 1987). A single injection of 0, 10, 50, 100 or 200 mg/kg bw benzo[*b*]fluoranthene into male strain A/J mice resulted in dose-related increases in the incidence and multiplicity of lung adenomas (Nesnow *et al.*, 1995; Ross *et al.*, 1995; Mass *et al.*, 1996; Nesnow e*t al.*, 1998a).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 0.1, 0.3 or 1.0 mg benzo[*b*]fluoranthene into Osborne-Mendel rats induced dose-related increases in the incidence of pulmonary squamous-cell carcinomas and sarcomas (Deutsch-Wenzel *et al.*, 1983).

Benzo[*ghi*]fluoranthene

Benzo[*ghi*]fluoranthene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one study in which female mice were treated by dermal application; no skin tumours were observed. The Working Group concluded that the

available data were inadequate to permit an evaluation of the carcinogenicity of benzo[*ghi*]fluoranthene in experimental animals. No new studies were available.

Benzo[*j*]fluoranthene

Previous evaluation

Benzo[*j*]fluoranthene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated a bioassay in which the compound was administered dermally to mice and concluded that benzo[*j*]fluoranthene induced a high incidence of skin tumours. It was also considered by a Working Group in February 1983 (IARC, 1983) that, in addition to the previous assay, evaluated studies in which benzo[*j*]fluoranthene was administered dermally to mice (repeated administration and initiation–promotion protocols) and by intrapulmonary injection to rats. The Working Group concluded that there was *sufficient evidence* that benzo[*j*]fluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In initiation–promotion studies in female CD-1 mice, total initiating doses of 0–3.0 μmol (Rice *et al.*, 1987), 0–2.0 μmol (Weyand *et al.*, 1992) and 0–1000 nmol (LaVoie *et al.*, 1993) benzo[*j*]fluoranthene resulted in dose-related increases in the incidence and multiplicity of skin papillomas. In female Crl:CD-1 (ICR)BR mice, total initiating doses of 0–1000 μg also resulted in dose-related increases in the incidence and multiplicity of skin papillomas (LaVoie *et al.*, 1982a).

Intraperitoneal administration (see also Table 3.7)

Mouse

In two studies in male and female newborn CD-1 mice, intraperitoneal injection of benzo[*j*]fluoranthene at doses of 0–1.1 μmol increased the incidence and multiplicity of lung (adenomas) and liver (hepatomas) tumours (LaVoie *et al*., 1987, 1994a).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 0.2, 1.0 or 5.0 mg benzo[*j*]fluoranthene into 35 Osborne-Mendel rats induced a dose-related increase in the incidence of pulmonary

squamous-cell carcinomas (0, 2.9, 8.6 and 51.4%, respectively) (Deutsch-Wenzel *et al.*, 1983).

Benzo[*k*]fluoranthene

Previous evaluation

Benzo[*k*]fluoranthene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which the compound was administered dermally to mice (repeatedly and initiation–promotion protocols), by subcutaneous injection to mice and by intrapulmonary injection to rats. On the basis of the available data, the Working Group concluded that there was *sufficient evidence* that benzo[*k*]fluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In an initiation–promotion study, total initiating doses of 4 μmol benzo[*k*]-fluoranthene induced squamous-cell papillomas in 37% [effective number not specified] of female CD-1 mice (Amin *et al.*, 1985b).

Intraperitoneal administration (see also Table 3.7)

Mouse

Intraperitoneal injection of 2.1 μmol benzo[*k*]fluoranthene into newborn CD-1 mice induced lung adenomas in 6.3% and 16.7% of males and females, respectively, and liver hepatomas and adenomas in 18.8% of males (LaVoie *et al.*, 1987).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 0.16, 0.83 or 4.15 mg benzo[*k*]fluoranthene into 35 Osborne-Mendel rats induced a dose-related increase in the incidence of pulmonary squamous-cell carcinomas (0, 0, 9.7 and 44.4%, respectively) (Deutsch-Wenzel *et al.*, 1983).

Benzo[*a*]fluorene

Previous evaluation

Benzo[*a*]fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*a*]fluorene was administered dermally and subcutaneously to mice; each of the studies gave negative results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*a*]fluorene to experimental animals. No new studies were available.

Benzo[*b*]fluorene

Previous evaluation

Benzo[*b*]fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated an initiation–promotion bioassay in mice, which gave positive results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*b*]fluorene to experimental animals. No new studies were available.

Benzo[*c*]fluorene

Previous evaluation

Benzo[*c*]fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*c*]fluorene was administered dermally to mice (repeated administration and initiation–promotion protocols). Both studies gave negative results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*c*]fluorene to experimental animals. Additional bioassays that have been published since that time are summarized below.

Oral administration (see also Table 3.6)

Mouse

Groups of 30 female A/J mice, 7 weeks of age, were fed benzo[*c*]fluorene (purity ≥98% by HPLC) at doses of 27 or 397 μmol/kg of diet for up to 260 days, at which time all surviving mice were killed. A group of 30 control mice was fed basal diet. Gross necropsies were performed on the lungs, liver, stomach, small intestine, kidney, ovaries, uterus and urinary bladder, and all stomachs were examined histologically. Lung adenomas were observed in 46% [13/28] of the mice fed 27 μmol/kg (0.57 ± 0.13 tumours/mouse) and 100% [29/29] of the mice fed 397 μmol/kg (46.0 ± 2.8 tumours/mouse) compared with 24% [7/29] of the control mice (0.31 ± 0.11 tumours/mouse). At

the highest dose, the number of tumours per mouse was significantly greater than that observed in the control group (p <0.001). There was a 15% incidence of squamous hyperplasia in the forestomach in both groups treated with benzo[*c*]fluorene compared with 3% in the control group. No carcinomas were found in the forestomach in any of the groups (Weyand *et al*., 2004).

Intraperitoneal administration (see also Table 3.7)

Mouse

A group of 30 female A/J mice, 7 weeks of age, was administered a single intraperitoneal injection of 1.75 mg (100 mg/kg bw; purity ≥98% by HPLC) in 250 μL tricaprylin. A control group of 30 mice received tricaprylin alone. All surviving mice were killed at 260 days. Gross necropsies were performed on the lungs, liver, stomach, small intestine, kidney, ovaries, uterus and urinary bladder, and all stomachs were examined histologically. Lung adenomas were observed in 92% [26/28] of the mice administered benzo[*c*]fluorene (4.0 ± 0.53 tumours/mouse) compared with 48% [12/29] of the controls (0.6 ± 0.14 tumours/mouse). The number of tumours per mouse in the treated group was significantly greater than that observed in the control group (p <0.01). There was an 8% incidence of squamous hyperplasia in the forestomach in the group treated with benzo[*c*]fluorene compared with 0% in the control group. No carcinomas were found in the forestomach in any of the groups (Weyand *et al*., 2004).

Benzo[*ghi*]perylene

Previous evaluation

Benzo[*ghi*]perylene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*ghi*]perylene was administered dermally to mice (repeated administration and initiation–promotion protocols), subcutaneously to mice and by intrapulmonary injection into rats. The study on intrapulmonary injection was considered to be inadequate for evaluation; the other studies gave negative results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*ghi*]perylene to experimental animals. No new studies were available.

Benzo[*c*]phenanthrene

Previous evaluation

Benzo[*c*]phenanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*c*]phenanthrene was administered dermally to mice (repeated administration and initiation–promotion protocols) and by

subcutaneous injection into mice and rats. Benzo[*c*]phenanthrene was considered to be active as a tumour initiator in the initiation–promotion assay. The other bioassays (repeated dermal application to mice and subcutaneous injection into mice and rats) were considered to be inadequate for evaluation. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*c*]phenanthrene to experimental animals. An additional bioassay that has been published since that time is summarized below.

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of male and female newborn CD-1 mice received three intraperitoneal injections of benzo[*c*]phenanthene in DMSO on days 1, 8 and 15 of life (total doses, 0, 50 or 150 nmol). The incidence of lung tumours (number of tumours/tumour-bearing animal) was increased in the high-dose groups: male control, 3% [1/31] (0.06); male low-dose, 6% [2/36] (0.06); male high-dose, 57% [16/28] (1.6); female control, 5% [2/44] (0.07); female low-dose, 9% [3/32] (0.09); and female high-dose, 65% [23/35]. The carcinogenicity of seven suspected activated metabolites of benzo[*c*]phenanthene was also examined. Some of these metabolites substantially increased the incidence of lung tumours in both sexes of mice and those of liver tumours in male mice. The activated metabolites also showed a significant activity in an initiation–promotion experiment in the same study (Levin *et al.*, 1986) and in a previous experiment (Levin *et al.*, 1980).

Benzo[*a*]pyrene

Previous evaluation

This compound was considered by earlier Working Groups in December 1972 (IARC, 1973), October 1982 (IARC, 1982) and February 1983 (IARC, 1983). The Working Group of December 1972 (IARC, 1973) concluded that benzo[*a*]pyrene produced tumours in all species (mouse, rat, hamster, guinea-pig, rabbit, duck, newt, monkey) for which data were reported following exposure by different routes (oral, dermal, inhalation, intratracheal, intrabronchial, subcutaneous, intraperitoneal, intravenous). It had both a local and a systemic carcinogenic effect, was an initiator of skin carcinogenesis in mice and was carcinogenic in single-dose studies and following prenatal and tranplacental exposure. The 1983 Working Group (IARC, 1983) did not evaluate the studies of carcinogenicity in animals published since 1972 but concluded that there is *sufficient evidence* that benzo[*a*]pyrene is carcinogenic to experimental animals. This conclusion was based on the data evaluated by the Working Group of December 1972 (IARC, 1973) and on a number of reviews, such as those of Dipple (1976), Freudenthal and Jones (1976), Bingham *et al.* (1980) and Conney (1982).

Studies described below are illustrative of the various routes of administration used in experimental animals or of various recently developed methods in carcinogenicity testing.

Inhalation exposure (see also Table 3.13)

Hamster

Groups of 24 male Syrian golden hamsters, 8–14 weeks of age, were exposed by inhalation to 0, 2.2, 9.5 or 46.5 mg/m^3 benzo[*a*]pyrene (mixed with an aerosol of 0.1% saline solution) for 4.5 h per day on 7 days per week for 10 weeks and then for 3 h per day for the rest of their life. Tumours (papillomas, polyps and squamous-cell carcinomas) were found in the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, oesophagus and forestomach) of mid- and high-dose animals but not in controls or low-dose animals. The tumour response was dose-related. No bronchogenic tumours were detected (Thyssen *et al*., 1981). In a similar liftetime study with small numbers of animals (three groups of 10 males exposed to 0, 9.8 or 44.8 mg/m^3 benzo[*a*]pyrene) and short exposure periods (10 and 16 weeks for the high- and low-dose groups, respectively), only one tumour was found (a tracheal polyp in a low-dose animal) (Thyssen *et al*., 1980).

Oral administration (see also Table 3.6)

Mouse

Following oral administration of benzo[*a*]pyrene by gavage or in the diet to different strains of mice, increased tumour responses were found in several organs including the lung, forestomach, liver and lymphoreticular system (see Table 3.6). Compared with controls, significant dose-related increases in the incidence of tumours and in the number of tumours per animal were observed in the lung and the forestomach of female strain A/J mice fed a gel diet containing 16 or 98 ppm benzo[*a*]pyrene for 260 days (Weyand *et al*., 1995). In female B6C3F1 mice fed diets containing 0, 5, 25 or 100 ppm benzo[*a*]pyrene for 2 years, significantly increased incidences of squamous-cell papillomas and/or carcinomas were observed in the oesophagus and tongue of high-dose animals and in the forestomach of mid- and high-dose animals (Table 3.6) (Culp *et al*., 1998).

Table 3.13. Carcinogenicity studies of inhalation exposure of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene									
Hamster, Syrian golden	M	10	NS (0.01% saline solution); particle size, generally 0.2–1.5 μm	0, 9.8, 44.8 mg/m^3, 4.5 h/day, 5 days/wk, 16 wk for the low-dose group and 10 wk for the high-dose group	Lifespan (average survival/group, 71–82 wk)	The only respiratory tract tumour found was a papillary polyp in the trachea of a low-dose animal	–	Short exposure period; small number of animals	Thyssen *et al.* (1980)
Hamster, Syrian golden	M	24 (+ animals added during the study)	NS (0.1% saline solution); particle size, >99% diameter 0.2–0.5 μm, >80% diameter 0.2–0.3 μm	0, 2.2, 9.5, 46.5 mg, 4.5 h/day, 7 days/wk, 10 wk; thereafter 3 h/day, 7 days/wk for life (total average doses: 0, 29, 127, 383 mg/animal)	Lifespan (average survival/group, 59–96 wk)	Respiratory tract T (P, polyps, SCC): 0/27, 0/27, 34.6% [9/26; 3 nasal, 8 laryngeal, 1 tracheal], 52% [13/25; 1 nasal, 13 laryngeal, 3 tracheal; no bronchogenic T] Upper digestive tract T (P, polyps, SCC): 0/27, 0/27, 26.9% [6/26; 6 pharyngeal, 1 forestomach], 56% [14/25; 14 pharyngeal, 2 oesophageal, 1 forestomach]	+	Types of tumour/site NS	Thyssen *et al.* (1981)

M, male; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; SCC, squamous-cell carcinoma; T, tumour; wk, week

[a] –, negative; +, positive

Transgenic mouse

In a study of male Eμ-*pim*-1 transgenic mice administered benzo[*a*]pyrene by gavage at doses of 0, 4.3, 13 or 39 mg/kg bw thrice weekly for 13 weeks and observed for a period of up to 287 days, a significantly increased incidence of lymphomas and forestomach tumours was found in the two highest-dose groups (Kroese *et al*., 1997). Administration of benzo[*a*]pyrene by gavage to mice that lack the nucleotide excision repair gene ($XPA^{-/-}$) resulted in a high incidence of lymphomas; the tumour response was significantly stronger than that in similarly treated $XPA^{+/-}$ and $XPA^{+/+}$ mice (de Vries *et al*., 1997). When treated with benzo[*a*]pyrene by gavage, $XPA^{-/-}/p53^{+/-}$ double transgenic mice developed tumours (mainly splenic lymphomas and forestomach tumours) much earlier and at a higher incidence than similarly treated single transgenic ($XPA^{-/-}$ or $p53^{+/-}$) and wild-type counterparts (C57/B1/6) (van Oostrom *et al*., 1999). These cancer-prone $XPA^{-/-}$ or $XPA^{-/-}/p53^{+/-}$-deficient mice also developed a high incidence of tumours (80–87%; mainly forestomach tumours) after being fed a diet that contained 75 ppm benzo[*a*]pyrene for 13 weeks followed by a 6-month observation period, whereas similarly treated wild-type counterparts showed only a 10% incidence of forestomach papillomas (Hoogervorst *et al*., 2003).

Rat

In a lifespan study of Sprague-Dawley rats fed diets that contained benzo[*a*]pyrene at levels that resulted in total doses of 0, 6 or 39 mg/kg bw per year, a slightly higher incidence of forestomach papillomas was observed in high-dose animals (10/64 versus 3/64 controls; $p < 0.1$) (Brune *et al*., 1981). The incidence and total number of mammary gland tumours (fibroadenomas, adenomas and adenocarcinomas) were markedly increased in female CD rats treated with benzo[*a*]pyrene (50 μmol [13.21 mg]) by gavage once a week for 8 weeks followed by an observation period of 41 weeks (El-Bayoumy *et al*., 1995).

Dermal application (see also Table 3.1)

Mouse

Benzo[*a*]pyrene was found to induce skin tumours (mainly papilloma and squamous cell carcinomas) in different strains of mice following repeated skin painting for prolonged periods of time.

Mice that lack the aryl hydrocarbon receptor ($AhR^{-/-}$) did not develop tumours after weekly dermal application of 200 μg/animal benzo[*a*]pyrene for 25 weeks, whereas over 90% of their similarly treated AhR-positive counterparts ($AhR^{+/+}$ or $AhR^{+/-}$) developed skin tumours, mainly squamous-cell carcinomas (Shimizu *et al*., 2000).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Benzo[*a*]pyrene has been found to possess tumour-initiating activity in the skin of a range of strains of mice subsequently treated with TPA in acetone or in acetone/DMSO or, in once case, with croton oil (DiGiovanni *et al*., 1980) as the tumour-promoting agent. The predominant type of skin tumour was papilloma.

Buccal pouch application (see also Table 3.11)

Hamster

A high incidence of forestomach papillomas (8/10) and one buccal pouch tumour (a squamous-cell carcinoma) were observed in male Syrian golden hamsters that received applications to the surface of both buccal pouches with 20 mM 5.2g/L benzo[*a*]pyrene dissolved in paraffin oil twice a week for up to 20 weeks. No tumours were observed in these organs in controls (Solt *et al*., 1987).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies of newborn mice of various strains, intraperitoneal injection of benzo[*a*]pyrene resulted in an increased incidence of (mainly benign) liver and lung tumours. In some of these studies, an increased incidence of forestomach tumours (papillomas and squamous-cell carcinomas) and lymphoreticular tumours (mainly reticulum-cell sarcomas) was also found (Vesselinovitch *et al.*, 1975a; Weyand *et al.*, 1995).

Rat

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

Subcutaneous administration (see also Table 3.3)

Mouse

In a series of experiments in female NMRI mice that received single subcutaneous injections of a range of doses of benzo[*a*]pyrene, malignant tumours (mainly fibrosarcomas) were found at the injection site. The tumour incidences were invariably dose-related but varied from one study to another and appeared to be affected by the

nature of the vehicle (tricaprylin, 0.9% saline solution or lutrol 9 (polyethylene-oxide)) and the concentration of benzo[*a*]pyrene in this vehicle (Pott *et al*., 1973a,b).

Transgenic mouse

Mice that lack the aryl hydrocarbon receptor ($AhR^{-/-}$) did not develop tumours after two subcutaneous injections of 2 mg benzo[*a*]pyrene/animal whereas 100% of their similarly treated AhR-positive counterparts ($AhR^{+/+}$ or $AhR^{+/-}$) developed malignant tumours at the injection site that were mainly fibrosarcomas (Shimizu *et al*., 2000).

Rat

Dose-related increases in the incidence of malignant tumours (mainly fibrosarcomas) were found at the site of injection in groups of female Wistar rats given a single subcutaneous injection of tricaprylin containing various concentrations of benzo[*a*]pyrene (Pott *et al*., 1973a,b). Rippe and Pott (1989) reported a high incidence of sarcomas at the injection site in female rats following a single subcutaneous injection of a suspension of benzo[*a*]pyrene in tricaprylin or DMSO.

Hamster

Groups of 25 male and 25 female Syrian hamsters of one randomly bred and 11 different inbred strains (one inbred strain comprised females only) received a single subcutaneous injection of 0.5 mg benzo[*a*]pyrene suspended in tricaprylin and were observed for life. Sarcomas at the site of injection were found in both sexes of all 12 strains; the incidence in males ranged from 12 to 64% and that in females from 17 to 64% (Homburger *et al*., 1972).

Monkey

No tumours were found in 17 Old World monkeys that received [an unspecified number of] subcutaneous injections of benzo[*a*]pyrene and were observed for up to 18 years (Adamson & Sieber, 1983).

Intratracheal instillation (see also Table 3.12)

After the landmark study on an effective method for the induction of tracheobronchial carcinomas in Syrian golden hamsters (Saffiotti *et al*., 1968) that received repeated intratracheal instillations of benzo[*a*]pyrene particles (attached to ferric oxide particles as a carrier) suspended in saline, this model has been used extensively in experimental studies of lung cancer. Studies of benzo[*a*]pyrene mixed with ferric oxide or other particles (or fibres) are summarized in Section 3.15. Studies on benzo[*a*]pyrene not

attached to ferric oxide particles (or other particulates) are summarized in Table 3.12 and are discussed below.

Mouse

An increased incidence and a larger number of lung tumours per animal were found in mice that received 20 weekly intratracheal instillations of a relatively low dose of benzo[*a*]pyrene (50 μg/instillation; total dose, 1 mg) and were observed for a maximum period of 2 years (Heinrich *et al*., 1986a).

Transgenic mouse

In *XPA*$^{-/-}$ mice, a stronger lung tumour response was found after four weekly intratracheal instillations of 100 μg benzo[*a*]pyrene (total dose, 0.4 mg) than in similarly treated *XPA*$^{+/-}$ and *XPA*$^{+/+}$ mice (Ide *et al*., 2000).

Rat

Following 20 weekly intratracheal instillations of 1 mg benzo[*a*]pyrene suspended in 0.9% saline solution, 7/36 (19%) female Wistar rats developed benign and malignant lung tumours (one adenoma, five squamous-cell carcinomas and one adenosquamous carcinoma) whereas no lung tumours were seen in 40 vehicle-treated controls (Pott *et al*., 1987). A very high incidence of malignant lung tumours (38/40) was also found in male and female Sprague-Dawley rats after 22 twice-weekly intratracheal instillations of 7 mg/kg bw benzo[*a*]pyrene, every other week (Steinhoff *et al*., 1991).

Hamster

Benzo[*a*]pyrene suspended in saline (with or without suspendents such as gelatine, lutrol or Tween 60) was found to induce a variety of benign and malignant respiratory tract tumours in male and female Syrian golden hamsters. Although the tumour response varied widely and ranged from only a few benign tumours (Sellakumar *et al*., 1976; Künstler, 1983; Feron *et al*., 1985) to a high incidence of malignant tracheobronchial and pulmonary carcinomas (Feron, 1972; Kobayashi, 1975; Kruysse & Feron, 1976), positive dose–response relationships were established (Feron *et al*., 1973; Feron & Kruysse, 1978; Pott *et al.*, 1978), and larger particles were found to be more effective than smaller ones (Stenbäck & Rowland, 1978; Feron *et al*., 1980).

Intrapulmonary administration (see also Table 3.4)

Rat

Malignant lung tumours (mainly squamous-cell carcinomas) were found in different strains of rat that received direct injections into the lung tissue of a fixed volume (generally 50 μL) of a mixture of beeswax/tricaprylin or beeswax/trioctanoin that contained different amounts of benzo[*a*]pyrene (30–1000 μg benzo[*a*]pyrene/animal) and were observed for life. The tumour incidence was invariably dose-related; low incidences (<10%) occurred at the lowest dose tested (30 μg) (Deutsch-Wenzel *et al*., 1983; Iwagawa *et al*., 1989; Wenzel-Hartung *et al*., 1990; Horikawa *et al*., 1991).

Tracheal graft (see also Table 3.14)

Rat

Malignant tumours (mainly squamous-cell carcinomas) were found in subcutaneously grafted rat tracheas exposed to beeswax pellets that contained 10–2490 μg benzo[*a*]-pyrene; tumour incidence ranged from 77% (40/52) to 100% (12/12) for the three-highest dose levels (1740, 2160 and 2490 μg, respectively); no tumours were observed in 52 controls (Nettesheim *et al*., 1977).

Intramammary administration (see also Table 3.5)

Rat

Intramammary injection or dispersion of benzo[*a*]pyrene over the exposed mammary glands led to a high incidence of malignant mammary gland tumours (mainly adeno-carcinomas and fibrosarcomas) in female Sprague-Dawley rats (Cavalieri *et al*., 1988a,b, 1991).

Intracolonic instillation (see also Table 3.14)

Mouse

No intestinal tumours were found in male or female mice of three different strains that were treated intracolonically with benzo[*a*]pyrene. However, treatment with benzo[*a*]-pyrene did cause significant increases in tumour incidence in various other organs including the lungs, forestomach, mammary glands, peritoneum, lymphoreticular tissue, oesophagus, anus and/or skin (Toth, 1980; Anderson *et al*., 1983).

Table 3.14. Carcinogenicity studies by miscellaneous routes of exposure to various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene										
Rat, Fischer 344	NS	10–52	Subcutaneous. tracheal graft exposed to pellet	NS (beeswax)	0 (untreated), 0 (beeswax controls), 10, 45, 300, 480, 900, 1250, 1740, 2160, 2490 μg/graft, 1×	4–22 mo	Tracheal (graft) T: 0/52 (untreated and beeswax controls combined), 0/24, 0/10, 1/14 (7%; 1 SCC), 0/12, 2/12 (17%; 1 SCC, 1 S), 7/13 (54%; 6 SCC, 1 S), 7/13 (54%; 6 SCC, 1 P), 8/10 (80%; 7 SCC, 3 undifferentiated C, 3 non-invasive C, 2 P), 12/12 (100%; 7 SCC, 1 AdC, 5 non-invasive C, 5 P), 40/52 (77%; 32 SCC, 2 AdC, 3 non-invasive C, 1 P, 2 S)	+		Nettesheim *et al.* (1977)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Human; nude mouse, BALB/c-A	M	6 or 17	Human bronchial grafts (from 4 patients) transplanted subcutaneously in mice and treated by local application (intraluminal injection)	NS (distilled water)	0 (untreated), 10 mg/graft (animal), 3× (once in wk 4, once in wk 12 and once in wk 20 after transplantation)	16–21 wk after transplantation	One bronchial T (SCC) in 13 treated grafts examined histopathologically; 4/13 treated grafts showed preneoplastic epithelial changes (polypoid structures with squamous metaplasia, cellular atypia, unclear irregularity and mitotic changes). In addition, high incidence of basal-cell and goblet/mucus hyperplasia, and squamous metaplasia occurred in the treated grafts. No such changes were seen in the 6 control grafts which all showed well-preserved bronchial structure. Spindle-cell sarcoma of mouse origin developed in 7/13 animals bearing treated bronchial transplants	+		Ito *et al.* (1982)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss albino	M, F	50 M, 50 F	Intracolonic instillation	98% (olive oil)	0, 200, 2000 μg/g bw (total doses); control and high-dose group, 10×/wk instillations of 0 and 200 μg, respectively; low-dose group, 1 instillation	120 wk	**Malignant lymphomas** M, 0/50, 6/50* (12%; 1 histiocytic, 4 lymphocytic, 1 mixed), 7/50** (14%; 2 histiocytic, 3 lymphocytic, 2 mixed) F, 11/49 (22%; 5 histiocytic, 6 lymphocytic), 21/50*** (42%; 5 histiocytic, 16 lymphocytic), 18/49 (36%; 6 histiocytic, 8 lymphocytic, 4 mixed) *(p <0.04), **(p <0.02), ***(p <0.053) **Oesophagus** M, no tumours; F, 0/49, 0/50 or 5/49 (10%) **Forestomach** M, 0/50, 2/50 (4%; 2 P), 10/50* (20%; 9 P, 1 SCC) F, 1/49 (2%; 1 SCC), 5/10 (20%; 3 P, 2 SCC), 11/49** (22%; 9 P, 2 SCC) *(p <0.005), **(p <0.006)	+	Anal and skin tumours probably due to release of benzo[*a*]pyrene through the anal orifice	Toth (1980)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
							Anus M, 0/50, 0/50, 7/50* (14%; 4 P, 3 SCC) F, 0/49, 1/50 (2%; 1 P), 6/49** (12%; 1 P, 4 SCC, 1 K) *(p <0.02), ** (p <0.04) **Skin** M, 1/50 (2%; 1 K), 0/50, 13/50* (26%; 5 P, 7 SCC, 1 K) F, 0/49, 2/50 (4%; 2 SCC), 11/49** (22%; 4 P, 5 SCC, 2 K) *(p <0.0001), ** (p <0.005)			Toth 1980 (contd)
Mouse, Swiss ICR/Ha	F	45–60	Intracolonic instillation	99% (olive oil, enzyme inducer β-naphtho-flavone)	0 (no treatment, olive oil or β-naphthoflavone in olive oil), 1 mg/animal, 1×/wk, 14 wk	18 mo	Lung T: 13/52 (25%; multiplicity, 1.4 ± 0.8), 27/37 (73%; multiplicity, 5.1 ± 3.9) (p <0.05 or less) Forestomach P: 4/20 (20%; multiplicity, 1.2 ± 0.5); 16/17 (94%; multiplicity, 1.9 ± 1.1; p <0.05 or less) Mammary gland C: 5/53 (9%), 10/43 (23%; p <0.05 or less)	+	Type of lung tumours NS; no colon tumours found	Anderson *et al.* (1983)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C57/Bl/6	F	45–60	Intracolonic instillation	99% (olive oil, enzyme inducer β-naphtho-flavone)	0 (no treatment, olive oil or β-naphthoflavone in olive oil), 1 mg/animal (in olive oil), 1×/wk, 14 wk	18 mo	Forestomach P: 7/34 (21%; multiplicity, 1.1 ± 0.4), 17/18 (94%; multiplicity, 3.2 ± 2.3; p <0.05 or less) Peritoneal S: 0/40, 5/32 (16%; p <0.05 or less) Lymphoma: 1/40 (2.5%), 9/32 (28%; p <0.05 or less)	+	No colon tumours found	Anderson *et al.* (1983)
Mouse, C57B1	F	10 or 76	Intravaginal application	NS (acetone)	Cotton swab soaked in acetone (controls) or 1% solution of benzo[*a*]pyrene in acetone, 2×/wk, 5 mo	5 mo	0/10, 17/76 (22%; invasive cervical C)	+		Näslund *et al.* (1987)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mongrel dogs, Beagle	NS	3 or 6	Submucosal injection or sustained-release implant	NS (NS) Vehicle (NS)	Experiment 1: 3 dogs, 15–45 mg/wk (total dose, 1.02 g) plus topical application of 380×10^6 mg TPA administered over 4 mo Experiment 2: 6 dogs, 1 or more sustained-release bronchial implants containing 31–50 mg followed after 6 mo by sustained-release implants containing 0.1% TPA for 6 mo	5.5 years 2.6 (2.3–2.9) years	No lung cancer found	–		Benfield *et al.* (1986)
Mouse, Swiss	M, F	43–56	Intrafetal injection	>99% (trioctanoin-acetone mixture (1:1))	0, 0.4, 4.0, 9.9, 19.8 nmol [0, 0.1, 1, 2.6, 5.2 µg]/animal, 1×	12 wk	Lung A (M + F combined): 0/37, 1/39 (3%), 10/42 (25%), 10/38 (26%), 12/31 (39%)	+		Rossi *et al.* (1983)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a,h*]anthracene										
Rat, NS	NS	68	Subcutaenous/ intraperitoneal (alternating)	NS (aqueous suspension)	1 mg in 2 mL, 2×/wk, ~44 wk	~44 wk	31/68 (46%; S)		No control	Boyland & Burrows (1935)
Frog, *Rana pipiens*	M, F	23, 10 and 192 controls	Intra-renal	NS (olive oil)	0.3–0.5 mg in 100 µL, 1×	<7 mo	6/23 (26%; kidney AdC) vs 0/10 solvent controls and 6/192 (3%) untreated controls	+	Small no. of solvent-treated controls; no statistics	Strauss & Mateyko (1964)
Dibenzo[*a,i*]pyrene										
Mouse, NS	F	30	Intrauterine	NS (NS)	500 µg (volume NS), 1×	32 wk	0/30	–	No control; limited design	Homburger & Tregier (1960)
Dibenzo[*a,l*]pyrene										
Hamster, Syrian golden	F	7, 4 controls	Tongue	NS (acetone)	0.25%, 0.01 µmol (3µg), 5×/wk, 30 wk	30 wk	6/7 (86%) oral cavity C (2.6 T/ animal) vs 0/4 solvent controls	+	No statistics	Schwartz *et al.* (2004)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, mixed C57Bl/6 and 129/Sv [wild-type and P450 1B1-null]	F	18 wild-type, 13 P450 1B1 null, 27 controls	Intragastric	99.8% (corn oil)	1.07 mg/kg (~32 µg) in 100 µL, 5×/wk, 3 wk	12 mo	Wild-type: 18/18 (12/17 ovary T (83% granulosa-cell T), 5/17 lymphoma (60% lymphoblastic, 40% follicular), 1/17 liver A, 8/17 skin hyperplasia, 5/17 uterine T (40% haemangioS, 60% endometrial cystic hyperplasia), 0/17 lung T); P450 1B1-null: 8/13 (0/13 ovary T, 1/13 follicular lymphoma, 1/13 liver haemangioma, 5/13 endometrial cystic hyperplasia, 5/13 lung A) vs 4/27 [1/27 follicular lymphoma, 1/27 liver A, 1/27 endometrial cystic hyperplasia, 1/27 lung A) solvent controls (both genotypes combined)	+	No histology for one wild-type mouse	Buters *et al*. (2002)

A, adenoma; AdC, adenocarcinoma; bw, body weight; C, carcinoma; F, female; K, keratoacanthoma; M, male; mo, months; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; vs, versus; wk, week

[a] –, negative; +, positive

Intravaginal application (see also Table 3.14)

Mouse

Intravaginal application of a 1% solution of benzo[*a*]pyrene in acetone twice a week for 5 months to C57BL mice produced invasive cervical carcinoma in 17/76 (22%) animals whereas no such tumours were seen in acetone-treated controls (0/10) (Näslund *et al*., 1987)

Intrafetal administration (see also Table 3.14)

Mouse

Groups of 43–56 male and female Swiss mice were injected intrafetally with 1 μL of a trioctanoin/acetone mixture (1:1) that contained benzo[*a*]pyrene at concentrations that produced doses of 0 (controls), 0.4, 4.0, 9.9 and 19.8 nmol [0, 0.1, 1, 2.61 and 5.23 μg]/animal respectively. Survivors were killed at 12 weeks of age. Lung adenomas were found in all treated groups at an incidence of 1/39 (3%), 10/42 (25%), 10/38 (26%) and 12/31 (39%) in the low-, low-mid-, high-mid- and high-dose groups, respectively. No lung tumours were found in 37 controls (Rossi *et al*., 1983)

Administration with particles and/or fibres (dusts) (see also Table 3.15)

Rat

Large numbers of malignant lung tumours (range, 17–21 tumours in 20 males and 16–18 tumours in 20 females) developed in Sprague-Dawley rats following 22 intratracheal instillations administered once a week every other week of 7 mg benzo[*a*]pyrene alone or mixed with 10–40 mg Bayferrox 130 (96.2% cubic α-Fe_2O_3) or 10–40 mg Bayferrox 920 (86.1% fibrous α-FeOOH) whereas no lung tumours were seen in controls and rats treated with Bayferrox 130 or Bayferrox 920 alone except for one benign and one malignant lung tumour in 50 females treated with Bayferrox 920 (Steinhoff *et al*., 1991).

Hamster

In a large number of studies of Syrian golden hamsters, repeated intratracheal instillation of benzo[*a*]pyrene mixed with ferric oxide (used as carrier particles) and suspended in saline or saline/gelatine was found to induce benign and malignant tumours in various segments of the respiratory tract (larynx, trachea, bronchi and lungs) (see Table 3.15). Although the tumour response was generally dose-related, wide variations in tumour incidence occurred between studies (see also review by Wolterbeek *et al*., 1995).

Table 3.15. Carcinogenicity studies in rats and hamsters exposed by intratracheal instillation to combinations of benzo[*a*]pyrene and 'particles/fibres'

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Sprague-Dawley	M, F	20 or 50	NS (physiological saline solution with or without Tween 60); Bayferrox 130, Bayferrox 920	0 (untreated), 0, 10–40 mg/kg bw Bayferrox 130 (96.2% cubic α-Fe_2O_3), 10–40 mg/kg bw Bayferrox 920 (86.1% fibrous α-FeOOH), 7 mg/kg bw, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 130, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 920, ~1×/2 wk, 44–~130 wk	Up to 920 days	Lung T (mainly malignant) M: 0/50, 0/50, 0/50, 0/50, 19 malignant T in 20 animals, 21 malignant and 1 benign T in 20 animals, 17 malignant and 1 benign T in 20 animals F: 0/50, 0/50, 0/50, 1 malignant and 1 benign T in 50 animals, 18 malignant and 1 benign T in 20 animals, 16 malignant T in 20 animals, 17 malignant and 2 benign T in 20 animals	+	Type of lung tumour NS	Steinhoff *et al.* (1991)
Hamster, Syrian golden	M, F	23–110 M; 18–107 F	NS ferric oxide (0.9% saline solution)	Experiment 1 0, 50 mg ferric oxide, 5 mg + 45 mg ferric oxide, 12.5 mg + 37.5 mg ferric oxide/ animal, 1× Experiment 2 (2 groups/dose level) 5 mg + 5 mg ferric oxide, 10 mg + 10 mg ferric oxide, 15 mg + 15 mg ferric oxide, 1×/wk, 15 wk	Lifespan (up to 140 wk)	Experiment 1 **Respiratory tract T** M: 0/45, 0/101, 3/92 (3%; 1 tracheal polyp, 1 P, 1 bronchial A), 3/27 (11%; 1 bronchial A, 1 bronchogenic SCC, 1 anaplastic C) F: 0/44, 0/89, 4/97 (4%; 1 tracheal polyp, 1 P, 1 bronchiolar A, 1 AdC), 6/33 (18%; 1 bronchial P, 1 A, 2 bronchogenic SCC, 1 anaplastic C, 2 bronchiolar A) **Forestomach P** M: 5/45 (11%; 6 T), 5/101 (5%; 5 T), 15/92 (16%; 35 T), 8/27 (30%; 16 T) F: 2/44 (5%; 2 T), 2/89 (2%; 3 T), 5/97 (5%; 5 T), 4/33 (12%; 6 T)	+		Saffiotti *et al.* (1972)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						Experiment 2 **Respiratory tract T (M + F combined)** 7/50 (14%; 2 tracheal P, 1 SCC, 1 bronchial P, 1 A, 2 SCC, 1 anaplastic C, 1 pulmonary SCC), 8/58 (14%; 2 tracheal polyps, 1 bronchial polyp, 2 SCC, 2 AdC, 2 pulmonary A, 2 AdC), 17/61 (28%; 2 tracheal polyps, 2 P, 5 SCC, 5 bronchial SCC, 1 pulmonary A, 1 SCC, 1 AdC, 1 anaplastic C), 25/60 (42%; 4 tracheal polyps, 3 P, 3 SCC, 4 anaplastic C, 1 bronchial P, 1 SCC, 2 anaplastic C, 4 AdC, 1 A, 2 pulmonary SCC, 2 anaplastic C, 6 A), 25/39 (64%; 1 tracheal P, 10 SCC, 1 anaplastic C, 3 bronchial P, 7 SCC, 11 anaplastic C, 2 AdC, 2 pulmonary SCC, 2 A), 35/55 (64%; 2 laryngeal SCC, 11 tracheal P, 1 polyp, 12 SCC, 1 carcinoS, 2 fibroS, 16 bronchial SCC, 10 anaplastic C, 6 AdC, 3 A, 2 pulmonary A)			Saffiotti *et al.* (1972) (contd)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						Forestomach T M: 8/22 (36%; 13 P, 1 SCC), 6/28 (21%; 9 P), 11/34 (32%; 28 P, 1 SCC), 11/30 (37%; 18 P), 5/22 (23%; 10 P), 1/28 (4%; 1 P) F: 9/28 (32%; 14 P), 6/30 (20%; 9 P), 5/27 (19%; 20 P), 8/30 (27%; 10 P, 1 SCC), 5/17 (29%; 11 P), 3/27 (11%; 3 P)			Saffiotti *et al.* (1972) (contd)
Hamster, Syrian golden	F	48	NS (tricaprylin, Tween 60/saline solution); atmospheric dust from Bochum, Germany (particle size <5 µm)	340 µg in tricaprylin, 340 µg in Tween 60/saline solution, 340 µg in Tween 60/saline solution + 850 µg atmospheric dust/animal, 45× within a period of 6.5 months (total dose, ~15 mg; dust, 38 mg)	Presum-ably lifespan	Respiratory tract T (mainly P or SCC of the larynx, trachea or bronchi): 2/48 (4%), 14/48 (29%), 16/48 (33%)	+		Pott *et al.* (1973b)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian	M, F	36; 193 controls	NS (0.9 % saline saline solution); ferric oxide	0, 3 mg + 3 mg ferric oxide, 3 mg + 6 mg ferric oxide, 3 mg + 9 mg ferric oxide, 1×/2wk, 20 wk	100 wk	**Respiratory tract T (M + F combined)** 26/67 (39%; 3 laryngeal polyp, 3 P, 3 SCC, 7 tracheal polyp, 6 P, 2 SCC, 2 bronchial polyp, 5 SCC, 9 AdC, 1 anaplasic C, 7 lung A, 1 AdC), 28/64 (44%; 1 laryngeal polyp, 3 P, 6 SCC, 3 tracheal polyp, 9 P, 3 SCC, 3 bronchial polyp, 1 P, 4 SCC, 3 AdC, 1 anaplastic C, 7 lung A, 4 AdC), 26/66 (39%; 3 laryngeal polyp, 6 SCC, 6 tracheal polyp, 11 P, 1 SCC, 1 bronchial polyp, 1 P, 4 SCC, 4 AdC, 2 anaplastic C, 6 lung A, 6 AdC) **Forestomach T** M: 17/32 (53%; 37 P), 10/31 (32%; 16 P, 1 SCC), 6/35 (17%; 15 P) F: 10/35 (29%; 30 P), 12/33 (36%; 25 P), 15/31 (48%; 33 P) vs 0/193 untreated controls	+		Sellakumar *et al.* (1973)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	73–83	NS (0.9% saline solution); ferric oxide or carotene-free cottonseed oil	3 mg + 3 mg ferric oxide, 1×/wk, 12 wk followed after 1 wk by 100, 1600 or 3300/µg vitamin A (retinyl acetate), intragastric 1×/wk [signs of vitamin A toxicity appeared in animals given 3300 µg after 24 wk; dose reduced to 2400 µg/wk]	~60 wk	**Respiratory tract T** *Low-vitamin A group* 48/83 (58%; 37 laryngeal/tracheal T: 10 P, 4 polyp, 20 SCC, 2 AdC, 1 undifferentiated C; 35 bronchial/ bronchiolar/pulmonary T: 2 P, 9 A, 8 SCC, 7 AdC, 4 AdSC, 3 undifferentiated C, 2 fibroS) *Mid-vitamin A group* 52/74 (70%; 32 laryngeal/tracheal T: 3 P, 4 polyp, 18 SCC, 1 AdC, 1 carcinoS, 6 fibroS; 38 bronchial/bronchiolar/pulmonary T: 1 P, 11 A, 7 SCC, 6 AdC, 3 AdSC, 3 undifferentiated C, 1 carcinoS, 6 fibroS) *High-vitamin A group* 59/73 (81%; 32 laryngeal/tracheal T: 7 P, 1 polyp, 13 SCC, 4 AdC, 1 AsSC, 1 undifferentiated C, 3 carcinoS, 2 fibroS; 52 bronchial/ bronchiolar/pulmonary T: 11 A, 13 SCC, 16 AdC, 2 undifferentiated C, 1 carcinoS, 9 fibroS)	+		Smith *et al.* (1975a)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	49–58	NS (0.15 M saline solution); ferric oxide or carotene-free cottonseed oil	3 mg + 3mg ferric oxide/animal, 1×/wk, 12 wk followed after 1 wk by 100, 1600 or 3300 μg vitamin A (retinyl acetate) intragastric 1×; half of the animals in each group were conventially housed (CH) while the other half was housed in laminar flow units (LFU), [signs of vitamin A toxicity appeared in animals given 3300 μg after 24 wk; doses reduced to 2400 μg/wk]	~100 wk	**Respiratory tract T** *Low-vitamin A group* CH: 34/57 (60%; 23 P, 11 polyps, 5 A, 26 SCC, 18 AdC, 3 ASC, 5 oat-cell C, 4 undifferentiated C, 2 carcinoS, 3 fibroS) LFU: 37/52 (71%; 25 P, 13 polyp, 14 A, 25 SCC, 11 AdC, 8 ASC, 2 undifferentiated C, 2 lymphoma) *Mid-vitamin A group*: CH: 45/58 (78%; 20 P, 14 polyp, 10 A, 31 SCC, 11 AdC, 2 oat-cell C, 4 undifferentiated C, 1 carcinoS, 6 fibroS, 1 haemangioS), LFU: 31/53 (59%; 16 P, 10 polyp, 2 A, 36 SeC, 16 AdC, 2 ASC, 2 oat-cell C, 2 undifferentiated C, 5 fibroS, 2 chondroS, 2 lymphoma, 5 reticulum-cell S)	+	Site of respiratory tract T NS	Smith *et al.* (1975b)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						High-vitamin A group: CH: 43/58 (74%; 19 P, 11 polyp, 18 A, 37 SCC, 8 AdC, 7 fibroS) LFU: 28/49 (57%; 21 P, 9 polyp, 7 A, 22 SCC, 14 AdC, 7 AdSC, 14 oat-cell C, 2 fibroS, 2 lymphoma, 2 reticulum-cell S) **Forestomach P (CH + LFU combined)** *Low-vitamin A group* [55/109] (50%) (multiplicity, 2.9 ± 0.2) *Mid-vitamin A group* [28/111] (25%) (multiplicity, 2.2 ± 0.3) *High-vitamin A group* [28/107] (26 %) (multiplicity, 3.1 ± 0.2)			Smith *et al.* (1975b) (contd)
Hamster, Syrian golden	M, F	48 or 90	NS (0.2% saline solution); ferric oxide, magnesium oxide	0 (untreated), 2 mg + 1 mg magnesium oxide/animal, 1×/wk, 20 wk, 3 mg + 3 mg ferric oxide/animal, 1×/wk, 15 wk	Lifespan (up to 120 wk)	Respiratory tract tumours (M + F combined): 0/89, 32/45 [71%] (11 laryngeal P, 3 SCC, 1 tracheal polyp, 20 P, 5 SCC, 1 AdC, 1 bronchial P, 3 A, 8 AdC, 1 adenoSC), 31/44 (70%; 10 laryngeal P, 4 SCC, 8 tracheal P, 12 SCC, 2 anaplastic C, 2 bronchial P, 4 A, 2 AdC, 17 SCC, 3 anaplastic C)	+		Stenbäck *et al.* (1975)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	50 untreated controls	>99% (saline, 0.5% gelatine in saline); manganese dioxide, silicon dioxide	0 (untreated), 0 (saline), 0 (gelatine in saline), 3 mg silicon dioxide in saline, 1.5 mg manganese dioxide in saline, 3 mg in saline, 3 mg in gelatine/saline, 3 mg + 3 mg silicon dioxide in saline, 1.5 mg + 1.5 mg manganese dioxide in saline/animal, 1×/wk, 20 wk	Lifespan (up to 100 wk)	All T (M + F combined): 2/100 (2%; 2 lymphoma), 1/48 (2%; 2 forestomach P), 2/45 (4%; 2 lymphoma), 0/48 (0%), 2/48 (4%; 1 forestomach P, 1 lymphoma), 18/46 (39%; 1 laryngeal P, 1 SCC, 4 tracheal P, 15 forestomach P), 11/47 (23%; 2 tracheal P, 1 SCC, 3 bronchial SCC, 1 splenic haemangioma, 1 adrenal cortical A, 1 lymphoma, 2 forestomach SCC), 25/48 (52%; 1 laryngeal SCC, 8 tracheal P, 2 SCC, 3 bronchial SCC, 6 lung A, 3 AdC, 10 forestomach P, 1 thyroid A, 1 uterine fibroma, 1 A, 1 lymphoma), 20/48 (42%; 1 laryngeal P, 3 tracheal P, 1 SCC, 1 bronchial SCC, 24 forestomach P, 1 ovarian fibroma, 1 thyroid A, 2 forestomach SCC, 1 squamous-cell fibroma)	+		Stenbäck & Rowland (1979)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamsters, Syrian golden	M, F	40	NS (saline); ferric oxide	10 mg + 8 mg ferric oxide/animal, 1×/2wk for 10 wk to animals kept on a low- (600 IU/kg) [M only], medium- (4000 IU/kg) or high- (100 000–200 000 IU/kg) vitamin A diet	M, up to 512 days, F, up to 380 days	**Respiratory tract T** M: 16/36 (44%; 21 T: 4 epidermoid P, 11 epidermoid C, 4 epidermoid/AdC, 1 AdC, 1 S), 14/39 (36%; 20 T: 4 epidermoid P, 7 epidermoid C, 5 epidermoid/AdC, 1 AdC, 3 S), 13/37 (35%; 17 T: 6 epidermoid P, 6 epidermoid C, 3 epidermoid/AdC, 2 S) F: 14/38 (37%; 22 T: 12 epidermoid P, 1 C *in situ*, 8 epidermoid C, 1 epidermoid/AdC), 12/37 (32%; 15 T: 4 epidermoid P, 6 epidermoid C, 2 epidermoid/AdC, 3 S)	+	No control; site of respiratory tract T NS	Beems (1984)
Hamster, Syrian golden	M, F	40	NS (saline); ferric oxide	4 mg + 4 mg ferric oxide/ animal, 1×/wk, 15 wk to animals on control (low-fat diet), high-saturated fat diet or high-unsaturated fat diet	656 days for M, 512 days for F	**Respiratory tract T** M: 10/33 (30%; 2 P, 6 epidermoid C, 1 epidermoid/AdC, 1 A, 1 S), 14/32 (44%; 7 P, 6 epidermoid C, 3 epidermoid/AdC, 1 AdC, 1 A, 1 S), 17/27 (63%; 9 P, 11 epidermoid C, 3 epidermoid AdC) F: 16/40 (40%; 5 P, 9 epidermoid C, 3 epidermoid/AdC, 1 A), 20/38 (53%; 13 P, 13 epidermoid C, 1 epidermoid/AdC, 1 A, 1 S), 22/37 (59%; 12 P, 10 epidermoid C, 9 epidermoid/AdC)	+	No control; site of respiratory tract NS	Beems & van Beek (1984)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	35	NS (saline, gelatine); glass microfibres (code 104; glasswool); size distribution by number: length, 95% <20 μm, 89% <12 μm, 59% <5 μm; diameter, 25% <2 μm, 88% <1 μm, 60% <0.5 μm, 31% <0.25 μm	0, 1 mg glass fibres, 1 mg, 1 mg + 1 mg glass fibres/animal, 1×/2 wk, 52 wk	85 wk	**Respiratory tract T** M: 0/31, 0/34, 6/34 (18%; 3 tracheal P, 1 SCC, 1 S, 1 bronchial P), 3/35 (9%; 2 tracheal P, 1 S) F: 0/28, 0/30, 1/29 (3%; 1 laryngeal C *in situ*), 1/31 (3%; 1 tracheal S)	±	Statistics NS	Feron *et al.* (1985)
Hamster, Syrian golden	M, F	35	NS (0.9 % saline solution); ferric oxide	0, 8 mg + 6 mg ferric oxide/animal, 1×/wk, 6 wk	82 wk	**Respiratory tract T** M: 0/32, 12/24 (50%; 15 T: 3 laryngeal P, 1 tracheal P, 1 SCC, 2 bronchial polyp, 2 SCC, 1AdC, 3 pulmonary SCC, 1 AdSC, 1 AdC) F: 0/35, 9/26 (35%; 12 T: 1 laryngeal P, 5 tracheal P, 2 bronchial polyp, 2 pulmonary SCC, 1 AdSC, 1 AdC)	+		Reynders *et al.* (1985)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamsters, Syrian golden	M, F	40 or 60	NS (saline); ferric oxide	8 mg + 8 mg ferric oxide, 1×/2wk, 16 wk given to animals on control (low-selenium/low-fat) diet, high-selenium/low-fat diet, low-selenium/high-fat diet or high-selenium/high-fat diet	429 days for M; 374 days for F	**Respiratory tract T** M: 34/57 (60%; 7 P, 4 C *in situ*, 15 epidermoid C, 13 epidermoid/AdC, 1 AdC, 5 lung A, 2 S), 27/38 (71%; 6 P, 1 C *in situ*, 14 epidermoid C, 8 epidermoid/AdC, 8 lung A, 1 S), 31/38 (82%; 6 P, 3 C *in situ*, 13 epidermoid C, 12 epidermoid/AdC, 1 AdC, 10 lung A, 1 S), 28/39 (72%; 4 P, 1 C *in situ*, 16 epidermoid C, 10 epidermoid/AdC,10 lung A) F: 37/57 (65%; 5 P, 2 C *in situ*, 14 epidermoid C, 19 epidermoid/AdC, 2 lung A, 3 S), 30/35 (86%; 11 P, 2 C *in situ*, 11 epidermoid C, 11 epidermoid/AdC, 4 lung A, 1 S), 28/36 (78%; 4 P, 5 C *in situ*, 11 epidermoid C, 9 epidermoid/AdC, 1 AdC, 5 lung A), 22/34 (65%; 5 P, 2 C *in situ*, 10 epidermoid C, 7 epidermoid/AdC, 1 AdC, 3 lung A)	+	No control; site of respiratory tract T NS	Beems (1986)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	40 or 60	NS (sterile saline); ferric oxide	8 mg + 8 mg ferric oxide, 1×/2wk, 16 wk given to animals on control diet or diet supplemented with 56 mg/kg carotene	429 days for M; 374 days for F	**Respiratory tract T** M: 34/57 (60%; 7 P, 4 C *in situ*, 11 epidermoid C, 13 epidermoid/AdC, 1 AdC, 5 lung A, 2 S), 26/38 (68%; 7 P, 3 C *in situ*, 10 epidermoid C, 10 epidermoid/AdC, 3 lung A, 1 S) F: 37/57 (65%; 5 P, 2 C *in situ*, 14 epidermoid C, 19 epidermoid/AdC, 2 lung A, 3 S), 25/36 (69%; 7 P, 3 C *in situ*, 10 epidermoid C, 14 epidermoid/AdC, 3 lung A, 1 S)	+	No control; site of respiratory tract T NS	Beems (1987)

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; C, carcinoma; F, female; M, male; NS, not specified; P, papilloma; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; vs, versus; wk, week

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

In Syrian golden hamsters given 20 weekly intratracheal instillations of a suspension of silicon dioxide mixed with benzo[*a*]pyrene in saline (3 mg benzo[*a*]pyrene + 3 mg silicon dioxide/instillation), a clearly higher incidence of respiratory tract tumours was observed than that in hamsters similarly treated with benzo[*a*]pyrene alone (23 tumours in 48 animals given the combination versus six tumours in 46 animals that received benzo[*a*]pyrene alone and no tumours in 48 saline-treated controls). Manganese dioxide did not elicit such an effect (Stenbäck & Rowland, 1979). Magnesium oxide was found to be as effective as ferric oxide as a carrier for benzo[*a*]pyrene suspended in saline in the induction of respiratory tract tumours in Syrian golden hamsters following repeated intratracheal instillation of such suspensions (Stenbäck *et al.*, 1975).

Repeated intratracheal instillation of benzo[*a*]pyrene mixed with glasswool microfibres suspended in gelatine/saline induced a few tracheal tumours in male and female Syrian hamsters; the incidence was comparable with that found after instillation of benzo[*a*]pyrene in gelatine/saline alone (Feron *et al*., 1985).

The tumour response in the respiratory tract (16/48 or 33%; mainly laryngeal and tracheobronchial papillomas and carcinomas) of Syrian golden hamsters administered 45 weekly intratracheal instillations of 850 µg atmospheric urban dust mixed with 340 µg benzo[*a*]pyrene and suspended in 2% Tween 60 in saline (total doses, 38 mg dust and about 15 mg benzo[*a*]pyrene) was not appreciably different from that (14/48 or 29%) of animals similarly treated with benzo[*a]*pyrene alone (Pott *et al*., 1973b).

Benzo[*e*]pyrene

Previous evaluation

Benzo[*e*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated two bioassays in which benzo[*e*]pyrene was administered dermally to mice (repeatedly and initiation–promotion protocols). On the basis of the available data, the Working Group concluded that benzo[*e*]pyrene was not an initiator of skin carcinogenesis in mice. Benzo[*e*]pyrene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the bioassays considered previously, plus two studies in which benzo[*e*]pyrene was administered intraperitoneally to neonatal mice and one study in which it was administered by pulmonary injection into rats. All of the studies are summarized in Tables 3.2, 3.4 and 3.7. On the basis of the available studies, the Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*e*]pyrene to experimental animals. No new studies were available.

Chrysene

Previous evaluation

Chrysene was considered in December 1972 (IARC, 1973) and February 1983 (IARC, 1983) by working groups that evaluated bioassays in which chrysene was administered dermally (repeatedly and initiation–promotion protocols) to mice, subcutaneously to mice and rats and intraperitoneally to mice. Chrysene was active when applied to mouse skin, when given subcutaneously to mice and when administered to newborn mice. On the basis of the available data, the working groups concluded that there was *limited evidence* for the carcinogenicity of chrysene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In mouse skin initiation–promotion studies in which TPA was subsequently applied as a promoter, total initiating doses of 0–1.5 μmol chrysene applied to the skin of female CD-1 mice increased the incidence of skin papillomas (Chang *et al.*, 1983; Rice *et al.*, 1988). A total initiating dose of 1.0 mg chrysene resulted in papillomas in 92% of animals (Rice *et al*., 1985). However, female CD-1 mice initiated with a single dose of 33 nmol chrysene (Amin *et al.*, 1990), and male and female SENCAR mice initiated with a single application of 1600 nmol (Bhatt & Coombs, 1990) did not develop skin tumours.

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies of newborn male and female Swiss-Webster BLU:Ha (ICR) mice, intraperitoneal injection of chrysene at doses of 0–1.4 μmol significantly increased the incidence of lung and liver tumours in male mice (Chang *et al.*, 1983). In another study, intraperitoneal injection of chrysene at total doses of 0, 0.03 or 0.92 μmol did not increase the incidence of lung tumours in male or female mice (Busby *et al.*, 1989). In newborn male and female CD-1 mice, intraperitoneal injection of total doses of 700 nmol chrysene increased the incidence of liver adenomas and carcinomas combined in male mice (Wislocki *et al.,* 1986), and the highest dose (2800 nmol) significantly increased the incidence of liver carcinomas and lung adenomas in male mice (Wislocki *et al.,* 1986).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 1 or 3 mg chrysene into Osborne-Mendel rats induced dose-related increases (0%, 14.3% and 28.6%) in the incidence of pulmonary squamous-cell carcinomas (Wenzel-Hartung *et al.*, 1990).

Coronene

Previous evaluation

Coronene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which coronene was administered dermally to mice (repeatedly) and one initiation–promotion assay in mice. The initiation–promotion assay gave positive results; the repeated application gave negative results. On the basis of these studies, the Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of coronene to experimental animals. No new data were available.

4*H*-Cyclopenta[*def*]chrysene

Dermal initiation–promotion (see also Table 3.2)

In an initiation–promotion study in female CD-1 mice, an initiating dose of 1.0 mg 4*H*-cyclopenta[*def*]chrysene prior to thrice-weekly applications of TPA resulted in 100% of the animals bearing skin papillomas with an average of 5.63 papillomas/animal (Rice *et al.*, 1985). In another initiation–promotion study in female CD-1 mice, total initiating doses of 0.15, 0.5 or 1.5 μmol 4*H*-cyclopenta[*def*]chrysene prior to thrice-weekly applications of TPA resulted in increased incidences of skin papillomas (13/20 (65%), 19/19 (100%) and 19/19 (100%)) in treated mice, with averages of 1.10, 6.84 and 8.47 tumours/mouse, respectively; papillomas were observed in 4/20 (20%) of the acetone controls (Rice *et al.*, 1988).

Cyclopenta[*cd*]pyrene

Previous evaluation

Cyclopenta[*cd*]pyrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two bioassays in which cyclopenta[*cd*]pyrene was administered dermally to mice, results were positive in one study. It also gave positive results as an initiator in three initiation–promotion studies on mouse skin. The Working Group concluded that there was *limited evidence* for the carcinogenicity of cyclopenta[*cd*]pyrene

in experimental animals. Animal bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

Cyclopenta[*cd*]pyrene applied to the skin of female Swiss mice at doses of 0, 22.2, 66.6 or 200 nmol twice a week resulted in dose-related increases in the incidence of skin tumours (Cavalieri *et al.*, 1983).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies of newborn male and female Swiss-Webster BLU:ha (ICR) mice, intraperitoneal injection of cyclopenta[*cd*]pyrene at doses of 0, 1.55, 3.09, 4.64, 6.19 or 7.73 μmol increased the incidence of lung adenomas and adenocarcinomas (males: 8%, 62%, 56%, 86%, 77% and 89%; females: 8%, 60%, 70%, 93%, 100% and 100%) and number of tumours/animal (males: 0.08, 1.12, 2.78, 9.29, 4.08 and 7.33; females: 0.08, 2.20, 3.20, 6.71, 13.57 and 5.33) in males and females combined (Busby *et al.*, 1988). Intraperitoneal injection of male Strain A/J mice with single doses of 0, 10, 50, 100 or 200 mg/kg bw cyclopenta[*cd*]pyrene resulted in an increased incidence of lung adenomas per mouse at doses above 10 mg/kg (Nesnow *et al.*, 1994, 1995; Ross *et al.*, 1995; Nesnow *et al.*, 1998a,b).

5,6-Cyclopenteno-1,2-benzanthracene

Dermal application (see also Table 3.1)

Mouse

Groups of 10 mice [strain, sex, age and body weight unspecified] were treated dermally with solutions of 5,6-cyclopenteno-1,2-benzanthracene in benzene [volume and number of treatments unspecified]. Three groups were treated with a 0.3 % solution (two groups) or a 0.1% solution (one group) of ‘pure’ compound (purity determined by melting-point); an additional group was treated with a 0.3% solution of compound that was purified through recrystallization of the corresponding picrate. No control group was used. The time of death of the last animal in each group was 224–321 days in the group treated with 0.3% 5,6-cyclopenteno-1,2-benzanthracene and 339 days in the group treated with the 0.1% dose. Among the groups treated with the 0.3% solution, the tumour incidence was 2/10, 1/10 and 0/10 skin papillomas and 1/10, 4/10 and 8/10 skin epitheliomas (the last value in each series corresponded to the tumours obtained with the

material purified as the picrate). The group treated at the 0.1% dose developed 0/10 skin papillomas and 4/10 skin epitheliomas (Cook, 1932). A subsequent, similarly designed experiment using 0.03, 0.1 and 0.3% solutions of 5,6-cyclopenteno-1,2-benzanthracene [purity unspecified] resulted in an incidence of 1/20, 0/10 and 5/40 skin papillomas and 1/20, 5/10 and 14/40 skin epitheliomas, respectively (Barry *et al*., 1935).

Dibenz[*a*,*c*]anthracene

Previous evaluation

Dibenz[*a*,*c*]anthracene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which dibenz[*a*,*c*]anthracene was administered dermally to mice, either *per se* or as part of initiation–promotion protocols. These studies are summarized in Tables 3.1 and 3.2, respectively. On the basis of the available data, the Working Group concluded that there was *limited evidence* that dibenz[*a*,*c*]anthracene was carcinogenic to experimental animals. Additional studies that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 39–40 female CD-1 white mice, approximately 45 days of age [body weight unspecified], received a single dermal application of 0, 25 or 50 μg dibenz[*a*,*c*]-anthracene [purified by chromatography and recrystallized] in 100 μL acetone. One week later, promotion began with twice-weekly applications of 0.64 μg TPA in 100 μL acetone for 29 weeks, followed by doses of 1 μg for the subsequent 38 weeks. Systematic post-mortem examinations were conducted on all animals and tumours were examined histologically. At the end of the experiment, 5/39 mice in the 25-μg dose group and 8/40 mice in the 50-μg dose group had developed skin papillomas compared with 3/40 mice in the TPA-treated control group (Chouroulinkov *et al*., 1983).

Subcutaneous administration (see also Table 3.3)

Mouse

Groups of 30 male and female C57BL/6J, DBA/2J and B6D2F$_1$ mice, approximately 5 weeks of age [body weight unspecified], received a single subcutaneous injection of 150 or 300 μg dibenz[*a*,*c*]anthracene [purity unspecified] in 50 μL trioctanoin. Controls in all groups (10 mice/group) received trioctanoin alone. The mice were monitored for 12 months. At the end of the experiment, no tumours were observed in any of the groups treated with 150 μg dibenz[*a*,*c*]anthracene. The incidence of subcutaneous fibrosarcoma

in the 300-μg dose groups was 1/30, 0/30 and 1/30 for C57BL/6, DBA/2 and B6D2F_1 mice, respectively. Tumour incidences were not reported for the control groups (Kouri *et al*., 1983).

Intraperitoneal administration (see also Table 3.7)

Mouse

A group of 24 newborn male B6C3F_1 mice received intraperitoneal injections of a total dose of 400 nmol [111 μg] dibenz[*a*,*c*]anthracene (purity >99% by HPLC) in 35 μL DMSO [1/7, 2/7 and 4/7 of the dose on days 1, 8 and 15 of life, respectively]. The mice were monitored for 12 months. At the end of the experiment, necropsies were performed and liver and lung masses were subjected to histology. The incidence of liver tumour was 9/24 (adenomas) dibenz[*a*,*c*]anthracene-treated mice compared with 2/24 (adenomas) and 1/24 (carcinoma) solvent-treated control mice. Lung tumours were not observed in either of the two groups (von Tungeln *et al*., 1999b).

Dibenz[*a,h*]anthracene

Previous evaluations

Dibenz[*a,h*]anthracene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenz[*a*,*h*]anthracene was tested by oral administration to mice, dermal application in mice and hamsters, by intratracheal injection into hamsters, by intramammary injection into rats, by subcutaneous injection into mice, rats and guinea-pigs, by intramuscular injection into pigeons and chickens, by intravenous injection into mice, pulmonary administration to mice and rats and by intrarenal injection into frogs. These studies are summarized in Tables 3.1–3.4, 3.6, 3.9, 3.10, 3.12 and 3.14. On the basis of the available data, the Working Group concluded that dibenz[*a*,*h*]anthracene was carcinogenic in experimental animals. Dibenz[*a,h*]anthracene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenz[*a,h*]anthracene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or that were not considered in an earlier monograph are summarized below.

Dermal application (see also Table 3.1)

Mouse

Groups of 50 female NMRI mice [age unspecified] were treated three times a week with 17 μL acetone that contained 0.1, 0.4 or 1.1 μg dibenz[*a,h*]anthracene (>99 % pure

by gas chromatography (GC) and HPLC) for 112 weeks (total doses, 37.8, 125 and 378 μg). A control group of 50 female mice was treated with acetone alone. In the treated groups, skin tumours were observed in 3/50 low-dose, 4/50 mid-dose and 16/50 high-dose mice; 2/48 mice treated with acetone developed skin tumours (Platt *et al*., 1990).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 16 female NMRI mice [age unspecified] received a single dermal application of 100 μL acetone that contained 83.5 μg dibenz[*a*,*h*]anthracene (>99 % pure by GC and HPLC) or 100 μL tetrahydrofuran that contained 167 μg dibenz[*a*,*h*]-anthracene. A control group of 30 female mice was treated with 100 μL acetone alone. One week later, all mice received twice weekly dermal applications of 10 nmol TPA in 100 μL acetone. The experiment was terminated after 24 weeks. In treated groups, skin tumours were observed in 6/16 (38%) low-dose and 15/16 (93%) high-dose mice; no skin tumour was observed in mice treated with acetone alone (Platt *et al*., 1990).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 50 male and female C57BL mice [age unspecified] received a single subcutaneous injection of 20 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 500 μL tricaprylin. Mice surviving more than 4 months were included in the final tumour count. A control group of 304 mice was treated with tricaprylin alone. The experiment was continued for 22 months. Sarcomas occurred in 28/48 mice treated with dibenz[*a*,*h*]-anthracene compared with 3/280 control mice (Steiner & Falk, 1951).

Groups of 100 female NMRI mice [age unspecified] received a single subcutaneous injection of 2.35, 4.7, 9.3, 18.7, 37.5 or 75.0 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 500 μL tricaprylin. A control group of 100 mice was available. The experiment was continued for 114 weeks. The incidence of sarcomas was 37/100, 39/100, 44/100, 56/100, 65/100 and 69/100 in the groups treated with 2.35, 4.7, 9.3, 18.7, 37.5 and 75.0 μg dibenz[*a*,*h*]anthracene, respectively. The incidence in the control group was not reported (Pfeiffer, 1977).

Groups of male and female B6 (30 mice), D2 (30 mice) or B6D2F_1 (57 or 60) mice [age unspecified] received a single subcutaneous injection of 150 or 300 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 50 μL tricaprylin. Masses >1 cm were considered to be positive. Control mice (10 per group) were treated with tricaprylin alone. The experiment was terminated after 12 months. In the treated groups, the incidence of fibrosarcomas was 16/30 low-dose and 14/30 high-dose B6 mice; 1/30 low-dose and 0/30

high-dose D2 mice; and 8/57 low-dose and 33/60 high-dose B6D2F$_1$ mice. The tumour incidence in the control group was not reported (Kouri *et al*., 1983).

Groups of 30 male C3H/HeJ, C57BL/6J, AKR/J and DBA/J2 mice [age unspecified] received single subcutaneous injections of 150 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 50 μL trioctanoin. Control groups of 10 mice were treated with the solvent alone. The experiment was continued for 9 months. The incidence of fibrosarcomas in the treated groups was 24/30 C3H/HeJ, 16/30 C57BL/6J, 0/30 AKR/J and 1/30 DBA/J2 mice. No fibrosarcomas were observed in the control groups (Lubet *et al*., 1983).

Groups of 50 female NMRI mice [age unspecified] received single subcutaneous injections into the interscapular region of 500 μL tricaprylin that contained 10, 11, 30, 86 or 112 μg dibenz[*a*,*h*]anthracene (>99 % pure by GC and HPLC). Two control groups of 50 female mice were treated with tricaprylin alone. Masses >1 cm were considered to be positive. The experiment was terminated after 112 weeks. The incidence of fibrosarcomas in dibenz[*a*,*h*]anthracene-treated groups was: 10 μg, 25/48; 11 μg, 16/47; 30 μg, 25/50; 86 μg, 31/49; and 112 μg, 38/48; the incidence in controls was 3/49 and 1/50 (Platt *et al*., 1990).

Groups of 40 male and 44 female newborn NMRI mice, 2 days of age, received a single subcutaneous injection of 50 μL of an aqueous solution (1% gelatine, 0.9% saline, 0.4% Tween 20) that contained 11 or 111 μg dibenz[*a*,*h*]anthracene (>99 % pure by GC and HPLC). A control group of 49 male and female mice was treated with the solvent alone. In the treated groups, the incidence of pulmonary tumours was 6/13 (tumour multiplicity, 3.3 tumours/mouse) low-dose and 16/17 (30.6 tumours/mouse) high-dose females and 6/22 (3.8 tumours/mouse) low-dose and 19/21 (27.3 tumours/mouse) high-dose males. The incidence in controls was 1/19 (1.0 tumours/mouse) females and 1/14 (2.0 tumours/mouse) males (Platt *et al*., 1990).

Rat

Groups of 12 female Sprague-Dawley rats, 30 days of age, received thrice weekly subcutaneous injections for a total of 20 times of 100 μL sesame oil:DMSO (9:1) that contained 300 μg dibenz[*a*,*h*]anthracene (single peak by GC/mass spectrometry (MS) and HPLC). A control group of 12 rats was treated with the solvent alone. The experiment was continued for 37 weeks. The incidence of sarcomas in rats treated with dibenz[*a*,*h*]-anthracene was 12/12 compared with 0/12 in the solvent-treated controls (Flesher *et al*., 2002).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 20 male A/J mice, 5–6 weeks of age, received single intraperitoneal injections of 1.25, 2.5, 5 or 10 mg/kg bw dibenz[*a*,*h*]anthracene (purity, 97%) in 100 or

200 μL tricaprylin. A control group of 20 mice was treated with the solvent alone. The experiment continued for 8 months (survival, >90%), at which time gross lung adenomas were counted. The incidence of lung adenoma was 100% at doses above 2.5 mg/kg dibenz[*a*,*h*]anthracene. Tumour multiplicity (adenomas/mouse) was 1.44, 3.05, 13.1 and 32.1 at 1.25, 2.5, 5 and 10 mg/kg dibenz[*a*,*h*]anthracene, and was significantly different ($p < 0.05$) from the solvent-treated control (0.6 lung adenomas/mouse) at doses above 1.25 mg/kg (Nesnow *et al*., 1995; Ross *et al*., 1995; Nesnow *et al*., 1998a,b).

Intrapulmonary administration (see also Table 3.4)

Rat

Groups of 35 inbred female Osborne-Mendel rats [age unspecified] received a single pulmonary implantation of 100 μg dibenz[*a*,*h*]anthracene (purity, 99.3%) in a mixture of beeswax and tricaprylin or the solvent alone. The animals were monitored for 123 weeks. Lung carcinomas were found in 20 rats administered dibenz[*a*,*h*]anthracene, but not in solvent-treated control rats (Wenzel-Hartung *et al*., 1990).

Intramammary administration (see also Table 3.5)

Rat

Groups of 20 female Sprague-Dawley rats, 50 days of age, received a single intramammary injection of 1.1 or 4.5 mg finely powdered dibenz[*a*,*h*]anthracene (purity >99%). The untreated contralateral breast served as the control. All rats were killed after 20 weeks. No tumours were observed in either the treated or contralateral control mammary glands (Cavalieri *et al*., 1988a).

Intravenous administration (see also Table 3.9)

Mouse

A group of equal numbers of male and female strain A mice [age unspecified] received a single intrapulmonary injection of 250 μg dibenz[*a*,*h*]anthracene (melting-point, 266.0–266.8 °C) suspended in 250 μL water. The control group received the solvent alone. Twenty weeks after treatment, the incidence of pulmonary tumours was 10/10 (30.5 tumours/mouse) in the experimental group compared with 4/19 (1.0 tumours/mouse) in the controls (Andervont & Shimkin, 1940; Shimkin & Stoner, 1975).

Intratracheal injection (see also Table 3.12)

Hamster

Groups of 48 male Syrian golden hamsters [age unspecified] received a total of 30 weekly intratracheal administrations of 50 or 250 μg dibenz[*a*,*h*]anthracene (purity >99% by thin-layer chromatography; TLC) mixed with an equal amount of haematite dust (Fe_2O_3) in 200 μL saline and were monitored for 120 weeks. The incidence of respiratory tract tumours was 0/46 low-dose animals and 2/46 high-dose animals. No respiratory tract tumour was observed in 82 untreated controls (Sellakumar & Shubik, 1974).

Groups of Syrian golden hamsters [initial number, sex and age not specified] received weekly intratracheal injections of 300 or 900 μg dibenz[*a*,*h*]anthracene in a saline solution that contained 0.4% Tween 80 for 20 weeks. Control animals were treated with the solvent alone. The incidence of respiratory tract tumours was 55% in low-dose animals, 65% in high-dose animals and 3% in animals treated with the solvent (Pott *et al*., 1978).

Dibenz[*a,j*]anthracene

Previous evaluation

Dibenz[*a,j*]anthracene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which dibenz[*a,j*]anthracene was administered dermally and subcutaneously to mice. These studies are summarized in Tables 3.1 and 3.3. On the basis of the available data, the Working Group concluded that there was *limited evidence* that dibenz[*a,j*]anthracene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 400 or 800 nmol [111 or 223 μg] benz[*a,j*]anthracene [purity not specified] in 200 μL acetone. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA [solvent unspecified] twice a week for 20 weeks. After 20 weeks of promotion, the skin tumour incidence [no histology] was 70% (1.3 papillomas/mouse) in mice treated with 400 nmol dibenz[*a,j*]-anthracene and 97% (3.0 papillomas/mouse) in those treated with 800 nmol dibenz[*a,j*]-anthracene compared with 19% (0.19 papillomas/mouse) in acetone-treated control mice (Sawyer *et al.*, 1987).

Groups of 24 shaved female SENCAR mice [age unspecified] received a single dermal application of 400 nmol [111 μg] dibenz[*a,j*]anthracene [purity unspecified] in 200 μL peroxide-free tetrahydrofuran. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA in 200 μL acetone twice a week for 14 weeks. After 14 weeks of promotion, the skin tumour incidence (histology on random samples) was 29% (0.58 papillomas/mouse) in mice treated with dibenz[*a,j*]anthracene compared with 5% (0.05 papillomas/mouse) in the tetrahydrofuran-treated control mice (p <0.05) (Harvey *et al*., 1988).

Groups of 30 shaved female SENCAR mice [age unspecified] received a single dermal application of 400 or 800 nmol [111 or 223 μg] dibenz[*a,j*]anthracene [purity unspecified] in 200 μL peroxide-free tetrahydrofuran. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA twice a week for 20 weeks. After 20 weeks of promotion, the skin tumour incidence [no histology] was 70% (1.27 papillomas/mouse) in mice treated with 400 nmol dibenz[*a,j*]-anthracene and 97% (3.00 papillomas/mouse) in those treated with 800 nmol dibenz[*a,j*]-anthracene compared with 16% (0.16 papillomas/mouse) in acetone-treated control mice (Sawyer *et al*., 1988). [The Working Group noted that these appear to be the same data as those given in Sawyer *et al*. (1987).]

Groups of 24 shaved female SENCAR mice [age unpecified] received a single dermal application of 400 or 800 nmol [111 or 223 μg] dibenz[*a,j*]anthracene [purity unspecified] in 200 μL peroxide-free tetrahydrofuran. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA twice a week for 20 weeks. After 20 weeks of promotion, the skin tumour incidence was 39% (0.86 papillomas/mouse) in mice treated with 400 nmol dibenz[*a,j*]anthracene and 65% (1.83 papillomas/mouse) in those treated with 800 nmol dibenz[*a,j*]anthracene compared with 5% (0.05 papillomas/mouse) in the tetrahydrofuran-treated control mice (Sawyer *et al*., 1988).

Dibenzo[*a,e*]fluoranthene

Previous evaluation

Dibenzo[*a,e*]fluoranthene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one study in which dibenzo[*a,e*]fluoranthene was applied dermally to mice and one initiation–promotion study in mice. Both studies gave positive results. On the basis of these data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of dibenzo[*a,e*]fluoranthene in experimental animals. No new studies were available.

13*H*-Dibenzo[*a,g*]fluorene

Dermal application (see also Table 3.1)

Rat

In two experiments that explored the anti-carcinogenic activity of certain organic compounds, a group of 10 male and 10 female CF1 mice, 12 weeks of age, received dermal applications of one drop (0.02 mL) of a 0.3% solution of 13*H*-dibenzo[*a,g*]-fluorene [purity unspecified] [~ 60 µg] in acetone on the shaved interscapular region twice a week for 31 weeks. The experiments were terminated after 48 weeks. In one of the two experiments, 6/20 animals (30%) developed skin tumours (six squamous-cell carcinomas and one sarcoma). In the other experiment, 9/20 animals (45%) had skin tumours (nine squamous-cell carcinomas and two sarcomas) (Riegel *et al*., 1951). [The Working Group noted the absence of controls.]

Dibenzo[*h,rst*]pentaphene

Previous evaluation

Dibenzo[*h,rst*]pentaphene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated one bioassay in mice that were injected subcutaneously with dibenzo[*h,rst*]pentaphene, this is summarized in Table 3.3. On the basis of this study, another Working Group (IARC, 1987) concluded that there was *limited evidence* in experimental animals for the carcinogenicity of dibenzo[*h,rst*]pentaphene. No new studies were available.

Dibenzo[*a,e*]pyrene

Previous evaluations

Dibenzo[*a,e*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenzo[*a*,*e*]pyrene was administered dermally and subcutaneously to mice. These studies, which are summarized in Tables 3.1 and 3.3, indicated the induction of skin papillomas and epitheliomas after dermal application and sarcomas after subcutaneous injection. Dibenzo[*a*,*e*]pyrene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenzo[*a*,*e*]-pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or that were not considered in an earlier monograph are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female Ha/ICR/Mil (Swiss albino) mice [age and body weight unspecified] received 10 dermal applications of 25 μL 0.1% dibenzo[*a*,*e*]pyrene [25 μg; recrystallized and chromatographically pure; purity confirmed by determination of melting-point] in dioxane at 2-day intervals [total dose, 250 μg/animal]. On day 28, dermal applications of 2.5% (2.3 mg) croton oil in acetone [volume, number, frequency and duration of treatments unspecified] were begun. A group of 30 mice was treated with croton oil solution alone. All survivors were killed after 6 months, at which time mortality rates were 2/28 and 4/30 and tumour incidence (skin papillomas) was 10/28 and 2/30 in the dibenzo[*a*,*e*]pyrene-treated and croton oil control groups, respectively. The total number of papillomas/mouse was 0.83 and 0.1 in the dibenzo[*a*,*e*]pyrene-treated and croton oil control mice, respectively (Hoffmann & Wynder, 1966; LaVoie *et al.*, 1979).

A group of 21 female SENCAR mice, 8 weeks of age, [weight unspecified] received a single dermal application of 800 nmol [242 μg] dibenzo[*a*,*e*]pyrene (purity >99% by HPLC) in 100 μL dioxane/DMSO (75:25). A vehicle control group was treated with 100 μL dioxane/DMSO alone. One week later, all mice were treated with 4.26 nmol (2.6 μg) TPA in 100 μL acetone twice a week for 25 weeks and were killed after the 25th week of promotion. Complete necropsies were performed. At the end of the experiment, 5/21 mice in the dibenzo[*a*,*e*]pyrene-treated group had developed seven skin papillomas (0.3 papillomas/mouse) and 2/23 mice in the control group had developed one skin papilloma (0.1 papilloma/mouse). The first skin papillomas appeared after 15 weeks in the dibenzo[*a*,*e*]pyrene-treated group compared with 20 weeks in the control group (Cavalieri *et al.*, 1989).

Intramamillary administration (see also Table 3.5)

Rat

A group of 19 female Sprague-Dawley rats, 8 weeks of age, [weight unspecified] received a single intramamillary injection of 4 μmol (1.2 mg)/gland dibenzo[*a*,*e*]pyrene (purity >99% by HPLC) dissolved in 100 μL trioctanoin (total dose, 32 μmol [9.6 mg]). One control group of 21 rats was treated with 100 μL solvent and another control group of 20 rats remained untreated. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter; all the remaining animals were killed at 40 weeks. Complete necropsies were performed. Survival rates (mean ± SD) were 38 ± 0, 37 ± 4 and 40 ± 0 weeks in the dibenzo[*a*,*e*]pyrene-treated, untreated and vehicle-control groups, respectively, and tumour latencies were 35 ± 0 and 25 ± 13 weeks in the dibenzo[*a*,*e*]pyrene-treated and untreated groups, respectively. At the end of the study, 1/19 rats in the dibenzo[*a*,*e*]pyrene-treated group and 2/20 rats in the untreated group had developed mammary epithelial tumours (one adenofibroma in the

dibenzo[*a*,*e*]pyrene-treated rat; one adenofibroma and one adenocarcinoma in the untreated rats). No tumours were observed in the vehicle-control group (Cavalieri *et al*., 1989).

Dibenzo[*a,h*]pyrene

Previous evaluations

Dibenzo[*a,h*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenzo[*a*,*h*]pyrene was administered dermally and subcutaneously to mice and by intramuscular injection to rats. These studies are summarized in Tables 3.1, 3.3 and 3.10. On the basis of the available data, the Working Group concluded that dibenzo[*a*,*h*]pyrene was carcinogenic in experimental animals. Dibenzo[*a*,*h*]pyrene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenzo[*a*,*h*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or were not considered in an earlier monograph are summarized below.

Dermal application (see also Table 3.1)

Mouse

A group of 40 random-bred female Swiss mice [age unspecified] received dermal applications of 119 μg dibenzo[*a*,*h*]pyrene (purity, 99.6%) in 16.7 μL acetone twice weekly for 30 weeks. A control group of 40 mice was treated with acetone alone. Tumours were recorded when they persisted for 4 weeks or more. All dibenzo[*a*,*h*]-pyrene-treated mice had died or were removed by 45 weeks and 35/39 had skin tumours (papillomas, keratoacanthomas and carcinomas). No skin tumours were observed in 29 control mice after a period of 70 weeks (Cavalieri *et al*., 1977).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 31 female CD-1 mice [age unspecified] received a single dermal application of 200 μg dibenzo[*a*,*h*]pyrene [purity unspecified] in acetone. A control group of 32 mice was treated with acetone alone. One week later, all mice received twice weekly dermal applications of 10 μg TPA for 26 weeks. Skin tumours occurred in 26/28 mice treated with dibenzo[*a*,*h*]pyrene compared with 2/32 solvent-treated control mice (Sardella *et al*., 1981).

Groups of 30 female CD-1 mice, 7–8 weeks of age, received a single dermal application of 15 μg (50 nmol) dibenzo[*a*,*h*]pyrene ('essentially' pure by chromatography, mass spectrometry (MS) and nuclear magnetic resonance (NMR)) in 200 μL 10% DMSO in tetrahydrofuran or the solvent alone. Seven days later, all mice received twice-weekly applications of 16 nmol TPA in 200 μL acetone. Papillomas >2 mm in diameter that persisted for ≥2 weeks were included in the cumulative total. After 16 weeks of TPA promotion, 55% of the dibenzo[*a*,*h*]pyrene-treated mice had papillomas (1.41 tumours/mouse) compared with 0% in the solvent-treated controls; after 24 weeks of TPA promotion, the respective values were 72% (3.97 tumours/mouse) and 0%. In a second experiment, groups of 30 female CD-1 mice received a single dermal application of either 60 μg (200 nmol) or 180 μg (600 nmol) dibenzo[*a*,*h*]pyrene followed by applications of TPA. After 16 weeks of TPA promotion, papillomas had occurred in 79% (4.72 tumours/mouse) and 72% (5.52 tumours/mouse) low- and high-dose animals, respectively, compared with 10% (0.01 tumours/mouse) of the solvent-treated controls (Chang *et al*., 1982).

A group of 24 female SENCAR mice, 8 weeks of age, received a single dermal application of 240 μg (800 nmol) dibenzo[*a*,*h*]pyrene (purity >99% by HPLC) in 100 μL dioxane: DMSO (75:25); a control group of 23 mice was treated with the solvent alone. One week later, all mice received twice weekly dermal applications of 4.26 nmol TPA in 100 μL acetone for 25 weeks. Papillomas occurred in 18/24 mice treated with dibenzo[*a*,*h*]pyrene (5.3 tumours/mouse) compared with 2/23 solvent-treated control mice (0.1 tumours/mouse) (Cavalieri *et al*., 1989).

Intraperitoneal administration (see also Table 3.7)

Mouse

60 Newborn male and female Swiss-Webster mice (BLU:Ha(ICR)) received intraperitoneal injections on days 1, 8 and 15 of life of 3.8, 7.6 and 15.2 μg dibenzo[*a*,*h*]-pyrene ('essentially' pure by chromatography, MS and NMR) in 5, 10 and 20 μL DMSO. Control mice were treated with the solvent alone. The experiment continued until the mice were 49–54 weeks old. Pulmonary tumours occurred in 13/14 treated females (4.78 tumours/mouse), 25/25 treated males (5.20 tumours/mouse), 11/39 control females (0.44 tumours/mouse) and 7/32 control males (0.80 tumours/mouse). Hepatic tumours occurred in 1/14 treated females (0.07 tumours/mouse) and 11/25 treated males (0.88 tumours/mouse), but not in solvent-treated controls. Two female mice treated with dibenzo[*a*,*h*]pyrene had skin sarcomas and one had an adenocarcinoma of the small intestine (Chang *et al*., 1982).

Intramamillary administration (see also Table 3.5)

Rat

A group of 20 female Sprague-Dawley rats, 8 weeks of age, received a single intramamillary injection of 1.2 μg (4 μmol)/gland dibenzo[*a*,*h*]pyrene (purity >99% by HPLC) in 100 μL tricaprylin (total dose, 9.6 μg for eight glands). A group of 21 control rats was treated with tricaprylin alone. Animals were killed when tumours were ≥2 cm in diameter. The experiment lasted 40 weeks. Fibrosarcomas occurred in 19/20 rats treated with dibenzo[*a*,*h*]pyrene compared with 0/21 control rats. Mammary gland adenofibromas and adenocarcinomas occurred in 4/20 rats treated with dibenzo[*a*,*h*]pyrene compared with 0/21 control rats (Cavalieri *et al.*, 1989).

Dibenzo[*a*,*i*]pyrene

Previous evaluations

Dibenzo[*a*,*i*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenzo[*a*,*i*]pyrene was administered dermally to mice and subcutaneously to mice and hamsters. The transfer of injection-site tissues following subcutaneous injection to mice was also analysed. These studies, which are summarized in Tables 3.1 and 3.3, indicated the induction of skin papillomas and epitheliomas [the Working Group considered these tumours to be squamous-cell carcinomas] after repeated dermal application and sarcomas after subcutaneous injection; in addition, it was shown that transfer of injection-site tissues to secondary hosts shortened the latent period. Dibenzo[*a*,*i*]pyrene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenzo[*a*,*i*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or that were not considered in an earlier monograph are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female Ha/ICR Swiss albino mice, 50–55 days of age [body weight unspecified], received 10 dermal applications of dibenzo[*a*,*i*]pyrene (95% pure by HPLC) in 100 μL acetone once on alternate days (total doses, 100 μg and 500 μg) or acetone alone. Ten days after initiation had been completed, all animals received thrice-weekly applications of 2.5 μg TPA in 100 μL acetone for 20 weeks. Tumours were counted weekly and at autopsy, and were examined histologically; however, skin tumour types were not reported. Mice treated with 100 μg dibenzo[*a*,*i*]pyrene had a 40% skin tumour

incidence (average of 0.5 skin tumours/mouse), whereas the group treated with 500 μg dibenzo[*a*,*i*]pyrene had an 85% skin tumour incidence (average of 5.8 skin tumours/mouse). No skin tumours were observed in the vehicle-control group (Hecht *et al.*, 1981).

Groups of 30 female CD-1 mice, 7–8 weeks of age [body weight unspecified], were treated with a single dermal application of 15, 60 or 180 μg dibenzo[*a*,*i*]pyrene ('essentially pure' on the basis of chromatography, MS and NMR) in 200 μL 10% DMSO in tetrahydrofuran or the solvent alone. One week later, the mice received twice-weekly applications of 10 μg TPA in 200 μL acetone. Papillomas >2 mm in diameter were included in the cumulative total when they persisted for ≥2 weeks. After a 16-week period of promotion, skin tumour incidence was 28, 67 and 79% in the low-, mid- and high-dose groups, respectively. The corresponding numbers of skin papillomas/mouse [no histology] were 0.52, 5.33 and 5.25. In a separate experiment, promotion with TPA was continued for 24 weeks in a group of 30 female mice previously treated with 15 μg dibenzo[*a*,*i*]pyrene; skin tumour incidence was 69%, and the number of skin papillomas/mouse was 2.07 ± 0.44 (mean ± SE). No skin tumours were observed in the solvent control groups for both 15-μg dose groups; the skin tumour incidence was 10% (0.10 papillomas/mouse) in the controls for the 60- and 180-μg dose groups (Chang *et al.*, 1982).

Groups of 24 female SENCAR mice, 8 weeks of age [body weight unspecified], received a single dermal application of 800 nmol [242 μg] dibenzo[*a*,*i*]pyrene (purity >99% by HPLC) in 100 μL dioxane:DMSO (75:25) or dioxane:DMSO only. One week later, all mice were treated with TPA (4.26 nmol [2.6 μg]/100 μL acetone] twice a week for 25 weeks, and were killed after the 25th week of promotion. At the end of the experiment, 15/24 mice in the dibenzo[*a*,*i*]pyrene-treated group had developed 63 skin papillomas (2.6 papillomas/mouse) and 2/23 mice in the control group had developed a skin papilloma (0.1 papillomas/mouse). The first skin papillomas appeared after 12 weeks in the dibenzo[*a*,*i*]pyrene-treated group compared with 20 weeks in the control group (Cavalieri *et al.*, 1989).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 50 mice [strain, sex, age and weight unspecified] received a single subcutaneous injection of 100 μg dibenzo[*a*,*i*]pyrene [purity unspecified] in tricaprylin [volume unspecified]. A control group of 25 mice was treated with tricaprylin alone. Survival rates after 75 weeks were 41/50 dibenzo[*a*,*i*]pyrene-treated animals and 24/25 controls. Tumour incidences (local sarcomas) at 75 weeks were 40/50 treated mice and 0/25 controls, respectively (Sardella *et al.*, 1981).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of newborn male and female Swiss-Webster [BLU:Ha(ICR)] mice received three intraperitoneal injections of 12.5, 25 and 50 nmol (3.8, 7.6 and 15.1 μg) dibenzo[*a,i*]pyrene ('essentially pure' on the basis of chromatography, MS and NMR) dissolved in 5, 10 and 20 μL DMSO, respectively, on days 1, 8 and 15 of life (total dose, 87.5 nmol [26.5 μg]). Control mice received injections of DMSO alone. The mice were weaned at 25 days of age, and killed at 49–54 weeks. At the end of the experiment, the incidence of pulmonary tumours was 38/39 (3.64 tumours/mouse) treated males, 7/32 (0.80 tumours/mouse) control males, 21/21 (5.80 tumours/mouse) treated females and 11/39 (0.44 tumours/mouse) control females. In addition, hepatic tumours were observed in 21/39 treated males [0.82 tumours/mouse], but not in the other groups. A representative number of pulmonary tumours and all hepatic tumours were examined histologically. Most of the hepatic tumours were type A or neoplastic nodules (Chang *et al*., 1982).

Intratracheal administration (see also Table 3.12)

Hamster

A group of 36 male Syrian golden hamsters, 9–10 weeks of age and weighing approximately 98 g, received four weekly intratracheal doses of 2 mg dibenzo[*a,i*]pyrene (purity > 99% by TLC; total dose, 8 mg), ground to a finely aggregated dust (1:1, w:w) with haematite (particle size <1 μm) and then suspended in 200 μL saline (0.9% aqueous). A second group of 48 male hamsters of the same strain and age was treated similarly with 24 weekly doses of 500 μg dibenzo[*a,i*]pyrene (total dose, 12 mg), and an additional group of 90 hamsters was untreated. No control group received intratracheal instillation of the vehicle. The animals were monitored daily, weighed once a week and died spontaneously or were killed when moribund. At 100 weeks, all treated animals and 71/90 controls had died. Respiratory insufficiency, due to extensive tumour involvement in the respiratory tract, accounted for the increased mortality rates in the treated groups. In the group treated with four doses of 2 mg dibenzo[*a,i*]pyrene, the incidence of tumours of the respiratory tract was 16/34; specific incidences were one tumour of the larynx, two of the trachea, 13 of the bronchi and one of the lung; no tumours were found at other sites. In the group treated with 24 doses of 500 μg dibenzo[*a,i*]pyrene, the incidence of tumours of the respiratory tract was 39/44; specific incidences were six tumours of the trachea, 37 of the bronchi and one of the lung. In addition, two malignant lymphomas occurred in this group. A total of 19 treatment-induced respiratory tract tumours developed in the group treated with four doses of 2 mg, and a total of 95 tumours in the group treated 24 times with 500 μg; squamous-cell carcinomas were the predominant histological type. No

tumours of the respiratory tract were observed in the untreated group, which had an incidence of 11/82 tumours at other sites (Sellakumar & Shubik, 1974).

Two groups of 24 male and 24 female Syrian golden hamsters, 6–7 weeks of age [weight unspecified], were treated intratracheally with dibenzo[*a*,*i*]pyrene (purity >99% by TLC) finely suspended in distilled water (particle size <25 μm). One group received 1 mg once a week for 12 weeks (total dose, 12 mg) and the other group received 500 μg once a week for 17 weeks (total dose, 8.5 mg). No control group was available. Animals were monitored weekly and those in poor condition were isolated and allowed to die spontaneously or were killed when moribund [follow-up time unspecified]; death was most frequently attributed to respiratory insufficiency due to extensive neoplastic involvement of the respiratory system. The incidence of respiratory tumours (males and females combined) was 36/48 in the group that received 12 doses of 1 mg and 39/48 in the group that received 17 doses of 500 μg; the earliest tumours appeared in the larynx and trachea at 8 weeks. Main bronchi tumours (62% at the 12 × 1-mg dose level and 82% at the 17 × 500-μg dose level) predominated, followed by tracheal tumours (19% at the 12 × 1-mg dose level and 13% at the 17 × 500-μg dose level). Tumours of the larynx, lung and pleura were observed at lower incidences. Morphologically, the most common tumours were squamous-cell carcinomas (Stenbäck & Sellakumar, 1974).

Intramamillary administration (see also Table 3.5)

Rat

A group of approximately 20 female Sprague-Dawley rats, 8 weeks of age, [weight unspecified], received a single intramamillary injection of 4 μmol (1.2 mg)/gland dibenzo[*a*,*i*]pyrene (purity >99% by HPLC) dissolved in 100 μL tricaprylin (eight glands; total dose, 32 μmol [9.6 mg]). One control group was treated with 100 μL solvent and another was untreated. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter. All the remaining animals were killed at 40 weeks and complete necropsies were performed. Survival rates (mean ± SD) were 30 ± 5, 37 ± 4 and 40 ± 0 weeks in the dibenzo[*a*,*i*]pyrene-treated, untreated and vehicle-control groups, respectively. Tumour latencies were 19 ± 2 and 25 ± 13 weeks in the dibenzo[*a*,*i*]pyrene-treated and untreated groups, respectively. At the end of the study, 18/19 rats in the dibenzo[*a*,*i*]pyrene-treated group had developed fibrosarcomas (2.4 tumours/tumour-bearing rat), 11/19 rats had mammary adenocarcinomas (1.4 tumours/tumour-bearing rat) and 1/19 had mammary adenofibromas (two tumours). In contrast, 2/20 rats in the untreated group had developed mammary epithelial tumours (one adenofibroma and one adenocarcinoma) but no fibrosarcomas. No tumours were observed in the vehicle-control group (Cavalieri *et al*., 1989).

Intrauterine administration (see Table 3.14)

Mouse

A group of 30 female mice [strain, age and weight unspecified] received a single intrauterine injection of 0.5 mg dibenzo[*a,i*]pyrene [vehicle, purity and volume unspecified]. After 32 weeks, none of the 30 treated animals developed tumours (Homburger & Tregier 1960).

Dibenzo[*a,l*]pyrene

Previous evaluations

Dibenzo[*a,l*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that restricted its evaluation to work published after 1966, since earlier data reported for dibenzo[*a,l*]pyrene had in fact been obtained from experiments that used dibenzo[*a,e*]fluoranthene. A single study was analysed that showed the induction of sarcomas following subcutaneous administration of dibenzo[*a,l*]pyrene to mice (Table 3.3). Dibenzo[*a,l*]pyrene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassay as that considered previously, and an additional study of dermal application to mice that resulted in the induction of skin tumours (see Table 3.1). On the basis of these data, the Working Group concluded that there was *sufficient evidence* that dibenzo[*a,l*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Oral administration (see also Table 3.6)

Fish

A group of 65 Japanese medaka fish (*Oryzias latipes*), 2 months of age, was fed a diet containing 100 ppm dibenzo[*a,l*]pyrene (purity >97% by HPLC; dissolved in menhaden oil before mixing) *ad libitum* once a day on 5 days per week for 28 days, followed by basal diet for an additional 9 months. A control group of 75 fish was fed basal diet only. At the end of the experiment, tumour incidence was 17/65 (26%) in the dibenzo[*a,l*]pyrene-treated group compared with 6/75 (8%) in the control group ($p < 0.05$). Hepatic neoplasia (12/65 (18%) versus 0/75; $p < 0.001$) predominated, followed by hepatocellular carcinoma (7/65 (11%) versus 0/75). Other types of neoplasia observed included bile duct adenoma, ovarian dysgerminoma, testicular neurofibrosarcoma and paravertebral ganglioneuroma (1/65 (1.5%), 2/65 (3%), 0/65 and 1/65 (1.5%), respectively, compared with 0/75, 5/75 (7%), 1/75 (1%) and 0/75 in the control group) (Reddy *et al*., 1999a).

A group of 260 Shasta strain rainbow trout (*Oncorhynchus mykiss*), 19 weeks of age, was fed a diet containing 500 ppm dibenzo[*a,l*]pyrene [purity unspecified] for 2 weeks

and then returned to basal diet for 11 months. Since the 500-ppm dose induced a high mortality rate [level unspecified] at the end of the treatment period, a second group of 260 fish, 21 weeks of age, was fed a diet containing a lower dose of dibenzo[*a*,*l*]pyrene (200 ppm) for an extended exposure period (4 weeks); this group was then fed basal diet for 9 months. Control groups were fed basal diet only. At the end of the study, tumour incidence in the 500-ppm group was 61% (liver), 91% (stomach) and 53% (swimbladder) (2.58, 5.67 and 2.25 tumours/tumour-bearing fish, respectively). The corresponding numbers in the 200-ppm group were 36% (liver), 48% (stomach) and 30% (swimbladder) (2.00, 3.80 and 2.40 tumours/tumour-bearing fish, respectively). No tumours were observed in the control groups. Stomach and swimbladder tumours were papillary adenomas in both dose groups. Among the liver tumours, hepatocellular carcinomas (64% relative incidence) predominated in the 500-ppm dose group. In the 200-ppm dose group, the relative incidences of hepatocellular carcinomas (43%) and adenomas (44%) were comparable. Cholangiocellular adenomas and mixed hepato/cholangiocellular carcinomas were also observed, at lower incidences, in both groups (Reddy *et al*., 1999b).

Dermal application (see also Table 3.1)

Mouse

Groups of 22–24 female SENCAR mice, 8 weeks of age, were treated with repeated applications of 1, 4 or 8 nmol [0.3, 1.2 or 2.4 μg] dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) in 100 μL acetone twice a week for 40 weeks. The highest dose was the maximum dose tolerated without the development of erythema. A control group of 27 mice was treated with 100 μL acetone alone. Mice were killed when tumours reached 2 cm in diameter. All surviving mice were killed by 48 weeks, and complete necropsies were performed; all tumours were histologically verified. At the end of the study, skin tumour incidence was 1/24 (three squamous papillomas) low-dose group, 19/23 (82%; 16/23 carcinomas; 1.8 tumours/tumour-bearing animal; 9/23 squamous papillomas; 1.9 tumours/tumour-bearing animal; and 2/23 sebaceous gland adenomas; 1.5 tumours/tumour-bearing animal) mid-dose group and 20/22 (90%; 20/22 carcinomas; 2.6 tumours/tumour-bearing animal; 16/22 squamous papillomas; 1.9 tumours/tumour-bearing animal; and 3/22 sebaceous gland adenomas; 1.7 tumours/tumour-bearing animal) high-dose group. In contrast, skin tumours were not observed in the control group. In addition, tumours were observed at other sites (totals of 5/24 (21%), 9/23 (39%) and 15/22 (68%) low-, mid- and high-dose animals and 1/27 (4%) controls). These tumours included lung adenomas, malignant lymphomas of the spleen and malignant lymphomas with multiple organ involvement; lung adenomas predominated in the low-dose group, as opposed to lymphomas of the spleen in the mid- and high-dose groups (Higginbotham *et al*., 1993).

A recent study compared the response of aryl hydrocarbon receptor-deficient ($AhR^{-/-}$) and -proficient ($AhR^{+/+}$) C57BL/6J mice to repeated dermal applications of dibenzo[*a*,*l*]-pyrene. A group of 15 $AhR^{-/-}$ and a group of 17 $AhR^{+/+}$ mice [sex, age and body weight

unspecified] received a single dermal application of 30 μg dibenzo[*a*,*l*]pyrene [purity unspecified; solvent and volume unspecified]. The initial treatment was followed by successive applications of 6 μg dibenzo[*a*,*l*]pyrene [solvent and volume unspecified] once a week for 20 weeks [the time between the first and second treatment was unspecified]. No vehicle control group was used. Skin tumours were first detected in $AhR^{+/+}$ mice at 11 weeks; a tumour incidence of 17/17 was reached in this group by 24 weeks. In contrast, skin tumours were first detected in $AhR^{-/-}$ mice at 21 weeks; a tumour incidence of 5/15 was reached in this group by 24 weeks and did not increase further during the follow-up period (up to 2 years). Multiplicities of skin tumours were 2.7 ± 1.4/mouse in the $AhR^{+/+}$ group (76% papillomas and 24% squamous-cell carcinomas) and 0.46 ± 0.83/mouse in the $AhR^{-/-}$ group (100% papillomas). The differences between the two groups were significant, both for tumour incidence (p <0.001) and tumour multiplicity (p <0.005) (Nakatsuru *et al.*, 2004).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 24 female SENCAR mice, 8 weeks of age, [body weight unspecified], received a single dermal application of 800 nmol [242 μg] dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) in 100 μL dioxane:DMSO (75:25). A vehicle-control group was treated with 100 μL dioxane:DMSO alone. One week after initiation, most of the mice in the dibenzo[*a*,*l*]pyrene-treated group had developed erythema at the site of application, which resolved 2 weeks later. One (control group) and 3 (dibenzo[*a*,*l*]pyrene-treated group) weeks after initiation, all mice were treated with 4.26 nmol [2.6 μg] TPA in 100 μL acetone twice a week for the duration of the experiment. The mice were killed 26 weeks after initiation. Complete necropsies were performed. At the end of the experiment, 22/24 (92%) mice in the dibenzo[*a*,*l*]pyrene-treated group had developed 92 skin papillomas (3.8 papillomas/mouse) and 2/23 (9%) mice in the control group had developed a skin papilloma (0.1 papillomas/mouse). The first skin papilloma appeared after 5 weeks in the dibenzo[*a*,*l*]pyrene-treated group compared with 20 weeks in the control group (Cavalieri *et al.*, 1989).

In another similarly designed initiation–promotion study, groups of 24 female SENCAR mice, 8 weeks of age, were treated dermally with 0, 33.3, 100 or 300 nmol [10.1, 30.2 or 90.7 μg] dibenzo[*a*,*l*]pyrene in 100 μL acetone followed 1 week later by promotion with 3.24 nmol [2.0 μg] TPA in 100 μL acetone. The mice treated with dibenzo[*a*,*l*]pyrene developed erythemas after the first treatment with TPA, and the severity of the reaction was directly related to the initiating dose. Due to this reaction, promotion was stopped until the 4th experimental week and was then resumed for 12 weeks. The mice were killed 15 weeks after initiation, and complete necropsies were performed. At the end of the experiment, 23/24 (96%), 22/24 (92%) and 24/24 (100%) mice in the low-, mid- and high-dose groups had developed skin tumours (6.75, 7.92 and

8.50 tumours/mouse, respectively), which were predominantly papillomas; a small number of suspected carcinomas were not verified histologically. In contrast, no skin tumours were observed in the control group. The first skin papilloma appeared at 4 weeks in the mid- and high-dose groups, and at 8 weeks in the low-dose group (Cavalieri *et al.*, 1991).

In a separate experiment, doses of 4, 20 or 100 nmol [1.2, 6.0 or 30.2 μg] dibenzo[*a*,*l*]pyrene were applied dermally to groups of 24 female NMRI mice at 8 weeks of age; promotion with 2.0 μg TPA in 100 μL acetone was begun 2 weeks later. An additional group of mice was treated once with 100 nmol dibenzo[*a*,*l*]pyrene and received no TPA. The experiment lasted 27 weeks. In the group that was not treated with TPA, 7/24 (29%) mice developed skin tumours (1 tumour/tumour-bearing animal), of which [4/7] were skin papillomas and [3/7] were squamous-cell carcinomas. Among animals treated with TPA, the incidence of skin tumours was 22/24 (92%), 20/24 (83%) and 20/24 (83%) (6.96, 5.29 and 3.29 tumours/mouse) in the low-, mid- and high-dose groups, respectively; no skin tumours were observed in the control group. Most of the tumours were skin papillomas; squamous-cell carcinomas represented <2.5% of the total. The inverse relationship between dose and tumorigenic response was attributed to interference in tumour initiation by the toxicity of the compound (Cavalieri *et al.*, 1991).

Lower doses of initiator and promoter also effectively induced skin tumours. Groups of 24 female SENCAR mice, 8 weeks of age, received single dermal applications of 0.25 or 1 nmol [75.5 or 302 ng] dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) in 100 μL acetone or the solvent alone. One week later, promotion was begun with twice-weekly dermal applications of 2.16 nmol [1.3 μg] TPA in 100 μL acetone; this dose was chosen to prevent the appearance of erythema in the dibenzo[*a*,*l*]pyrene-treated group. The mice were killed 27 weeks after initiation, at which time approximately 30% of mice in the 0.25-nmol group and 80% of mice in the 1-nmol group had developed skin papillomas; in addition, two skin carcinomas were observed in the high-dose group. The first tumour developed in this group after 5 weeks. Tumour incidence was not reported for the control group (Higginbotham *et al*., 1993).

In another study that involved multiple dermal applications of dibenzo[*a*,*l*]pyrene, groups of 20 female CD-1 mice, 50–55 days of age [body weight unspecified], received total doses of 1, 4, 10 and 25 nmol [0.3, 1.2, 3.0 and 7.6 μg] dibenzo[*a*,*l*]pyrene [purity unspecified] dissolved in 100 μL acetone applied to the skin as 10 subdoses on alternate days. One control group was treated with acetone alone. Ten days after the last treatment, promotion was begun by applying 2.5 μg TPA in 100 μL acetone three times a week for 20 weeks. The incidence of skin tumours was 18/19 (95%; 5.0 tumours/mouse), 20/20 (100%; 17.8 tumours/mouse), 18/20 (90%; 11.3 tumours/mouse) and 20/20 (100%; 15.0 tumours/mouse) in the lowest- to the highest-dose groups compared with 3/20 (15%; 0.15 tumours/mouse) in the control group (p <0.001 at all doses) (LaVoie *et al.*, 1993).

Other similarly designed initiation–promotion studies in female SENCAR mice entailed single dermal applications of 1.33–12 nmol [0.4–3.6 μg] dibenzo[*a*,*l*]pyrene

followed by twice-weekly applications of 1.62 nmol [1 μg] TPA for 25–28 weeks. High tumour incidence and multiplicity were observed (Gill *et al.*, 1994; Marston *et al.*, 2001), which increased in a dose-related manner (Gill *et al.*, 1994).

Additional initiation–promotion studies were conducted with other strains of mice. In one study, a group of 16 female outbred NMRI mice, 7 weeks of age [body weight unspecified], received a single dermal application of 40 nmol [12 μg] dibenzo[*a*,*l*]pyrene (purity ≥99.7%) in 100 μL acetone, followed 1 week later by twice-weekly applications of 10 nmol [6.2 μg] TPA in 100 μL acetone for 30 weeks. A strong increase in skin tumour rates was observed 8–9 weeks after exposure to dibenzo[*a*,*l*]pyrene. At 18 weeks, the incidence of skin tumours (papillomas and carcinomas combined) in the dibenzo[*a*,*l*]pyrene-treated group was 15/16 (94%; 6.5 tumours/mouse). No skin tumours were observed in a vehicle-control group (Luch *et al.*, 1999).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 30–35 male A/J mice, 5–6 weeks of age and weighing approximately 20 g, received a single intraperitoneal injection of 0.3, 1.5, 3.0 or 6.0 mg/kg bw [6, 30, 60 or 120 μg] dibenzo[*a*,*l*]pyrene (analytical grade) suspended in tricaprylin or tricaprylin alone [volume unspecified]. In a separate experiment, groups of mice of the same strain, sex and age were given higher doses (12, 18 or 24 mg/kg bw [240, 360 or 480 μg]) in a similar manner. The mice were killed 8 months after treatment, and lungs were removed and examined for the presence of tumours. The treatment was associated with an increased incidence of hepatocellular necrosis and inflammation, although no dose-related effect was apparent. Lung adenomas were observed in 14/33 (42%), 33/34 (97%), 34/34 (100%) and 35/35 (100%) mice treated with 6–120 μg dibenzo[*a*,*l*]pyrene at increasing dose levels compared with 15/30 (50%) vehicle controls. The corresponding numbers of lung adenomas/mouse (0.42 ± 0.56, 4.30 ± 2.86, 7.50 ± 3.79 and 16.1 ± 7.26 (mean ± SD)) increased in a dose-dependent manner in the dibenzo[*a*,*l*]pyrene-treated groups; 0.67 ± 0.80 lung adenomas/mouse were observed in the control group (p <0.001 for doses ≥30 μg compared with the control group). Treatment at the three highest doses (240–480 μg) did not affect survival up to 8 months, but a 22% weight loss was observed in the group treated with 480 μg dibenzo[*a*,*l*]pyrene; 5/5 mice in this group developed lung adenomas (36.67 ± 10.64 tumours/mouse) (Prahalad *et al.*, 1997).

A similarly designed study of groups of 20 mice of the same strain and age that received doses of 0–6 mg/kg dibenzo[*a*,*l*]pyrene gave comparable results (Nesnow *et al.*, 1998a).

Groups of 25–40 male and female newborn Crl:CD-1(ICR)BR mice received three intraperitoneal injections of dibenzo[*a*,*l*]pyrene [purity unspecified] (total doses, 40 or 400 nmol [12.1 or 121 μg]). The mice received 1/8 (12%), 1/4 (25%) and 5/8 (62%) of the total dose dissolved in 10, 20 and 50 μL DMSO on days 1, 8 and 15 of life,

respectively. One group of control mice was left untreated and another was administered DMSO only. The mice were weaned at 3–4 weeks of age and killed at 51 ± 1 weeks, except for the high-dose dibenzo[*a*,*l*]pyrene-treated group, in which high mortality imposed termination of the experiment after 17 weeks. At the end of the experiment, the incidence of pulmonary tumours was 84.8% in low-dose males (2.85 ± 0.44 tumours/mouse (mean ± standard error of the mean [SEM])), 41.2% in high-dose males (0.65 ± 0.21 tumours/mouse), and 31.4% (0.54 ± 0.17 tumours/mouse) and 25.0% (0.33 ± 0.14 tumours/mouse) in male untreated and solvent-treated mice, respectively. In addition, 84.8% of low-dose males had hepatic tumours (5.67 ± 0.86 tumours/mouse) and 30.3% had tumours at other sites (0.58 ± 0.17 tumours/mouse); 35.3% of high-dose males had hepatic tumours (1.00 ± 0.38 tumours/mouse) and 23.5% had tumours at other sites (0.35 ± 0.19 tumours/mouse); extrapulmonary tumours were not detected in any of the control groups. In female mice, the incidence of pulmonary tumours at 17 weeks was 89.5% in the low-dose (2.95 ± 0.67 tumours/mouse), 35.7% in the high-dose (0.57 ± 0.29 tumours/mouse), and 37.0% (0.67 ± 0.19 tumours/mouse) and 10.0% (0.67 ± 0.19 tumours/mouse) in the untreated and solvent-treated groups, respectively; the incidence of hepatic tumours was 10.5% in the low-dose (0.11 ± 0.07 tumours/mouse) and 14.3% in the high-dose groups (21 ± 0.15 tumours/mouse). Tumours at other sites were observed in 47.4% of low-dose (0.53 ± 0.14 tumours/mouse) and 42.9% of high-dose females (0.50 ± 0.17 tumours/mouse). Female controls did not develop any extrapulmonary tumours. Lung and liver tumours were predominantly adenomas; in addition to the lung and liver, the kidneys, intestine, ovaries and skin were found to be prone to dibenzo[*a*,*l*]pyrene-induced tumorigenicity (Platt *et al.*, 2004).

Intramamillary administration (see also Table 3.5)

Rat

A group of 19 female Sprague-Dawley rats, approximately 8 weeks of age [body weight unspecified], received a single intramamillary injection of 4 μmol [1.2 mg]/gland dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) dissolved in 100 μL trioctanoin (eight glands; total dose, 32 μmol [9.6 mg]). One control group of 21 rats was treated with 100 μL solvent and another control group was untreated. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter. Of the dibenzo[*a*,*l*]pyrene-treated rats, 10/19 (53%) died within the first 9 weeks of treatment apparently as a result of toxicity; the remaining rats in this group were killed after 15 weeks because of poor health. Survival rates (mean ± SD) were 37 ± 4 and 40 ± 0 weeks in the untreated and vehicle-control groups, respectively. Complete necropsies were performed. Tumour latencies were 9 ± 1 and 25 ± 13 weeks in the dibenzo[*a*,*l*]-pyrene-treated and untreated groups, respectively. At the end of the study, tumour incidence was 100% in the dibenzo[*a*,*l*]pyrene-treated group. Within this group, 7/9 (78%) rats developed fibrosarcomas (2.1 tumours/tumour-bearing rat), 8/9 (89%) rats had

mammary adenocarcinomas (3.8 tumours/rat) and 8/9 (89%) rats had squamous-cell carcinomas of the skin (2.4 tumours/rat); mammary adenofibromas were not observed in this group. In contrast, 2/20 (10%) rats in the untreated group developed mammary epithelial tumours (one adenofibroma and one adenocarcinoma) but no fibrosarcomas or skin tumours. Similarly, no tumours were observed in the vehicle-control group (Cavalieri *et al*., 1989).

Groups of 20 female Sprague-Dawley rats, 8 weeks of age [body weight unspecified], received a single intramamillary injection of 0.25 or 1 μmol [75.6 or 302 μg]/gland dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) dissolved in 50 μL trioctanoin (eight glands; total doses, 2 or 8 μmol [605 or 2420 μg]). One control group of 19 rats was treated with 50 μL solvent. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter; surviving animals were killed after 24 weeks. Complete necropsies were performed on all animals. Survival rates (mean ± SD) were 17 ± 2, 20 ± 2 and 24 ± 0 weeks in the high- and low-dose dibenzo[*a*,*l*]pyrene-treated and control groups, respectively; the corresponding tumour latencies were 11 ± 1, 14 ± 2 and 22 ± 0 weeks. At the end of the study, 19/19 (100%) and 20/20 (100%) rats in the high- and low-dose groups had developed mammary adenocarcinomas (9.1 and 6.3 tumours/rat, respectively), but no mammary adenofibromas, compared with 0/18 (adenocarcinomas) and 1/18 (5%) (adenofibromas) controls. In addition, 14/19 (74%) rats in the high-dose group and 4/20 (20%) rats in the low-dose group developed fibrosarcomas (2.4 and 1.3 tumours/tumour-bearing animal, respectively), and one animal from each treatment group developed a squamous-cell carcinoma of the skin; fibrosarcomas and squamous-cell carcinomas were not observed in the control group (Cavalieri *et al*., 1991).

Tongue application (see also Table 3.14)

Hamster

A group of seven female Syrian hamsters [age and body weight unspecified] received applications on the tongue of 0.01 μmol (3 μg) dibenzo[*a*,*l*]pyrene [purity unspecified] in acetone five times a week for 30 weeks, at which time 6/7 (86%) animals had developed oral cavity carcinomas (2.6 tumours/animal) compared with 0/4 solvent-treated controls. (Schwartz *et al.*, 2004).

Intragastric administration (see also Table 3.14)

Mouse

A group of 18 female wild-type (mixed genetic background of C57Bl/6 and 129/Sv) and 13 female P450 1B1-null mice, 7 weeks of age, was treated intragastrically with daily doses of 1.07 mg/kg (~32 μg/mouse) dibenzo[*a*,*l*]pyrene (99.8 % pure [criteria for purity

unspecified]) dissolved in 100 μL corn oil on 5 days a week for 3 weeks. An additional group of mice of each genotype was treated with corn oil alone. The mice were monitored twice weekly and were killed whenever a sudden weight loss (>20% in a week) or the appearance of tumours (>1 cm) were detected. All surviving mice were killed at 12 months. Complete autopsies and histopathological analyses were performed on 17 of the wild-type mice and all of the P450 1B1-null mice. Survival rates at the end of the experiment were 61% (11/18) in the wild-type mice and 92% (12/13) in the P450 1B1-null mice. Tumour incidence was 18/18 (100%) wild-type mice and 8/13 (61%) P450 1B1-null mice compared with 4/27 (15%) solvent-control mice of both genotypes combined (one lung adenoma, one liver adenoma, one follicular lymphoma and one endometrial cystic hyperplasia in four wild-type mice [numbers of control mice of each genotype unspecified]). Wild-type mice treated with dibenzo[*a,l*]pyrene developed ovarian tumours (12/17; predominantly (83%) granulosa-cell tumours), lymphomas (5/17; 60% lymphoblastic and 40% follicular), a liver adenoma (1/17), verruciform hyperplasia of the skin (8/17) and uterine tumours (5/17; 60% endometrial cystic hyperplasia and 40% haemangiosarcomas); lung tumours were not observed in this group. P450 1B1-null mice developed a follicular lymphoma (1/13; 8%), a liver haemangioma (1/13; 8%), endometrial cystic hyperplasia (5/13; 38%) and lung adenomas (5/13; 38%); tumours of the ovary were not observed in this group. The differences between the two genotypes were statistically significant for tumours of the ovary, skin and lung (Buters *et al*., 2002).

Dibenzo[*e,l*]pyrene

Dermal application (see also Table 3.1)

Mouse

Two groups of 20 female Swiss albino Ha/ICR/Mil mice, 7–8 weeks of age [body weight unspecified], received thrice-weekly dermal applications of either a 0.05% or a 0.07% solution [volume unspecified] of dibenzo[*e,l*]pyrene (purified by column chromatography and recrystallization; purity verified by determination of melting-point in dioxane) for 12 months. An additional group of 20 mice was treated with dioxane only. Skin tumours were not observed in any of the groups at 15 months, when the study was terminated (Hoffmann & Wynder, 1966).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Two groups of 30 Swiss albino Ha/ICR/Mil mice [sex, age and body weight unspecified] received 10 dermal applications of 25 μL of 0.1% chromatographically pure (recrystallized) dibenzo[*e,l*]pyrene in dioxane [25 μg; total dose, 250 μg/animal] at 2-day

intervals. On day 28, 2.5% (2.3 mg) croton oil in acetone was applied to the skin [volume, number, frequency and duration of treatments not given]. A group of 30 mice was treated with croton oil solution alone. All survivors were killed after 6 months, at which time mortality rates were 7/30 (23%) and 4/30 (13%), and the number of skin papilloma-bearing mice was 0/30 and 2/30 (7%) in the dibenzo[*e*,*l*]pyrene-treated and control groups, respectively (Hoffmann & Wynder, 1966).

1,2-Dihydroaceanthrylene

Subcutaneous administration (see also Table 3.3)

Mouse

A group of nine female mice [strain, age and body weight unspecified] received a single subcutaneous injection of 5 mg 1,2-dihydroaceanthrylene (crystalline) [solvent and volume unspecified]. No control group was used in the study. All mice were alive and tumour-free after 14 months. By 17 months, five mice were still alive with no evidence of tumours. The last two mice were killed after 20 months with no evidence of tumours (Shear, 1938).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 31 male and 23 female newborn CD-1 mice received intraperitoneal injections of 1,2-dihydroaceanthrylene (purified by HPLC; purity assessed by GC–MS) in DMSO three times over 2 weeks (total volume, 35 μL; total doses, 0.86, 2.14 and 4.29 μmol [175, 437.5 and 875 μg]). Injections given were on postnatal days 1 [1/7 of the total dose in 5 μL], day 8 [2/7 of the total dose in 10 μL] and 15 [4/7 of the total dose in 20 μL]. A group of 24 male and 34 female control mice was treated in the same manner with DMSO alone. The mice were killed at 9 months of age and necropsied, and tumours were evaluated histologically. The incidence of liver tumours in male mice was 0/24, 1/17 (6%), 1/25 (4%) and 0/31 at the 0-, 175-, 437.5- and 875-μg dose levels, respectively. Liver tumours were not observed in female mice at any of the dose levels (groups of 34, 23, 21 and 13 mice, respectively). Lung tumour incidence was 0/24 (adenomas) and 1/24 (4%; adenocarcinomas) at 0 μg, 2/17 (12%; adenomas) and 2/17 (12%; adenocarcinomas) at 175 μg, 2/25 (8%; adenomas) and 0/25 (adenocarcinomas) at 437.5 μg and 5/31 (16%; adenomas) and 1/31 (3%; adenocarcinomas) at 875 μg in male mice; that in female mice was 1/34 (3%; adenomas) and 0/34 (adenocarcinomas) at 0 μg, 0/23 (adenomas) and 1/23 (4%; adenocarcinomas) at 175 μg, 1/21 (5%; adenomas) and 0/21 (adenocarcinomas) at 437.5 μg and 1/13 (8%; adenomas) and 1/13 (8%; adenocarcinomas) at 875 μg. When the data from male and female mice were combined, the incidence of combined adenoma and

adenocarcinoma (8/44; 18%) in the 875-μg group was significantly different (p <0.025) from that in the control group (2/58), as was the number of tumours/mouse (0.03 ± 0.02 versus 0.20 ± 0.07; p <0.01) (Wang *et al*., 1999).

In a second experiment, a group of 10 male and 23 female newborn BLU:Ha mice was treated with 1,2-dihydroaceanthrylene, using the protocol described above, at a total dose of 0.86 μmol (175 μg). A group of 22 male and 25 female control mice was treated with DMSO alone. The mice were killed at 6 months of age and necropsied, and tumours were evaluated histologically. The incidence of lung tumours was 0/10 treated male and 1/23 (4%; lung adenomas) treated female mice, and 1/22 (5%; adenomas) control males and 1/25 (4%; adenomas) control females (Wang *et al*., 1999).

1,4-Dimethylphenanthrene

Previous evaluation

1,4-Dimethylphenanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two initiation–promotion bioassays in which 1,4-dimethylphenanthrene was applied dermally to mice; these are summarized in Table 3.2.

The Working Group concluded that the available data were inadequate to permit an evaluation of the carcinogenicity to experimental animals of 1,4-dimethylphenanthrene *per se*. No new studies were available.

Fluoranthene

Previous evaluation

Fluoranthene was evaluated by earlier working groups in February 1983 (IARC, 1983) and March 1987 (IARC, 1987). Fluoranthene alone was not carcinogenic in experimental species following exposure via dermal or subcutaneous routes. The study of subcutaneous administration was considered to be inadequate. Fluoranthene had no carcinogenic effect in two studies of dermal application and was inactive as an initiator in the mouse skin initiation–promotion assay. However, when co-administered with benzo[*a*]pyrene, fluoranthene significantly increased the incidence of tumour-bearing mice and produced an excess number of skin tumours (primarily squamous-cell carcinomas) compared with that induced by the same dose of benzo[*a*]pyrene alone. The Working Group of March 1987 (IARC, 1987) concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of fluoranthene. Additional bioassays that have been published since 1983 are summarized below.

Dermal application (see also Table 3.1)

Mouse

In a co-carcinogenicity study, groups of 20 male C3H/HeJ mice [age unspecified] received twice-weekly dermal applications of a solution of 0.1% fluoranthene in combination with 0.001% benzo[*a*]pyrene in toluene for 104 weeks. The combined treatment induced skin tumours in one (8%) mouse; mice treated with fluoranthene alone or benzo[*a*]pyrene alone and vehicle controls did not develop skin tumours (Warshawsky *et al.*, 1993).

Intraperitoneal administration (see also Table 3.7)

Mouse

In newborn male and female Swiss-Webster BLU:ha (ICR) mice, intraperitoneal injections of total doses of 3.46 or 17.30 μmol fluoranthene on postnatal days 1, 8 and 15 increased the incidence of lung tumours (primarily adenomas) at 24 weeks in male mice at both doses and in female mice at 17.30 μmol (Busby *et al*., 1984).

Similar intraperitoneal injection of a total dose of 1.27 μmol fluoranthene increased the incidence of lung tumours (primarily adenomas) in both sexes at 26 weeks (Busby *et al*., 1989).

Newborn male and female CD-1 mice received intraperitoneal injections of 1/7, 2/7 and 4/7 of total doses of 3.46, 8.65 and 17.30 μmol fluoranthene dissolved in 5 μL DMSO on postnatal days 1, 8 and 15, respectively, and were killed at 6 and 9 months. At that time, the incidence of lung tumours (primarily adenomas) was increased in both sexes. A low incidence of liver tumours was noted in male mice at 6 months, whereas the incidence of liver adenomas was increased in male mice at 9 months (Wang & Busby, 1993).

In newborn male and female CD-1 mice, similar intraperitoneal injection of total doses of 3.46 or 17.30 μmol fluoranthene induced a significantly increased incidence of lung tumours (primarily carcinomas) in both males and females at 6 months. At both doses, the incidence of liver adenoma in male mice was increased (LaVoie *et al*. 1994b).

Fluorene

Previous evaluation

Fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated studies in which fluorene was administered to mice by dermal application and subcutaneous injection and to female rats in the diet. The studies were considered to be inadequate for evaluation. No new studies were available.

Indeno[1,2,3-*cd*]pyrene

Previous evaluations

Indeno[1,2,3-*cd*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated a bioassay in which indeno[1,2,3-*cd*]pyrene was administered to mice by dermal application (repeatedly and initiation–promotion). Positive responses were obtained. Indeno[1,2,3-*cd*]pyrene was also administered by subcutaneous injection to mice in an experiment that did not include control animals and induced sarcomas. On the basis of these data, the Working Group concluded that indeno[1,2,3-*cd*]pyrene was a complete carcinogen. Indeno[1,2,3-*cd*]pyrene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that indeno[1,2,3-*cd*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

A total initiating dose of 1.0 mg indeno[1,2,3-*cd*]pyrene in acetone applied to the skin of 25 female outbred albino Crl:CD-1 (ICR) BR mice followed by thrice-weekly applications of 2.5 μg TPA for 20 weeks induced squamous-cell papillomas in 90% of the animals, with an average of 2.83 skin tumours/mouse; papillomas occurred in fewer than 5% of TPA controls (Rice *et al.* 1986). In a similar experiment with a total initiating dose of 4.0 μmol [1105 μg] indeno[1,2,3-*cd*]pyrene, 72% of treated female CD-1 mice developed papillomas; TPA controls did not develop skin tumours (Rice *et al.* 1990).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies with newborn male and female mice CD-1, intraperitoneal injection of total doses of 0 or 2.1 μmol indeno[1,2,3-*cd*]pyrene on postnatal days 1, 8 and 15 induced lung adenomas in 9.1% [1/11] of treated male mice; DMSO control mice did not develop tumours (LaVoie *et al.*, 1987).

Intrapulmonary administration (see also Table 3.4)

Rat

In Osborne-Mendel rats, intrapulmonary implantation of indeno[1,2,3-*cd*]pyrene at doses of 0, 0.16, 0.83 or 4.15 mg induced dose-related increases in the incidence of

pulmonary squamous-cell carcinomas and sarcomas (0% [0/35], 11.4% [4/35], 22.9% [8/35] and 60.0% [21/35]) (Deutsch-Wenzel *et al.*, 1983).

1-Methylchrysene

Previous evaluation

1-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 1-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 1-Methylchrysene was considered to be active as a tumour initiator but not when applied repeatedly to mouse skin. The Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of 1-methylchrysene to experimental animals. No new studies were available.

2-Methylchrysene

Previous evaluation

2-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 2-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 2-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 2-methylchrysene to experimental animals. No new studies were available.

3-Methylchrysene

Previous evaluation

3-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 3-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Table 3.1 and 3.2. 3-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 3-methylchrysene to experimental animals. No new data were available.

4-Methylchrysene

Previous evaluation

4-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 4-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 4-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 4-methylchrysene to experimental animals. No new studies were available.

5-Methylchrysene

Previous evaluation

5-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated numerous positive bioassays in which 5-methylchrysene was administered dermally (repeatedly and initiation–promotion protocols) and by subcutaneous injection to mice. Selected studies are summarized in Tables 3.1 and 3.2. On the basis of the available data, the Working Group concluded that there was *sufficient evidence* for the carcinogenicity of 5-methylchrysene to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Application of total initiating doses of 0–100 nmol and 0–1.5 μmol 5-methylchrysene to the skin of female CD-1 mice followed by thrice-weekly applications of 2.5 μg TPA significantly increased the incidence (65–100%) and multiplicity of skin papillomas. Controls treated with solvent and TPA alone had a low incidence (0–10%) of skin papillomas (Amin *et al.*, 1985c; Hecht *et al.*, 1985; El-Bayoumy *et al.*, 1986, Hecht *et al.*, 1987; Rice *et al.*, 1988; Amin *et al.*, 1990, 1992).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies with newborn male and female ICR mice, intraperitoneal injection of 5-methylchrysene at total doses of 0 or 56 nmol on postnatal days 1, 8 and 15 significantly increased the incidence of lung (males, 4% and 20%; females, 7% and 21%) and liver (males, 2% and 23%; females, 2% and 12%) adenomas at 35 weeks (Hecht *et al.*, 1985).

Male strain A/J mice injected intraperitoneally with 0, 10, 50, 100 or 200 mg/kg bw 5-methylchrysene had dose-related increases in the incidence (55%, 65%, 100%, 100% and 100%) and multiplicity (0.6, 1.8, 39.0, 93.1 and too numerous to count) of lung adenomas (You *et al.*, 1994; Nesnow *et al.*, 1995; Ross *et al.*, 1995; Nesnow *et al.,* 1998a).

6-Methylchrysene

Previous evaluation

6-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 6-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 6-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 6-methylchrysene to experimental animals. No new studies were available.

2-Methylfluoranthene

Previous evaluation

2-Methylfluoranthene was considered by a Working Group in February 1983 (IARC, 1983). On the basis of the available data (see Tables 3.1 and 3.2), the Working Group concluded that there was *limited evidence* that 2-methylfluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of newborn male and female CD-1 mice received intraperitoneal injections of 2-methylfluoranthene in DMSO on postnatal days 1, 8 and 15 (total doses, 3.46 or 17.3 μmol). Controls received DMSO alone. The bioassay was terminated when the mice were 1 year old. At the end of the study, the incidence of lung tumours in male (23/24; 96%) and female (11/16; 69%) mice and the number of tumours per mouse were significantly increased following treatment with 17.3 μmol. The incidence of liver tumours in male mice (22/24; 92%) was significantly increased by the high dose compared with controls (3/29; 10%); high-dose female mice had a lower incidence of liver tumours (5/16; 31%) than high-dose males. Alterations of hepatic foci were noted in males at the low dose (LaVoie *et al.*, 1994b).

3-Methylfluoranthene

Previous evaluation

3-Methylfluoranthene was considered by a Working Group in February 1983 (IARC, 1983). On the basis of the available data (see Table 3.2), the Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of 3-methylfluoranthene to experimental animals. Additional bioassays that have been published since that time are summarized below.

Intraperitoneal injection (see also Table 3.7)

Mouse

Groups of newborn male and female CD-1 mice received intraperitoneal injections of 3-methylfluoranthene in DMSO on postnatal days 1, 8 and 15 (total doses, 3.46 or 17.3 μmol). Controls received DMSO alone. The bioassay was terminated when the mice were 1 year old. At the end of the study, the incidence of lung tumours in control and treated male (5/29, 17%; 6/24, 25%; and 5/26; 19%) and female (4/34, 12%; 5/33, 15%; and 6/28, 21%) mice was similar. The incidence of liver tumours in male mice (15/26; 55%) was significantly increased at the high dose compared with controls (3/29; 10%) (LaVoie *et al.*, 1994b).

1-Methylphenanthrene

Previous evaluation

1-Methylphenanthrene was considered by a Working Group in February 1983 (IARC, 1983) and was classified as having inadequate evidence of carcinogenicity. In one bioassay in which 1-methylphenanthrene was applied dermally to mice (initiation–promotion protocol), it was inactive as an initiator (Table 3.2). No new studies were available to the Working Group.

Naphtho[1,2-*b*]fluoranthene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Total initiating doses of 0, 1.0 or 4.0 μmol naphtho[1,2-*b*]fluoranthene [purity unspecified] applied to the skin of female CD-1 mice followed by thrice-weekly applications of 2.5 μg TPA induced squamous-cell papillomas in 65% and 100% of the treated animals with averages of 2.5 and 6.6 skin tumours/mouse, respectively.

Papillomas occurred in 10% of TPA controls with an average of 0.1 tumours/mouse (Weyand *et al*., 1990).

Naphtho[2,1-*a*]fluoranthene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Total initiating doses of 0, 1.0 or 4.0 μmol naphtho[2,1-*a*]fluoranthene [purity unspecified] applied to the skin of female CD-1 mice followed by thrice weekly applications of 2.5 μg TPA induced squamous-cell papillomas in 90% and 100% of the treated animals with averages of 5.9 and 7.3 skin tumours/mouse, respectively. Papillomas occurred in 10% of TPA controls with an average of 0.1 tumours/mouse (Weyand *et al*., 1990).

Naphtho[2,3-*e*]pyrene

Dermal application (see also Table 3.1)

Mouse

A group of 20 female Swiss-Albino Ha/ICR/Mil mice [age unspecified] received dermal applications of 100 μg naphtho[2,3-*e*]pyrene in dioxane three times a week [duration of treatment not specified]. No tumours developed (Hoffmann & Wynder, 1966; LaVoie *et al.,* 1979).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 30 female Swiss-Albino Ha/ICR/Mil mice [age unspecified] received 10 dermal applications of 25 μg naphtho[2,3-*e*]pyrene in dioxane every other day (total dose, 250 μg). A control group received dioxane alone. Ten days after the last application, all mice received dermal applications of 2.5% croton oil in acetone for 20 weeks. After 20 weeks of promotion, the incidence of mice bearing skin tumours was 33% in the treated group compared with 7.6% in the control group (Hoffman & Wynder, 1966; LaVoie *et al.,* 1979).

Perylene

Previous evaluation

Perylene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two bioassays in which perylene was applied dermally to mice (repeated dose and initiation–promotion protocols), both of which gave negative results; these are summarized in Tables 3.1 and 3.2. On the basis of the available studies, the Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of perylene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application

Mouse

Female CD-1 mice [number and age not specified] received dermal applications of 1% perylene [purity, solvent and volume not specified] three times a week for 1 year. The incidence of skin tumours [number not specified] did not differ from that in the control group (Anderson & Anderson, 1987).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female Crl/CD-1 (ICR)Br mice [age unspecified] received a total of 10 dermal applications of 0 or 100 μg perylene (purity >99% by HPLC) in 100 μL acetone on alternate days. Ten days after the last application, all mice received thrice weekly dermal applications of 2.5 μg TPA for 25 weeks. At the end of the treatment period, the incidence of skin tumours (5%) in mice treated with perylene (0.1 tumours/mouse) did not differ from that in the control mice (0.1 tumours/mouse) (El-Bayoumy *et al*., 1982).

Intraperitoneal administration

Mouse

Female strain A mice [number and age not specified] received intraperitoneal injections 0, 200, 500 or 1000 mg/kg bw perylene [purity not specified] three times a week for 8 weeks and the incidence of lung tumours was determined 16 weeks after the last injection. None of the treatments with perylene affected the number of lung tumours [incidence not given] (Anderson & Anderson, 1987).

Phenanthrene

Previous evaluation

Phenanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which phenanthrene was fed to rats, administered dermally and by subcutaneous injection to mice or given by intraperitoneal injection to neonatal mice. One study on tumour initiation gave positive results; all of the other studies gave either negative results or were considered to be inadequate; these are summarized in Tables 3.1–3.3, 3.6 and 3.7. On the basis of the available studies, the Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of phenanthrene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

A group of 20 male C3H/HeJ mice, 6–8 weeks of age, received twice-weekly skin applications of 50 μL of a 0.1% toluene solution of phenanthrene (>99% pure by HPLC; 50 μg per treatment) for 104 weeks. A control group of 50 male mice was treated with toluene alone. Lesions (≥1 mm^3) that persisted for at least 1 week were diagnosed as papillomas. After 104 weeks, one benign skin tumour was observed in the 12 surviving experimental mice and no benign or malignant skin tumours in the 39 surviving control mice. Gross examination of internal organs indicated no tumours in either the experimental or control groups (Warshawsky *et al*., 1993).

Intrapulmonary administration (see also Table 3.4)

Rat

Groups of 35 inbred female Osborne-Mendel rats, 3 months of age and weighing on average 256 g, received a single pulmonary implantation of 1, 3 or 10 mg phenanthrene (purity, 99.9%) in a mixture of beeswax and tricaprylin. The animals were monitored for 132–135 weeks. A single lung carcinoma was found in the high-dose phenanthrene-treated group. No lung carcinomas developed in solvent-treated control rats (Wenzel-Hartung *et al*., 1990).

Picene

Dermal application (see also Table 3.1)

Mouse

In early experiments, picene was applied dermally to mice. The results were negative (Kennaway, 1924a,b, 1930; Kennaway & Hieger, 1930). [The experiments are poorly described; the data are not included in the Table.]

Groups of 50 female NMRI mice [age unspecified] received thrice-weekly dermal applications of 0.4, 1.33 or 4.04 nmol [0.1, 0.4 or 1.1 μg] picene (>99% pure) in 17 μL acetone for 112 weeks (total doses, 38, 125 or 378 μg). A control group of 50 female mice was treated with the solvent alone. Skin tumours were observed in 3/49 mice treated with 0.1 μg picene, 11/48 mice treated with 0.4 μg picene, 11/50 mice treated with 1.1 μg picene and 2/48 mice treated with the solvent [no histopathology] (Platt *et al*., 1990).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female CD-1 mice, 8 weeks of age, received a single skin application of 10 μmol [2.8 mg] picene (purified by preparative layer chromatography) in benzene [volume unspecified]. One week later, the mice received twice-weekly skin applications of 10 μmol TPA for 34 weeks. [The dose of TPA was probably 10 μg.] A control group of 30 female mice of the same strain and age was treated twice weekly with 10 μmol TPA only for 34 weeks. At the end of the promotion period, all mice in the picene-treated and control groups were still alive. At that time, 8/30 (27%) picene-treated mice had developed skin papillomas (0.60 tumours/mouse [no histology]). One skin tumour occurred in 1/30 (3%) TPA controls by week 25, but it had regressed by the end of the study, when no tumours were found in the control group (Scribner, 1973).

Groups of female NMRI mice [age unspecified] received a single dermal application of 300 nmol [83.5 μg] picene (>99% pure) in 100 μL acetone (16 mice), 600 nmol [167 μg] picene in 100 μL tetrahydrofuran (16 mice) or 10000 nmol [2800 μg] picene in 100 μL benzene (30 mice). A control group of 30 female mice was treated with 100 μL acetone alone. One week later, all mice received twice-weekly dermal applications of 10 nmol (6.2 μg) TPA in 100 μL acetone. The experiment was terminated after 24 weeks. Skin tumours were not observed in mice treated with 83.5 or 167 μg picene or in the acetone-treated control mice; the incidence in mice treated with 2800 μg picene was 19% (0.29 tumours/mouse) [no histopathology] (Platt *et al*., 1990).

Subcutaneous administration (see also Table 3.3)

Mouse

Groups of 50 female NMRI mice [age unspecified] received a single subcutaneous injection into the interscapular region of 36, 40, 108, 308 or 399 nmol [10, 11, 30, 86 or 111 μg] picene (>99 % pure) in 500 μL tricaprylin. Two control groups of 50 female mice were treated with tricaprylin alone. Animals were palpated weekly; masses >1 cm were considered to be positive. The experiment was terminated after 112 weeks. [No histopathology appeared to be performed.] The incidence of fibrosarcomas was: 10 μg picene, 9/50 (18%); 11 μg picene, 12/46 (26%); 30 μg picene, 17/49 (35%); 86 μg picene, 31/49 (63%); 111 μg picene, 29/50 (58%); and controls, 3/49 (6%) and 1/50 (2%) (Platt *et al*., 1990).

Groups of 45 male and 50 female newborn NMRI mice, 2 days of age, received a single subcutaneous injection of 40 or 400 nmol [11 or 111 μg] picene (>99% pure) in 50 μL of an aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20). A control group of 49 male and female mice was treated with the solvent alone. The mice were separated by sex at 30 days of age and housed for a total of 40 weeks. A necropsy was performed, lung nodules were counted and histopathology was performed. The incidence of pulmonary tumours [type unspecified] was 4/16 (25%; 11 μg picene; 2.8 tumours/tumour-bearing mouse) and 8/23 (35%; 111 μg picene; 2.1 tumours/tumour-bearing mouse) in females, and 1/22 (4%; 11 μg picene; 1.0 tumours/tumour-bearing mouse) and 2/13 (15%; 111 μg picene; 1.5 tumours/tumour-bearing mouse) in males. The incidence in controls was 1/19 (5%; 1.0 tumours/tumour-bearing mouse) in females and 2/14 (14%; 2.0 tumours/tumour-bearing mouse) in males (Platt *et al*., 1990).

Rat

Groups of 12 female Sprague-Dawley rats, 30 days of age, received a total of 20 thrice-weekly subcutaneous injections of 300 μg picene (recrystallized, sharp melting-point, single peak by GC/MS and HPLC) in 100 μL sesame oil: DMSO (9:1). A control group of 12 rats was treated with the solvent alone. The experiment was continued for 37 weeks. The incidence of sarcomas in the rats treated with picene was 0/12 compared with 0/12 solvent-treated controls (Flesher *et al*., 2002).

Pyrene

Previous evaluation

Pyrene was first considered in February 1983 (IARC, 1983) by a Working Group that evaluated several bioassays in which pyrene was applied dermally to mice; no tumors were observed. It was also tested in initiation–promotion studies in mice with

inconclusive results. A study of subcutaneous injection into mice was considered to be inadequate, and a study of intratracheal administration in hamsters gave negative results. On the basis of the available studies, a Working Group in 1987 (IARC, 1987) concluded that the data were inadequate to permit an evaluation of the carcinogenicity of pyrene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.16)

Mouse

In a co-carcinogenicity study in male C3H/HeJ mice, a solution of 0.1% pyrene applied to the skin simultaneously with 0.001% benzo[*a*]pyrene did not induced skin tumours; one mouse (8%) treated with pyrene alone developed a skin tumour; mice treated with benzo[*a*]pyrene alone and vehicle (toluene) controls did not develop skin tumours (Warshawsky *et al.*, 1993).

Table 3.16. Incidence of benign and malignant skin tumours in mice treated with some PAHs and mixtures of PAHs

Compound	Malignant skin tumours	Benign and malignant skin tumours combined
Toluene	0/20	0/39
Benzo[*a*]pyrene	19/20 (95%)	0/14
Benzo[*a*]pyrene + chrysene	13/16 (81%)	3/13 (23%)
Benzo[a]pyrene + phenanthrene + pyrene	18/19 (95%)	19/19 (100%)
Benzo[*a*]pyrene + anthracene	1/13 (8%)	1/13 (8%)
Benzo[*a*]pyrene + fluoranthene		1/12 (8%)
Benzo[*a*]pyrene + phenanthrene		1/17 (6%)
Benzo[*a*]pyrene + pyrene		0/13
Benzo[*a*]pyrene + anthracene, chrysene, fluoranthene, phenanthrene and pyrene		8/17* (47%)
Anthracene		0/14
Chrysene		1/15 (7%)
Fluoranthene		0/15
Phenanthrene		1/12 (8%)
Pyrene		1/13 (8%)
Anthracene, chrysene, fluoranthene, phenanthrene and pyrene		3/13 (23%)

From Warshawsky *et al.* (1993)
PAH, polycyclic aromatic hydrocarbon
*Significantly different from benzo[*a*]pyrene alone

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies with newborn male and female CD-1 and Swiss-Webster BLU:Ha (ICR) mice and 5–6-week-old male strain A mice, intraperitoneal injection of pyrene at doses of 0–9.65 μmol did not significantly increase the incidences of lung adenomas (Wislocki *et al.,* 1986; Busby *et al.*, 1989; Ross *et al.*, 1995). However, in one study, the highest initiating dose (2800 nmol) significantly increased the incidence of liver adenomas (Wislocki *et al.,* 1986).

Triphenylene

Previous evaluation

Triphenylene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two bioassays in which triphenylene was applied dermally to mice and these are summarized in Table 3.1. The Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of triphenylene to experimental animals. No new studies were available.

Laboratory mixtures of PAHs

Dermal application

Mouse

Groups of IRC mice [number and age unspecified] received dermal applications of 0.005% benzo[*a*]pyrene alone or in combination with 0.1% pyrene, 0.5% fluoranthene, 0.1% phenanthrene or 0.015% benz[*a*]anthracene [number of applications, volume applied and treatment period unspecified]. The total number of tumour-bearing mice appeared to be decreased by phenanthrene and benz[*a*]anthracene, and the total number of carcinoma-bearing mice appeared to be decreased by benz[*a*]anthracene and increased by pyrene and fluoranthene [given the limited documentation, the data are very difficult (if not impossible) to evaluate] (Hoffmann & Wynder, 1963).

Groups of male Swiss mice [age unspecified] received dermal applications every 4th day of two drops of a benzene solution that contained 0.3% benzo[*a*]pyrene [purity unspecified] (40 mice), 0.3% perylene [purity unspecified] (20 mice) or a mixture of 0.3% benzo[*a*]pyrene and 0.3% perylene (40 mice). The experiment was continued for ~22 weeks, at which time ~50% of the animals treated with benzo[*a*]pyrene alone or with the mixture had died. Tumours with a diameter >0.5 mm were scored, but these were not verified histologically. The incidence of skin tumours was: benzo[a]pyrene alone, 36/40 (90%); perylene alone, 0/20 (0%); and benzo[*a*]pyrene + perylene, 13/40 (33%) (Finzi *et*

al., 1968). [The addition of perylene to benzo[*a*]pyrene significantly reduced the tumorigenic response observed with benzo[*a*]pyrene alone (Fisher's exact two-tailed test).]

Groups of 50 female ICR/Ha mice [age unspecified] received thrice-weekly dermal applications for 52 weeks of 100 µL acetone that contained 5 µg benzo[*a*]pyrene alone or in combination with 15 µg benzo[*e*]pyrene, 12 µg pyrene or 21 µg benzo[*ghi*]perylene. Each of the compounds was 'highly pure' as assessed by TLC, multichannel ultraviolet-visible (UV-VIS), spectrophotometery, fluorescence and MS. Additional groups [number unspecified] were treated in a similar manner with 15 µg benzo[*e*]pyrene, 12 µg pyrene, 21 µg benzo[*ghi*]perylene or the solvent alone. The incidence of skin papilloma and squamous-cell carcinoma (verified histologically) after 52 weeks was, respectively: benzo[*a*]pyrene, 13/42 (31%) and 10/42 (24%); benzo[*a*]pyrene + benzo[*e*]pyrene, 34/39 (87%) and 27/39 (69%); benzo[*a*]pyrene + pyrene, 27/41 (66%) and 19/41 (46%); and benzo[*a*]pyrene + benzo[*ghi*]perylene, 20/37 (54%) and 17/37 (46%) (Van Duuren *et al.*, 1973). [Each mixture significantly increased the incidence of papilloma and squamous-cell carcinoma compared with benzo[*a*]pyrene alone, with the exception of squamous-cell carcinomas following treatment with benzo[*ghi*]perylene (Fisher's exact two-tailed test).]

Groups of 50 female ICR/Ha mice, 6–8 weeks of age, received thrice-weekly dermal applications for 368 or 440 days of 100 µL acetone that contained 5 µg benzo[*a*]pyrene alone or in combination with 4, 12 or 40 µg pyrene, 7 or 21 µg benzo[*ghi*]perylene, 5 or 15 µg benzo[*e*]pyrene or 40 µg fluoranthene. Fluoranthene was purified by zone refinement and the other compounds were purified by recrystallization. Each was characterized by its melting-point. Additional groups of 50 mice were treated similarly with 12 or 40 µg pyrene, 21 µg benzo[*ghi*]perylene, 15 µg benzo[*e*]pyrene, 40 µg fluoranthene or acetone alone. The incidence of skin papilloma and squamous-cell carcinoma (verified histologically) is given in Table 3.17 (Van Duuren & Goldschmidt, 1976). [Each of the following mixtures significantly increased the incidence of papilloma and squamous-cell carcinoma compared with benzo[*a*]pyrene alone: 12 µg pyrene + benzo[*a*]pyrene (368 days), 40 µg pyrene + benzo[*a*]pyrene (440 days), 15 µg benzo[*e*]pyrene + benzo[*a*]pyrene (368 days) and 40 µg fluoranthene + benzo[*a*]pyrene (440 days) (Fisher's exact two-tailed test).]

Groups of 30 female Swiss mice [age unspecified] received twice-weekly dermal applications for 48 weeks of 2.2, 6.6 or 20 nmol benzo[*a*]pyrene [purity unspecified], 22.2, 66.6 or 200 nmol cyclopenta[*cd*]pyrene [purity unspecified], 2.2 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta[*cd*]pyrene, 6.6 nmol benzo[*a*]pyrene + 66.6 nmol cyclopenta[*cd*]pyrene, 20 nmol benzo[*a*]pyrene + 200 nmol cyclopenta[*cd*]pyrene, 2.2 nmol benzo[*a*]pyrene + 66.6 nmol cyclopenta[*cd*]pyrene, 6.6 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta[*cd*]pyrene, 2.2 nmol benzo[*a*]pyrene + 200 nmol cyclopenta[*cd*]-pyrene or 20 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta[*cd*]pyrene in 50 µL acetone. The experiment lasted 61 weeks. The following mixtures gave a tumour incidence that was significantly greater than the sum of the individual components at the same doses: 6.6 nmol benzo[*a*]pyrene + 66.6 nmol cyclopenta[*cd*]pyrene, 2.2 nmol benzo[*a*]pyrene +

Table 3.17. Incidence of skin tumours in female ICR/Ha mice following dermal application of benzo[*a*]pyrene and binary mixtures of benzo[*a*]pyrene and some PAHs

Compound	Papilloma	Squamous-cell carcinoma
B[*a*]P (368 days)	14/50 (28%)	10/50 (20%)
B[*a*]P + 4 μg pyrene (368 days)	12/50 (24%)	6/50 (12%)
B[*a*]P + 12 μg pyrene (368 days)	25/50 (50%)*	20/50 (40%)*
B[*a*]P +7 μg benzo[*g,h,i*]perylene (368 days)	19/50 (38%)	10/50 (20%)
B[*a*]P + 21 μg benzo[*g,h,i*]perylene (368 days)	20/50 (40%)	18/50 (36%)
B[*a*]P + 5 μg B[*e*]P (368 days)	24/50 (50%)	9/50 (18%)
B[*a*]P + 15 μg B[*e*]P (368 days)	33/50 (66%)*	27/50 (54%)*
B[*a*]P (440 days)	16/50 (32%)	12/50 (24%)
B[*a*]P + 40 μg pyrene (440 days)	35/50 (70%)*	26/50 (52%)*
B[*a*]P + 40 μg fluoranthene (440 days)	39/50 (78%)*	37/50 (74%)*

From Van Duuren & Goldschmidt (1976)
B[*a*]P, benzo[*a*]pyrene; B[*e*]P, benzo[*e*]pyrene; PAH, polycyclic aromatic hydrocarbon
*Significantly different from benzo[*a*]pyrene alone at 368 or 440 days

66.6 nmol cyclopenta[*cd*]pyrene and 6.6 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta-[*cd*]pyrene (Table 3.18; Cavalieri *et al*., 1983).

Groups of 20 shaved male C3H/HeJ mice [age unspecified] received twice-weekly dermal applications of 0.2% benzo[*a*]pyrene (purity, 99.5%), a mixture of 0.2% benzo[*a*]pyrene and 0.2% chrysene (purity, 99.5%) or a mixture of 0.2% benzo[*a*]pyrene, 0.2% phenanthrene (purity >99%) and 0.26% pyrene (purity, 99.5%) in toluene, or toluene alone. [The volume was not specified. Histopathology appeared to be performed. There was no statistical analysis. None of the treatments resulted in a tumour incidence that differed from that induced by benzo[*a*]pyrene alone (Fisher's exact two-tailed test). Tumour latency appeared to be shortened with the mixtures, but this was not examined statistically] (Table 3.16; Warshawsky *et al*., 1993).

Groups of 20 shaved male C3H/HeJ mice, 6–8 weeks of age, received twice-weekly dermal applications of 50 μL toluene solutions that contained 0.1% anthracene (purity, 99.5%), 0.1% chrysene, 0.1% fluoranthene (purity, >99%), 0.2% phenanthrene or 0.1% pyrene, or a mixture of 0.1% anthracene, 0.1% chrysene, 0.1% fluoranthene, 0.1% phenanthrene and 0.1% pyrene. Additional groups were treated in an identical manner with 0.001% benzo[*a*]pyrene (18 mice), each of the solutions in combination with 0.001% benzo[*a*]pyrene (20 mice per group) or the solvent alone (50 mice). The incidence of benign and malignant skin tumours after treatment for 14 weeks is given in Table 3.16 (Warshawsky *et al*., 1993). [None of the mixtures, with the exception of the

mixture of anthracene, chrysene, fluoranthene, phenanthrene, pyrene and benzo[*a*]pyrene, resulted in a tumour incidence that differed from that induced by benzo[*a*]pyrene alone. The tumour incidence induced by the mixture of anthracene, chrysene, fluoranthene, phenanthrene and pyrene did not differ from that induced by the mixture of anthracene, chrysene, fluoranthene, phenanthrene, pyrene and benzo[*a*]pyrene (Fisher's exact two-tailed test).]

Table 3.18. Tumour incidence and multiplicity in mice treated with benzo[*a*]pyrene, cyclopenta[*cd*]pyrene and combinations of the two compounds

Benzo[*a*]pyrene (nmol)	Cyclopenta[*cd*]-pyrene (nmol)	Tumour incidence (%)	Squamous-cell carcinoma/tumour-bearing animal
2.2		7	1.0
6.6		7	1.0
20		57	2.6
0	22.2	7	0.0
0	66.6	7	1.0
0	200	83	2.9
2.2	22.2	3	5.0
2.2	66.6	30*	1.0
2.2	200	97	3.8
6.6	22.2	31*	1.8
6.6	66.6	69*	7.2
20	22.2	83	3.8
20	200	87	5.1

From Cavalieri *et al.* (1983)
*Significantly greater that the sum of tumour incidence for the individual compounds at the same doses

Dermal initiation–promotion

Mouse

Groups of 30 shaved female Charles River CD-1 mice [age unspecified] received a single dermal application of 200 μL acetone that contained 100 μg benzo[*e*]pyrene (>99% pure), 100 μg pyrene [purity unspecified] or 100 μg fluoranthene [purity unspecified] or the solvent alone. After 5 min, the mice received a dermal application of 200 nmol benzo[*a*]pyrene [purity, solvent and volume unspecified]. One week later, the mice received twice-weekly dermal applications of 10 μg TPA for 30 weeks. The incidence of papillomas per mouse (± SD) was: benzo[*a*]pyrene, 100 ± 12; benzo[*e*]pyrene + benzo-[*a*]pyrene, 130 ± 13; pyrene + benzo[*a*]pyrene, 135 ± 18; and fluoranthene + benzo-

[*a*]pyrene, 123 ± 12 (Slaga *et al*., 1979). [Each of the mixtures significantly increased the number of papillomas per mouse compared with that observed with benzo[*a*]pyrene alone (ANOVA, followed by Dunnett's test). Since the compounds were not tested individually, it cannot be ascertained whether the increase is due to an additive response or to an effect of the compounds on benzo[*a*]pyrene.] Additional experiments were conducted with 7,12-dimethylbenz[*a*]anthracene (DMBA) instead of benzo[*a*]pyrene and the opposite effect was observed, i.e. the number of papillomas per mouse decreased in the mixture-treated group compared with groups treated with DMBA alone.

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 200 nmol benzo[*e*]pyrene [purity unspecified] in 200 μL acetone or the solvent alone. After 5 min, the mice received a single application of 200 nmol benzo[*a*]pyrene [purity unspecified] in 200 μL acetone and, 1 week later, twice-weekly dermal applications of 3.4 nmol TPA in 200 μL acetone for 16 weeks. The incidence of papillomas per mouse was 3.3 for benzo[*a*]pyrene-treated mice (88% papilloma incidence) and 4.3 for benzo[*e*]pyrene + benzo[*a*]pyrene-treated mice (94% tumour incidence). The standard deviation for each of these values was <16%. Random papillomas were verified histologically (DiGiovanni *et al*., 1982). [Benzo[*e*]pyrene caused a statistically significant increase in the number of papillomas per mouse compared with that observed with benzo[*a*]pyrene alone (ANOVA, followed by Dunnett's test). Since benzo[*e*]pyrene was not tested individually, it cannot be ascertained whether the increase was due to an additive response or to an effect of benzo[*e*]pyrene on benzo[*a*]pyrene.] Additional experiments were conducted using DMBA, and the opposite effect was observed, i.e. the number of papillomas per mouse decreased in the mixture-treated group compared with the group treated with DMBA alone. Further experiments were conducted with 3-methylcholanthrene instead of benzo[*a*]pyrene and the results were inconclusive.

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 200 or 400 nmol dibenz[*a,c*]anthracene [purity unspecified] in 200 μL acetone or the solvent alone. After 5 min, the mice received an application of 200 nmol benzo[*a*]pyrene in 200 μL acetone and, 1 week later, twice-weekly dermal applications of 3.4 nmol TPA in 200 μL acetone for 16 weeks. The incidence of papillomas per mouse was 3.3 for benzo[*a*]pyrene (88% papilloma incidence) [the Working Group noted that this was the same group of animals as that used in the previous experiment], 3.2 for 200 nmol dibenz[*a,c*]anthracene + benzo[*a*]pyrene (97% tumour incidence) and 2.9 for 400 nmol dibenz[*a,c*]anthracene + benzo[*a*]pyrene (88% tumour incidence). The standard deviation for each of these values was <20%. Dibenz[*a,c*]-anthracene had 'little to no' tumour-initiating activity (DiGiovanni *et al*., 1982). [Treatment with 400 nmol dibenz[*a,c*]anthracene caused a statistically significantly increase in the number of papillomas per mouse compared with that observed with benzo[*a*]pyrene alone (ANOVA, followed by Dunnett's).] Additional experiments were conducted with DMBA and 3-methylcholanthrene. Dibenz[*a,c*]anthracene appeared to

inhibit the tumour multiplicity induced by these compounds to an even greater extent than for tumours induced by benzo[*a*]pyrene.

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 200 nmol benzo[*e*]pyrene or dibenzo[*a,c*]anthracene in 200 μL acetone or the solvent alone. After 5 min, the mice received a single dermal application of 200 nmol 5-methylchrysene [purity unspecified] or dibenz[*a,h*]anthracene [purity unspecified] in 200 μL acetone, followed 1 week later by twice-weekly applications of 3.4 nmol TPA in 200 μL acetone for 16 weeks. The incidence of papillomas per mouse was 2.9 for 5-methylchrysene (63% papilloma incidence), 2.6 for 5-methylchrysene + benzo[*e*]pyrene (64% tumour incidence) and 2.9 for 5-methylchrysene + dibenz[*a,c*]-anthracene (69% tumour incidence); 6.8 for dibenz[*a,h*]anthracene (91% papilloma incidence), 3.5 for dibenz[*a,h*]anthracene + benzo[*e*]pyrene (74% tumour incidence) and 4.1 for dibenz[*a,h*]anthracene + dibenz[*a,c*]anthracene (71% tumour incidence). The standard deviation for each of these values was <20% (DiGiovanni *et al*., 1982). [Benzo[*e*]pyrene and dibenzo[*a,c*]anthracene caused a statistically significantly increase in the number of papillomas per mouse compared with that observed with dibenzo[*a,h*]-anthracene alone, but had no effect upon the tumour multiplicity induced by 3-methyl-cholanthrene (ANOVA, followed by Dunnett's test)]. Additional experiments were conducted with 7- and 12-methylbenz[*a*]anthracene. The tumour multiplicity with 7-methylbenz[*a*]anthracene was decreased, but that with 12-methylbenz[*a*]anthracene was not affected.

Subcutaneous administration

Mouse

Groups of 50 male and 50 female C57 black mice, 3–4 months of age, received a single subcutaneous injection of 5.0 mg chrysene [purity unspecified], 5.0 mg benz[*a*]anthracene [purity unspecified], 20 μg dibenz[*a,h*]anthracene [purity unspecified] or mixtures of 2.5 mg chrysene + 2.5 mg benz[*a*]anthracene or 5.0 mg benz[*a*]anthracene + 20 μg dibenz[*a,h*]anthracene in 500 μL tricaprylin. A total of 304 control mice were given a single injection of 0.2–2.0 mL tricaprylin. Mice that survived 4 months after the treatment were evaluated for tumour incidence and the experiment was continued for 22 months. The incidence of sarcomas (histologically verified) is given in Table 3.19 (Steiner & Falk, 1951). [The addition of benz[*a*]anthracene to dibenz[*a,h*]anthracene significantly decreased (Fisher's exact two-tailed test) the response observed with dibenz[*a,h*]anthracene alone based upon the number of mice alive 4 months after treatment. The difference was not significant when the comparison was made based upon the number of animals alive when the first tumour occurred. The response observed with chrysene and benz[*a*]anthracene appeared to be additive, and was also marginally greater than that observed with benz[*a*]anthracene alone when the comparison was made based upon the number of animals alive 4 months after treatment. It was significantly greater

when the comparison was made based upon the number of animals alive when the first tumour appeared.]

Table 3.19. Incidence of sarcomas in mice after subcutaneous injection of some PAHs and binary mixtures of PAHs

Compound	Sarcoma[a]	Sarcoma[b]
Chrysene (5 mg)	4/39 (10.3%)	4/24 (16.7%)
Benz[*a*]anthracene (5.0 mg)	8/46 (17.4%)	8/44 (18.2%)
Dibenz[*a,h*]anthracene (20 μg)	28/48 (58.3%)	28/48 (58.3%)
Chrysene (2.5 mg) + benz[*a*]anthracene (2.5 mg)	14/41 (36.6%)	15/34 (44.1%)*
Benz[*a*]anthracene (5.0 mg) + dibenz[*a,h*]anthracene (20 μg)	11/39 (28.2%)**	11/30 (36.7%)
Tricaprylin (0.2–2.0 mL)	3/280 (1.1%)	3/233 (1.3%)

From Steiner & Falk (1951)
PAH, polycyclic aromatic hydrocarbon
[a]Based upon those alive 4 months after treatment
[b]Based upon those alive when the first tumour occurred
*Significantly different from benz[*a*]anthrancene alone
**Significantly different from dibenz[*a,h*]anthrancene alone

Groups of 40–50 male and 40–50 female C57 black mice [age unspecified] received a single subcutaneous injection of 20 or 40 μg dibenz[*a,h*]anthracene [purity unspecified], 5 or 10 mg benz[*a*]anthracene [purity unspecified], 90 μg benzo[*a*]pyrene [purity unspecified], 5 mg chrysene [purity unspecified], 5 mg anthracene [purity unspecified] or 5 mg phenanthrene [purity unspecified], or mixtures of 20 or 40 μg dibenz[*a,h*]anthracene + 5 or 10 mg benz[*a*]anthracene, 90 μg benzo[*a*]pyrene + 5 mg benz[*a*]anthracene, 20 μg dibenz[*a,h*]anthracene + 5 mg chrysene, 20 μg dibenz[*a,h*]anthracene + 5 mg anthracene or 20 μg dibenz[*a,h*]anthracene and 5 mg phenanthrene. The number of mice alive when the first sarcoma appeared was used to calculate the tumour incidence. The experiments were continued for 22–28 months. The incidence of sarcomas (histologically verified) is given in Table 3.20 (Steiner, 1955). [The addition of benz[*a*]anthracene, chrysene, anthracene or phenanthrene to dibenz[*a,h*]anthracene did not significantly affect the incidence of sarcomas observed with treatment with dibenz[*a,h*]anthracene alone (Fisher's exact two-tailed test). Similarly, the addition of benz[*a*]anthracene to benzo[*a*]-pyrene did not significantly affect the indicence of sarcomas observed with benzo[*a*]-pyrene alone.]

In a subsequent experiment, mice were administered 50, 200 or 1000 μg benz[*a*]-anthracene with or without 20 μg dibenz[*a,h*]anthracene (Steiner, 1955) (see Table 3.20). [The only significant difference occurred when dibenz[*a,h*]anthracene was combined with 50 μg benz[*a*]anthracene.]

Groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 275 μg dibenz[*a,h*]anthracene [purity unspecified] in ethyl laurate [volume

unspecified] either alone or in combination with phenanthrene [purity unspecified] in molar ratios (phenanthrene:dibenz[*a*,*h*]anthracene) of 12:1, 24:1 or 48:1. The incidence of sarcomas 30 months after treatment is given in Table 3.21 (Falk *et al*., 1964). [Although there was a decrease in the incidence of sarcomas with the addition of phenanthrene, the difference was not significant (Fisher's exact two-tailed test). There was also a decreasing trend when the ratio of phenanthrene:dibenzo[*a*,*h*]anthracene was increased, but this was not significant either.]

Table 3.20. Incidence of sarcomas in mice after subcutaneous injection of some PAHs and binary mixtures of PAHs

Compound	Sarcoma
Experiment 1	
Dibenz[*a,h*]anthracene (20 μg)	7/21 (33%)
Dibenz[*a,h*]anthracene (40 μg)	6/18 (33%)
Benz[*a*]anthracene (5 mg)	20/36 (56%)
Benz[*a*]anthracene (10 mg)	5/16 (31%)
Benzo[*a*]pyrene (90 μg)	16/21 (76%)
Chrysene (5 mg)	5/22 (23%)
Anthracene (5 mg)	0/26 (0%)
Phenanthrene (5 mg)	0/27 (0%)
Dibenz[*a,h*]anthracene (20 μg) + benz[*a*]anthracene (5 mg)	14/35 (40%)
Dibenz[*a,h*]anthracene (40 μg) + benz[*a*]anthracene (10 mg)	16/27 (59%)
Benzo[*a*]pyrene (90 μg) + benz[*a*]anthracene (5 mg)	16/27 (59%)
Dibenz[*a,h*]anthracene (20 μg) + chrysene (5 mg)	9/25 (36%)
Dibenz[*a,h*]anthracene (20 μg) + anthracene (5 mg)	13/29 (45%)
Dibenz[*a,h*]anthracene (20 μg) + phenanthrene (5 mg)	14/26 (54%)
Experiment 2	
Dibenz[*a,h*]anthracene (20 μg)	7/21 (33%)
Benz[*a*]anthracene (50 μg)	5/44 (11%)
Benz[*a*]anthracene (200 μg)	11/45 (24%)
Benz[*a*]anthracene (1000 μg)	15/44 (34%)
Benz[*a*]anthracene (50 μg) + dibenz[*a,h*]anthracene (20 μg)	18/28 (64%)*
Benz[*a*]anthracene (200 μg) + dibenz[*a,h*]anthracene (20 μg)	14/22 (64%)
Benz[*a*]anthracene (1000 μg) + dibenz[*a,h*]anthracene (20 μg)	13/25 (52%)

From Steiner (1955)
PAH, polycyclic aromatic hydrocarbon
*Significantly different from dibenz[*a,h*]anthracene (20 μg) alone

Additional groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 60 μg dibenz[*a*,*h*]anthracene in ethyl laurate either alone or in combination with phenanthrene in a molar ratio (phenanthrene:dibenz[*a*,*h*]anthracene) of 24:1. The incidence of sarcomas 30 months after treatment (verified histologically) is given in Table 3.21 (Falk *et al*., 1964). [Although there was a decrease in incidence of

sarcomas with the addition of phenanthrene, the difference was not significant (Fisher's exact two-tailed test).]

Table 3.21. Incidences of sarcomas in mice after subcutaneous injection of some PAHs and binary mixtures of PAHs

Compound	Sarcoma
Experiment 1	
Dibenzo[*a*,*h*]anthracene (275 μg)	~16/30[a] (53%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (12:1)	~14/30[a] (47%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (24:1)	~9/30[a] (30%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (48:1)	~10/30[a] (33%)
Experiment 2	
Dibenzo[*a*,*h*]anthracene (60 μg)	~9/30[a] (30%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (12:1)	~3/30[a] (10%)
Experiment 3	
Dibenzo[*a*,*h*]anthracene (30 μg × 4)	~12/30[b] (40%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1)	~13/30[b] (43%)
Dibenzo[*a*,*h*]anthracene (60 μg × 2)	19/30[b] (63%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1)	16/30[b] (53%)
Dibenzo[*a*,*h*]anthracene (120 μg × 1)	24/30[b] (80%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1)	14/30*[b] (47%)
Dibenzo[*a*,*h*]anthracene (150 μg)	16/30[b] (53%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1) 100 μL triethylene glycol	28/30*[b] (93%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1) 200 μL triethylene glycol	30/30*[b] (100%)
Benzo[*a*]pyrene (400 μg)	27/30[b] (90%)
Benzo[*a*]fluorene + benzo[*a*]pyrene (0.1:1)	2/30*[b] (67%)
Perylene + benzo[*a*]pyrene (0.1:1)	5/30*[b] (17%)
Chrysene + benzo[*a*]pyrene (0.15:1)	3/30*[b] (10%)
Benzo[*k*]fluoranthene + benzo[*a*]pyrene (1:1)	5/30*[b] (17%)
Benzo[*mno*]fluoranthene + benzo[*a*]pyrene (1:1)	3/30*[b] (10%)
Anthracene + phenanthrene + pyrene + benzo[*a*]pyrene (10:10:10:1)	1/30*[b] (3%)

From Falk *et al.* (1964)
PAH, polycyclic aromatic hydrocarbon
* Significantly different from the control group
[a] After 30 months
[b] After 18 months

In a subsequent experiment, groups of 30 male C57BL mice [age unspecified] received four subcutaneous injections of 30 μg dibenz[*a*,*h*]anthracene in ethyl laurate at 2-month intervals, two subcutaneous injections of 60 μg dibenz[*a*,*h*]anthracene in ethyl laurate at 4-month intervals or a single subcutaneous injection of 120 μg dibenz[*a*,*h*]-anthracene in ethyl laurate, either alone or in combination with phenanthrene in a molar ratio (phenanthrene:dibenz[*a*,*h*]anthracene) of 15:1. The incidence of sarcomas 18 months

after treatment is given in Table 3.21 (Falk *et al*., 1964). [There was a significant decrease in the incidence of sarcomas with the addition of phenanthrene in a single injection (Fisher's exact two-tailed test).]

In a further experiment, groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 500 μg dibenz[*a,h*]anthracene in 100 μL triethylene glycol either alone or in combination with phenanthrene in a molar ratio (phenanthrene: dibenz[*a,h*]anthracene) of 15:1. One additional group was administered the mixture in 200 μL triethylene glycol. The incidence of sarcomas 18 months after treatment (verified histologically) is given in Table 3.21 (Falk *et al.*, 1964). [There was a significant increase in the incidence of sarcomas with the addition of phenanthrene when triethylene glycol was used as the vehicle (Fisher's exact two-tailed test).]

In another experiment, groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 400 μg benzo[*a*]pyrene [purity unspecified] in tricaprylin [volume unspecified] either alone or in combination with benzo[*a*]fluorene [purity unspecified] (molar ratio of benzo[*a*]fluorene:benzo[*a*]pyrene, 0.1:1), perylene [purity unspecified] (molar ratio of perylene:benzo[*a*]pyrene, 0.1:1), chrysene [purity unspecified] (molar ratio of chrysene:benzo[*a*]pyrene, 0.15:1), benzo[*k*]fluoranthene [purity unspecified] (molar ratio of benzo[*k*]fluoranthene:benzo[*a*]pyrene, 1:1), benzo-[*mno*]fluoranthene [purity unspecified] (molar ratio of benzo[*mno*]fluoranthene: benzo[*a*]pyrene, 1:1) or a mixture of anthracene, phenanthrene and pyrene [purities unspecified] (molar ratio of anthracene:phenanthrene:pyrene:benzo[*a*]pyrene, 10:10:10:1). The incidence of sarcomas 18 months after treatment (verified histologically) is given in Table 3.21 (Falk *et al*., 1964). [There was a significant decrease in the incidence of sarcomas with the addition of all compounds (Fisher's exact two-tailed test).]

Additional groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 400 μg benzo[*a*]pyrene in tricaprylin [volume unspecified] in combination with acenaphthylene [purity unspecified] (molar ratio of acenaphthylene:benzo[*a*]pyrene, 5:1), fluorene [purity unspecified] (molar ratio of fluorene: benzo[*a*]pyrene, 1:1), coronene [purity unspecified] (molar ratio of coronene: benzo[*a*]pyrene, 1:1), benzo[*ghi*]perylene [purity unspecified] (molar ratio of benzo[*ghi*]-perylene:benzo[*a*]pyrene, 0.3:1), dibenzo[*b,e*]pyrene [purity unspecified] (molar ratio of dibenzo[*b,e*]pyrene:benzo[*a*]pyrene, 0.15:1), dibenzo[*a,l*]pyrene [purity unspecified] (molar ratio of dibenzo[*a,l*]pyrene:benzo[*a*]pyrene, 0.1:1), dibenz[*a,h*]anthracene [purity unspecified] (molar ratio of dibenz[*a,h*]anthracene:benzo[*a*]pyrene, 0.1:1), indeno[1,2,3-*cd*]pyrene [purity unspecified] (molar ratio of indeno[1,2,3-*cd*]pyrene:benzo[*a*]pyrene, 0.1:1) or anthanthrene [purity unspecified] (molar ratio of anthanthrene:benzo[*a*]pyrene, 0.1:1). The observation period and resultant tumour data were not reported; however, the authors stated that "no inhibitory effects on benzo[*a*]pyrene carcinogenesis could be observed" (Falk *et al*., 1964).

Groups of 100 female C57BL mice [age unspecified] received a single subcutaneous injection of 3.12, 6.25, 12.5, 25.0, 50.0 or 100.0 μg benzo[*a*]pyrene [purity unspecified] in

500 μL tricaprylin (Series A). Additional groups (Series B) were similarly treated with 2.35, 4.7, 9.3, 18.7, 37.5 or 75.0 μg dibenz[*a,h*]anthracene [purity unspecified]. Further groups of mice (Series C) were treated with a mixture of 10 PAHs that included benzo[*e*]pyrene, benz[*a*]anthracene, phenanthrene, anthracene, pyrene, fluoranthene, chrysene, perylene, benzo[*ghi*]perylene and coronene [purities unspecified]. Additional groups were treated with mixtures of benzo[*a*]pyrene and dibenz[*a,h*]anthracene (Series D) and mixtures of these two compounds and the other 10 PAHs (Series E), at the same mass ratios as those used in the preceding studies. The study was continued for 114 weeks, at which time fewer than 10% of the control mice were alive. The incidence of sarcomas increased in a dose–responsive manner in mice treated with benzo[*a*]pyrene, from 9/100 at 3.12 μg to 83/100 at 100 μg. Dibenz[*a,h*]anthracene also increased the incidence of sarcomas in a dose–responsive manner, from 37/100 at 2.35 μg to 69/100 at 75 μg. In mice treated with the mixture of 10 PAHs, the incidence of sarcomas varied between 4/100 and 13/100, and a dose–response was not evident. The mixture of benzo[*a*]pyrene and dibenz[*a,h*]anthracene was 1.4 times more effective at inducing sarcomas than dibenz[*a,h*]anthracene alone, a difference that was not statistically significant. The shape of the dose–response curve for benzo[*a*]pyrene and dibenz[*a,h*]-anthracene was similar to that for dibenz[*a,h*]anthracene alone. Administration of the mixture of benzo[*a*]pyrene and dibenz[*a,h*]anthracene and the 10 PAHs gave a sarcoma response that was similar to that induced by dibenz[*a,h*]anthracene alone (Pfeiffer, 1973, 1977).

Intraperitoneal administration

Mouse

Groups of 20 male A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 200 μL tricaprylin that contained a mixture of five PAHs: 30 or 75 mg/kg benzo[*a*]pyrene (purity, ≥98%), 30 or 75 mg/kg bw benzo[*b*]fluoranthene (purity, 99%), 2.5 or 10 mg/kg bw dibenz[*a,h*]anthracene (purity, 97%), 10 or 30 mg/kg bw 5-methyl-chrysene (purity, 99%) and 30 or 100 mg/kg bw cyclopenta[*cd*]pyrene (purity, 99%) using a 2^5 factorial design. Eight months after treatment, the number of lung adenomas was assessed. No histopathology was carried out. Survival in the 32 groups ranged from 70 to 100% (mean, 85%). Body weights were initially affected by treatment. The incidence of lung adenomas was 100% in each group, with the exception of the solvent-treated control group, which had a 26% incidence. Tumour multiplicity ranged from 16.8 to 63.8 lung adenomas per mouse, compared with 0.32 lung adenomas per mouse in the tricaprylin controls, a difference that was significant (p <0.01). Two groups exhibited a statistically significant (p <0.05) increase in tumour multiplicity compared with that expected from summation of the tumour response of the individual PAHs. [The identity of these mixtures was not given]. Thirteen groups exhibited a statistically significant (p<0.05) decrease in tumour multiplicity compared with that expected from summation of

the tumour response of the individual PAHs. [The identity of these mixtures was not given] (Nesnow *et al*., 1998a).

In a subsequent experiment, the effect of pyrene on a quintary mixture was examined. Specifically, a group of 20 male A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 100 mg/kg bw pyrene (purity, 99.7%), 30 mg/kg bw benzo[*a*]-pyrene, 30 mg/kg bw benzo[*b*]fluoranthene, 2.5 mg/kg bw dibenz[*a,h*]anthracene, 30 mg/kg bw 5-methylchrysene and 100 mg/kg bw cyclopenta[*cd*]pyrene. After 8 months, all of the mice had lung adenomas, with a mean multiplicity of 30.5 adenomas per mouse. This was a statistically significant reduction compared with the multiplicity of 47.1 adenomas per mouse in mice treated with the mixture of 5 PAHs. Mice administered pyrene (10–200 mg/kg) had a 15–40% incidence of adenomas, with a multiplicity of 0.3–0.6 adenomas per mouse, which did not differ significantly from those in the control group (Nesnow *et al*., 1998a).

Environmental mixtures of PAHs

Dermal application

Mouse

Groups of 50 male Swiss CD-1 and C3H/HeJ mice [age unspecified] received twice-weekly dermal applications of two types of asphalt and two types of coal-tar pitch used on roofs for 78 weeks. The materials, which were chosen on the basis of their common use and extremes of classification, were Type I ('dead level') and Type III ('steep') asphalt and Type I ('regular roofing') and Type III ('low fuming' or 'low burn') coal-tar pitch (see Table 3.22 for PAH content). Fumes were collected at two temperatures (232 °C and 316 °C), and condensed material was collected using a glass cryogenic system. The collections at 316 °C gave 9–16 times more volatile material from the asphalts and 2–7 times more volatile material from the coal-tar pitches as compared with the yield from 232 °C collections. The materials were applied in 50 μL of a mixture of cyclohexane and acetone (1:1). The coal-tar pitch condensate solutions were adjusted to give a concentration of benzo[*a*]pyrene of approximately 0.01% (100 μg/mL), which resulted in 30–84 mg/mL of condensed fumes being applied, depending upon the specific coal-tar pitch. The asphalt condensate solutions were adjusted to give 50% total solids (500 mg/mL). The benzo[*a*]pyrene content of these solutions was ≤3 μg/mL. Benzo[*a*]pyrene [purity not specified] (100 μg/mL) was used as a positive control; and other control mice were treated with the solvent. Additional groups were exposed on alternate weeks to Type I coal-tar pitch condensate and Type III asphalt condensate. One arm of the experiment included exposure to simulated solar light. This arm is not considered further. The incidence of malignant tumours (squamous-cell carcinoma and fibrosarcoma) was much lower in CD-1 mice (~5%) compared with C3H/HeJ mice (~60%), and fibrosarcomas were more common in C3H/HeJ mice. C3H/HeJ mice treated

with the condensates had decreased survival compared with the controls, except for those exposed to 232 °C Type I asphalt condensate. CD-1 mice treated with 232 °C Type I and 232 °C Type III coal-tar pitch condensate had lower survival than the control group. C3H/HeJ mice exposed to the 316 °C asphalt condensates had a decreased tumour latency compared with those exposed to 232 °C asphalt condensates. The temperature of the coal-tar pitch condensates had no effect on tumour latency in C3H/HeJ mice. The temperature of the asphalt or coal-tar pitch condensates had no effect on tumour latency in CD-1 mice. Compared with C3H/HeJ mice exposed to benzo[*a*]pyrene alone, C3H/HeJ mice treated with each of the condensates (except for 232 °C Type I and Type III asphalt) had a decreased tumour latency. In CD-1 mice, treatment with 232 °C Type I and Type III asphalt increased tumour latency, while 316 °C Type III coal-tar pitch condensate decreased tumour latency (Niemeier *et al.*, 1988).

Table 3.22. Concentration of PAHs (μg/mL) in solutions for dermal application

PAH	Asphalt[a]				Pitch[a]			
	Type I		Type III		Type I		Type III	
	232 °C	316 °C	232 °C	316 °C	232 °C	316 °C	232 °C	316 °C
Naphthalene	22	4	17	49	>1800	1770	288	620
Fluorene	26	22	39	28		740		
Anthracene/phenanthrene	180	53	300	69	>960	2960	>2580	>5200
Fluoranthene	86	20	97	7	>2940	2350	>960	>2800
Pyrene	70	9	63	8	>2070	1790	>720	>2300
Benz[*a*]anthracene	11	10	8	6	570	330	330	800
Chrysene/Triphenylene	25	19	13	14	460	300	290	710
Benzofluoranthenes	3	4	5	–	230	230	250	260
Benzo[*e*]pyrene	6	8	4	1	42	51	45	45
Benzo[*a*]pyrene	2	2	3	–	96	85	102	90
Indeno[*cd*]pyrene	3	3	2	–	33	2	11	7
Benzo[*ghi*]perylene	1	2	1	–	28	2	7	1
Dibenzanthracenes	2	–	2	–	12	–	4	–
Coronene	–	–	–	–	–	–	–	–
Dibenzopyrenes	–	–	–	–	–	–	–	–

From Niemeier *et al*. (1988)
PAH, polycylic aromatic hydrocarbon
[a] –, not tested

Groups of 30 shaved male C3H/HeJ mice, 8 weeks of age, received twice-weekly dermal applications for 104 weeks of 50 μL cyclohexane:acetone (1:1) that contained 25 mg standard commercial Type III 'steep' asphalt. Additional groups were similarly treated with 25 mg asphalt that had been heated to 316 °C, 25 mg of a combination of asphalt that had been heated to 316 °C and the fumes that were released and collected by condensation and 25 mg of the fumes that resulted from heating asphalt to 316 °C. There

was no consistent effect of treatment on survival. Histopathology was conducted. After 104 weeks, the only exposure that resulted in a significant induction of skin tumours was asphalt fumes, which gave a total of 12 skin papillomas and 25 skin carcinomas in 21 tumour-bearing mice compared with no tumours in the solvent-treated controls (see Table 3.23). The collected fume condensates were then fractionated into five fractions, designated A, B, C, D and E, and these were applied to additional groups of C3H/HeJ mice (either alone or in combination) in amounts corresponding to their original contribution to the fume condensate. Only fraction B (or mixtures containing fraction B), which contained benzothiophenes, anthracenes and/or phenanthrenes, fluorenes, pyrenes and/or fluoranthenes, benzofurans and fluorenones, and fraction C (or mixtures containing fraction C), which contained various ketones, pyrenes and/or fluoranthenes, chrysenes and fused-ring thiophenes, demonstrated significant activity (see Table 3.23). In an additional experiment, groups of 30 male SENCAR mice, 8 weeks of age, received twice-weekly dermal applications for 104 weeks of 50 μL cyclohexane:acetone (1:1) that contained 25 mg condensed asphalt fume or the solvent alone. After 104 weeks, the condensed fume exposure resulted in a significant induction of skin tumours with a total of 21 skin papillomas and 18 skin carcinomas in 20 tumour-bearing mice compared with no tumours in the solvent-treated controls (see Table 3.23) (Sivak *et al.*, 1997).

Table 3.23. Tumour incidence in male C3H/HeJ and SENCAR mice exposed dermally to asphalts

Compound	No. surviving 104 weeks	Total no. of papillomas/ group	Total no. of carcinomas/ group	No. of TBAs	No. of tumours/ TBA
Experiment 1: C3H/HeJ					
Type III 'steep asphalt'	15/30 (50%)	1	3	4	1.0
Heated asphalt (less fumes)	18/30 (60%)	0	0	0	0
Heated asphalt (plus fumes)	21/30 (70%)	0	0	0	0
Heated asphalt fumes	2/30 (7%)	12*	25*	21	1.8
Solvent control	11/30 (37%)	0	0	0	0
Fraction A	19/30 (63%)	0	0	0	0
Fraction B	12/30 (40%)	2	10*	11	1.1
Fraction C	6/30 (20%)	4	18*	20	1.1
Fraction D	7/30 (23%)	0	0	0	0
Fraction E	13/30 (43%)	0	0	0	0
Experiment 2: SENCAR					
Heated asphalt fume	5/30 (17%)	21*	18*	20	2.0
Solvent control	18/30 (60%)	0	0	0	0

From Sivak *et al.* (1997)
TBA, tumour-bearing animal
*Significantly different from the control group

Dermal initiation–promotion studies

Mouse

Groups of 30 shaved female CD-1 mice [age unspecified] received dermal applications of narrow temperature range distillates from solvent-refined coal (SRC) heavy-end coal liquids. Specifically, 17 mg of 800–850 °F+ [427–454 °C+] distillate from SRC-I process solvent, 17 mg of 800–850 ºF [427–454 °C+] distillate from SRC-II process solvent or 17 mg >850 ºF [>454 °C+] distillate from SRC-II process solvent (see Table 3.24 for PAH content) in 50 μL of a mixture of acetone and methylene chloride (1:1) once to the back. Additional mice were treated with subfractions of the materials, which were obtained by chromatographing the materials on alumina to give fractions that contained primarily aliphatic and olefinic compounds (A1), neutral PAHs (A2), nitrogen-containing PAHs (A3) and hydroxy PAHs (A4). These subfractions were applied in amounts corresponding to their original contribution to the mixture. Control mice were treated with the acetone and methylene chloride mixture. Positive-control mice were given a single dose of 50 μg benzo[*a*]pyrene in 50 μL of the same solvent. Two weeks later, all mice were given twice-weekly dermal applications of 5 μg TPA in 50 μg acetone for 24 weeks. After 24 weeks of TPA, the incidence of skin tumours (no histopathology) was 76% (2.24 tumours/mouse) for 800–850 ºF+ [427–454 °C+] distillate from SRC-I process solvent, 72% (1.43 tumours/mouse) for 800–850 ºF [427–454 °C+] distillate from SRC-II process solvent and 79% (4.59 tumours/mouse) for >850 ºF [>454 °C+] distillate from SRC-II process solvent. The control group had an incidence of 14% (0.17 tumours/mouse; see Table 3.25). Subfraction A2 showed the greatest tumour-initiating activity, followed by A3, then A1 and A4 (Mahlum *et al.*, 1984; Springer *et al.*, 1988). [Each of the crude distillates was more active than controls based upon incidence (Fisher's exact one-sided test) or tumours/mouse (ANOVA, Dunnett's test). Fractions A2 and A3 also tended to be significantly different from controls based upon incidence and multiplicity. The >850 ºF [>454 °C+] SRC-II was significantly different from the other two fractions based upon tumours/mouse.]

Groups of 30 or 40 shaved female SENCAR mice [age unspecified] received dermal applications of either coal tar-based paints or petroleum asphalt-based paints. Three coal tar-based paints (designated E, F and G) were used and these typically contained measurable quantities (>1 mg/g) of naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, 2-methylphenanthrene, fluoranthene, pyrene, chrysene + benz[*a*]anthracene and benzo[*a*]pyrene + benzo[*e*]pyrene. Four petroleum asphalt-based paints (designated A, B, C and D) were used; two of these (A and D) were assessed for their PAH content and, with the exception of naphthalene, none (<0.01 mg/g) was detected. The coal tar-based paints were applied once at 0.2–20 μL/animal. Two weeks later, animals were treated thrice weekly for 20 weeks with 1.0 μg TPA in 200 μL acetone. Control mice were treated with acetone alone, and benzo[*a*]pyrene (10 μg) was used as a positive control. Each of the coal tar-based paints caused an [significant; Fisher's exact one-tailed

test] increase in the incidence of papilloma and carcinoma, typically at doses ≥0.6 μL. Benzo[*a*]pyrene caused an [significant; Fisher's exact one-tailed test] increase in the incidence of papilloma. The petroleum asphalt-based paints were applied once at 200 μL/animal. Two weeks later, animals were treated thrice weekly for 20 weeks with 1.0 μg TPA in 200 μL acetone. Control mice were treated with acetone alone. None of the petroleum asphalt-based paints caused an increase in the incidence of papilloma and carcinoma, with one exception. In an additional experiment, groups of 40 mice were treated weekly for 30 weeks with 2 μL coal tar E or 200 μL petroleum asphalt D. Control mice were treated with the solvent (200 μL mineral spirits) alone. Coal tar caused an [significant; Fisher's exact one-tailed test] increase in the incidence of carcinoma (Robinson *et al.*, 1984).

Table 3.24. Levels of several PAHs in the neutral PAH fractions (ppm)

Compound	SRC-I[a] (800–850 °F+ [427–454 °C+])	SRC-II[a] (800–850 °F [427–454 °C+])	SRC-II[a] (>850 °F [>454 °C+])
Naphthalene	–	–	–
Acenaphthylene	–	10	12
Acenaphthene	–	–	–
Fluorene	–	41	–
Phenanthrene	60	211	58
Fluoranthene	–	232	30
Pyrene	30	2275	235
9,10-Dimethylanthracene	–	22	–
2,4-Methylpyrene or benzo[*i*]fluorene	–	5742	–
1-Methylpyrene	–	1307	–
Benz[*a*]anthracene	387	2997	–
Chrysene	563	7324	31
Methylchrysene	3372	3320	215
Benzo[*b*]fluoranthene	2316	9592	2361
Benzo[*k*]fluoranthene	306	86	3972
Dimethylbenzanthracene	95	4532	–
Benzo[*e*]pyrene	2305	6135	5755
Benzo[*a*]pyrene	1707	3530	3637
Indeno[1,2,3-*cd*]pyrene	428	–	7232
Dibenz[*a,c*]anthracene or dibenz[*a,h*]anthracene	7232	–	1997
Benzo[*ghi*]perylene	366	–	15311
Coronene	–	–	1072

From Mahlum *et al* (1984); Springer *et al.* (1988)
PAH, polycyclic aromatic hydrocarbon; SRC, solvent-refined coal
[a] –, not determined by gas chromatography because of their low volatility

Groups of 20 shaved female SENCAR mice, 6 weeks of age, received a single application of 200 μL standard crude coal-tar solution. Additional groups of 20 mice received 39 nmol 7,12-dimethylbenz[*a*]anthracene [purity unspecified], 746 nmol 3-methylcholanthrene [purity unspecified], 396 nmol benzo[*a*]pyrene [purity unspecified] or 352 nmol 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene [purity unspecified] in 200 μL acetone or 200 μL acetone alone. Seven days later, all mice were treated twice weekly with 3.24 nmol TPA. The experiment lasted 11 weeks. [Histopathology was not performed, and statistical analyses were not conducted]. All animals developed skin tumours, and the average number of tumours/mouse was 24.3, 15.0, 9.8, 6.6 and 3.3 in those treated with 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, benzo[*a*]-pyrene, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene and coal-tar solution, respectively. A dose of 500 μL coal-tar solution gave a tumour incidence similar to that of the 200 μL treatment (Mukhtar *et al*., 1986).

Table 3.25. Skin tumour incidence in female CD-1 mice treated dermally with SRC[a]

Compound	Incidence (%)	Tumours/mouse
Control	14	0.17 ± 0.09
800–850 °F+ [427–454 °C+], SRC-I	76*	2.24 ± 0.39*
A1	20	0.30 ± 0.13
A2	88*	3.04 ± 0.52*
A3	60*	0.76 ± 0.16*
A4	12	0.12 ± 0.07
800–850 °F [427–454 °C+], SRC-II	72*	1.43 ± 0.26*
A1	27	0.27 ± 0.08
A2	92*	1.93 ± 0.23*
A3	61*	0.97 ± 0.20*
A4	25	0.27 ± 0.10
>850 °F [>454 °C+], SRC-II	79*	4.59 ± 0.62*
A1	43*	0.93 ± 0.34
A2	100*	6.89 ± 0.59*
A3	83*	2.07 ± 0.30*
A4	41*	0.40 ± 0.12

From Mahlum *et al.* (1984); Springer *et al.* (1988)
PAH, polycyclic aromatic hydrocarbon; SRC, solvent-refined coal
[a] A1, fractions containing primarily aliphatic and olefinic compounds; A2, neutral PAHs; A3, nitrogen-containing PAHs; A4, hydroxy-PAHs
* Significantly different from control

Groups of 30 shaved female CD-1 mice [age unspecified] received dermal applications of complex mixtures obtained from an SRC-II process. Specifically, five distillates were obtained from distillation of a full boiling-range (300–850 ºF+ [149–

454 °C+]) blend of atmospheric flash bottoms and recycle process solvent. The fractions were 300–700 ºF [149–371 °C+], 700–750 ºF [371–399 °C+], 750–800 ºF [399–427 °C+], 800–850 ºF [427–454 °C+] and >850 ºF [>454 °C+] (see Table 3.26 for distribution of PAHs). The distillates (5 mg) were applied once in 50 μL of a mixture of acetone and methylene chloride (1:1) to the shaved backs of the mice. Additional groups of 30 mice were treated with subfractions of the materials, obtained by chromatographing on alumina to give fractions that contained primarily aliphatic hydrocarbons (AH), PAH, nitrogen-containing PAH (NPAH) and hydroxy PAH (HPAH). These subfractions were applied in amounts corresponding to their original contribution to the mixture. Control mice were treated with the acetone and methylene chloride mixture alone. Positive-control mice were given a single dose of 50 μg benzo[*a*]pyrene in 50 μL of the same solvent. Additional groups of 30 mice were treated with each of the distillates in combination with 50 μg benzo[*a*]pyrene. Two weeks later, all mice were given twice-weekly dermal applications of 5 μg TPA in 50 μg acetone for 24 weeks. After 24 weeks of TPA, the incidence of skin tumours from the 300–700 °F [149–399 °C+] mixture did not result in a statistically significant increase in tumour multiplicity compared with controls (see Table 3.27). The other mixtures caused significant tumour-initiating activity with the order increasing as follows: 700–750 °F [371–399 °C+], 750–800 °F [399–427 °C+], 800–850 °F [427–454 °C+], >850 °F [>454 °C+]. When the mixtures were co-administered with benzo[a]pyrene, the 700–750 °F [371–399 °C+], 750–800 °F [399–427 °C+] and 800–850 °F [427–454 °C+] distillates decreased tumour multiplicity

Table 3.26. Concentrations of selected PAH components in SRC-II distillates (mg/g)

Compound	700–750 °F [371–399 °C]	750–800 °F [399–427 °C]	800–850 °F [427–454 °C]	>850 °F [>454 °C]
Fluoranthene	15	1.3		
Pyrene	140	18	1.2	
Benzo[*b*]fluorene	130	37	2.9	
1-Methylpyrene	17	8.0	0.53	
Benz[*a*]anthracene	2.0	3.1	1.5	
Chrysene	1.0	3.8	3.7	0.014
6- or 4-Methylchrysene	0.081	4.7	18	0.010
Benzo[*j* or *b*]fluoranthene			4.8	1.1
Benzo[*k*]fluoranthene			2.0	0.14
Benzo[*e*]pyrene			3.1	2.6
Benzo[*a*]pyrene			1.8	1.7
Indeno[1,2,3-*cd*]pyrene				3.3
Benzo[*ghi*]perylene				7
Coronene				0.49

From Springer *et al.* (1989)
PAH, polycyclic aromatic hydrocarbon; SRC, solvent-refined coal

compared with mice treated with benzo[*a*]pyrene alone. When AH, PAH, NPAH and HPAH subfractions from the 700–750 ºF [371–399 °C+] distillate were applied to the mouse in an amount similar to their contribution to the original mixture, only the PAH subfraction induced a significant increase in tumour multiplicity. When the subfractions from the 700–750 °F [371–399 °C+] distillate were co-administered with benzo[*a*]pyrene, the PAH and NPAH subfractions decreased tumour multiplicity compared with mice treated with benzo[*a*]pyrene alone (Springer *et al.*, 1989).

Table 3.27. Tumour incidence in female CD-1 mice exposed dermally to complex mixtures of PAHs

Compound	No. of mice/group	No. of tumours/mouse (± SEM)
Solvent	30	0.17 ± 0.07
300–700 °F [149–371 °C]	30	0.37 ± 0.13
700–750 °F [371–399 °C]	30	0.57 ± 0.14[a]
750–800 °F [399–427 °C]	30	0.60 ± 0.18[a]
800–850 °F [427–454 °C]	30	1.23 ± 0.43[a]
>850 °F [>454 °C]	29	4.52 ± 0.43[a]
B[*a*]P	30	7.07 ± 0.67
300–700 °F [149–371 °C] + B[*a*]P	30	6.63 ± 0.50
700–750 °F [371–399 °C] + B[*a*]P	29	4.14 ± 0.49[b]
750–800 °F [399–427 °C] + B[*a*]P	29	2.93 ± 0.33[b]
800–850 °F [427–454 °C] + B[*a*]P	30	3.00 ± 0.36[b]
>850 ºF [>454 °C] + B[*a*]P	30	6.33 ± 0.75
Solvent	29	0.24 ± 0.07
750–800 °F [399–427 °C]	29	0.69 ± 0.09[c]
AH from 750–800 °F [399–427 °C]	30	0.13 ± 0.07
PAH from 750–800 °F [399–427 °C]	30	0.67 ± 0.08[c]
NPAH from 750–800 °F [399–427 °C]	30	0.27 ± 0.06
HPAH from 750–800 °F [399–427 °C]	30	0.23 ± 0.07
B[*a*]P	29	7.21 ± 0.65
750–800 °F [399–427 °C] + B[*a*]P	30	2.23 ± 0.29[d]
AH from 750–800 °F [399–427 °C] + B[*a*]P	30	5.73 ± 0.78
PAH from 750–800 °F [399–427 °C] + B[*a*]P	30	2.50 ± 0.32[d]
NPAH from 750–800 °F [399–427 °C] + B[*a*]P	27	2.81 ± 0.51[d]
HPAH from 750–800 °F [399–427 °C] + B[*a*]P	27	6.44 ± 0.53

From Springer *et al.* (1989)
AH, aliphatic hydrocarbons; B[*a*]P, benzo[*a*]pyrene; HPAH, hydroxy PAH; NPAH, nitrogen-containing PAH; PAH, polycyclic aromatic hydrocarbon; SEM, standard error of the mean
[a] Significantly different from control (as reported by authors)
[b] Significantly different from B[*a*]P (ANOVA, followed by Dunnett's test)
[c] Significantly different from control (ANOVA, followed by Dunnett's test)
[d] Significantly different from B[*a*]P (ANOVA, followed by Dunnett's test)

Groups of 30 shaved female CD-1 mice, 7–8 weeks of age, received dermal applications on 5 days per week for 2 weeks of 50 mg of a 1.5% coal-tar ointment (Lorinden). Beginning 1 week after the coal-tar treatment, one group of mice received thrice-weekly dermal applications for 40 weeks of 50 mg of a 0.1% dithranol cream (promotion), while the second group of mice remained untreated. An additional group of mice was treated with dithranol only. After 40 weeks of treatment, skin papillomas (histologically verified) occurred in four mice administered coal tar followed by dithranol, in no mice treated with coal tar only and in no mice treated with dithranol only. The incidence of tumours in mice treated with coal tar and dithranol was significantly different (log-rank test) from that in mice treated with only coal tar or only dithranol (see Table 3.28). Benzo[*a*]pyrene, with dithranol promotion, which was used as a positive initiation control, gave papillomas in 14 mice (Phillips & Alldrick, 1994).

Table 3.28. Tumour incidence in female CD-1 mice treated dermally

Compound	No. of survivors at 40 weeks	No. of mice with skin tumours
Coal tar + dithranol	27/30 (90%)	4*
B[*a*]P + dithranol	28/30 (93%)	14*
Coal tar	30/30 (100%)	0
Dithranol	28/30 (93%)	0

From Phillips & Alldrick (1994)
B[*a*]P, benzo[*a*]pyrene
*Significantly different from coal tar alone

Groups of shaved female SENCAR mice, 6–7 weeks of age, received dermal applications of 1 mg medium crude coke-oven coal tar (standard reference material (SRM) 1597) that contained 10.4 μg benzo[*a*]pyrene in 125 μL toluene (30 mice) [the 10.4 μg benzo[*a*]pyrene/1 mg SRM 1597 is an error; the correct value is 95.8 ng (see Table 3.29)], 200 nmol (50.4 μg) benzo[*a*]pyrene [purity not specified] in 200 μL toluene (35 mice), 1 mg SRM 1597 + 200 nmol benzo[*a*]pyrene in 100 μL toluene (35 mice) or 200 μL toluene alone (10 mice). In a separate experiment, groups of shaved female SENCAR mice received dermal applications of 1 mg SRM 1597 in 125 μL toluene (30 mice), 2 nmol (600 ng) dibenzo[*a,l*]pyrene [purity, solvent and volume not specified] (35 mice), 1 mg SRM 1597 + 2 nmol dibenzo[*a,l*]pyrene [solvent and volume not specified] (35 mice) or 200 μL toluene alone (10 mice). Two weeks later, all mice received twice-weekly dermal applications of 1 μg TPA in 200 μL acetone for 25 weeks. Tumours were subjected to routine histopathology. After 25 weeks of TPA, 24/26 (92%) mice (4.92 tumours/mouse) treated with SRM 1597, 27/30 (90%) mice (8.03

tumours/mouse) treated with benzo[a]pyrene, 29/29 (100%) mice (8.72 tumours/mouse) treated with SRM 1597 + benzo[*a*]pyrene and 0/8 mice (0.12 tumours/mouse) treated with toluene developed skin tumours that were almost exclusively papillomas. Mice administered benzo[*a*]pyrene and SRM 1597 + benzo[*a*]pyrene had a significantly greater tumour multiplicity than those treated with SRM 1597 alone. There was no significant difference between treatment with benzo[*a*]pyrene and SRM 1597 + benzo[*a*]pyrene. After 25 weeks of TPA, 26/27 (96%) mice (3.41 tumours/mouse) treated with SRM 1597, 30/30 (100%) mice (7.87 tumours/mouse) treated with dibenzo[*a,l*]pyrene, 29/30 (97%) mice (4.67 tumours/mouse) treated with SRM 1597 + dibenzo[*a,l*]pyrene and 2/9 (22%) mice (0.25 tumours/mouse) treated with toluene developed skin tumours that were almost exclusively papillomas. Mice administered dibenzo[*a,l*]pyrene had a significantly greater tumour multiplicity than those treated with SRM 1597 + dibenzo[*a,l*]pyrene. Mice administered SRM 1597 + dibenzo[*a,l*]pyrene had a significantly greater number of tumours/mouse than those treated with SRM 1597. The authors concluded that SRM 1597 inhibited dibenzo[*a,l*]pyrene tumorigenicity but had no effect upon that of benzo[*a*]pyrene. Their mechanistic data did not provide a reason for this because SRM 1597 inhibited DNA adduct formation with both compounds, but did not induce cytochrome P450 1A1 or 1B1 (Marston *et al.*, 2001).

Table 3.29. Certified concentrations of selected PAHs in SRM 1597

Compound	Concentration (mg/kg)
Naphthalene	1160
Phenanthrene	462
Anthracene	101
Fluoranthene	322
Pyrene	235
Benz[*a*]anthracene	98.6
Chrysene	71.7
Triphenylene	12.1
Benzo[*a*]pyrene	95.8
Perylene	26.1
Indeno[1,2,3-*cd*]pyrene	60.2
Benzo[*ghi*]perylene	53.7

From Marston *et al.* (2001)
PAH, polycyclic aromatic hydrocarbon; SRM, standard reference material

Oral administration

Mouse

Groups of 40 female A/J mice, 8 weeks of age, were administered 1.0, 10.0 or 55.0 mg coal-tar paint in 200 μL 2% Emulphor by gavage thrice weekly for 8 weeks. Due to the viscosity of the solution, the 55.0 mg dose was given twice daily at 27.5 mg per treatment. The coal-tar paint consisted of 67% coal-tar pitch and 33% xylene. The coal-tar paint had been applied to glass panels that were kept in a dust-free chamber for 4 months. The paint was scraped from the panels and particulate suspensions were made in the Emulphor solution. Benzo[*a*]pyrene (250 μg; 99% pure) was used as a positive control. Groups of 20 control mice were treated with the solvent either once a day or twice a day. The experiment lasted 7 months. Histopathogy was conducted. The two highest doses of coal-tar paint resulted in a significant induction of lung tumours, based upon both incidence and multiplicity (see Table 3.30). Benzo[*a*]pyrene also resulted in a significant induction of lung tumours. The two highest doses of coal-tar paint resulted in a significant induction of forestomach papilloma and carcinoma (see Table 3.31), as did benzo[*a*]pyrene. The benzo[*a*]pyrene content of the coal-tar paint could account for the induction of forestomach tumours, but not of the lung tumours (Robinson *et al*., 1987).

Table 3.30. Lung tumour incidence in female A/J mice treated orally with coal-tar paint (CTP)

Compound	No. of survivors	% of mice with lung tumours	Multiplicity
Solvent control	38/40	29	0.32 ± 0.09
CTP 1.0 mg	37/40	35	0.46 ± 0.13
CTP 10.0 mg	37/40	97*	4.27 ± 0.39**
CTP 55.0 mg	36/40	72*	4.33 ± 2.70**
Benzo[*a*]pyrene	36/40	61*	1.42 ± 0.40**

From Robinson *et al.* (1987)
Significantly different from control *p <0.01; **p <0.001

Groups of approximately 30 female A/J mice, 6 weeks of age, were fed a gel diet that contained 0.10 or 0.25% manufactured gas plant (MGP) residue (coal tar) for 260 days. Additional groups were fed gel diets that contained 16 or 98 ppm benzo[*a*]pyrene. During the 260-day feeding period, the mice fed MGP diet consumed 0.65 and 1.53 g coal tar, which contained 1.8 and 4.2 mg benzo[*a*]pyrene. The mice fed benzo[*a*]pyrene consumed a total of 11 and 67 mg benzo[*a*]pyrene. Lungs and forestomachs were examined histologically. After 260 days of feeding, the incidence of lung tumours was 19/27 (70%) mice fed 0.10% MGP diet (1.19 tumours/mouse), 29/29 (100%) mice fed 0.25% MGP diet (12.17 tumours/mouse), 9/25 (36%) mice fed 16 ppm benzo[*a*]pyrene diet

(0.48 tumours/mouse) and 14/27 (52%) mice fed 98 ppm benzo[*a*]pyrene diet (0.59 tumours/mouse). Control mice fed the gel diet had a lung tumour incidence of 4/19 (21%) (0.19 tumours/mouse). The lung tumour incidence in the mice fed MGP diet and those fed 98 ppm benzo[*a*]pyrene was significantly different from that in the control group. The lung tumour multiplicity in mice fed MGP diet was significantly different from that in the control group, whereas that in mice fed benzo[*a*]pyrene did not differ from that in the control group. Forestomach tumours did not occur in mice fed MGP diet or control mice, whereas they were induced in mice fed 16 ppm (5/25 (20%); 0.22 tumours/mouse) and 98 ppm (27/27 (100%); 4.22 tumours/mouse) benzo[*a*]pyrene (Weyand *et al*., 1995). [Benzo[*a*]pyrene does not seem to be responsible for the induction of lung tumours, and the quantity of benzo[*a*]pyrene in the MGP diet was insufficient to induce forestomach tumours.]

Table 3.31. Incidence of forestomach tumours in female A/J mice treated orally with coal-tar paint (CTP)

Compound	No. of survivors	% of mice with forestomach tumours	% of mice with forestomach papilloma	% of mice with forestomach carcinoma
Solvent control	38/40 (95%)	0	0	0
CTP 1.0 mg	37/40 (92%)	0	0	0
CTP 10.0 mg	37/40 (92%)	0	0	0
CTP 55.0 mg	36/40 (90%)	42**	36**	19*
Benzo[*a*]pyrene	36/40 (90%)	92**	67**	61*

From Robinson *et al.* (1987)
Significantly different from control *p <0.01; **p <0.001

Groups of 48 female B6C3F$_1$ mice, 5 weeks of age, were fed diets that contained 0.01, 0.03, 0.1, 0.3, 0.6 or 1.0% of a coal-tar mixture designated Coal Tar Mixture 1 (CT-1) for 104 weeks. This mixture was a composite from seven MGP waste sites and had a benzo[*a*]pyrene concentration of 1837 mg/kg (see Table 3.32). Additional groups of female mice were fed diets that contained 0.03, 0.1 or 0.3% of a coal-tar mixture designated Coal Tar Mixture 2 (CT-2) for 104 weeks. This mixture was a composite from two of the seven waste sites plus a third site that had a very high benzo[*a*]pyrene content. The benzo[*a*]pyrene concentration of this coal tar mixture was 2760 mg/kg. For comparison, additional mice were fed diets that contained 0.0005, 0.0025 or 0.01% benzo[*a*]pyrene. None of the mice fed 0.6 and 1.0% CT-1 survived the 104-week feeding; survival in mice fed 0.3% CT-1 and CT-2 was ~15–20%. All other groups had a survival similar to the control groups (~70%). Liver neoplasms (hepatocellular adenomas, carcinomas or both) did not occur in the control group but occurred in all groups of mice fed the coal tar, and the incidence was significant in mice fed 0.3% CT-1 and 0.3% CT-2 (see Table 3.33); all tumours were verified by histopathology. Alveolar/bronchiolar

Table 3.32. Polycyclic aromatic hydrocarbon composition of coal tar mixtures (CT)

Compound	CT-1 (mg/kg)	CT-2 (mg/kg)
Acenaphthene	2049	1270
Acenaphthylene	390	5710
Anthracene	2524	2900
Benz[*a*]anthracene	2374	3340
Benzo[*b*]fluoranthene	2097	2890
Benzo[*k*]fluoranthene	699	1010
Benzo[*ghi*]perylene	1493	2290
Benzo[*a*]pyrene	1837	2760
Chrysene	2379	2960
Dibenz[*a,h*]anthracene	267	370
Dibenzofuran	1504	1810
Fluoranthene	965	6370
Fluorene	3692	4770
Indan	1133	490
Indeno[1,2,3-*cd*]pyrene	1353	1990
1-Methylnaphthalene	6550	5660
2-Methylnaphthalene	11 289	10 700
Naphthalene	22 203	32 300
Phenanthrene	7640	10 100
Pyrene	5092	7220

From Culp *et al.* (1998)

Table 3.33. Tumour incidence in female B6C3F$_1$ mice fed coal-tar mixtures (CT)

Compound	Hepatocellular adenoma and/or carcinoma	Alveolar/bronchiolar adenoma and/or carcinoma	Forestomach papilloma and/or carcinoma	Small intestine adenocarcinoma
Control	0/47	2/47 (4%)	0/47	0/47
CT-1 0.01%	4/48 (8%)	3/48 (6%)	2/47 (4%)	0/46
CT-1 0.03%	2/46 (4%)	4/48 (8%)	6/45 (13%)	0/45
CT-1 0.1%	3/48 (6%)	4/48 (8%)	3/47 (6%)	0.47
CT-1 0.3%	14/45* (31%)	27/47* (57%)	14/46* (30%)	0/42
CT-1 0.6%	1/42 (2%)	25/47* (53%)	15/45* (33%)	22/36* (64%)
CT-1 1.0%	5/43 (12%)	21/45* (47%)	6/41 (15%)	36/41* (88%)
CT-2 0.03%	7/47 (15%)	4/48 (8%)	3/47 (6%)	0/47
CT-2 0.1%	4/47 (8%)	10/48* (21%)	2/47 (4%)	0/47
CT-2 0.3%	10/45* (22%)	23/47* (49%)	13/44* (30%)	1/37 (3%)

From Culp *et al.* (1998)
*Significantly different from controls, $p < 0.05$

adenomas, carcinomas or both were present in all groups fed coal tar. The incidence was significant in those fed 0.3%, 0.6% and 1.0% CT-1 and 0.1% and 0.3% CT-2 compared

with the control group. Papillomas and/or carcinomas of the forestomach squamous epithelium occurred in all groups fed coal tar, and the incidence was significant in those fed 0.3% CT-1, 0.6% CT-1 and 0.3% CT-2 compared with the control group. Adenocarcinoma of the small intestine was present in mice fed 0.6% and 1.0% CT-1, and the incidence was significant compared with the control group. The coal-tar mixtures also induced significant dose-related increases in haemangiosarcomas, histiocytic sarcomas and sarcomas. Mice fed benzo[*a*]pyrene had an increased incidence of papillomas and/or carcinomas of the tongue, oesophagus and forestomach. A comparison of the results obtained from mice fed benzo[*a*]pyrene indicated that the benzo[*a*]pyrene in coal-tar mixtures could be responsible for the forestomach tumours. The lung and liver tumours appeared to be due to other components contained in the coal-tar mixtures (Culp *et al.*, 1998).

Intraperitoneal injection

Mouse

Groups of approximately 30 male and 30 female B6C3F1 mice, 15 days of age, were administered a single intraperitoneal injection of MGP residue (coal tar). One group received 7.98 mg MGP-4, a product obtained from a single MGP site (see Table 3.34) for characterization), in corn oil [volume not specified]. The benzo[*a*]pyrene content of this mixture was 1.56 g/kg. Additional groups received 1.995, 3.99 or 7.98 mg MGP-7 (see Table 3.31 for characterization), a product formulated by mixing equal amount of residues from seven different MGP sites, including MGP-4, in corn oil. The benzo[*a*]pyrene content of this mixture was 1.84 g/kg. Further groups of mice received 125, 250 or 375 µg benzo[*a*]pyrene [purity not specified] in corn oil, or corn oil alone (approximately 60 of each sex). Tumorigenicity was assessed at 26, 39 and 52 weeks. Livers, lungs and forestomachs were examined histologically. Forestomach tumours were not detected and there was only a very low incidence of pulmonary tumours. With one exception, liver tumours occurred only in male mice. When assessed 26 weeks after treatment, only a low incidence of liver tumours was observed in male mice treated with MGP residue, and none were detected in male mice treated with benzo[*a*]pyrene. Thirty-nine weeks after treatment, the incidence of liver tumours in male mice was 4/34 (9%), 0/33 and 23/28 (82%) for groups that received 1.995, 3.99 and 7.98 mg MGP-7, 10/22 (45%) for the group that received 9.98 mg MGP-4, 6/26 (23%), 13/34 (38%) and 15/23 (65%) for the mice that received 125, 250 and 375 µg benzo[*a*]pyrene and 0/59 for corn oil controls. Fifty-two weeks after treatment, the incidence of liver tumours in male mice was 4/34 (12%), 8/32 (25%) and 17/29 (59%) for groups that received 1.995, 3.99 and 7.98 mg MGP-7, 12/28 (43%) for the group that received 9.98 mg MGP-4, 13/29 (45%), 14/27 (52%) and 19/24 (79%) for the mice that received 125, 250 and 375 µg benzo[a]pyrene and 3/63 (5%) for corn oil controls (Rodriguez *et al*., 1997). [At 39 weeks, the low and high doses of MGP gave significantly different results from the corn oil control. At

52 weeks, the medium and high doses gave significantly different results from the control. The benzo[*a*]pyrene was administered in great excess of that found in the coal tar (125–375 μg as compared with <15 μg benzo[*a*]yrene in coal tar); thus, the liver tumour response observed with coal tar may be due to compounds other than its benzo[*a*]pyrene content.]

Groups of 30 male B6C3F$_1$ mice, 15 days of age, were administered a single intraperitoneal injection of a mixture of 17 PAHs including indan (0.2% of the mixture), naphthalene (23.8%), 2-methylnaphthalene (23.2%), 1-methylnaphthalene (13.3%), acenaphthylene (7.7%), acenaphthene (0.6%), dibenzofuran (0.7%), fluorene (4.3%), phenanthrene (10.5%), anthracene (3.4%), fluoranthene (2.4%), pyrene (4.3%), benz[*a*]anthracene (1.4%), chrysene (1.5%), benzo[*b*]fluoranthene (0.8%), benzo[*k*]fluoranthene (0.9%) and benzo[*a*]pyrene (0.9%). This mixture was modelled on the PAHs identified in a MGP residue (coal tar) designated MGP-4, a product obtained from a single MGP site. Absence from the mixture (but present in MGP-4; see Table 3.34) were indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene. The mixture was given at a level of 193 mg/kg in the presence of 26.75–126.75 μg benzo[*a*]-pyrene. Additional mice were treated with 193, 535 or 1041 mg/kg of the mixture. Fifty-two weeks after treatment, none of the mice had liver tumours (Goldstein *et al*., 1998).

Table 3.34. Composition of coal tar mixtures (% of quantified total aromatic compounds)

Compound	MGP-4	MGP-7
Indan	0.2	1.4
Naphthalene	29.8	27.3
2-Methylnaphthalene	21.4	13.9
1-Methylnaphthalene	12.3	8.1
Acenaphthylene	6.9	3.9
Acenaphthene	0.5	2.5
Dibenzofuran	0.6	1.8
Fluorene	3.9	4.5
Phenanthrene	9.7	9.4
Anthracene	3.1	3.1
Fluoranthene	2.7	6.1
Pyrene	3.9	6.3
Benz[*a*]anthracene	1.3	2.9
Chrysene	1.3	2.9
Benzo[*b*]fluoranthene	0.8	2.6
Benzo[*k*]fluoranthene	0.3	0.9
Benzo[*a*]pyrene	0.9	2.3
Indeno[1,2,3-*cd*]pyrene	2.8	1.6
Dibenz[*a,h*]anthracene	1.0	0.4
Benzo[*ghi*]perylene	3.9	1.8

From Goldstein *et al.* (1998)

Inhalation exposure

Mouse

Groups of female NMRI mice [number and age unspecified] were exposed by inhalation to coal oven flue gas for 16 h per day on 5 days per week. The flue gas was obtained from a domestic coal oven. Since the PAH-content of the flue gas was very low, a tar pitch was added to the coal embers in the oven, which resulted in an increase to 0.3 μg benzo[*a*]pyrene/m^3 (Table 3.35). Subsequently, the PAH-content was raised further by mixing the coal oven exhaust with the gaseous components developed by continuous heating of pitch at approximately 750 °C in a nitrogen atmosphere. These modifications resulted in the following exposures: adult NMRI mice, coal oven gas mixed with pyrolysed pitch (COP) with a benzo[*a*]pyrene content of ~0.3 μg/m^3 for 9 months, followed by COP with a benzo[*a*]pyrene content of ~60 μg/m^3 for 15 months (Series 1); adult NMRI mice, COP with a benzo[*a*]pyrene content of ~50 μg/m^3 for 12 months (Series 2); and neonatal NMRI mice, COP with a benzo[*a*]pyrene content of ~90 μg/m^3 for 10 months (Series 3). Adult NRMI mice exposed to COP with a low benzo[*a*]pyrene content (~0.3 μg/m^3) followed by a high benzo[*a*]pyrene content (~60 μg/m^3) (Series 1) had a lung tumour incidence of 79.0% (7.0 ± 7.9 tumours/mouse) compared with an incidence of 32.0% (0.7 ± 1.7 tumours/mouse) in clean air control mice (p <0.05). Adult NRMI mice exposed to COP with a benzo[*a*]pyrene content ~50 μg/m^3 (Series 2) had a lung tumour incidence of 70.0% (3.8 ± 5.2 tumours/mouse) compared with an incidence of 12.5% (0.2 ± 0.5 tumours/mouse) in clean air control mice (p <0.05). NRMI mice exposed, beginning as neonates, to COP with a benzo[*a*]pyrene content ~90 μg/m^3 (Series 3) had a lung tumour incidence of 85.7% (7.9 ± 8.8 tumours/mouse) compared with an incidence of 3.5% (0.03 ± 0.19 tumours/mouse) in clean air control mice (p <0.05) (Heinrich *et al*., 1986a,b).

Table 3.35. Concentration of particle-bound PAH in diluted coal oven/pyrolysed pitch exhaust

Compound	Concentration (μg/mL)
Fluoranthene	47.5
Pyrene	33.8
Benz[*a*]anthracene	18.8
Chrysene	18.3
Benzofluoranthenes	27.2
Benzo[*e*]pyrene	11.2
Benzo[*a*]pyrene	14.7
Indeno[1,2,3-*cd*]pyrene	9.1
Benzo[*ghi*]perylene	10.1
Coronene	2.1

Heinrich *et al* (1986b)

Rat

Groups of female Wistar rats [number and age unspecified] were exposed by inhalation to coal oven flue gas for an average of 16 h per day on 5 days per week. The flue gas was obtained from a domestic coal oven. Since the PAH-content of the flue gas was very low, for some exposures, a tar pitch was added to the coal embers in the oven, which resulted in an increase of PAH content to 0.3 μg benzo[*a*]pyrene/m^3. Subsequently, the PAH-content was raised further by mixing the coal oven exhaust with the gaseous components developed by continuous heating of pitch at approximately 750 °C in a nitrogen atmosphere. These modifications resulted in female Wistar rats being exposed to COP with a benzo[*a*]pyrene content of ~0.3 μg/m^3 for 10 months, followed by COP with a benzo[*a*]pyrene content of ~90 μg/m^3 for 12 months. Wistar rats exposed to COP as described above, followed by clean air for up to 8 months had an incidence of 16/116 (14%) benign lung tumours and 5/116 (4%) malignant lung tumours. Control rats (115) that had been exposed to only clean air did not have any benign or malignant tumours [benign tumours, p <0.0001; malignant tumours, p <0.0306; combined benign and malignant tumours (21/116; 18%), p <0.0001] (Heinrich *et al*., 1986a,b).

Seventy-two groups of female Wistar rats [age unspecified] were exposed for 18 h per day on 5 days per week for 43 weeks to an aerosol that contained PAHs that was produced by heating pitch to ~750 °C under a nitrogen atmosphere, diluting the high-temperature vapour with 200 °C clean air and then 12 °C clean air. The mass concentration of the condensation aerosol was ~2.5 mg/m^3 and the benzo[*a*]pyrene content was 50 μg/m^3. The exposure period was followed by 57 weeks of exposure to clean air, at which time the PAH-exposed rats had a lung tumour incidence of 31% compared with 0% in the clean-air control rats. Additional groups of rats were exposed for 43 weeks to aerosols that contained PAHs with a benzo[*a*]pyrene content of 20 or 90 μg/m^3 [particle mass not specified]. After 57 additional weeks of exposure to clean air, the lung tumour incidence was 3 and 56%, respectively (Heinrich, 1989).

Groups of 72 female Wistar rats, 10 weeks of age, were exposed to a tar-pitch condensation aerosol for 17 h per day on 5 days per week for 43 or 86 weeks. The animals were then maintained on clean air for an additional 86 or 43 weeks. The tar-pitch condensation aerosol was generated by heating hard coal-tar pitch to 750 °C under a nitrogen atmosphere and diluting the high-temperature tar-pitch vapour with clean air at 12 °C. The resulting PAH-rich material, which was free of any carbon core, was administered to the rats by inhalation at concentrations of 1.1 and 2.6 mg/m^3, an amount that contained 20 and 50 μg benzo[*a*]pyrene/m^3. At the end of the 129-week experimental period, the incidence of lung tumours (histologically verified, primarily squamous carcinoma) was 4% and 33% in rats exposed to 1.1 and 2.6 mg tar-pitch/m^3 for 43 weeks, and 39% and 97% in rats exposed to 1.1 and 2.6 mg tar pitch/m^3 for 86 weeks, respectively. No lung tumours occurred in animals exposed to clean air only (Heinrich *et al*., 1994a,b). [With the exception of 20 μg benzo[*a*]pyrene at 43 weeks, all the exposures were significant (Fisher's exact one-tailed test)].

Hamster

Groups of female Syrian golden hamsters [number and age unspecified] were exposed by inhalation to coal oven flue gas for an average of 16 h per day on 5 days per week. The flue gas was obtained from a domestic coal oven in which the PAH-content was raised further by mixing the coal oven exhaust with the gaseous components developed by continuous heating of pitch at approximately 750 °C in a nitrogen atmosphere. This modification resulted in the exposure of Syrian golden hamsters to COP with a benzo[*a*]pyrene content of ~50 μg/m^3 for 18 months. This was followed by 11 months of clean air. Syrian golden hamsters exposed to COP with a benzo[*a*]pyrene content ~50 μg/m^3 did not develop lung tumours; however, 50% of the animals had bronchiolo-alveolar nodular squamous metaplasia and 14% had papillomas in the larynx/trachea region. These lesions did not occur in hamsters exposed to clean air (Heinrich *et al*., 1986a,b).

3.1 References

Adamson, R.H. & Sieber, S.M. (1983) Chemical carcinogenesis studies in nonhuman primates. In: Langenbach, R., Nesnow, S & Rice, J.M., eds, *Organ and Species Specificity in Chemical Carcinogenesis*, New York, Plenum Press, pp. 129–156

Albert, R.E., Miller, M.L., Cody, T., Andringa, A., Shukla, R. & Stuart Baxter, C. (1991) Benzo[*a*]pyrene-induced skin damage and tumor promotion in the mouse. *Carcinogenesis*, **12**, 1273–1280

Amin, S., Huie, K. & Hecht, S.S. (1985a) Mutagenicity and tumor initiating activity of methylated benzo[b]fluoranthenes. *Carcinogenesis*, **6**, 1023–1025

Amin, S., Hussain, N., Balanikas, G., Huie, K. & Hecht, S.S. (1985b) Mutagenicity and tumor initiating activity of methylated benzo[k]fluoranthenes. *Cancer Lett*., **26**, 343–347

Amin, S., Huie, K., Melikian, A.A., Leszczynska, J.M. & Hecht, S.S. (1985c) Comparative metabolic activation in mouse skin of the weak carcinogen 6-methylchrysene and the strong carcinogen 5-methylchrysene. *Cancer Res*., **45**, 6406–6412

Amin, S., Hecht, S.S., Di Raddo, P. & Harvey, R.G. (1990) Comparative tumor initiating activities of cyclopentano and methyl derivatives of 5-methylchrysene and chrysene. *Cancer Lett*., **51**, 17–20

Amin, S., Weyand, E.H., Huie, K., Boger, E., Nueber, E., Hecht, S.S. & LaVoie, E.J. (1991) Effects of fluorine substitution on benzo[b]fluoranthene tumorigenicity and DNA adduct formation in mouse skin. In: Cooke, M., Loening, K. & Merritt, J., eds, *Polynuclear Aromatic Hydrocarbons: Measurements, Means and Metabolism*, Columbus, OH, Battelle Press, pp. 25–31

Amin, S., Desai, D. & Hecht, S.S. (1992) Comparative tumorigenicity of dimethylchrysenes in mouse skin. *Chem. Res. Toxicol*., **5**, 237–241

Anderson, R.S. & Anderson, L.M. (1987) Lack of effect of perylene as an initiator of lung tumors or as a promoter of skin tumors in mice (Abstract). *Fed. Proc*., **46**, 746

Anderson, L.M., Priest, L.J., Deschner, E.E. & Budinger, J.M. (1983) Carcinogenic effects of intracolonic benzo[a]pyrene in β-naphthoflavone-induced mice. *Cancer Lett.*, **20**, 117–123

Andervont, H.B. & Shimkin M.B. (1940) Biologic testing of carcinogens. II. Pulmonary tumor induction technique. *J. natl Cancer Inst.*, **1**, 225–239

Andrews, F.J., Halliday, G.M. & Muller H.K. (1991) A role for prostaglandins in the suppression of cutaneous cellular immunity and tumour development in benzo(a)pyrene- but not dimethylbenz(a)anthracene-treated mice. *Clin. exp. Immunol*., **85**, 9–13

Badary, O.A., Al-Shabanah, O.A., Nagi, M.N., Al-Rikabi, A.C. & Elmazar, M.M.A. (1999) Inhibition of benzo(a)pyrene-induced forestomach carcinogenesis in mice by thymoquinone. *Eur. J. Cancer Prev*., **8**, 435–440

Badger, G.M., Cook, J.W., Hewet, C.L., Kennaway, E.L., Kennaway, N.M., Martin, R.H. & Robinson, A.M. (1940) The production of cancer by pure hydrocarbons. V. *Proc. roy. Soc. London*, **B129**, 439–467

Barry, G. & Cook, C.W. (1934) A comparison of the action of some polycyclic aromatic hydrocarbons in producing tumours of connective tissue. *Am. J. Cancer*, **20**, 58–69

Barry, G., Cook, J.W., Haslewood, G.A.D., Hewet, C.L., Hieger, I. & Kennaway, E.L. (1935) The production of cancer by pure hydrocarbons. Part III. *Proc. roy. Soc. London*, **B117**, 318–351

Beems, R.B. (1984) Modifying effect of vitamin A on benzo[a]pyrene-induced respiratory tract tumours in hamsters. *Carcinogenesis*, **5**, 1057–1060

Beems, R.B. (1986) Dietary selenium- and benzo[a]pyrene-induced respiratory tract tumours in hamsters. *Carcinogenesis*, **7**, 485–489

Beems, R.B. (1987) The effect of β-carotene on BP-induced respiratory tract tumors in hamsters. *Nutr. Cancer*, **10**, 197–204

Beems, R.B. & van Beek, L. (1984) Modifying effects of dietary fat on benzo[a]pyrene-induced respiratory tract tumours in hamsters. *Carcinogenesis*, **5**, 413–417

Benfield, J.R., Hammond, W.G., Paladugu, R.R., Pak, H.Y., Azumi, N. & Teplitz, R.L. (1986) Endobronchial carcinogenesis in dogs. *J Thorac. Cardiovasc. Surg.*, **92**, 880-889

Berenblum, I. & Haran, N. (1955) The influence of croton oil and of polyethylene glycol-400 on carcinogenesis in the forestomach of the mouse. *Cancer Res*., **15**, 510–516

Bhatt, T.S. & Coombs, M.M. (1990) The carcinogenicity of cyclopenta[a]phenanthrene and chrysene in the Sencar mouse. *Polycyc. aromat. Compds*, **1**, 51–58

Biancifiori, C. & Caschera, F. (1962) The relation between pseudopregnancy and the chemical induction by four carcinogens of mammary and ovarian tumours in BALB/c mice. *Br. J. Cancer*, **16**, 722–730

Bingham, E. & Falk, H.L. (1969) Environmental carcinogens. The modifying effect of cocarcinogens on the treshold response. *Arch. environ. Health*, **19**, 779–783

Bingham, E., Trosset, P.R. & Warshawsky, D. (1979) Carcinogenic potential of petroleum hydrocarbons: A critical review of the literature. *J. Environ. Pathol. Toxicol*., **3**, 483–563

Bock, F.G. & King, D.W. (1959) A study of the sensitivity of the mouse forestomach toward certain polycyclic hydrocarbons. *J. natl Cancer Inst*., **23**, 833–839

Boyland, E. & Burrows, H. (1935) The experimental production of sarcoma in rats and mice by a colloidal aqueous solution of 1:2:5:6-dibenzanthracene. *J. Pathol. Bacteriol*., **41**, 231–238

Boyland, E. & Sims, P. (1967) The carcinogenic activities in mice of compounds related to benz[*a*]anthracene. *Int. J. Cancer*, **2**, 500–504

Brune, H., Deutsch-Wenzel, R.P., Habs, M., Ivankovic, S. & Schmähl, D. (1981) Investigations of the tumorigenic response to benzo(a)pyrene in aqueous caffeine solution applied orally to Sprague-Dawley rats. *J. Cancer Res. clin. Oncol*., **102**, 153–157

Bryan, W.R. & Shimkin, M.B. (1943) Quantitative analysis of dose–response data obtained with three carcinogenic hydrocarbons in strain C3H male mice. *J. natl Cancer Inst*., **3**, 503–531

Buening, M.K., Wislocki, P.G., Levi, W., Yagi, H., Thakker, D.R., Akagi, H., Koreeda, M., Jerina, D.M. & Conney, A.H. (1978) Tumorigenicity of the optical enantiomers of the diastereomeric benzo[*a*]pyrene 7,8-diol-9,10-epoxides in newborn mice: Exceptional activity of (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Proc. natl Acad. Sci. USA*, **75**, 5358–5361

Buening, M.K., Levin, W., Karle, J.M., Yagi, H., Jerina, D.M. & Conney, A.H. (1979) Tumorigenicity of bay-region epoxides and other derivatives of chrysene and phenanthrene in newborn mice. *Cancer Res*., **39**, 5063–5068

Buening, M.K., Levin, W., Wood, A.W., Chang, R.L., Lehr, R.E., Taylor, C.W., Yagi, H., Jerina, D.M. & Conney, A.H. (1980) Tumorigenic activity of benzo(e)pyrene derivatives on mouse skin and in newborn mice. *Cancer Res*., **40**, 203–206

Busby, W.F., Jr, Goldman, M.E., Newberne, P.M. & Wogan, G.N. (1984) Tumorigenicity of fluoranthene in a newborn mouse lung adenoma bioassay. *Carcinogenesis*, **5**, 1311–1316

Busby, W.F., Jr, Stevens, E.K., Kellenbach, E.R., Cornelisse, J. & Lugtenburg, J. (1988) Dose–response relationships of the tumorigenicity of cyclopenta[cd]pyrene, benzo[a]pyrene and 6-nitrochrysene in a newborn mouse lung adenoma bioassay. *Carcinogenesis*, **9**, 741–746

Busby, W.F., Jr, Stevens, E.K., Martin, C.N., Chow, F.L. & Garner, R.C. (1989) Comparative lung tumorigenicity of parent and mononitropolynuclear aromatic hydrocarbons in the BLU:Ha newborn mouse assay. *Toxicol. appl. Pharmacol*., **99**, 555–563

Buters, J.T.M., Mahadevan, B., Quintanilla-Martinez, L., Gonzalez, F.J., Greim, H., Baird, W.M. & Luch, A. (2002) Cytochrome P450 1B1 determines susceptibility to dibenzo[*a*,*l*]pyrene-induced tumor formation. *Chem. Res. Toxicol*., **15**, 1127–1135

Cavalieri, E., Mailander, P. & Pelfrene, A. (1977) Carcinogenic activity of anthanthrene on mouse skin. *Z. Krebsforsch.*, **89**, 113–118

Cavalieri, E., Rogan, E., Toth, B. & Munhall, A. (1981) Carcinogenicity of the environmental pollutants cyclopenteno[*cd*]pyrene in mouse skin. *Carcinogenesis*, **2**, 277–281

Cavalieri, E., Munhall, A., Rogan, E., Salmasi, S. & Patil, K. (1983) Syncarcinogenic effect of the environmental pollutants cyclopenteno[cd]pyrene and benzo[a]pyrene in mouse skin. *Carcinogenesis*, **4**, 393–397

Cavalieri, E., Rogan, E. & Sinha, D. (1988a) Carcinogenicity of aromatic hydrocarbons directly applied to rat mammary gland. *J. Cancer clin. Oncol*., **114**, 3–9

Cavalieri, E., Rogan, E., Cremonesi, P., Higginbotham, S. & Salmasi, S. (1988b) Tumorigenicity of 6-halogenated derivatives of benzo[a]pyrene in mouse skin and rat mammary gland. *J. Cancer Res. clin. Oncol*., **114**, 10–15

Cavalieri, E., Rogan, E., Higginbotham, S., Cremonesi, P. & Salmasi, S. (1989) Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of dibenzo[*a*]pyrenes: The very potent environmental carcinogen dibenzo[*a,l*]pyrene. *J. Cancer Res. clin. Oncol.*, **115**, 67–72

Cavalieri, E.L., Higginbotham, S., RamaKrishna, N.V.S., Devanesan, P.D., Todorovic, R., Rogan, E.G. & Salmasi, S. (1991) Comparative dose–response tumorigenicity studies of dibenzo[*a,l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene, benzo[*a*]pyrene and two dibenzo[*a,l*]pyrene dihydrols in mouse skin and rat mammary gland. *Carcinogenesis*, **12**, 1939–1944

Chang, R.L., Levin, W., Wood, A.W., Lehr, R.E, Kumar, S., Yagi, H.. Jerina, D.M. & Conney, A.H. (1982) Tumorigenicity of bay-region diol-epoxides and other benzo-ring derivatives of dibenzo(*a,h*)pyrene and dibenzo(*a,i*)pyrene on mouse skin and newborn mice. *Cancer Res*., **42**, 25–29

Chang, R.L., Levin, W., Wood, A.W., Yagi, H., Tada, M., Vyas, K.P., Jerina, D.M. & Conney, A.H. (1983) Tumorigenicity of enantiomers of chrysene 1,2-dihydrodiol and of the diastereomeric bay-region chrysene 1,2-diol-3,4-epoxides on mouse skin and in newborn mice. *Cancer Res*., **43**, 192–196

Chouroulinkov, I., Coulomb, H., MacNicoll, A.D., Grover, P.L. & Sims, P. (1983) Tumour-initiating activities of dihydrodiols of dibenz[*a,c*]anthracene. *Cancer Lett*., **19**, 21–26

Clayson, D.B., Pringle, J.A.S., Bonser, G.M. & Wood, M. (1968) The technique of bladder implantation: Futher results and an assessment. *Br. J. Cancer*, **22**, 825–832

Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. *Cancer Res*., **42**, 4875–4917

Cook, J.W. (1932) The production of cancer by pure hydrocarbons. Part II. *Proc. roy. Soc. London*, **B111**, 485–496

Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S. & Beland, F.A. (1998) A comparison of the tumors induced by coal tar and benzo[*a*]pyrene in a 2-year bioassay. *Carcinogenesis*, **19**, 117–124

Deutsch-Wenzel, R. P., Brune, H., Grimmer, G., Dettbarn, G. & Misfeld, J. (1983) Experimental studies in rat lungs on the carcinogenicity and dose–response relationships of eight frequently occurring environmental polycyclic aromatic hydrocarbons. *J. natl Cancer Inst*., **71**, 539–544

DiGiovanni, J., Slaga, T.J. & Boutwell, R.K. (1980) Comparison of the tumor-initiating activity of 7,12-dimethylbenz[a]anthracene and benzo[a]pyrene in female SENCAR and CD-1 mice. *Carcinogenesis*, **1**, 381–389

DiGiovanni, J., Rymer, J., Slaga, T.J. & Boutwell, R.K. (1982) *Anti*carcinogenic and *co*carcinogenic effects of benzo[e]pyrene and dibenz[a,c]anthracene on skin tumor initiation by polycyclic hydrocarbons. *Carcinogenesis*, **3**, 371–375

Dipple, A. (1976) Polynuclear aromatic carcinogens. In: Searle, C.E., ed, *Chemical Carcinogens*, Washington DC, American Chemical Society, pp. 41–163

Druckrey, H. & Schmähl, D. (1955) [Carcinogenic effect of anthracene.] *Naturwissenschaften*, **42**, 159–160 (in German)

Dunlap, C.E. & Warren, S. (1946) The carcinogenic activity of some new derivatives of aromatic hydrocarbons. *Cancer Res.*, **6**, 454–465

El-Bayoumy, K., Hecht, S.S. & Hoffmann, D. (1982) Comparative tumor initiating activity on mouse skin of 6-nitrobenzo[*a*]pyrene, 6-nitrochrysene, 3-nitroperylene, 1-nitropyrene and their parent hydrocarbons. *Cancer Lett*., **16**, 333–337

El Bayoumy, K., Amin, S. & Hecht, S.S. (1986) Effects of 6-nitro substitution on 5-methylchrysene tumorigenicity, mutagenicity and metabolism. *Carcinogenesis*, **7**, 673–676

El-Bayoumy, K., Chae, Y-H., Upadhyaya, P., Rivenson, A., Kurtzke, C., Reddy, B. & Hecht, S.S. (1995) Comparative tumorigenicity of benzo[*a*]pyrene, 1-nitropyrene and 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine administration by gavage to female CD rats. *Carcinogenesis*, **16**, 431–434

Epstein, S.S., Joshi, S., Andrea, J., Forsyth, J. & Mantel, N. (1967) The null effect of antioxidants on the carcinogenicity of 3,4,9,10-dibenzpyrene to mice. *Life Sci*., **6**, 225–233

Estensen, R.D. & Wattenberg, L.W. (1993) Studies of chemopreventive effects of *myo*-inositol on benzo[a]pyrene-induced neoplasia of the lung and forestomach of female A/J mice. *Carcinogenesis*, **14**, 1975–1977

Estensen, R.D., Jordan, M.M., Wiedmann, T.S., Gallbraith, A.R., Steele, V.E. & Wattenberg, L.W. (2004) Effect of chemopreventive agents on separate stages of progression of benzo[*a*]pyrene induced lung tumors in A/J mice. *Carcinogenesis*, **25**, 197–201

Falk, H.L., Kotin, P., & Thompson, S. (1964) Inhibition of carcinogenesis. The effect of polycyclic hydrocarbons and related compounds. *Arch. environ. Health*, **9**, 169–179

Feron, V.J. (1972) Respiratory tract tumors in hamsters after intratracheal instillations of benzo(a)pyrene alone and with furfural. *Cancer Res*., **32**, 28–36

Feron, V.J. (1979) Effects of exposure to acetaldehyde in Syrian golden hamsters simultaneously treated with benzo(a)pyrene or diethylnitrosamine. *Prog. exp. Tumor Res*., **24**, 162–176

Feron, V.J. & Kruysse, A. (1978) Effects of exposure to furfural vapour in hamsters simultaneously treated with benzo[*a*]pyrene or diethylnitrosamine. *Toxicology*, **11**, 127–144

Feron, V.J., de Jong, D. & Emmelot, P. (1973) Dose–response correlation for the induction of respiratory-tract tumours in Syrian golden hamsters by intratracheal instillation of benzo(a)pyrene. *Eur. J. Cancer*, **9**, 387–390

Feron, V.J., van den Heuvel, P.D., Koëter, H.B.W.M. & Beems, R.B. (1980) Significance of particle size of benzo(*a*)pyrene for the induction of respiratory tract tumours in hamsters. *Int. J. Cancer*, **25**, 301–307

Feron, V.J., Scherrenberg, P.M., Immel, H.R. & Spit, B.J. (1985) Pulmonary response of hamsters to fibrous glass: Chronic effects of repeated intratracheal instillation with or without benzo[a]pyrene. *Carcinogenesis*, **6**, 1495–1499

Finzi, C., Daudel, P. & Prodi, G. (1968) Interference among polycyclic hydrocarbons in experimental skin carcinogenesis. *Eur. J. Cancer*, **3**, 497–501

Flesher, J.W., Horn, J., & Lehner, A.F. (2002) Comparative carcinogenicity of picene and dibenz[*a,h*]anthracene in the rat. *Biochem. biophys. Res. Commun*., **290**, 275–279

Freudenthal, R.I. & Jones, P.w., eds (1976) *Carcinogenesis – A comprehensive survey, Vol. 1. Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis*, New York, Raven Press

Geddie, J.E., Amin, S., Huie, K. & Hecht, S.S. (1987) Formation and tumorigenicity of benzo[b]fluoranthene metabolites in mouse epidermis. *Carcinogenesis*, **8**, 1579–1584

Gill, H.S., Kole, P.L., Wiley, J.C., Li, K.-M., Higginbotham, S., Rogan, E.G. & Cavalieri, E.L. (1994) Synthesis and tumor-initiating activity in mouse skin of dibenzo[*a,l*]pyrene *syn*- and *anti*-fjord-region diolepoxides. *Carcinogenesis*, **15**, 2455–2460

Godleski, J.J., Melnicoff, M.J., Sadri, S. & Garbeil, P. (1984) Effects of inhaled ammonium sulfate on benzo[a]pyrene carcinogenesis. *J. Toxicol. environ. Health*, **14**, 225–238

Goldstein, L.S., Weyand, E.H., Safe, S., Steinberg, M., Culp, S.J., Gaylor D.W., Beland, F.A. & Rodriguez, L.V. (1998) Tumors and DNA adducts in mice exposed to benzo[*a*]pyrene and coal tars: Implications for risk assessment. *Environ. Health Perspect.*, **106** (Suppl. 6), 1325–1330

Graffi, A., Vlamynck, E., Hoffmann, F. & Schulz, I. (1953) [Studies on the tumour producing effects of different chemicals in combination with croton oil.] *Arch Geschwulstforsch.*, **5**, 110–126 (in German)

Grant, G. & Roe, F.J.C. (1963) The effect of phenanthrene on tumour induction by 3,4-benzopyrene administered to newly born mice. *Br. J. Cancer*, **17**, 261–265

Habs, M., Schmäl, D. & Misfeld, J. (1980) Local carcinogenicity of some environmentally relevant polycyclic aromatic hydrocarbons after longlife topical application to mouse skin. *Arch. Geschwulstforsch*., **50**, 266–274

Habs, M., Jahn, S.A.A. & Schmäl, D. (1984) Carcinogenic activity of condensate from coloquint seeds (*Citrullus colocynthis*) after chronic epicutaneous administration to mice. *J. Cancer Res. clin. Oncol*., **108**, 154–156

Hadler, H.I., Darchun, V. & Lee, K. (1959) Initiation and promotion activity of certain polynuclear hydrocarbons. *J. natl Cancer Inst*., **23**, 1383–1387

Hakura, H., Tsutsui, Y., Sonoda, J., Kai, J., Imade, T., Shimada, M., Sugihara, I. & Mikami, T. (1998) Comparison between in vivo mutagenicity and carcinogenicity in multiple organs by benzo[*a*]pyrene in the *lacZ* transgenic mouse (Muta^{TM} Mouse). *Mutat. Res*., **398**, 123–130

Harvey, R.G., Cortez, C., Sawyer, T.W. & DiGiovanni, J. (1988) Synthesis of the tumorigenic 3,4-dihydrodiol metabolites of dibenz[*a,j*]anthracene and 7,14-dimethyldibenz[*a,j*]anthracene. *J. med. Chem*., **31**, 1308–1312

Hecht, S.S., Bondinell, W.E. & Hoffmann, D. (1974) Chrysene and methylchrysenes. Presence in tobacco smoke and carcinogenicity. *J. natl Cancer Inst.*, **53**, 1121–1132

Hecht, S.S., Loy, M. & Hoffman, D. (1976). On the structure and carcinogenicity of the methylchrysenes. In: Freudenthal, R.I. & Raven P.W.J., eds, *Polynuclear aromatic hydrocarbons: chemistry, metabolism, and carcinogenesis*. Raven Press, New York, pp. 325–340

Hecht, S.S., LaVoie, E.J., Bedenko, V., Pingaro, L., Katayama, S., Hoffmann, D., Sardella, D.J., Boger, E. & Lehr, R.E. (1981) Reduction of tumorigenicity and of dihydrodiol formation by fluorine substitution in the angular rings of dibenzo(*a,i*)pyrene. *Cancer Res.*, **41**, 4341–4345

Hecht, S.S., Radok, L., Amin, S., Huie, K., Melikian, A.A., Hoffmann, D., Pataki, J. & Harvey, R.G. (1985) Tumorigenicity of 5-methylchrysene dihydrodiols and dihydrodiol epoxides in newborn mice and on mouse skin. *Cancer Res*., **45**, 1449–1452

Hecht, S.S., Amin, S., Huie, K., Melikian, A.A. & Harvey, R.G. (1987) Enhancing effect of a bay region methyl group on tumorigenicity in newborn mice and mouse skin of enantiomeric bay region diol epoxides formed stereoselectively from methylchrysenes in mouse epidermis. *Cancer Res*., **47**, 5310–5315

Hecht, S.S., Amin, S., Lin, J.M., Rivenson, A., Kurtzke, C. & El Bayoumy, K. (1995) Mammary carcinogenicity in female CD rats of a diol epoxide metabolite of fluoranthene, a commonly occurring environmental pollutant. *Carcinogenesis*, **16**, 1433–1435

Heinrich, U. (1989) Exhaust-specific carcinogenic effects of polycyclic aromatic hydrocarbons and their significance for the estimation of the exhaust exposure-related lung cancer risk. In: Mohr, U., Bates D.V., Dungworth, D.L., Lee, P.N., McClellan, R.O. & Roe, F.J.C., eds. Assessment of Inhalation Hazards: Integration and Extrapolation Using Diverse Data. Berlin, Springer-Verlag, pp. 301–313

Heinrich, U., Pott, F., Mohr, R., Fuhst, R. & König, J. (1986a) Lung tumours in rats and mice after inhalation of PAH-rich emissions. *Exp. Pathol*., **29**, 29–34

Heinrich, U., Pott, F. & Rittinghausen, S. (1986b) Comparison of chronic inhalation effects in rodents after long-term exposure to either coal oven flue gas mixed with pyrolized pitch or diesel engine exhaust. In: Ishinishi, N., Koizumi, A., McClellen, R.O. & Stöber, W., eds,

Carcinogenic and Mutagenic Effects of Diesel Engine Exhaust, Amsterdam, Elsevier, pp. 441–457

Heinrich, U., Dungworth, D.L., Pott, F., Dasenbrock, C., Levsen, K., Koch, W., Creutzenberg, O. & Schulte, A. (1994a) The carcinogenic effects of carbon black particulates and tar-pitch condensation aerosol after inhalation exposure of rats. *Ann. occup. Hyg.*, **38** (Suppl. 1), 351–356

Heinrich, U., Peters, L., Creutzenberg, O., Dasenbrock, C. & Hoymann, H.-G. (1994b) Inhalation exposure of rats to tar/pitch condensation aerosol or carbon black alone or in combination with irritant gases. In: Dungworth, D.L., Mauderly, J.L. & Oberdörster, G., eds, *Toxic and Carcinogenic Effects of Solid Particles in the Respiratory Tract*, Washington DC, ELSI Press, pp. 433–441

Henry, M.C., Port, C.D., Bates, R.R. & Kaufman, D.G. (1973) Respiratory tract tumors in hamsters induced by benzo(*a*)pyrene. *Cancer Res.*, **33**, 1585–1592

Heston, W.E. & Schneidermann, M.A. (1953) Analysis of dose–response in relation to mechanism of pulmonary tumor induction in mice. *Science*, **117**, 109–111

Higginbotham, S., RamaKrishna, N.V.S., Johansson, S.L., Rogan, E.G. & Cavalieri, E.L. (1993) Tumor-initiating activity and carcinogenicity of dibenzo[*a,l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene at low doses in mouse skin. *Carcinogenesis*, **14**, 875–878

Hill, W.T., Stanger, D.W., Pizzo, A., Riegel, B., Shubik, P. & Wartman, W.B. (1951) Inhibition of 9,10-dimethyl-1,2-benzanthracene skin carcinogenesis in mice by polycyclic hydrocarbons. *Cancer Res.*, **11**, 892–897

Hoffmann, D. & Wynder, E.L. (1963) Studies on gasoline engine exhaust. *J. Air Pollut. Control Assoc.*, **13**, 322–327

Hoffmann, D. & Wynder, E.L., (1966) [Contribution on the carcinogenic effect of dibenzopyrenes.] *Z. Krebsforsch.*, **68**, 137–149 (in German)

Hoffmann, D., Rathkamp, G., Nesnow, S. & Wynder, E.L. (1972) Fluoranthenes: Quantitative determination in cigarette smoke, formation by pyrolysis, and tumor-initiating activity. *J. natl Cancer Inst.*, **49**, 1165–1175

Homburger, F. & Baker, J.R. (1969) Accelerated carcinogen testing. *Progr. exp. Tumor Res.*, **11**, 384–394

Homburger, F. & Treger, A. (1967) Acceleration of growth of chemically induced tumors by use of tranplantation technic. *Cancer Res.*, **27**, 1205–1213

Homburger, F. & Treger, A. (1970) Transplantation technique for acceleration of carcinogenesis by benz(a)anthracene or 3,4,9,10-dibenzpyrene [benzo(r,s,t)pentaphene]. *J. natl Cancer Inst.*, **44**, 357–360

Homburger, F. & Treger, A. (1960) Modifying factors in carcinogenesis. *Progr. exp. Tumor Res.*, **1**, 311–328

Homburger, F., Russfield, A.B., Baker, J.R. & Treger, A. (1962) Experimental chemotherapy in chemically induced mouse tumors and their transplants. *Cancer Res.*, **22**, 368–374

Homburger, F., Hsueh, S.-S., Kerr, C.S. & Russfield, A.B. (1972) Inherited susceptibility of inbred strains of Syrian hamsters to induction of subcutaneous sarcomas and mammary and gastrointestinal carcinomas by subcutaneous and gastric administration of polynuclear hydrocarbons. *Cancer Res.*, **32**, 360–366

Hoogervorst, E.M., de Vries, A., Beems, R.B., van Oostrom, C.T.M., Wester, P.W., Vos, J.G., Bruins, W., Roodbergen, M., Cassee, F.R., Vijg, J., van Schooten, F.-J. & van Steeg, H. (2003) Combined oral benzo[*a*]pyrene and inhalatory ozone exposure have no effect on lung tumor development in DNA repair-deficient *Xpa* mice. *Carcinogenesis*, **24**, 613–619

Horikawa, K., Sera, N., Otofuji, T., Murakami, K., Tokiwa, H., Iwagawa, M., Izumi, K. & Otsuka, H. (1991) Pulmonary carcinogenicity of 3,0- and 3,7-dinitrofluoranthene, 3-nitrofluoranthene and benzo[*a*]pyrene in F344 rats. *Carcinogenesis*, **12**, 1003–1007

Horton, A.W. & Christian, G.M. (1974) Cocarcinogenic versus incomplete carcinogenic activity among aromatic hydrocarbons: Contrast between chrysene and benzo(*b*)triphenylene. *J. natl. Cancer Inst.*, **53**, 1017–1020

Huggins, C. & Yang, N.C. (1962) Induction and extinction of mammary cancer. *Science*, **137**, 257–262

IARC (1973) *IARC Monographs on the Evaluation of the Carcinogenic Risk of the Chemical to Man*, Vol. 3, *Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds*, Lyon

IARC (1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 32, *Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data*, Lyon

IARC (1987) *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon

Ide, F., Lida, N., Nakatsuru, Y., Oda, H., Tanaka, K. & Ishikawa, T. (2000) Mice deficient in the nucleotide excision repair gene *XPA* have elevated sensitivity to benzo[*a*]pyrene induction of lung tumors. *Carcinogenesis*, **21**, 1263–1265

Ito, M., Tamada, J. & Aoki, M. (1982) Induction of squamous cell carcinoma with 3,4-benzo[*a*]pyrene in the human bronchus transplanted into nude mice. *Gann*, **73**, 141–146

Iwagawa, M., Maeda, T., Izumi, K., Otsuka, H., Nishifuji, K., Onishi, Y. & Aoki, S. (1989) Comparative dose–response studies on pulmonary carcinogenicity of 1,6-dinitropyrene and benzo[a]pyrene in F344 rats. *Carcinogenesis*, **10**, 1285–1290

Johnson, S. (1968) Effect of thymectomy on the induction of skin tumours by dibenzanthracene, and of breast tumours by dimethylbenzanthracene in mice of the IF strain. *Br. J. Cancer*, **22**, 755–761

Kennaway, E.L. (1924a) On cancer-producing tars and tar-fractions. *J. ind. Hyg*., **5**, 462–488

Kennaway, E.L. (1924b) On cancer-producing factor in tar. *Br. med. J*., **i**, 564–567

Kennaway, E.L. (1930) Further experiments on cancer producing substances. *Biochem. J*., **24**, 497–504

Kennaway, E.L. & Hieger, I. (1930) Carcinogenic substances and their fluorescence spectra. *Br. med. J*., **1**, 1044–1046

Ketkar, M., Reznik, G., Misfeld, J. & Mohr, U. (1977) Investigations on the carcinogenic burden by air pollution in man. The effect of a single dose of benzo(a)pyrene in Syrian golden hamsters. *Cancer Lett*., **3**, 231–235

Ketkar, M., Reznik, G., Schneider, P. & Mohr, U. (1978) Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo(a)pyrene in bovine serum albumin in Syrian golden hamsters. *Cancer Lett*., **4**, 235–239

Ketkar, M., Green, U., Schneider, P. & Mohr, U. (1979) Investigations on the carcinogenic burden by air pollution in man. Intratracheal instillation studies with benzo[*a*]pyrene in a mixture of Tris buffer and saline in Syrian golden hamsters. *Cancer Lett.*, **6**, 279–284

Klein, M. (1960) A comparison of the initiating and promoting actions of 9,10-dimethy1-1,2-benzanthracene and 1,2,5,6-dibenzanthracene in skin tumorigenesis. *Cancer Res.*, **20**, 1179–1183

Klein, M. (1963) Susceptibility of strain B6AF$_1$/J hybrid infant mice to tumorigenesis with 1,2-benzanthracene, deoxycholic acid, and 3-methylcholanthrene. *Cancer Res.*, **23**, 1701–1707

Kleinenberg, H.E., (1938) On the blastogenic effect of 3,4,8,9-dibenzpyrene and some of its derivatives. I. Tumours caused by subcutaneous injection of dibenzpyrene. *Arch. Biol. Nauk.*, **51**, 127–136

Kleinenberg, H.E., (1939) Investigations on the blastomogenic effect of 3,4,8,9-dibenzpyrene and some of its derivatives. II. Tumours called forth by painting the skin with dibenzpyrene. *Arch. Biol. Nauk.*, **56**, 39–47

Kobayashi, N. (1975) Production of respiratory tract tumors in hamsters by benzo[*a*]pyrene. *Gann,* **66**, 311–315

Kouri, R.E., Wood, A.W., Levin, W., Rude, T.H., Yagi, H., Mah, H.D., Jerina, D.M. & Conney, A.H. (1980) Carcinogenicity of benzo[a]pyrene and thirteen of its derivatives in C3H/fCum mice. *J. natl Cancer Inst.*, **64**, 617–623

Kouri, R.E., Connolly, G.M., Nebert, D.W. & Lubet, R.A. (1983) Association between susceptibility to dibenzanthracene induced fibrosarcoma formation and the Ah locus. *Int. J. Cancer*, **32**, 765–768

Kroese, E.D., Dortant, P.M., van Steeg, H., van Oostrom, C.T.M., van der Houven van Oordt, C.W., van Kranen, H.J., de Vries, A., Wester, P.W. & van Krijel, C.F. (1997) Use of Eμ-*PIM*-1 transgenic mice for short-term *in vivo* carcinogenicity testing: Lymphoma induction by benz[*a*]pyrene, but not by TPA. *Carcinogenesis*, **18**, 975–980

Kruysse, A. & Feron, V.J. (1976) Repeated exposure to cyclopentenone vapour: Long-term study in Syrian golden hamsters. *Zbl. Bakt. Hyg. Abt. Orig. B*, **163**, 448–457

Künstler, K. (1983) Failure to induce tumors by intratracheal instillation of automobile exhaust condensate and fractions thereof in Syrian golden hamsters. *Cancer Lett.*, **18**, 105–108

Lacassagne, A., Zajdela, F., Buu-Hoï, N.P. & Chalvet, H. (1957) [On the increased carcinogenic activity of 3,4,9,10-dibenzopyrene and some of its derivatives.] *C.R. Acad. Sci.*, **244**, 273–274 (in French)

Lacassagne, A., Buu-Hoï, N.P. & Zajdela, F. (1958) [Relationships between molecular structure and carcinogenic activity in three series of hexacyclic aromatic hydrocarbons.] *C.R. Acad. Sci.*, **246**, 1477–1480 (in French)

Lacassagne, A., Buu-Hoï, N.P., Zajdela, F. & Lavit-Lamy, D. (1963) [High carcinogenic activity of 1,2,3,4-dibenzopyrene and 1,2,4,5-dibenzopyrene.] *C.R. Acad. Sci.*, **256**, 2728–2730 (in French)

Lacassagne, A., Buu-Hoï, N.P., Zajdela, F. & Lavit-Lamy, D. (1964) [Carcinogenic activity of substituted derivatives of 1.2:3.4-dibenzopyrene, 1.2:4.5-dibenzopyrene, and 1.2:4.5:8.9-tribenzopyrene] *C.R. Acad. Sci.*, **259**, 3899–3902 (in French)

Lacassagne, A., Buu-Hoï, N.P., Zajdela, F. & Vingiello, F.A. (1968) The true dibenzo[*a*,*l*]pyrene, a new, potent carcinogen. *Naturwissenschaften*, **55**, 43

LaVoie, E., Bedenko, V., Hirota, N., Hecht, S.S. & Hoffmann, D. (1979) A comparison of the mutagenicity, tumor-initiating activity and complete carcinogenicity of polynuclear aromatic hydrocarbons. In: Jones, P.W. & Leber, P., eds, *Polynuclear Aromatic Hydrocarbons*, Ann Arbor, MI, Ann Arbor Science, pp. 705–721

LaVoie, E.J., Tulley-Freiler, L., Bedenko, V. & Hoffmann, D. (1981) Mutagenicity, tumor-initiating activity, and metabolism of methylphenanthrenes. *Cancer Res.*, **41**, 3441–3447

LaVoie, E.J., Amin, S., Hecht, S.S., Furuya, K. & Hoffmann, D. (1982a) Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. *Carcinogenesis*, **3**, 49–52

LaVoie, E.J., Bedenko, V., Tulley-Freiler, L. & Hoffmann, D. (1982b) Tumor-initiating activity and metabolism of polymethylated phenanthrenes. *Cancer Res.*, **42**, 4045–4049

LaVoie, E.J., Coleman, D.T., Tonne, R.L. & Hoffmann, D. (1983) Mutagenicity, tumor initiating activity and metabolism of methylated anthracenes. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons*, Columbus, OH, Battelle Press, pp. 785–798

LaVoie, E.J., Coleman, D.T., Rice, J.E., Geddie, N.G., & Hoffmann, D. (1985) Tumor-initiating activity, mutagenicity, and metabolism of methylated anthracenes. *Carcinogenesis*, **6**, 1483–1488

LaVoie, E.J., Braley, J., Rice, J.E. & Rivenson, A. (1987) Tumorigenic activity of non-alternant polynuclear aromatic hydrocarbons in newborn mice. *Cancer Lett.*, **34**, 15–20

LaVoie, E.J., He, Z.-M., Meegalla, R.L. & Weyand, E.H. (1993) Exceptional tumor-initiating activity of 4-fluorobenz[*j*]fluoranthene on mouse skin: Comparison with benzo[*j*]fluoranthene, 10-fluoro-benzo[j]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,l*]pyrene and 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett.*, **70**, 7–14

LaVoie, E.J., Cai, Z.W., Meegalla, R.L. & Weyand, E.H. (1993b) Evaluation of the tumor-initiating activity of 4-, 5-, 6-, and 7-fluorobenzo[b]fluoranthene in mouse skin. *Chem.-biol. Interact.*, **89**, 129–139

LaVoie, E.J., He, Z.M., Wu, Y., Meschter, C.L. & Weyand, E.H. (1994a) Tumorigenic activity of the 4,5- and 9,10-dihydrodiols of benzo[j]fluoranthene and their syn- and anti-diol epoxides in newborn mice. *Cancer Res.*, **54**, 962–968

LaVoie, E.J., Cai, Z.W., Meschter, C.L. & Weyland, E.H. (1994b) Tumorigenic activity of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene in newborn CD-1 mice. *Carcinogenesis*, **15**, 2131–2135

Levin, W., Wood, A.W., Wislocki, P.G., Kapituinik, J., Yagi, H., Jerina, D.M. & Conney, A.H. (1977) Carcinogenicity of benzo ring derivatives of benzo(a)pyrene on mouse skin. *Cancer Res.*, **37**, 3357–3361

Levin, W., Wood, A.W., Chang, R.L., Ittah, Y., Croisy-Delcey, M., Yagi, H., Jerina, D.M. & Conney, A.H. (1980) Exceptionally high tumor-initiating activity of benzo(c)phenanthrene bay-region diol-epoxides on mouse skin. *Cancer Res.*, **40**, 3910–3914

Levin, W., Chang, R.L., Wood, A.W., Yagi, H., Thakker, D.R., Jerina, D.M. & Conney, A.H. (1984) High stereoselectivity among the optical isomers of the diastereomeric bay-region diol epoxides of benz(a)anthracene in the expression of tumorigenic activity in murine tumor models. *Cancer Res.*, **44**, 929–933

Levin, W., Chang, R.L., Wood, A.W., Thakker, D.R., Yagi, H., Jerina, D.M. & Conney, A.H. (1986) Tumorigenicity of optical isomers of the diastereomeric bay-region 3,4-diol-1,2-epoxides of benzo(c)phenanthrene in murine tumor models. *Cancer Res.*, **46**, 2257–2261

Lijinsky, W. & Garcia, H. (1972) Skin carcinogenesis tests of hydrogenated derivatives of anthanthrene and other polynuclear hydrocarbons. *Z. Krebsforsch.*, **77**, 226–230

Lijinsky, W., Garcia, H., Terracini, B. & Saffiotti, U. (1965) Tumorigenic activity of hydrogenated derivatives of dibenz[*a,h*]anthracene. *J. natl Cancer Inst*., **34**, 1–6

Lijinsky, W., Garcia, H. & Saffiotti, U. (1970) Structure–activity relationships among some polynuclear hydrocarbons and their hydrogenated derivatives. *J. natl Cancer Inst*., **44**, 641–649

Lorenz, E. & Stewart, H.L. (1947) Tumors of alimentary tract induced in mice by feeding olive oil emulsions containing carcinogenic hydrocarbons. *J. natl Cancer Inst*., **7**, 227–238

Lorenz, E. & Stewart, H.L. (1948) Tumors of alimentary tract in mice fed carcinogenic hydrocarbons in mineral oil emulsions. *J. natl Cancer Inst.*, **9**, 173–180

Lubet, R.A., Connolly, G.M., Nebert, D.W. & Kouri, R.E. (1983) Dibenz[a,h]anthracene-induced subcutaneous tumors in mice. Strain sensitivity and the role of carcinogen metabolism. *Carcinogenesis*, **4**, 513–517

Luch, A., Friesel, H., Seidel, A. & Platt, K.-L. (1999) Tumor-initiating activity of the (+)-(*S*,*S*)- and (–)-(*R*,*R*)-enantiomers of *trans*-11,12-dihydroxy-11,12-dihydrodibenzo[*a*,*l*]pyrene in mouse skin. *Cancer Lett*., **136**, 119–128

Mahlum, D.D., Wright, C.W., Chess, E.D. & Wilson, B.W. (1984) Fractionation of skin tumor-initiating activity in coal liquids. *Cancer Res*., **44**, 5176–5181

Marston, C.P., Pereira, C., Ferguson, J., Fischer, K., Hedstrom, O., Dashwood, W.-M. & Baird, W.M. (2001) Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumor initiation, PAH-DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. *Carcinogenesis*, **22**, 1077–1086

Mass, M.J., Jeffers, A.J., Ross, J.A., Nelson, G., Galati, A.J., Stoner, G.D. & Nesnow, S. (1993) Ki-*ras* oncogene mutations in tumors and DNA adducts formed by benz[*j*]aceanthrylene and benzo[*a*]pyrene in the lungs of strain A/J mice. *Mol. Carcinog.*, **8**, 186–192

Mass, M.J., Abu-Shakra, A., Roop, B.C., Nelson, G., Galati, A.J., Stoner, G.D., Nesnow, S. & Ross, J.A. (1996) Benzo[b]fluoranthene: Tumorigenicity in strain A/J mouse lungs, DNA adducts and mutations in the Ki-ras oncogene. *Carcinogenesis*, **17**, 1701–1704

Masuda, Y. & Kagawa, R. (1972) A novel synthesis and carcinogenicity of dibenzo[*a*,*l*]pyrene. *Chem. pharm. Bull*., **20**, 2736–2737

Miescher, G. (1942) [Experimental studies on the formation of tumours by photosensitization.] *Schweiz. med. Wochenschr*., **72**, 1082–1084 (in German)

Miller, J.A. & Miller, E.C. (1963) The carcinogenicities of fluoro derivatives of 10-methyl-1,2-benzanthracene. II. Substitution in the K-region and the 3-, 6-, and 7-positions. *Cancer Res*., **23**, 229–239

Mukhtar, H., Das, M. & Bickers, D.R. (1986) Skin tumor initiating activity of therapeutic crude coal tar as compared to other ploycyclic aromatic hydrocarbons in SENCAR mice. *Cancer Lett*., **31**, 147–151

Nakatsuru, Y., Wakabayashi, K., Fujii-Kuriyama, Y., Ishikawa, T., Kusama, K. & Ide, F. (2004) Dibenzo[*a*,*l*]pyrene-induced genotoxic and carcinogenic responses are dramatically suppressed in aryl hydrocarbon receptor-deficient mice. *Int. J. Cancer*, **112**, 179–183

Näslund, I., Rubio, C.A. & Auer, G.U. (1987) Nuclear DNA changes during pathogenesis of squamous cell carcinoma of the cervix in 3,4-benzopyrene-treated mice. *Anal. quant. Cytol*., **9**, 411–418

Nesnow, S., Gold, A., Sangaiah, R., Triplett, L.L. & Slaga, T.J. (1984a) Mouse skin tumor-initiating activity of benz[*e*]aceanthrylene and benz[*l*]aceanthrylene in SENCAR mice. *Cancer Lett*., **22**, 263–268

Nesnow, S., Triplett, L.L. & Slaga, T.J. (1984b) Tumor initiating activities of 1-nitropyrene and its nitrated products in SENCAR mice. *Cancer Lett*., **23**, 1–8

Nesnow, S., Gold, A., Sangaiah, R. & Slaga, T.J. (1993) Mouse skin tumor-initiating activity of benz[*j*]aceanthrylene in SENCAR mice. *Cancer Lett*., **73**, 73–76

Nesnow, S., Ross, J.A., Nelson, G., Wilson, K., Roop, B.C., Jeffers, A.J., Galati, A.J., Stoner, G.D., Sangaiah, R., Gold, A. & Mass, M.J. (1994) Cyclopenta[cd]pyrene-induced tumorigenicity, Ki-ras codon 12 mutations and DNA adducts in strain A/J mouse lung. *Carcinogenesis*, **15**, 601–606

Nesnow, S., Ross, J.A., Stoner, G.D. & Mass M.J. (1995) Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. *Toxicology*, **105**, 403–413

Nesnow, S., Mass, M.J., Ross, J.A., Galati, A.J., Lambert, G.R., Gennings, C., Carter, W.H., Jr & Stoner, G.D. (1998a) Lung tumorigenic interactions in strain A/J mice of five environmental polycyclic aromatic hydrocarbons. *Environ. Health Perspect.*, **106** (Suppl. 6), 1337–1346

Nesnow, S., Ross, J.A., Mass, M.J. & Stoner, G.D. (1998b) Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. *Exp. Lung Res*., **24**, 395–405

Nettesheim, P., Griesemer, R.A., Martin, D.H. & Caton, J.E., Jr (1977) Induction of preneoplastic and neoplastic lesions in grafted rat tracheas continuously exposed to benzo(*a*)pyrene. *Cancer Res*., **37**, 1271–1278

Niemeier, R.W., Thayer, P.S., Menzies, K.T., Von Thuna, P., Moss, C.E. & Burg, J. (1988). A comparison of the skin carcinogenicity of condensed roofing asphalt and coal tar pitch fumes. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons: A Decade of Progress*. *Tenth International Symposium*, Columbus, OH, Battelle Press, pp. 609–647

O'Gara, R.W., Kelly, M.G., Brown, J. & Mantel, N. (1965) Induction of tumors in mice given a minute single dose of dibenz[*a,h*]anthracene or 3-methylcholanthrene as newborns. A dose–response study. *J. natl Cancer Inst*., **35**, 1027–1042

Old, L.J., Boyse, E.A., Clarke, D.A. & Carswell, E.A. (1962) Antigenic properties of chemically induced tumours. II. Antigens of tumor cells. *Ann. N.Y. Acad. Sci.*, **101**, 80–106

van Oostrom, C.T.M. van, Boeve, M., van den Berg, J., de Vies, A., Dollé, M.E.T., Beems, R.B., van Kreijl, C.F., Vijg, J. & van Steeg, H. (1999) Effect of heterozygous loss of *p*53 on benzo[a]pyrene-induced mutations and tumors in DNA repair-deficient XPA mice. *Environ. mol. Mutag*., **34**, 124–130

Pai, S.R. & Ranadive, K.J. (1965) Biological testing of analogues of 3,4-benzpyrene. *Indian J. med. Res*., **53**, 638–644

Pataki, J. & Huggins, C. (1969) Molecular site of substituents of benz(a)anthracene related to carcinogenicity. *Cancer Res*., **29**, 506–509

Peacock, P.R. (1935) Studies of fowl tumours induced by carcinogenic agents. *Am. J. Cancer*, **25**, 37–48

Pfeiffer, E.H. (1973) Investigations on the carcinogenic burden by air pollution in man. VII. Studies on the oncogenetic interaction of polycyclic aromatic hydrocarbons. *Zbl. Bakteriol.*, **B158**, 69–83

Pfeiffer, E.H. (1977) Oncogenic interaction of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons in mice. In: Mohr, U., Schmäl, D. & Tomatis, L., eds, *Air Pollution and Cancer in Man* (IARC Scientific Publications No.16), Lyon, pp. 69–77

Phillips, D.H. & Alldrick, A.J. (1994) Tumorigenicity of a combination of psoriasis therapies. *Br. J. Cancer*, **69**, 1043–1045

Pisareva, T.A., Ber, L.S., & Sorin, J.E., (1940) [Activity of cancerogenic compounds of Soviet production. Proceedings of the 1st meeting of oncologists, 25–30 march 1938, Kiev.]. *Tr. Perv. Syezda Onkol.*, 31–33 (in Russian)

Platt, K.L., Pfeiffer, E., Petrovic, P., Friesel, H., Beermann, D., Hecker, E. & Oesch, F. (1990) Comparative tumorigenicity of picene and dibenz[*a,h*]anthracene in the mouse. *Carcinogenesis*, **11**, 1721–1726

Platt, K.L., Dienes, H.P., Tommasone, M. & Luch, A. (2004) Tumor formation in the neonatal mouse bioassay indicates that the potent carcinogen dibenzo[*def,p*]chrysene (dibenzo[*a*,*l*]pyrene) is activated in vivo via its *trans*-11,12-dihydrodiol. *Chem.-biol. Interact.*, **148**, 27–36

Pollia, J.A. (1939) Investigations on the possible carcinogenic effect of anthracene and chrysene and some of their compounds. I. The effect of painting on the skin of mice. *J. ind. Hyg. Toxicol.*, **21**, 219–220

Pollia, J.A. (1941) Investigation on the possible carcinogenic effect of anthracene and chrysene and some of their compounds. II. The effect of subcutaneous injection in rats. *J. ind. Hyg. Toxicol.*, **23**, 449–451

Pott, F., Brockhaus, A., & Huth, F. (1973a) [Tests on the production of tumours in animal experiments with polycyclic aromatic hydrocarbons.] *Zbl. Bakt. Hyg. Abt. Orig. B*, **157**, 34–43 (in German)

Pott, F., Tomingas, R. & Reiffer, F.J. (1973b) Experimental studies on the carcinogenicity and the retention of benzo(a)pyrene in application region after intratracheal and subcutaneous injection. *Zbl. Bakt. Hyg. Abt. Orig. B*, **158**, 97–108

Pott, F., Mohr, U., & Brockhaus, A. (1978) [Studies on the combined effects of benzopyrene and dibenz[*a*]anthracene with SO_2 and NO_2 inhalation on the golden hamster.] In: Rothe, H., ed. [*Air hygiene and silicosis research*], Essen, Girardet, pp. 225–226 (in German)

Pott, F., Ziem, U., Reiffer, F.-J., Huth, F., Ernst, H. & Mohr, U. (1987) Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats. *Exp. Pathol.*, **32**, 129–152

Prahalad, A.K., Ross, J.A., Nelson, G.B., Roop, B.C., King, L.C., Nesnow, S. & Mass, M.J. (1997) Dibenzo[*a*,*l*]pyrene-induced DNA adduction, tumorigenicity, and Ki-*ras* oncogene mutations in strain A/J mouse lung. *Carcinogenesis*, **18**, 1955–1963

Prichard, R.W., Eubanks, J.W. & Hazlett, C.C. (1964) Age and breed effects on induction of sarcomas by dibenz[*a,h*]anthracene in pigeons. *J. natl Cancer Inst.*, **32**, 905–915

Ranadive, K.J. & Karande, K.A. (1963) Studies on 1,2,5,6-dibenzanthracene-induced mammary carcinogenesis in mice. *Br. J. Cancer*, **17**, 272–280

Rask-Nielsen, R. (1950) On the susceptibility of the thymus, lung, subcutaneous and mammary tissues in strain Street mice to direct application of small doses of four different carcinogenic hydrocarbons. *Br. J. Cancer*, **4**, 108–116

Raveh, D., Slaga, T.J. & Huberman, E. (1982) Cell-mediated mutagenesis and tumor-initiating activity of the ubiquitous polycyclic hydrocarbon, cyclopenta[*c,d*]pyrene. *Carcinogenesis*, **3**, 763–766

Reddy, A.P., Spitsbergen, J.M., Mathews, C., Hendricks, J.D. & Bailey, G.S. (1999a) Experimental hepatic tumorigenicity by environmental hydrocarbon dibenzo[*a*,*l*]pyrene. *J. environ. Pathol. Toxicol. Oncol*., **18**, 261–269

Reddy, A.P., Harttig, U., Barth, M.C., Baird, W.M., Schimerlik, M., Hendricks, J.D. & Bailey, G.S. (1999b) Inhibition of dibenzo[*a*,*l*]pyrene-induced multi-organ carcinogenesis by dietary chlorophyllin in rainbow trout. *Carcinogenesis*, **20**, 1019–1926

Reynders, J.B., Immel, H.R., Scherrenberg, P.M., Feron, V.J. & Beems, R.B. (1985) Respiratory tract tumors in hamsters after severe focal injury to the trachea and intratracheal instillation of benzo[a]pyrene. *Cancer Lett*., **29**, 93–99

Rice, J.E., Makowski, G.S., Hosted, T.J. & LaVoie, E.J. (1985) Methylene-bridged bay region chrysene and phenanthrene derivatives and their keto-analogs: Mutagenicity in Salmonella typhimurium and tumor-initiating activity on mouse skin. *Cancer Lett.*, **27**, 199–206

Rice, J.E., Hosted, T.J., Jr, DeFloria, M.C., LaVoie, E.J., Fischer, D.L. & Wiley, J.C., Jr (1986) Tumor-initiating activity of major in vivo metabolites of indeno[1,2,3-cd]pyrene on mouse skin. *Carcinogenesis*, **7**, 1761–1764

Rice, J.E., Weyand, E.H., Geddie, N.G., DeFloria, M.C. & LaVoie, E.J. (1987) Identification of tumorigenic metabolites of benzo[j]fluoranthene formed in vivo in mouse skin. *Cancer Res*., **47**, 6166–6170

Rice, J.E., Jordan, K., Little, P. & Hussain, N. (1988) Comparative tumor initiating activity of methylene-bridged and bay-region methylated derivatives of benz[*a*]anthracene and chrysene. *Carcinogenesis*, **9**, 2275–2278

Rice, J.E., Weyand, E.H., Burrill, C. & LaVoie, E.J. (1990) Fluorine probes for investigating the mechanism of activation of indeno[1,2,3-cd]pyrene to a tumorigenic agent. *Carcinogenesis*, **11**, 1971–1974

Riegel, B., Wartman, W.B., Hill, W.T., Reeb, B.B., Shubik, P. & Stanger, D.W. (1951) Delay of methylcholanthrene skin carcinogenesis in mice by 1,2,5,6-dibenzofluorene. *Cancer Res*., **11,** 301–303

Rippe, R.M. & Pott, D. (1989) [Investigations on the carcinogenicity of nitro-PAH (nitrosamines) with respect to their relevance for the carcinogenic effect of diesel engine exhaust.] In: [*Society for promotion of air hygiene and silicosis research*], Düsseldorf, Stefan W. Albers, pp. 65–89

Robinson, M., Bull, R.J., Munch, J. & Meier, J. (1984) Comparative carcinogenic and mutagenic activity of coal tar and petroleum asphalt paints used in potable water supply systems. *J. appl. Toxicol.*, **4**, 49–56

Robinson, M., Laurie, R.D., Bull, R.J. & Stober, J.A. (1987) Carcinogenic effects in A/J mice of particulate of a coal tar paint used in potable water systems. *Cancer Lett*., **34**, 49–54

Rodriguez, L.V., Dunsford, H.A., Steinberg, M., Chaloupka, K.K., Zhu, L., Safe, S., Womack, J.E. & Goldstein, L.S. (1997) Carcinogenicity of benzo[*a*]pyrene and manufactured gas plant residues in infant mice. *Carcinogenesis*, **18**, 127–135

Roe, F.J.C. (1962) Effect of phenanthrene on tumour-initiation by 3,4-benzopyrene. *Br. J. Cancer*, **16**, 503–506

Roe, F.J.C. & Salaman, M.H. (1955) Further studies on incomplete carcinogenesis: Triethylene melamine (TEM), 1,2-benzanthracene and β-propiolactone as initiators of skin tumour formation in the mouse. *Br. J. Cancer*, **9**, 177–203

Roe, F.J.C. & Waters, M.A. (1967) Induction of hepatoma in mice by carcinogens of the polycyclic hydrocarbon type. *Nature*, **214**, 299–300

Roe, F.J.C., Mitchley, B.C.V. & Walters, M. (1963) Tests for carcinogenesis using newborn mice, 1,2-benzanthracene, 2-naphthylamine, 2-naphthylhydroxylamine and ethyl methane sulphonate. *Br. J. Cancer*, **17**, 255–260

Roller, M., Kamino, K. & Rosenbruch, M. (1992) Carcinogenicity testing of bladder carcinogens and other organic compounds by the intraperitoneal and intravesicular route. In: Seemayer, N.H. & Hadnagy, W., eds, *Environmental Hygiene III*, Berlin, Springer-Verlag, pp. 95–98

Ross, J.A., Nelson, G.B., Wilson, K.H., Rabinowitz, J.R., Galati, A., Stoner, G.D., Nesnow, S. & Mass, M.J. (1995) Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Res.*, **55**, 1039–1044

Rossi, L., Barbieri, O., Sanguineti, M., Staccione, A., Santi, L.F. & Santi, L. (1983) Carcinogenic activity of benzo[*a*]pyrene and some of its synthetic derivatives by direct injection into the mouse fetus. *Carcinogenesis*, **4**, 153–156

Roussy, G. & Guéin, M. (1942) [Comparative activity of three principal synthetic hydrocarbon carcinogens.] *Bull. Cancer*, **30**, 66–73 (in French)

Russell, H. (1947) An unsuccessful attempt to induce gliomata in rabbits with cholanthrene. *J. Pathol. Bacteriol*., **59**, 481–483

Saffiotti, U., Cefis, F. & Kolb, L.H. (1968) A method for the experimental induction of bronchogenic carcinoma. *Cancer Res*., **28**, 104–124

Saffiotti, U., Montesano, R., Sellakumar, A.R., Cefis, F. & Kaufman, D.G. (1972) Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo(a)pyrene and ferric oxide. *Cancer Res*., **32**, 1073–1081

Salaman, M.H. & Roe, F.J.C. (1956) Further tests for tumour-initiating activity: N,N-Di-(2-chloroethyl)-p-aminophenyl-butyric acid (CB1348) as an initiator of skin tumour formation in the mouse. *Br. J. Cancer*, **10**, 363–378

Sardella, D.J., Boger, E. & Ghoshal, P.K. (1981) Active sites in hexacyclic carcinogens probed by the fluorine substitution methodology. In: Cooke, M. & Dennis, A.J., eds, *Polycyclic Aromatic Hydrocarbons: Chemical Analysis and Biological Fate*, Columbus, OH, Battelle Press, pp. 529–538

Sawyer, T.W., Chang, K., Harvey, R.G. & DiGiovanni, J. (1987) Further investigations into the effect of non-benzo ring bay-region methyl substituents on tumor-initiating activity of polycyclic hydrocarbons. *Cancer Lett*., **36**, 317–324

Sawyer, T.W., Baer-Dubowska, W., Chang, K., Crysup, S.B., Harvey, R.G. & DiGiovanni, J. (1988) Tumor-initiating activity of the bay-region dihydrodiols and diol-epoxides of dibenz[*a,j*]anthracene and cholanthrene on mouse skin. *Carcinogenesis*, **9**, 2203–2207

Schmähl, D. (1955) [Examination of the carcinogenic action of naphthalene and anthracene in rats.] *Z. Krebsforsch*., **60**, 697–710 (in German)

Schoental, R. (1959) Carcinogenic activity of 3:4:9:10-dibenzopyrene. *Acta unio. int. contra cancrum*, **15**, 216–219

Schwartz, J., Baker, V., Larios, E., Desai, D. & Amin, S. (2004) Inhibition of experimental tobacco carcinogen induced head and neck carcinogenesis. *Oral Oncol.*, **40**, 611–623

Scribner, J.D. (1973) Tumor initiation by apparently noncarcinogenic polycyclic aromatic hydrocarbons. *J. natl Cancer Inst*., **50**, 1717–1719

Sellakumar, A. & Shubik, P. (1974) Carcinogenicity of different polycyclic hydrocarbons in the respiratory tract of hamsters. *J. natl Cancer Inst*., **53**, 1713–1719

Sellakumar, A.R., Montesano, R., Saffiotti, U. & Kaufman, D.G. (1973) Hamster respiratory carcinogenesis induced by benzo(a)pyrene and different dose levels of ferric oxide. *J. natl Cancer Inst*., **50**, 507–510

Sellakumar, A., Stenbäck, F. & Rowland, J. (1976) Effects of different dusts on respiratory carcinogenesis in hamsters induced by benzo(a)pyrene and diethylnitrosamine. *Eur. J. Cancer*, **12**, 313–319

Sellakumar, A., Stenbäck, F. & Rowland, J. (1979) Effects of different dusts on respiratory carcinogenesis in hamsters induced by benzo(a)pyrene and diethylnitrosamine. *Eur. J. Cancer*, **12**, 313–319

Shear, M.J. (1936) Studies in carcinogenesis. I. The production of tumors in mice with hydrocarbons. *Am. J. Cancer*, **26**, 322–332

Shear, M.J. (1938) Studies in carcinogenesis. V. Methyl derivatives of 1:2-benzanthracene. *Am. J. Cancer*, **33**, 499–537

Shimizu, Y., Nakatsuru, Y., Ichinose, M., Takahashi, Y., Kume, H., Mimura, J., Fujii-Kuriyama, Y. & Ishihawa, T. (2000) Benzo[*a*]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc. natl Acad. Sci. USA*, **97**, 779–782

Shimkin, M.B. & Stoner, G.D. (1975) Lung tumours in mice: Application to carcinogenesis bioassay. In: Klein, G. & Weinhouse, S., eds, *Advances in Cancer Research*, Vol. 1, New York, Raven Press, pp. 1–58

Shubik, P. & Della Porta, G. (1957) Carcinogenesis and acute intoxication with large doses of polycyclic hydrocarbons. *Am. med. Assoc. Arch. Pathol*., **64**, 691–703

Shubik, P., Pietra, G. & Della Porta, G. (1960) Studies of skin carcinogenesis in the Syrian golden hamster. *Cancer Res*., **20**, 100–105

Sivak, A., Niemeier, R., Lynch, D., Beltis, K., Simon, S., Salomon, R., Latta, R., Belinky, B., Menzies, K., Lunsford, A., Cooper, C., Ross, A. & Bruner, R. (1997) Skin carcinogenicity of condensed asphalt roofing fumes and their fractions following dermal application to mice. *Cancer Lett*., **117**, 113–123

Slaga, T.J., Huberman, E., Selkirk, J.K., Harvey, R.G. & Bracken, W.M. (1978) Carcinogenicity and mutagenicity of benz(a)anthracene diols and diol-epoxides. *Cancer Res.*, **38**, 1699–1704

Slaga, T.J., Jecker, L., Bracken, W.M. & Weeks, C.E. (1979) The effects of weak or non-carcinogenic polycyclic hydrocarbons on 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene skin tumor-initiation. *Cancer Lett*., **7**, 51–59

Slaga, T.J., Gleason, G.L., Mills, G., Ewald, L., Fu, P.P., Lee, H.M. & Harvey, R.G. (1980) Comparison of the skin tumor-initiating activities of dihydrodiols and diol-epoxides of various polycyclic aromatic hydrocarbons. *Cancer Res.*, **40**, 1981–1984

Smith, D.M., Rogers, A.E., Herndon, B.J. & Newberne, P.M. (1975a) Vitamin A (retinyl acetate) and benzo(*a*)pyrene-induced carcinogenesis in hamsters fed a commercial diet. *Cancer Res*., **35**, 11–16

Smith, D.M., Rogers, A.E. & Newberne, P.M. (1975b) Vitamin A and benzo(*a*)pyrene carcinogenesis in the respiratory tract of hamsters fed a semisynthetic diet. *Cancer Res*., **35**, 1485–1488

Snell, K.C. & Stewart, H.L. (1962) Pulmonary adenomatosis induced in DBA/2 mice by oral administration of dibenz[*a,h*]anthracene. *J. natl Cancer Inst*., **28**, 1043–1051

Solt, D.B., Polverini, P.J. & Calderon, L. (1987) Carcinogenic response of hamster buccal pouch epithelium to 4 polycyclic aromatic hydrocarbons. *J. oral Pathol*., **16**, 294–302

Sparnins, V.L., Mott, A.W., Barany, G. & Wattenberg, L.W. (1986) Effects of allyl methyl trisulfide on glutathione S-transferase activity and BP-induced neoplasia in the mouse. *Nutr. Cancer*, **8**, 211–215

Springer, D.L., Mahlum, D.D., Westerberg, R.B., Hopkins, K.L., Frazier, M.E., Later, D.W. & Weimer, W.C. (1988) Carcinogenesis, metabolism and DNA binding studies of complex organic mixtures. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons: Chemistry, Characterization and Carcinogenesis*, Columbus, OH, Battelle Press, pp. 881–891

Springer, D.L., Mann, D.B., Dankovic, D.A., Thomas, B.L., Wright, C.W. & Mahlum, D.D. (1989) Influences of complex organic mixtures on tumor-initiating activity, DNA binding and adducts of benzo[*a*]pyrene. *Carcinogenesis*, **10**, 131–137

Stanton, M.F., Miller, E., Wrench, C. & Blackwell, R. (1972) Experimental induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate. *J. natl Cancer Inst*., **49**, 867–877

Steiner, P.E. (1955) Carcinogenicity of multiple chemicals simultaneously administered. *Cancer Res*., **15**, 632–635

Steiner, P.E. & Edgcomb, J.H. (1952) Carcinogenicity of 1,2-benzanthracene. *Cancer Res*., **12**, 657–659

Steiner, P.E. & Falk, H.L. (1951) Summation and inhibition effects of weak and strong carcinogenic hydrocarbons: 1,2-Benzanthracene, chrysene, 1,2,5,6-dibenzanthracene, and 20-methylcholanthrene. *Cancer Res*., **11**, 56–63

Steinhoff, D., Mohr, U. & Hahnemann, S. (1991) Carcinogenesis studies with iron oxides. *Exp. Pathol*., **43**, 189–194

Stenbäck, F. & Rowland, J. (1978) Role of particle size in the formation of respiratory tract tumors induced by benzo(a)pyrene. *Eur. J. Cancer*, **14**, 321–326

Stenbäck, F. & Rowland, J. (1979) Experimental respiratory carcinogenesis in hamsters: Environmental, physicochemical and biological aspects. *Oncology*, **36**, 63–71

Stenbäck, F. & Sellakumar, A. (1974) Lung tumor induction by dibenz(*a*,*i*)pyrene in the Syrian golden hamster. *Z. Krebsforsch*., **82**, 175–182

Stenbäck, F., Sellakumar, A. & Shubik, P. (1975) Magnesium oxide as carrier dust in benzo[*a*]pyrene-induced lung carcinogenesis in Syrian hamsters. *J. natl Cancer Inst*., **54**, 861–866

Stevenson, J.L. & von Haam, E. (1965) Carcinogenicity of benzo(a)anthracene and benz(c)phenanthrene derivatives. *Am. ind. Hyg. Assoc. J*., **26**, 475–478

Strauss, E. & Mateyko, G.M. (1964) Chemical induction of neoplasms in the kidney of *Rana pipiens. Cancer Res*., **24**, 1969–1978

Tawfic, H.N. (1965) Studies on ear-duct tumors in rats. II. Inhibitory effect of methylcholanthrene and 1,2-benzanthracene on tumor formation by 4-methyl-amino-stilbene. *Acta pathol. jpn*, **15**, 255–260

Thyssen, J., Althoff, J., Kimmerle, G. & Mohr, U. (1980) Investigations on the carcinogenic burden of air pollution in man. XIX. Effect of inhaled benzo(a)pyrene in Syrian golden hamsters: A pilot study. *Zbl. Bakt. Hyg. Abt. Orig. B*, **171**, 441–444

Thyssen, J., Althoff, J., Kimmerle, G. & Mohr, U. (1981) Inhalation studies with benzo[*a*]pyrene in Syrian golden hamsters. *J. natl Cancer Inst*., **66**, 575–577

Toth, B. (1980) Tumorigenesis by benzo(a)pyrene administered intracolonically. *Oncology*, **37**, 77–82

von Tungeln, L.S., Xia, Q. Herreno-Sainz, D., Bucci, T.J., Heflich, R.H. & Fu, P.P. (1999a) Tumorigenicity of nitropolycyclic aromatic hydrocarbons in the neonatal B6C3F$_1$ mouse bioassay and characterization of *ras* mutations in liver tumors from treated mice. *Cancer Lett*., **146**, 1–7

von Tungeln, L.S., Xia, Q., Bucci, T., Heflich, R.H. & Fu, P.P. (1999b) Tumorigenicity and liver tumor *ras*-protooncogene mutations in CD-1 mice treated with 1- and 3-nitrobenzo[*a*]pyrene and their *trans*-7,8-dihydrodiol and aminobenzo[*a*]pyrene metabolites. *Cancer Lett*., **137**, 137–143

Van Duuren, B.L. & Goldschmidt, B.M. (1976) Co-carcinogenic and tumor-promoting agents in tobacco carcinogenesis. *J. natl Cancer Inst*., **56**, 1237–1242

Van Duuren, B.L., Langseth, L., Goldschmidt, B.M. & Orris, L. (1967) Carcinogenicity of epoxides, lactones and peroxy compounds. VI. Structure and carcinogenic activity. *J. natl Cancer Inst*., **39**, 1217–1228

Van Duuren, B.L., Sivak, A., Langseth, L., Goldschmidt, B.M. & Segal, A. (1968) Initiators and promotors in tobacco carcinogenesis. *Natl Cancer Inst. Monogr.*, **28**, 173–180

Van Duuren, B.L., Sivak, A., Goldschmidt, B.M., Katz, C. & Melchionne, S. (1970) Initiating activity of aromatic hydrocarbons in two-stage carcinogenesis. *J. natl Cancer Inst*., **44**, 1167–1173

Van Duuren, B.L., Katz, C. & Goldschmidt, B.M. (1973) Cocarcinogenic agents in tobacco carcinogenesis. *J. nal. Cancer Inst*., **51**, 703–705

Vesselinovitch, S.D., Kyriazis, A.P., Mihailovich, N. & Rao, K.V.N. (1975a) Factors influencing augmentation and/or acceleration of lymphoreticular tumors in mice by benzo(*a*)pyrene treatment. *Cancer Res*., **35**, 1963–1969

Vesselinovitch, S.D., Kyriazis, A.P., Mihailovich, N. & Rao, K.V.N. (1975b) Conditions modifying development of tumors in mice at various sites by benzo(*a*)pyrene. *Cancer Res*., **35**, 2948–2953

Voronjansky, G.S., Pisareva, T.A. & Sorin, J.E. (1939) The histological picture of the tumours caused by the synthetic cancerogenic hydrocarbons of Soviet make (dibenzepyrene and hydrodibenzepyrene). *Arch. Biol. Nauk.*, **55**, 89–97

de Vries, A., van Oostrom, C.T.M., Dortant, P.M., Beems, R.B., van Kreijl, C.F., Capel, P.J.A. & van Steeg, H. (1997) Spontaneous liver tumours and benzo[*a*]pyrene-induced lymphomas in *XPA*-deficient mice. *Mol. Carcinog.*, **19**, 46–53

Wang, J.S. & Busby, W.F., Jr (1993) Induction of lung and liver tumors by fluoranthene in a preweanling CD-1 mouse bioassay. *Carcinogenesis*, **14**, 1871–1874

Wang, J.-S., He, X., Mulder, P.P.J., Boere, B.B., Cornelisse, J., Lugtenburg, J. & Busby, W.F., Jr (1999) Comparative tumorigenicity of the cyclopenta-fused polycyclic aromatic hydrocarbons aceanthrylene, dihydroaceanthrylene and acephenanthrylene in preweanling CD-1 and BLU:Ha mouse bioassays. *Carcinogenesis*, **20**, 1137–1141

Waravdekar, S.S. & Ranadive, K.J. (1958) Biologic testing of 3,4,9,10-benzpyrene. *J. natl Cancer Inst*., **21**, 1151–1159

Warshawsky, D. & Barkley, W. (1987) Comparative carcinogenic potencies of 7H-dibenzo[*c,g*]carbazole, dibenz[*a,j*]acridine and benzo[*a*]pyrene in mouse skin. *Cancer Lett*., **37**, 337–344

Warshawsky, D., Barkley, W. & Bingham, E. (1993) Factors affecting carcinogenic potential of mixtures. *Fundam. appl. Toxicol*., **20**, 376–382

Wenzel-Hartung, R., Brune, H., Grimmer, G., Germann, P., Timm, J. & Wosniok, W. (1990) Evaluation of the carcinogenic potency of four environmental polycyclic aromatic compounds following intrapulmonary application in rats. *Exp. Pathol.*, **40**, 221–227

Weyand, E.H., Amin, S., Huie, K., Boger, E., Neuber, E., Hecht, S.S. & LaVoie, E.J. (1989) Effects of fluorine substitution on the DNA binding and tumorigenicity of benzo[b]fluoranthene in mouse epidermis. *Chem.-biol. Interact.*, **71**, 279–290

Weyand, E.H., Patel, S., LaVoie, E.J., Cho, B. & Harvey, R.G. (1990) Relative tumor initiating activity of benzo[a]fluoranthene, benzo[b]fluoranthene, naphtho[1,2-b]fluoranthene and naphtho[2,1-a]fluoranthene on mouse skin. *Cancer Lett.*, **52**, 229–233

Weyand, E.H., He, Z.M., Ghodrati, F., Wu, Y., Marshall, M.V. & LaVoie, E.J. (1992) Effect of fluorine substitution on benzo[j]fluoranthene genotoxicity. *Chem.-biol. Interact.*, **84**, 37–53

Weyand, E.H., Chen, Y.-C., Wu, Y., Koganti, A., Dunsford, H.A. & Rodriguez, L.V. (1995) Differences in tumorigenic activity of a pure hydrocarbon and a complex mixture following ingestion: Benzo[*a*]pyrene vs manufactured gas plant residue. *Chem. Res. Toxicol.*, **8**, 949–954

Weyand, E.H., Parimoo, B., Reuhl, K.R., Goldstein, L.S., Wang, J.Q. & Harvey, R.G. (2004) 7*H*-Benzo[*c*]fluorene: A potent systemic lung carcinogen. *Polycycl. arom. Compds*, **24**, 1–20

Wijnhoven, S.W.P., Kool, H.J.M., van Oostrom, C.T.M., Beems, R.B., van der Horst, G.T.J., Vrieling, H. & van Steeg, H. (2000) The relationship between benzo[*a*]pyrene-induced mutagenesis and carcinogenesis in repair-deficient Cockayne syndrome group B mice. *Cancer Res.*, **60**, 5681–5687

Wislocki, P.G., Buening, M.K., Levin, W., Lehr, R.E., Thakker, D.R., Jerina, D.M. & Conney, A.H. (1979) Tumorigenicity of the diastereomeric benz[a]anthracene 3,4-diol-1,2-epoxides and the (+)- and (–)-enantiomers of benz[a]anthracene 3,4-dihydrodiol in newborn mice. *J. natl Cancer Inst.*, **63**, 201–204

Wislocki, P.G., Bagan, E.S., Lu, A.Y., Dooley, K.L., Fu, P.P., Han-Hsu, H., Beland, F.A. & Kadlubar, F.F. (1986) Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. *Carcinogenesis*, **7**, 1317–1322

Wodinsky, I., Helinsky, A. & Kensler, C.J. (1964) Susceptibility of Syrian hamsters to induction of fibrosarcomas with a single injection of 3,4,9,10-dibenzpyrene. *Nature*, **203**, 308–309

Wodinsky, I., Helinski, A. & Kensler, C.J. (1965) Experimental tumorigenesis in the hamster cheek pouch. *Nature*, **207**, 770–772

Wolterbeek, A.P.M., Schoevers, E.J., Rutten, A.J.J.L. & Feron, V.J. (1995) A critical appraisal of intratracheal instillation of benzo[*a*]pyrene to Syrian golden hamsters as a model in respiratory tract carcinogenesis. *Cancer Lett.*, **89**, 107–116

Wood, A.W., Chang, R.L., Levin, W., Ryan, D.E., Thomas, P.E., Mah, H.D., Karle, J.M., Yagi, H., Jerina, D.M. & Conney, A.H. (1979) Mutagenicity and tumorigenicity of phenanthrene and chrysene epoxides and diol epoxides. *Cancer Res.*, **39**, 4069–4077

Wood, A.W., Levin, W., Chang, R.L., Huang, M.-T., Ryan, D.E., Thomas, P.E., Lehr, R.E., Kumar, S., Koreeda, M., Akagi, H., Ittah, Y., Dansette, P., Yagi, H., Jerina, D. & Conney, A.H. (1980) Mutagenicity and tumor initiating activity of cyclopenta(*c*,*d*)pyrene and structurally related compounds. *Cancer Res.*, **40**, 642–649

Wynder, E.L. & Hoffmann, D. (1959) A study of tobacco carcinogenesis. VII. The role of higher polycyclic hydrocarbons. *Cancer*, **12**, 1079–1086

You, L., Wang, D., Galati, A.J., Ross, J.A., Mass, M.J., Nelson, G.B., Wilson, K.H., Amin, S., Stoner, J.C., Nesnow, S. & Stoner, G.D. (1994) Tumor multiplicity, DNA adducts and K-ras

mutation pattern of 5-methylchrysene in strain A/J mouse lung. *Carcinogenesis*, **15**, 2613–2618

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Toxicokinetics

4.1.1 *Absorption, distribution, metabolism and excretion*

(a) Overview

This section provides an overview of the toxicokinetics of polycyclic aromatic hydrocarbons (PAHs). Other more comprehensive reviews of the toxicokinetics of PAHs include those by the Environmental Protection Agency (1991), the Agency for Toxic Substances and Disease Registry (ATSDR, 1995) and the International Programme on Chemical Safety (IPCS, 1998), as well as reviews on the metabolism and bioactivation of PAHs by Conney (1982), Cooper *et al*. (1983), Shaw and Connell (1994), Penning *et al*. (1999), Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) and Xue and Warshawsky (2005). Little is known about the toxicokinetics of mixtures of or individual 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 derive 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 formation of mutations and the initiation of cancer. The structures of the DNA adducts that are formed provide an inference of the precursor metabolites. 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 molecular weight, several of the physicochemical properties that are critical to their biological activity vary greatly. Five properties in particular have a decisive influence on the biological activity of PAHs: their vapour pressure, their adsorption onto surfaces of solid carrier particles, their absorption into liquid carriers, their lipid/aqueous partition coefficient in tissues and their limits of solubility in the lipid and aqueous phases of tissues.

These properties are intrinsically linked with 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.

Transporters may play a role in the biological activity of PAHs. Adenosine triphosphate (ATP)-binding cassette (ABC) transporters (49 genes characterized in humans) transport specific molecules across lipid membranes including hydrophobic compounds and metabolites (Schinkel & Jonker, 2003). P-Glycoprotein transports mainly non-metabolized compounds and multidrug resistance-associated protein-1 (MRP1) and -2 conjugates of foreign compounds (Leslie *et al.*, 2001; Haimeur *et al.*, 2004). Several ABC transporters are polymorphic (Sakaeda *et al.*, 2004). Benzo[*a*]pyrene conjugates may be substrates for ABC transporters, such as the GSH conjugate of benzo[*a*]pyrene-7,8-diol-9,10-oxide which is a substrate for MRP2 (Srivastava *et al.*, 2002) and benzo-[*a*]pyrene-3-glucuronide which is a substrate for breast cancer resistance protein (BCRP) (Ebert *et al.*, 2005). PAHs are ubiquitous in the environment due to volcanic eruptions and forest fires, and their presence in various media (i.e. air and soil) constitutes a background level of exposure. Additional exposure occurs through the ingestion of grilled or cured meats (see Section 1). These exposures should be taken into consideration when assessing health risks due to exposure to PAHs.

(b) Absorption through the respiratory tract

Vapour pressure is a major determinant for the distribution of a 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 weight (Lohmann & Lammel, 2004), so that two-ringed naphthalenes are mostly found in the gas phase whereas five-ringed 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). Because the most carcinogenic PAHs of greater size are, to a large degree, particle-associated, there is considerable potential for covariance with an inflammatory response that is induced by the carrier particles alone. Typical carbonaceous carrier particles of PAHs that have no adsorbed genotoxic material have been shown to be carcinogenic, particularly in rats (see IARC, 2010). This mechanism is, however, outside the scope of this monograph. Gas/particle partitioning is also of great importance during inhalation exposure in order 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- ringed 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 from particles 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 for the bioavailability of the substance in the organism. The timing of the release from carrier particles in particular affects the toxicity of inhaled PAHs at the site of entry. 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. A rapid release from carrier particles gives a close correlation between the deposition pattern of inhaled aerosols and the site-of-entry exposures to particle-associated PAHs. Slower release alters the exposures, and shows a clearance pattern of inhaled particles. 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).

Following deposition and desorption from their carrier particles, PAHs are absorbed through the epithelial barriers onto which they are deposited. The slow diffusion of highly lipophilic substances into the tissues is fundamental to the behaviour of PAHs in biological systems. This is a strictly physicochemical mechanism that needs to be considered in all measurements of the kinetics of PAHs in tissues. A highly lipophilic substance dissolves readily in the first lipid membrane it encounters, but is then transported slowly into the next layer (Gerde *et al*., 1993a). This is due to the low concentration of lipophilic solute in the aqueous gaps between cell membranes, which results in a high concentration of solute in the epithelium at the site of entry, comparatively slow absorption into the circulation and a low concentration in all tissues distal to the site of entry. The absorption process is strongly dependent on the lipophilicity of the PAHs and their metabolites: with higher lipophilicities, the mobility of the substances by diffusion into tissues is lower and, with thicker entrance epithelium, the half-life of absorption into the capillary bed of the submucosa is longer. 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; 1993a,b,c; Gerde & Scott, 2001). The relative thickness of the epithelium of the conducting airways compared with the thin epithelium of the alveolar region has been proposed as a contributing factor to this regional difference in duration of absorption following deposition. Slow absorption through the epithelium of the conducting airways probably leads rapidly to saturation in the mucous lining layer and airway epithelium with increasing levels of exposure (Gerde *et al.*, 1991a,b). A probable consequence is an increase in the fraction of undissolved/undesorbed PAHs that is transported to the gastrointestinal tract by the mucociliary escalator.

A major effect of the metabolic conversion of PAHs of lower molecular weight 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 epithelia 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 lung cancer (Rojas *et al.*, 2004).

The low mobility of the highly lipophilic PAHs in tissues is an important factor that complicates the toxicokinetics/pharmacokinetics of such substances. The fundamental condition of perfusion-limited compartments in physiologically based pharmacokinetic models does not hold for highly lipophilic PAHs. According to this condition, a solute is in equilibrium between the tissues of an organ and the blood stream that leaves that organ (Bischoff & Dedrick, 1970). However, because of low mobility, the lipophilic solute does not have time to reach equilibrium before the blood exits the tissue. This effect is more pronounced for slowly perfused tissues. A typical example of such a tissue is adiposal fat which, despite a very high tissue/blood partition coefficient, contains low concentrations of PAHs shortly after exposure but has the highest relative concentrations longer after exposures (Withey *et al*., 1993a). For physiologically based pharmacokinetic models to predict better the systemic distribution of PAHs and their metabolites, the delayed equilibration between blood and tissue needs to be taken into account.

Another aspect of modelling that needs further study is an accurate description of site-of-entry dosimetry. Because of diffusion-limited absorption, the local dose to the epithelium of the site of entry is probably considerably higher than that predicted by the well-mixed compartment assumption in classical physiologically based pharmacokinetic models. Even if the absorption rates in classical models are adjusted with an empirical mass-transfer coefficient from the environment to the blood, the models cannot predict the limited distribution of the highly exposed cell populations at the site of entry. Conversely, models that describe site-of-entry dosimetry with greater resolution can predict both absorption rates and local tissue doses with reasonable accuracy (Gerde & Scott, 2001), but such models are rather complex and cannot describe the systemic distribution of the solutes in any detail. For these reasons, no validated physiologically based pharmacokinetic models for the deposition, absorption and systemic distribution of PAHs are available.

Results from studies of rats exposed by inhalation to radiolabelled benzo[*a*]pyrene indicate that inhaled PAHs can be rapidly absorbed by the respiratory tract (for reviews, see Environmental Protection Agency, 1991; ATSDR, 1995; IPCS, 1998). For example, in a study in which pregnant rats were exposed to 200 and 800 mg/m^3 [^{14}C]-labelled benzo[*a*]pyrene aerosol for 95 min on gestation day 17, radiolabel was detected, as the parent compound and metabolites, in maternal blood samples collected immediately after exposure (Withey *et al*., 1993a). The detection of high levels of radioactivity in the lung and liver of these rats is consistent with the occurrence of mucociliary clearance from the

respiratory tract and ingestion into the oesophagus and gastrointestinal tract, as well as rapid absorption, metabolism and biliary excretion of metabolites (Withey *et al.*, 1994).

The relatively longer retention of PAHs released in the conducting airways (compared with the air-exchange region) may allow for substantial metabolism within this region of deposition. In a study in which anaesthetized dogs were instilled intratracheally with single doses of [^{3}H]-labelled benzo[*a*]pyrene dissolved in a saline/phospholipid suspension, absorption into the trachea showed a half-time of about 73 min (Gerde *et al*., 1997). Three hours after instillation, a total of about 90% of the radioactivity retained in the tracheal epithelium was associated with metabolites of benzo[*a*]pyrene. Results from similar experiments with pyrene indicated that, although pyrene is more rapidly absorbed in the tracheal mucosa than benzo[*a*]pyrene, significant metabolism occurred within the tracheal epithelium (Gerde *et al*., 1998). Similar results that indicate extensive metabolism in the lung have been reported for rats exposed by inhalation for 4 h to aerosols of carbon black with adsorbed benzo[*a*]pyrene (Ramesh *et al*., 2001). In these experiments, only metabolites of benzo[*a*]pyrene, and not the parent material, were detected in lung tissue sampled at 30, 60, 120 or 240 min after cessation of exposure.

(c) Absorption through the gastrointestinal tract

PAHs are absorbed by the gastrointestinal tract via both diffusion across cellular membranes, based on their lipophilicity, and normal absorption of dietary lipids (O'Neill *et al*., 1991). Absorption of specific PAHs, such as benzo[*a*]pyrene, has been demonstrated following oral administration of radiolabelled compounds to laboratory animals (for review, see Environmental Protection Agency, 1991; ATSDR, 1995; IPCS, 1998). Results from animal studies indicate that absorption is rapid (Rees *et al*., 1971; Modica *et al*., 1983), that fractional absorption of lower-molecular-weight PAHs, such as two-ringed naphthalene, may be more complete than that of higher-molecular-weight PAHs, such as five-ringed 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).

(d) Absorption through the skin

Evidence of 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 in the workplace (van Rooij *et al*., 1993a) or coal-tar ointments (van Rooij *et al*., 1993b; Godschalk *et al*., 1998). Results from animal studies indicate that dermal absorption of PAHs can be rapid and extensive. For example, the reported half-times of disappearance of radiolabel from the skin of rats treated with 2, 6 or 15 mg/kg [^{14}C]-labelled pyrene in acetone applied to a 4-cm^2 area ranged from about 0.5 to 0.8 days, and about 50% of the applied radiolabel was recovered in urine and faeces collected within 6 days of application (Withey *et al*., 1993b).

(e) Distribution

Results from studies in rats 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 levels of PAHs and their metabolites following 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*., 1991,1993a). For example, immediately after a 95-min inhalation exposure of pregnant rats to [^{3}H]-labelled benzo[*a*]pyrene aerosols, levels of radiolabel associated with parent compound in the tissues decreased in the following order: lung > blood > liver > kidney > fat > fetus; 6 h after cessation of exposure, the order was fat > lung > kidney > liver > blood > fetus (Withey *et al*., 1993a). Similarly, about 7 h after a 95-min inhalation exposure of male rats to [^{14}C]-labelled pyrene aerosol at a concentration of 800 mg/m^{3}, levels of radiolabel associated with parent compound decreased in the following order: fat > kidney > lung > liver > testes > spleen > brain (Withey *et al*., 1994).

In other studies that involved inhalation exposure of rats for 1 h to [^{3}H]-benzo[*a*]-pyrene aerosols (500 μg/L; mass median aerodynamic diameter [MMAD], 1–2 μm), clearance of radiolabel from the respiratory tract was biphasic, with 50% of the radiolabel cleared within 2–3 h (Mitchell, 1982). Elimination half-times for radiolabel that remained in the lung after this time ranged from about 25 to 50 h, depending on the location in the lung. Half an hour after termination of exposure, concentrations of radiolabel were higher in the stomach and small intestine than in any other tissue, although significant amounts were detected in the liver and kidneys. During the first day after exposure, the amount of radiolabel in the faeces was about 10-fold greater than that in the urine. In experiments with rats exposed to pyrene aerosols (500 μg/L; MMAD, 0.3–0.8 μm), similar rapid clearance from the respiratory tract and distribution to the gastrointestinal tract, liver and kidneys was observed (Mitchell & Tu, 1979).

In experiments with rats exposed by inhalation to radiolabelled benzo[*a*]pyrene adsorbed onto ultrafine particles (e.g. gallium oxide or diesel exhaust particles; MMAD, ~0.1 μm), similar rapid clearance from the respiratory tract was observed. The relative amount of radiolabel in the stomach was higher following exposure to benzo[*a*]pyrene adsorbed onto gallium oxide or diesel exhaust particles than after exposure to pure aerosols of benzo[*a*]pyrene (Sun *et al*., 1982, 1984). In rats exposed to [^{3}H]-benzo[*a*]-pyrene-coated diesel engine exhaust particles (MMAD, ~0.14 μm; Sun *et al*., 1984), the amounts of radiolabel retained in the lung during the second, slow phase of lung clearance were higher than those in the study of gallium oxide-associated ^{3}H-benzo[*a*]pyrene study (Sun *et al*., 1982). [The results from 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 compo-

sitional characteristics of the particulate matter, as well as by the chemical properties of the PAHs themselves.]

(f) Metabolism

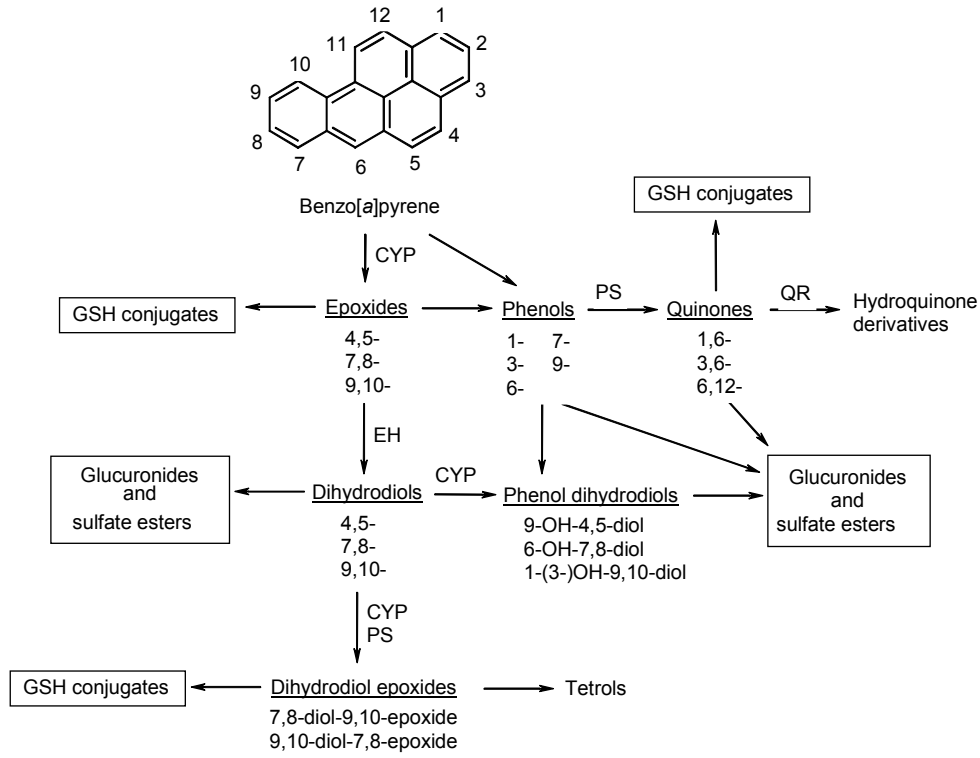
The metabolism of benzo[*a*]pyrene has been studied extensively in human and animal tissues, and generally serves as a model for the metabolism of other PAHs (for review, see Environmental Protection Agency, 1991; ATSDR 1995; IPCS 1998). A metabolic schema for benzo[*a*]pyrene is presented in Figure 4.1 which shows pathways to the formation of epoxides, phenols, quinones, hydroquinones, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, tetrols and other potentially reactive intermediates. Table 4.1 contains examples of PAHs and PAH metabolites that are substrates for human enzymes. The tissue localization of human enzymes indicates that metabolic activation can occur in key target organs. It should be noted that differences between in-vivo and in-vitro studies of metabolism have been observed in some cases for benzo[*a*]pyrene and other PAHs.

The mechanism of PAH oxidation by cytochrome P450 (CYP) mono-oxygenase is complex and can involve an odd electron abstraction rebound mechanism as well as a one-electron radical cation mechanism (Cavalieri *et al.*, 1988; Guengerich, 2001; Cavalieri & Rogan, 2002; Mulder *et al.*, 2003).

Benzo[*a*]pyrene is initially metabolized by CYP mono-oxygenases 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 enzyme, 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 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 GSH-*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.

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



Adapted from Cooper *et al.* (1983); ATSDR (1995); IPCS (1998).
CYP, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; PS, prostaglandin H synthase; QR, quinone reductase

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 (phosphate) (with or without phosphate) (NAD(P)H) and copper, reactive oxygen species that cause

Table 4.1. PAHs and PAH metabolites as substrates of human enzymes and localization in human tissues outside the liver (extrahepatic metabolism)

Enzyme	PAH substrates	Human tissues
CYP1A1	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene and its 11,12-diol Benzo[*b*]fluoranthene-9,10-diol Benzo[*a*]pyrene and its 7,8-diols Cyclopenta[*cd*]pyrene Dibenzo[*a*,*l*]pyrene Dibenzo[*a*,*l*]pyrene-11,12-diol 5-Methylchrysene 5-Methylchrysene-1,2-diol	Lung of smokers (mRNA, prot., act.). Oesophagus Stomach Small intestine Colon Skin Placenta of smokers Fetal liver
CYP1A2	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*b*]fluoranthene-9,10-diol Benzo[*a*]pyrene-7,8-diol Cyclopenta[*cd*]pyrene Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene and its 11,12-diol 5-Methylchrysene-1,2-diol	Lung (mRNA +/–, prot., +/–, no act.) Oesophagus Stomach Colon
CYP1B1	Benzo[*g*]chrysene-11,12-diol Benzo[*b*]fluoranthene-9,10-diol Benzo[*a*]pyrene and its 7,8-diol Dibenzo[*a*,*l*]pyrene Dibenzo[*a*,*l*]pyrene-11,12-diol 5-Methylchrysene-1,2-diol	Lung (mRNA, prot., act.) Small intestine Colon Skin Fetal liver
CYP2A		Oesophagus
CYP2A6		Lung (mRNA, prot. +/–, no act.) Nasal mucosa Trachea
CYP2A13[a, b, c]		Nasal mucosa (highest expression) Trachea Lung
CYP2B6	Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene	Lung (mRNA, prot., act.) Nasal mucosa Trachea
CYP2C	Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene	Lung (mRNA +/–, prot. +/–) Nasal mucosa Stomach Small intestine Early placenta Fetal liver (2C8)

Table 4.1 (contd)

Enzyme	PAH substrates	Human tissues
CYP2D6		Lung (mRNA +/–, prot. +/–, no act.) Small intestine Early placenta Fetal liver
CYP2E1	Dibenz[*a*,*h*]anthracene	Lung (mRNA, prot., act.) Oesophagus Small intestine Fetal liver
CYP2F1[a]		Lung (mRNA)
CYP2J2		Lung (mRNA, prot., act.) Nasal mucosa Oesophagus Stomach Small intestine Colon
CYP2S1[a]		Lung (mRNA, prot.) Trachea Stomach Small intestine
CYP3A	Dibenz[*a*,*h*]anthracene (3A3)	Nasal mucosa
CYP3A4	Cyclopenta[*c*,*d*]pyrene Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene 5-Methylchrysene	Lung (mRNA +/–, prot.) Stomach Small intestine Colon Fetal liver
CYP3A5[a,d]	Dibenz[*a*,*h*]anthracene	Lung (mRNA, prot., act.) Oesophagus Small intestine Colon Kidney Fetal liver
CYP3A7		Lung (mRNA +/–) Early placenta Fetal liver
CYP4B1[a]		Lung (mRNA, prot.) Placenta
PS	Benzo[*a*]pyrene Cyclopenta[*cd*]pyrene	Most tissues

Table 4.1 (contd)

Enzyme	PAH substrates	Human tissues
Aldo-keto-reductase (AKR)	Benz[*a*]anthracene Benzo[*g*]chrysene Benzo[*a*]pyrene 5-Methylchrysene	Most tissues
AKR1C1	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Liver Hep G2 cells[1,2,3] Lung adenocarcinoma A549 cells[1,2,3] Trachea[1], colon[1], bladder[1], small intestine[1], heart[1], aorta[1], stomach[1], testis[1], ovary[1]
AKR1C2	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Liver Hep G2 cells[1,2,3] Lung adenocarcinoma A549 cells[1,2,3] Trachea[1], colon, bladder[1], small intestine[1], heart[1], aorta[1], stomach[1], testis[1], prostate[1]
AKR1C3	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol-	Liver Hep G2 cells[1,2,3] Lung adenocarcinoma A549 cells[1,2,3] Trachea[1], colon[1], bladder[1], small intestine[1], heart[1], aorta[1], stomach[1], testis[1], mammary gland, prostate[1]
AKR1C4	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Liver-specific[1,2,3]
AKR1A1	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Kidney, liver[1], salivary gland[1], trachea[1], stomach[1], duodenum[1], pancreas, fetal lung[1], prostate[1], placenta[1], mammary gland[1], lung[1], lung adenocarcinoma[1], bladder[1], oesophagus[1], ovary[1], testis[1]

From Hakkola *et al.* (1998a,b); Palackal *et al.* (2001a); Hukkanen *et al.* (2002); Palackal *et al.* (2002a); Ding & Kaminsky (2003); Steckelbroeck *et al.* (2004); Shimada & Fujii-Kuriyama (2004)
+/–, conflicting evidence; act., catalytic activities; CYP, cytochrome P450; mRNA, messenger ribonucleicacid; PAH, polycyclic aromatic hydrocarbon; prot., protein; PS, prostaglandin H synthetase

[a] Preferentially expressed in lung
[b] Preferentially expressed in nasal mucosa
[c] Preferentially expressed in trachea
[d] Main isoform in kidney
[1] RNA analysis, array data with AKR1C proto or isoform specific reverse transcriptase polymerase chain reaction
[2] Protein
[3] Enzyme activity

DNA fragmentation and mutation of p53 (Flowers *et al*., 1996, 1997; Penning *et al*., 1999; Yu *et al.*, 2002a). 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.

(g) 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).

4.1.2 *Enzymes involved in the metabolism of PAHs and their genetic variability*

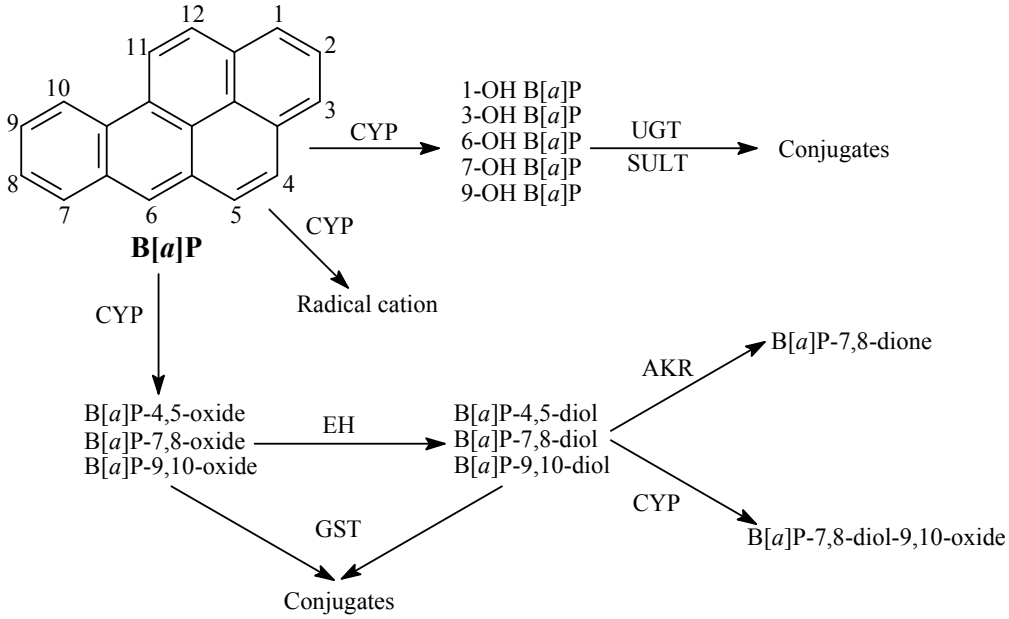
(a) Individual enzymes

(i) *Drug-metabolizing enzymes involved in the metabolism of PAHs*

PAHs are metabolized to water-soluble conjugates by the combined action of phase I enzymes that are involved in their functionalization (e.g. CYP, epoxide hydrolase) and phase II enzymes that form conjugates with the functionalized metabolites (e.g. GST, uridine 5′-diphosphate (UDP)-glucuronosyltransferase (UGT) and sulfotransferase (SULT)). In addition, AKRs and NAD(P)H quinone oxidoreductase 1 (NQO1) play important roles in the further metabolism of diols and quinones, respectively (Conney, 1982; Gonzalez *et al.*, 1991; Guengerich & Shimada, 1991; Hecht, 1998; Penning *et al.*, 1999; Hecht, 2002a).

CYPs are the major phase I enzymes that initially catalyse mono-oxygenation of PAHs (such as benzo[*a*]pyrene) to phenols and epoxides (Figure 4.2; Conney, 1982; Shimada *et al.*, 1989a,b; Guengerich & Shimada, 1991). The oxygenated (intermediate) products are further metabolized to more polar products by several conjugating enzymes such as epoxide hydrolase, GST, UGT and SULT (Nebert *et al.*, 1999). AKRs catalyse the oxidation of *trans*-dihydrodiols to catechols which are non-enzymatically oxidized to *ortho*-quinones, while NQO1 catalyses the two-electron reduction of *para*-quinones to hydroquinones. The catechols or hydroquinones in each instance can undergo conjugation. The conjugated metabolites thus formed are usually more soluble in water than the parent compounds and can be excreted from the body.

Figure 4.2. Metabolism of benzo[*a*]pyrene by CYPs and other drug-metabolizing enzymes

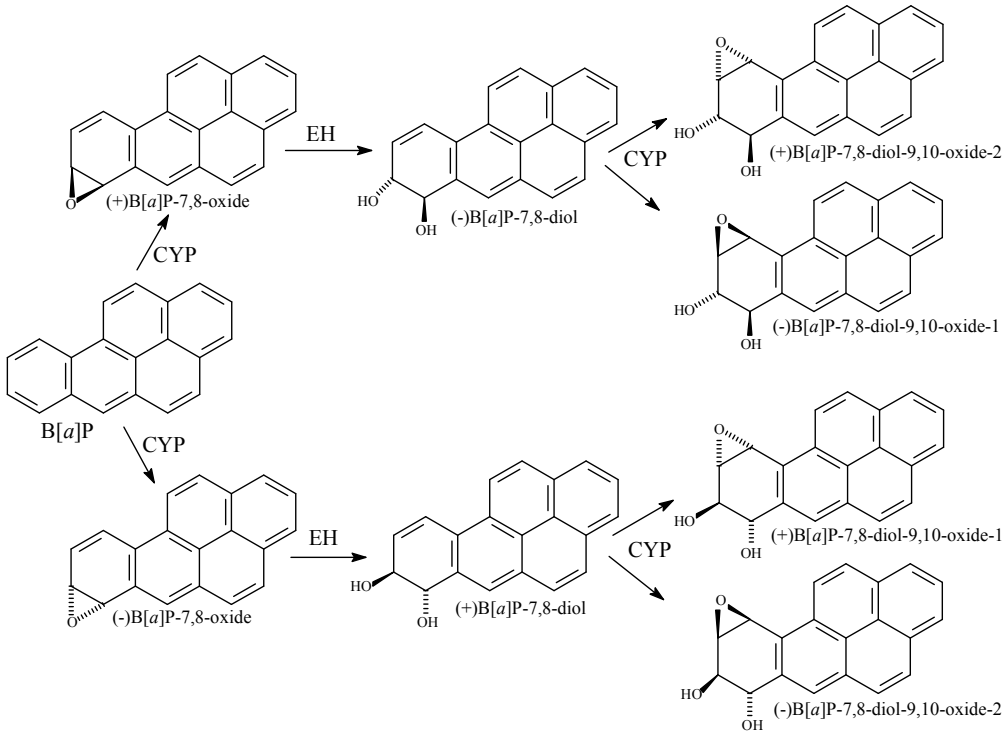


AKR, aldo-keto reductase; B[*a*]P, benzo[*a*]pyrene; CYP, cytochrome P450; EH, microsomal epoxide hydrolase; GST, glutathione *S*-transferase; SULT, sulfotransferase; UGT, Uridine 5′-diphosphate-glucuronosyltransferase

(ii) *Metabolic activation of PAHs by CYPs*

Bay-region diol epoxides represent major electrophilic PAH metabolites at the core of one of the proposed mechanisms of PAH activation (see Section 4.2.1). The pathways that lead to the formation of bay-region epoxides of benzo[*a*]pyrene were investigated extensively in rat liver microsomes and reconstituted systems containing purified rat CYPs and epoxide hydrolase (Figure 4.3) and serve as a model for other bay-region PAHs (Thakker *et al.*, 1977a,b; Lu & West, 1979; Levin *et al.*, 1980; Conney, 1982; Levin *et al.*, 1986). Benzo[*a*]pyrene is first oxidized by liver microsomes of 3-methylcholanthrene-treated rats to (+)- and (–)-benzo[*a*]pyrene-7,8-oxides; the rate of conversion of the (+) enantiomer is much higher than that of the (–) form. Subsequently, microsomal epoxide hydrolase hydrolyses these oxides to (–)- and (+)-benzo[*a*]pyrene-7,8-diols (Thakker *et al.*, 1977a,b; Levin *et al.*, 1980), which are finally activated by CYP isoforms to the highly reactive bay-region epoxides, (–)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-1, (+)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-2, (+)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-1 and (–)-benzo[*a*]pyrene-7, 8-diol-9,10-epoxide-2 (Thakker *et al.*, 1977b; Kapitulnik *et al.*, 1978a; Levin *et al.*, 1980). Although these four diol epoxides were

Figure 4.3. Metabolic activation of benzo[*a*]pyrene by CYPs and epoxide hydrolase



B[*a*]P, benzo[*a*]pyrene; CYP, cytochrome P450; EH, epoxide hydrolase

highly mutagenic in Ames *Salmonella* tester strains and Chinese hamster V79 cells, (+)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-2 was identified as the most reactive in producing tumours in newborn mice (Wood *et al.*, 1976; Buening *et al.*, 1978). Since (+)-benzo[*a*]-pyrene-7,8-diol-9,10-epoxide-2 had almost the same level of carcinogenicity as benzo[*a*]-pyrene itself or (–)-benzo[*a*]pyrene-7,8-diol, this diol epoxide is considered to be an ultimate carcinogenic metabolite (Kapitulnik *et al.*, 1978a; Conney, 1982; Levin *et al.*, 1986).

The bay-region theory has also been applied to other PAHs (Conney, 1982; Pelkonen & Nebert, 1982; Adams *et al.*, 1995). On the basis of this common mechanism, it is important to know that the major CYP isoforms involved in the formation of the respective arene oxides and diol epoxides are CYP1A1, CYP1A2 and CYP1B1.

(iii) *CYP1A1 and activation of PAHs*

CYP1A1 was thought to be uniquely responsible for the metabolic activation of most of the carcinogenic PAHs (Lu & West, 1979; Conney, 1982; Pelkonen & Nebert, 1982; Guengerich, 1988; Shimada *et al.*, 1989b; Ryan & Levin, 1990). CYP1A1 was first termed aryl hydrocarbon hydroxylase because it had high activity for benzo[*a*]pyrene 3-

hydroxylation (Nebert *et al.*, 1975; Nebert & Jensen, 1979), and was considered to be the major enzyme responsible for the metabolic activation of benzo[*a*]pyrene and other carcinogenic PAHs until CYP1B1 enzymes were found in mice, rats and humans (Pottenger & Jefcoate, 1990; Otto *et al.*, 1991; Pottenger *et al.*, 1991; Sutter *et al.*, 1991; Savas *et al.*, 1994; Sutter *et al.*, 1994). CYP1A1 has relatively similar substrate specificities to CYP1B1 (see below) in the metabolism of PAHs and other carcinogens, although some striking differences are observed, depending on the substrates (Guengerich *et al.*, 1986; Shimada *et al.*, 1992; Guengerich & Shimada, 1998; Shimada *et al.*, 1998a, 2001a). CYP1A1 also catalyses the oxidation of several xenobiotic compounds, including 7-ethoxyresorufin, theophylline, caffeine, 7-ethoxycoumarin and chlorzoxazone (Shimada *et al.*, 1997a), and the endogenous steroids, 17β-estradiol and estrone (Shimada *et al.*, 1999a, 2001b).

Recently, Uno *et al*. (2004) showed that CYP1A1 is important in the detoxification and protection against the oral toxicity of benzo[*a*]pyrene rather than in its metabolic activation in studies using CYP1A1 knockout mice. CYP1A1-null mice died within 30 days after oral administration of 125 mg/kg bw benzo[*a*]pyrene, while wild-type mice did not show any signs of toxicity for 1 year. In addition, benzo[*a*]pyrene–DNA adduct levels were found to be higher in CYP1A1-null mice than in the wild-type mice (Uno *et al.*, 2004; Nebert, 2005a).

CYP1A1 is expressed in human lung and other tissues including the prostate, peripheral blood cells (lymphocytes, monocytes), mammary gland, pancreas, thymus, small intestine, colon and uterus, but not in the liver, in adults (Shimada *et al.*, 1996; Ding & Kaminsky, 2003); it is also expressed in fetal liver (Kitada *et al.*, 1991). CYP1A1 is induced by PAHs, polyhalogenated biphenyls and polyhalogenated dibenzofurans and dioxins such as 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), through the AhR (Pelkonen & Nebert, 1982; Gonzalez *et al.*, 1991; Hankinson, 1995). In this paradigm, PAHs induce their own metabolism.

(iv) *CYP1A2 and activation of PAHs*

CYP1A2 is mainly expressed in the liver (Shimada *et al.*, 1994). It is regulated in part by AhR and is induced by AhR ligands, such as PAHs and TCDD (Hankinson, 1995; Sogawa *et al.*, 2004). Tobacco-smoke condensates and broiled foods, such as grilled meat and fish, induce CYP1A2 (Jaiswal *et al.*, 1987; Gonzalez, 1988; Guengerich & Shimada, 1991). The levels of CYP1A2 protein are ~10–15% that of total CYP in human adult livers on average, and the expression levels vary about 40-fold among individuals (Shimada *et al.*, 1994).

CYP1A2 catalyses the metabolic activation of a variety of aryl and heterocyclic amines to reactive metabolites (Shimada & Okuda, 1988; Shimada *et al.*, 1989a,b; Shimada & Guengerich, 1991). The procarcinogens that are activated by CYP1A2 include 2-aminoanthracene, 2-acetylaminofluorene, 2-aminofluorene, 6-aminochrysene and other aromatic amines. CYP1A2 catalyses the activation of PAH diols to reactive metabolites, although at slower rates than CYP1A1 and CYP1B1 (Shimada *et al.*, 2001a).

CYP1A2 also catalyses the oxidation of several xenobiotic compounds, including acetaminophen, antipyrine, caffeine, 7-ethoxyresorufin, lidocaine, phenacetin, theophylline and *R*-warfarin (Guengerich & Shimada, 1991; Shimada *et al.*, 1994, 1997a).

(v) *CYP1B1 and activation of PAHs*

In 1990, mouse CYP1B1 was purified from C3H/10T1/2 CL8 cells derived from an embryonic fibroblast and the purified enzyme was found to metabolize 7,12-dimethylbenz[*a*]anthracene (DMBA) (Pottenger & Jefcoate, 1990; Pottenger *et al.*, 1991). Spectral interaction studies suggested that purified mouse CYP1B1 can interact with several PAHs, such as benzo[*a*]pyrene, benz[*a*]anthracene, DMBA, 3-methylcholanthrene and 1-ethynylpyrene (Savas *et al.*, 1994, 1997). Human *CYP1B1* cDNA was isolated and was introduced into a yeast expression vector (Sutter *et al.*, 1991, 1994; Shimada *et al.*, 1996). Yeast microsomes that contained the expressed CYP1B1 protein activated diverse procarcinogens including PAHs, aryl and heterocyclic amines and nitroarenes to mutagenic products as determined by an *umu* gene expression system in the *Salmonella typhimurium* NM2009 tester strain (Shimada *et al.*, 1996; Guengerich & Shimada, 1998; Shimada *et al.*, 1998a, 2001a). A close resemblance was found in the substrate specificities of CYP1A1 and CYP1B1 towards various procarcinogens and promutagens, particularly when dihydrodiol derivatives of PAHs were used as substrates (Shimada *et al.*, 1998a, 2001a). Other human CYPs such as 2C9, 2C19 and 3A4 were all relatively weak at activating these PAH compounds.

Buters *et al.* (1999) reported that cultured embryonic fibroblasts isolated from CYP1B1-null mice were unable to metabolize DMBA and were resistant to its cytotoxic and carcinogenic effects. Similarly, recombinant human CYP1A1 and 1B1 were reported to differ in their regio- and stereochemical selectivity for the activation of dibenzo[*a,l*]pyrene; CYP1B1 was reported to play a more important role than CYP1A1 in the formation of fjord region dibenzo[*a,l*]pyrene-11,12-diol-13,14-epoxides (Luch *et al.*, 1997, 1999a). In support of these findings, Buters *et al*. (2002) reported that dibenzo[*a,l*]-pyrene does not produce any type of malignant or benign tumour in the ovaries or lymphoid tissues of *CYP1B1*-null mice. CYP1B1 is therefore thought to play a key role in the metabolic activation of the two prototype carcinogens, dibenzo[*a,l*]pyrene and DMBA, to toxic and carcinogenic metabolites.

Shimada *et al*. (1999b) reported that CYP1B1, together with epoxide hydrolase, catalyses the conversion of benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol at much higher rates that CYP1A1. The catalytic activity of CYP1B1 for the formation of benzo[*a*]-pyrene-7,8-diol was about 10-fold higher than that of CYP1A1. Other human CYPs (including CYP1A2, 2E1 and 3A4) had very low or undetectable activities for the formation of benzo[*a*]pyrene-7,8-diol.

Native human CYP1B1 protein has not yet been isolated from human tissue samples; the enzyme is predominantly expressed in extrahepatic organs, probably at low levels (Sutter *et al.*, 1994; Shimada *et al.*, 1996). A human CYP1B1 cDNA clone was isolated from a human cDNA library and characterized (Sutter *et al.*, 1991, 1994; Tang *et al*.,

1996). The isolated clone that maps to chromosome 2 at 2p21-22 contained a 5.1-kb sequence and a single open-reading frame of 1629 base pairs that predicts a protein of 543 amino acids (Sutter *et al.*, 1994; Shimada *et al.*, 1996; Tang *et al*., 1996). The similarity of amino acid sequence of human CYP1B1 to that of rat and mouse CYP1B1 enzymes was determined to be 80% and 81%, respectively, and that of CYP1A1 and CYP1A2 was reported to be ~40% in three animal species examined (Otto *et al.*, 1991; Savas *et al.*, 1994; Sutter *et al.*, 1994; Walker *et al.*, 1995).

In adults, CYP1B1 mRNA is expressed at significant levels in the mammary gland, prostate, uterus, kidney, adrenal and pituitary glands, ovary, colon, thymus, spleen, lung, small intestine and heart (Shimada *et al.*, 1996; Spink *et al.*, 1998). In human fetal tissues, CYP1B1 mRNA is also detected in the heart, brain, lung and kidney (Shimada *et al.*, 1996). CYP1B1 is expressed constitutively in several types of human cell, including mammary epithelial cells, mammary stromal fibroblasts, broncho-alveolar cells, lymphocytes, bone-marrow stromal cells and monocytes and macrophages (Shimada *et al.*, 1996; Baron *et al.*, 1998; Spink *et al.*, 1998; Spencer *et al.*, 1999; Shimada, 2000; Shimada & Fujii-Kuriyama, 2004). Human CYP1B1 is also reported to be expressed significantly in several tumours including breast cancer cells, uterine myoma and renal adenocarcinoma cells (Liehr *et al.*, 1995; Huang *et al.*, 1996; Spink *et al.*, 1997; Baron *et al.*, 1998; Spink *et al.*, 1998; Spencer *et al.*, 1999).

Human CYP1B1 plays an important role in the activation of diverse procarcinogens (Shimada *et al.*, 1996; Shimada, 2000; Toide *et al.*, 2003; Shimada & Fujii-Kuriyama, 2004). It catalyses the monoxygenation of such procarcinogens as PAHs and their dihydrodiol derivatives, heterocyclic and aryl amines and aminoazo dyes, and nitroaromatic hydrocarbons. These include benzo[*a*]pyrene, benzo[*a*]pyrene-4,5-diol, (+)-benzo[*a*]pyrene-7,8-diol, (–)-benzo[*a*]pyrene-7,8-diol, dibenzo[*a,l*]pyrene, dibenzo[*a,l*]-pyrene-11,12-diol, benz[*a*]anthracene, benz[*a*]anthracene-3,4-diol, benzo[*g*]chrysene-11,12-diol, benzo[*b*]fluoranthene-9,10-diol, benzo[*c*]phenanthrene-3,4-diol, chrysene-1,2-diol, dibenzo[*a,l*]pyrene-11,12-diol, 7,12-dimethylbenz[*a*]anthracene-3,4-diol, 5,6-dimethyl-chrysene-1,2-diol, 5-methylchrysene, 5-methylchrysene-1,2-diol, 6-aminochrysene-1,2-diol, 2-amino-3-methylimidazo[4,5-*f*]quinoline, 2-amino-3,4-dimethylimidazo[4,5-*f*],-quinoline, 2-amino-3,8-dimethylimidazo[4,5*f*]quinoxaline, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]-indole, 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine, 2-aminoanthracene, 2-amino-fluorene, 4-aminobiphenyl, 6-aminochrysene, 2-aminofluorene, 3-methoxy-4-amino-azobenzene, 2-nitropyrene and 6-nitrochrysene (Shimada *et al.*, 1996; Shimada, 2000; Shimada & Fujii-Kuriyama, 2004).

(vi) *CYP2C9 and activation of PAHs*

Other CYPs (CYP2C9 and CYP3A4) play minor roles in PAH metabolism. CYP2C9 is mainly located in the liver and the levels of its expression are estimated to account for an average of 20% of total CYP in the liver (Shimada *et al.*, 1994). It catalyses the metabolism of clinically used drugs such as flurbiprofen, phenytoin, tolbutamide and warfarin (Shimada *et al.*, 1994; Yamazaki & Shimada, 1997; Yamazaki *et al.*, 1997,

1998a). CYP2C9 also mediates 3-hydroxylation of benzo[*a*]pyrene and the metabolic activation of several PAH diols to active metabolites that induce *umu* gene expression in *Salmonella* tester strains (Yun *et al.*, 1992; Shimada *et al.*, 2001a). These activities, however, are lower than those induced by CYP1 enzymes (Shimada *et al.*, 2001a).

(vii) *CYP3A4 and activation of PAHs*

CYP3A4 is the most abundant CYP in human liver, and the levels of its expression in adults average about 30% of total CYP (Guengerich *et al.*, 1986; Shimada *et al.*, 1994). The enzyme is induced by various barbiturates, antibiotics and macrolide antibiotics such as rifampicin and troleandomycin (Gonzalez, 1988; Guengerich & Shimada, 1991; Wilkinson, 1996; Guengerich, 1999). CYP3A4 metabolizes about 50% of clinically used drugs (Guengerich *et al.*, 1986; Wilkinson, 1996; Guengerich, 1999). Procarcinogens activated by CYP3A4 include aflatoxin B_1, aflatoxin G_1, sterigmatocystin, benzo-[*a*]pyrene-7,8-diol and other PAH diols, and 6-aminochrysene (Shimada & Guengerich, 1989; Shimada *et al.*, 1989a; Guengerich & Shimada, 1991).

(viii) *Induction of CYP1A1, 1A2 and 1B1 by PAHs*

The AhR regulates the induction of CYP1A1, CYP1A2 and CYP1B1 and other drug-metabolizing enzymes by carcinogenic PAHs and other chemicals and plays an important role in the toxicity and carcinogenesis of these chemicals (Nebert, 1978, 1980; Gonzalez, 1990; Okey *et al.*, 1994; Hankinson, 1995; Mimura & Fujii-Kuriyama, 2003). Planar PAHs bind to the AhR in cytosol, which dissociates from chaperone proteins and heterodimerizes with AhR nuclear translocator (ARNT) in the nucleus. The ligand-occupied heterodimer then binds to the xenbiotic response elements of the *CYP1A1*, *CYP1A2* and *CYP1B1* genes to cause an increase in gene transcription. Genetically engineered $AhR^{-/-}$ mice are resistant to TCDD-induced teratogenesis (Mimura *et al.*, 1999; Mimura & Fujii-Kuriyama, 2003) and benzo[*a*]pyrene-induced carcinogenesis (Shimizu *et al.*, 2000). Mechanisms that underly the resistance of these animals might be due to their inability to express significant levels of CYP and other enzymes that play major roles in the activation of xenobiotic and endobiotic chemicals (Shimizu *et al.*, 2000; Mimura & Fujii-Kuriyama, 2003). The levels of expression of CYP1A1 and 1B1 are of particular interest, since these CYPs principally catalyse the activation of carcinogenic PAHs to reactive metabolites that initiate cancer (Shimada, 2000; Williams & Phillips, 2000; Murray *et al.*, 2001; Shimada & Fujii-Kuriyama, 2004).

Recently, potent carcinogenic PAHs, such as benzo[*a*]pyrene, DMBA, dibenzo[*a,l*]-pyrene, 3-methylcholanthrene, 1,2,5,6-dibenz[*a,h*]anthracene benzo[*b*]fluoranthene and benz[*a*]anthracene, have been shown to induce liver and lung CYP1A1 and 1B1 mRNAs significantly in $AhR^{+/+}$ mice but not in $AhR^{-/-}$ mice (Shimada *et al.*, 2002). Using male and female $AhR^{+/+}$ and $AhR^{-/-}$ C57BL/6J mice, Shimada *et al*. (2003a) found tissue-specific induction of CYP1A1 and 1B1 mRNAs by PAHs, 3,4,3′,4′-tetrachlorobiphenyl and the polychlorinated biphenyl mixtures, Kanechlor-300, -400 and -500. In both male and female mice, CYP1A1 mRNA was found to be constitutively expressed at very low

levels in various organs of $AhR^{+/+}$ and $AhR^{-/-}$ mice and was induced in various organs by PAHs and 3,4,3′,4′-tetrachlorobiphenyl only in $AhR^{+/+}$ mice. In contrast, CYP1B1 mRNA was expressed constitutively at significant levels in various organs, except for liver and lung, in both male and female $AhR^{+/+}$ and $AhR^{-/-}$ mice. Levels of induction of CYP1B1 by PAHs and tetrachlorobiphenyl in $AhR^{+/+}$ mice were not so significant in organs in which the constitutive levels of CYP1B1 were high.

The extents of induction of hepatic mRNAs of CYP1A1, 1A2 and 1B1 were also determined in $AhR^{+/+}$ mice that had been treated intraperitoneally with each of 23 PAHs or 3,4,3′,4′-tetrachlorobiphenyl at a dose level of 100 mg/kg bw (Shimada *et al.*, 2003b). Both CYP1A1 and 1B1 were highly induced by the PAHs that are potent carcinogens in experimental animals (Conney, 1982; Pelkonen & Nebert, 1982; Shimada *et al.*, 2002, 2003a). These PAHs include benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*a*]pyrene, 3-methylcholanthrene, dibenz[*a,h*]anthracene, benz[*a*]anthracene and dibenzo[*a,l*]pyrene. Other PAHs, such as dibenz[*a,j*]acridine, dibenz[*a,c*]anthracene, DMBA, 5-methylchrysene, 6-aminochrysene, chrysene and dibenz[*a,e*]pyrene, also induced mRNAs of liver CYP1A1 and 1B1. CYP1A2 was constitutively expressed in the livers of the mice but was induced to a much lesser extent than CYP1A1 and CYP1B1. Liver microsomal 7-ethoxyresorufin *O*-deethylation activities were well correlated with levels of liver CYP1A1 and 1B1 mRNAs in $AhR^{+/+}$ mice.

(ix) *Inhibitors of CYP1A1, 1A2 and 1B1*

Since most of the carcinogenic PAHs require metabolic activation by CYP and other enzymes to evoke their carcinogenic potentials, chemical inhibitors of CYP and other enzymes may potentially act as chemopreventive agents (Cai *et al.*, 1997; Shimada *et al.*, 1997b, 1998b; Chun *et al.*, 1999; Shimada, 2000; Chun *et al.*, 2001; Chun & Kim, 2003; Guengerich, 2003).

Some of these inhibitors of CYP1A1 and 1B1 suppress the tumorigenesis that is induced by a variety of chemical carcinogens (Gelboin *et al.*, 1970; Kinoshita & Gelboin, 1972; Slaga *et al.*, 1977a; El-Bayoumy *et al.*, 1992; Jang *et al.*, 1997; Kleiner *et al.*, 2002, 2003; El-Bayoumy & Sinha, 2004). In a rat mammary tumour model using DMBA, *para*-1,4-phenylene-bis(methylene)selenocyanate [also called xyleneselenocyanate] has been shown to have chemopreventive activity (El-Bayoumy *et al.*, 1992; Ip *et al.*, 1994; Prokopczyk *et al.*, 2000) and to inhibit the formation of DMBA–DNA adducts in the rat mammary gland (El-Bayoumy *et al.* 1992). The synthetic organoselenium compounds were found to be potent inhibitors of human CYP1A1 and CYP1B1, and were selective for CYP1B1 (Shimada *et al.*, 1997b). Similarly, 1-ethynylpyrene inhibits covalent binding of DMBA and benzo[*a*]pyrene to epidermal DNA (Viaje *et al.*, 1990) and prevents tumour formation caused by DMBA and benzo[*a*]pyrene in mouse skin (Alworth *et al.*, 1991); 1-ethynylpyrene inhibited CYP1B1 to a greater extent than CYP1A1 (Shimada *et al.*, 1998b). Resveratrol, which is found in red grapes and has been shown to have cancer preventive activity, is a potent inhibitor of human CYP1A1 and 1B1 (Jang *et al.*, 1997; Chun *et al.*, 1999; Potter *et al.*, 2002).

(x) *Epoxide hydrolase*

Microsomal epoxide hydrolase catalyses the hydrolysis of various epoxides and reactive epoxide intermediates of numerous endobiotic and xenobiotic chemicals into less reactive and more polar dihydrodiols (Omiecinski *et al.*, 2000; Hosagrahara *et al.*, 2004). Although it is mainly known to be a protective enzyme by decomposing numerous epoxide intermediates, it also mediates activation of PAHs to highly reactive diol epoxides, in conjunction with CYPs (Conney, 1982; Gonzalez *et al.*, 1991; Hecht, 2002a). Thus, microsomal epoxide hydrolase plays a role in both the inactivation and activation of xenobiotic chemicals (Lu & West, 1979; Pelkonen & Nebert, 1982).

(xi) *Glutathione* S-*transferases*

GSTs are constitutively expressed in a wide variety of mammalian tissues. Characteristic patterns of *GST* genes have a superfamily of at least 16 genes that consist of six subfamilies, named *alpha* (*GSTA*), *mu* (*GSTM*), *omega* (*GSTO*), *pi* (*GSTP*), *theta* (*GSTT*) and *zeta* (*GSTZ*) (Williams & Phillips, 2000; Strange *et al.*, 2001; Daly, 2003). GSTs encoded by polymorphic members of the *mu* (*GSTM1*), *pi* (*GSTP1*) and *theta* (*GSTT1*) gene families play important roles in the detoxication of a variety of reactive toxic and carcinogenic compounds, including PAH epoxides and diol epoxides (Hecht, 2002a). Isoforms involved in the formation of glutathionyl conjugates with PAH diol epoxides have been assigned (Jernström *et al.*, 1996; Sundberg *et al.*, 1998, 2002) and their rank order of importance based on catalytic efficiency (catalytic constant (k_{cat})/Michaelis constant (K_m)) varies by PAH diol epoxide and individual stereochemistry. For example, for the conjugation of the (–)-*anti*-diol epoxide dibenzo[*a*,*l*]pyrene (*R*-configuration at the benzylic oxirane carbon in the fjord-region), the preference is GSTA1-1 > GSTM1-1 > GSTP1-1. In contrast, the rank order of catalytic efficiency for the conjugation of (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide is GSTP1-1 > GSTM1-1 > GSTA1-1.

(xii) *Uridine 5′-diphosphate-glucuronosyltransferase*

UGTs catalyse the glucuronidation of a variety of endogenous and exogenous compounds to more polar metabolites that are excreted from the body (Hecht, 2002b; Daly, 2003). Glucuronidation is a major pathway of detoxification of numerous carcinogens such as PAHs and aryl and heterocyclic amines (Hecht, 2002a). The UGT1A family is the most relevant for PAH diol conjugation. Using the (+)-7*S*,8*S*-*trans*-dihydrodiol and the (–)7*R*,8*R*-*trans*-dihydrodiol of benzo[*a*]pyrene, the liver UGT1A1 and UGT2B7 isoforms conjugated the 7*S*,8*S*-enantiomer to form the 7*S*-glucuronide as the major diastereomer, while UGT1A8 and UGT1A10, the extrahepatic enzymes present in the aerodigestive tract, preferentially conjugated the 7*R*,8*R*-enantiomer to form the 7*R*-glucuronide and 8*R*-glucuronide (Fang *et al.*, 2002). The K_m values of individual UGT enzymes, which were taken to reflect affinity, gave the following rank order for the conjugation of (–)-7*R*,8*R*-dihydroxy-7,8-dihydrobenzo[*a*]pyrene: UGT1A10 > UGT1A9 > UGT1A1 > UGT1A7; in contrast, the K_m values of individual UGT enzymes gave the

following rank order for the conjugation of (+)-7*S*,8*S*-dihydroxy-7,8-dihydrobenzo[*a*]pyrene: UGT1A10 > UGT1A9 > UGT2B7 ≈ UGT1A1 > UGT1A7 (Fang *et al.*, 2002).

(xiii) *Sulfotransferases*

Cytosolic SULTs catalyse the sulfonation of a variety of carcinogens including PAHs (Hecht, 2002a; Moreno *et al.*, 2005). Sulfonation is generally thought to be a detoxication process; however, the enzyme also activates certain promutagens, e.g. 5-methylchrysene (Blanchard *et al.*, 2004), DMBA, cyclopenta[*c,d,*]pyrene and benzo[*a*]pyrene (Watabe *et al.*, 1982; Surh *et al.*, 1987, 1993; Surh & Tannenbaum, 1995) by a mechanism that involves the conjugation of benzylic alcohols. Six cytosolic SULTs are known to be expressed in humans, including phenol SULTs, hydroxy SULTs and estrogen SULTs (Raftogianis *et al.*, 1997; Daly, 2003; Moreno *et al.*, 2005). Phenol SULTs include SULT1A1, SULT1A2 and SULT1A3. SULT1A1 is highly expressed in numerous organs and catalyses the sulfonation of xenobiotic chemicals including PAHs and endogenous compounds (Raftogianis *et al*, 1997; Moreno *et al.*, 2005). SULT2A is the hydroxy SULT most heavily implicated in the activation of benzylic alcohols (Blanchard *et al*., 2004).

(xiv) *Aldo-keto reductases*

AKRs, the soluble NAD(P)H-dependent oxidoreductases, comprise a gene superfamily consisting of 14 families with 114 members among prokaryotes and eukaryotes (Penning, 2004; http://www.med.upenn.edu/akr). The AKR1 family contains several human enzymes, including the AKR1As (aldehyde reductases), AKR1Bs (aldose reductases), AKR1Cs (hydroxysteroid/dihydrodiol dehydrogenases) and AKR1Ds (steroid 5β-reductases) (Hyndman *et al.*, 2003). Human AKRs have been shown to be involved in the metabolism of PAHs (Palackal *et al*., 2001b, 2002a; Jiang *et al.*, 2005a). The enzymes have dihydrodiol dehydrogenation activities and can convert PAH dihydrodiols to reactive and redox-active *ortho*-quinones, which are key intermediates in one proposed mechanism of PAH activation. Such reactions are principally catalysed by human AKR1A1, 1C1, 1C2, 1C3 and 1C4 (Burczynski *et al*., 1998; Palackal *et al*., 2001b, 2002a).

Differences exist in the specificity of AKR1 enzymes for PAH *trans*-dihydrodiols. For example, AKR1A1 preferentially oxidizes the stereospecific oxidation of (–)-benzo-[*a*]pyrene-7*R*,8*R*-dihydrodiol whereas the AKR1C isoforms oxidize both stereoisomers of PAH *trans*-dihydrodiol mixtures and have a preference for methylated PAH *trans*-dihydrodiols (Palackal *et al*., 2001b).

AKR1C1 is induced by bi- and monofunctional inducers and by reactive oxygen species consistent with regulation by an anti-oxidant response element (Burczynski *et al.*, 1999a). In addition, PAH *ortho*-quinone products are ligands for the AhR and can induce CYP1A1 in human hepatoma HepG2 cells (Burczysnki & Penning, 2000).

AKR1C1 enzymes are also potently inhibited by non-steroidal anti-inflammatory drugs and may exert some of their chemopreventive properties by inhibition at this level (Penning & Talalay, 1983).

(xv) *NAD(P)H quinone oxidoreductase 1*

NQO1, formerly referred to as DT-diaphorase, catalyses two-electron reduction of quinones (Ross & Siegel, 2004). This conversion is important in detoxification since the resulting hydroquinone is eliminated as a sulfate or glucuronide conjugate; in addition, one electron-reduction of the quinone to the highly reactive semiquinone anion radical mediated by P450-NADPH oxidoreductases is avoided. Paradoxically, if the hydroquinone is not conjugated, it can be oxidized back to the quinone-generating reactive oxygen species, which would be a toxification event. For example, NQO1 converts benzene-derived quinones to less active hydroquinones and is considered to affect benzene-induced haematotoxicity (Ross, 2000; Ross & Siegel, 2004). However, certain quinones that are used to treat tumours, such as mitomycin C and streptonigrin, are activated by NQO1 to more toxic metabolites (Siegel *et al*., 1990; Beall *et al*., 1996; Ross & Siegel, 2004). Using benzo[*a*]pyrene as a representative PAH, extended dicarbonyls are produced, e.g. the 1,6-, 3,6- and 6,12-diones, from CYP isoforms. In addition, PAH *ortho*-quinones are formed from the oxidation of non-K region *trans*-dihydrodiols, e.g. benzo[*a*]pyrene-7,8-dione, by AKRs. Of these, only the extended diones are substrates for NQO1 (Flowers-Geary *et al*., 1992). NQO1 has been shown to prevent the formation of PAH quinone–DNA adducts (Joseph & Jaiswal, 1994) and NQO1-deficient mice show an increased incidence of skin cancer when exposed to benzo[*a*]pyrene and DMBA (Long *et al*., 2000, 2001). The human enzyme is induced by bi-functional (PAHs and chlorinated dibenzodioxins) and monofunctional inducers (Dinkova-Kostova *et al*., 2004; Ross & Siegel, 2004).

(b) Genetic variability in PAH-metabolizing enzymes

All of the genes involved in PAH activation/detoxification are polymorphic and may define individual susceptibility to exposure to PAHs. Polymorphisms can exist in the promoter regions, introns and exons of a given gene and therefore can effect transcription, mRNA processing, functional activity or enzyme stability, respectively. Many of the polymorphisms (gene deletion, multiple point mutation, single nucleotide variation, with an incidence > 1% in the population) have been studied in isolation, yet end-points of exposure to PAHs such as metabolic profile, DNA adducts (type and amount) and tumour incidence may result from multigene interactions. Relating a single nucleotide polymorphism within a single gene to these end-points can be problematic unless it is considered that complex *gene x gene x gene x PAH exposure* paradigms exist. The importance of single nucleotide polymorphisms exceeds the enzymes listed in this section and could include those involved in DNA-adduct repair as well as those involved in DNA-lesion by-pass. In addition, the frequency of allelic variation and its distribution within the human population should be considered. This section is limited to a discussion of the polymorphisms that have been identified in individual PAH-metabolizing enzymes. When these polymorphisms have been used to determine correlations between single

nucleotide polymorphism and incidence of disease in a human population, these studies are described in a later section (Section 4.3).

(i) *Genetic variability of* CYP1A1

The *CYP1A1* gene has seven exons and the cDNA is about 70% identical to that of *CYP1A2* (Jaiswal *et al.*, 1985, 1987).

At least 11 alleles of *CYP1A1*, including wild-type *CYP1A1*1A*, have been identified (see http://www.imm.ki.se/CYPalleles; see GeneAtlas website). *CYP1A1*2B*, **2C*, **3*, **4*, **5*, **6*, **7*, **8*, **9*, **10* and **11* showed amino acid changes (Nagata & Yamazoe, 2002). The extent to which these amino acid changes cause alterations in catalytic activities for the oxidation of xenobiotics, including PAHs, is still unknown. For example, a variant (Ile462Val) of CYP1A1 did not show any major differences in catalytic specificities towards various substrates when compared with the wild-type enzyme CYP1A1*1 (Zhang *et al.*, 1996; Persson *et al.*, 1997).

Two mutually linked polymorphisms of the human *CYP1A1* gene, the *MspI* polymorphism (**2A*) located in the 3'-flanking region of the gene and the *Ile-Val* polymorphism (**2B*) at amino acid residue 462 in the haeme-binding region, were reported to be associated with susceptibility to tobacco smoking-associated squamous-cell carcinoma of the lung in Japanese populations (Hayashi *et al.*, 1991; Kawajiri & Fujii-Kuriyama, 1991; Nakachi *et al.*, 1993; Kawajiri *et al.*, 1996; Masson *et al.*, 2005). However, this association has not been found in other ethnic groups (Tefre *et al.*, 1991; Hirvonen *et al.*, 1992). Other studies on the role of genetic polymorphisms of *CYP1A1* in cancer susceptibility in humans are discussed in Section 4.3.1. In many cases, race-related differences have been reported in the occurrence of genetic polymorphisms in *CYP1A1* (Kiyohara *et al.*, 2002a).

(ii) *Genetic variability of* CYP1A2

At least 24 genetic polymorphisms of *CYP1A2* have been identified (see http://www.imm.ki.se/CYPalleles). The *CYP1A2*7* allele contains a splicing defect at the site of intron 6 (G3534A) (Allorge *et al.*, 2003). This mutation was found in a 71-year-old, nonsmoking, Caucasian woman who showed very high concentrations of clozapine, a substrate of CYP1A2, when she was treated with a standard dose (Allorge *et al.*, 2003). *CYP1A2*1C* has been shown to decrease the clearance of caffeine in Japanese smokers *in vivo* (Nakajima *et al.*, 1999). Allele frequency in 116 Japanese showed 0.77 and 0.23 for the wild-type and variant *CYP1A2* alleles, respectively. Another study showed that subjects with *CYP1A2*1K* (-T739G, -C729T and -C163A) had significantly decreased CYP1A2 activity *in vivo* as determined by the caffeine clearance test (Aklillu *et al.*, 2003).

Alleles *CYP1A2*2*, **3*, **4*, **5*, **6*, **8*, **9*, **10*, **11*, **12*, **13*, **14*, **15* and **16* show amino acid changes (Nakajima *et al.*, 1999; Nagata & Yamazoe, 2002). In-vitro studies revealed that a Phe186Leu (*CYP1A2*11*) variant caused a decrease in enzymatic activity

toward 7-ethoxyresorufin *O*-deethylation (Murayama *et al.*, 2004). Little is known about the effects of other mutations of *CYP1A2* on its catalytic properties (Chida *et al*., 1999).

(iii) *Genetic variability of* CYP1B1

Several genetic polymorphisms occur in the human *CYP1B1* gene (see http:www.imm.ki.se/CYPalleles). At least 23 variant forms of *CYP1B1* exist in humans, 17 of which show amino acid changes (Nagata & Yamazoe, 2002). Null alleles of *CYP1B1* are reported to relate to hereditary glaucoma (Stoilov *et al.*, 1997, 1998). To determine the catalytic alterations due to mutations, *CYP1B1* variants with amino acid changes at 48 (Arg48Gly), 119 (Ala119Ser), 432 (Leu432Val) and 453 (Asn453Ser) have been studied extensively in different laboratories (Stoilov *et al.*, 1997; Bailey *et al.*, 1998; Fritsche *et al.*, 1999; Shimada *et al.*, 1999a; Bejjani *et al.*, 2000; Watanabe *et al.*, 2000; Shimada *et al.*, 2001b). 17β-Estradiol 4-hydroxylation activities have been measured in different protein expression systems. Although there are some differences in catalytic activities in the variant forms of *CYP1B1*, it is not clear whether these mutations cause major alterations in the catalytic activities of variant *CYP1B1s* (Stoilov *et al.*, 1997; Bailey *et al.*, 1998; Fritsche *et al.*, 1999; Bejjani *et al.*, 2000; Watanabe *et al.*, 2000; Aklillu *et al.*, 2002). For example, the maximum velocity (V_{max})/K_m ratio of 17β-estradiol 4-hydroxylation activities in eight *CYP1B1* variants with combinations of amino acid changes at 48, 119, 432 and 453 changed only from 0.12 to 0.25 $\mu M^{-1}min^{-1}$ (Shimada *et al.*, 2001b).

Shimada *et al*. (2001a) examined the catalytic specificities of four enzymes coded by *CYP1B1* variants, *CYP1B1*1* (wild-type, $Arg^{48}Ala^{119}Leu^{432}Asn^{453}$), *CYP1B1*2* ($Gly^{48}Ser^{119}Leu^{432}Asn^{453}$), *CYP1B1*3* ($Arg^{48}Ala^{119}Val^{432}Asn^{453}$) and *CYP1B1*6* ($Gly^{48}Ser^{119}Val^{432}Asn^{453}$) in the activation of PAHs, PAH diols and 9-hydroxybenzo[*a*]pyrene. Essentially similar catalytic specificities were determined in four *CYP1B1* variants when (+)-, (–)- and (±)-benzo[*a*]pyrene-7,8-diol, 5-methylchrysene-1,2-diol, 7,12-DMBA-3,4-diol, dibenzo[*a,l*]pyrene-11,12-diol, benzo[*b*]fluoranthene-9,10-diol, benzo[*c*]chrysene, 5,6-dimethylchrysene-1,2-diol, benzo[*c*]phenanthrene-3,4-diol, 7,12-DMBA, benzo[*a*]-pyrene, 5-methylchrysene and benz[*a*]anthracene were used as substrates.

(iv) *Genetic variability of* CYP2C9

Genetic polymorphisms of *CYP2C9* have been reported and more than 24 alleles have been identified (see http://www.imm.ki.se/CYPalleles) (Yamazaki *et al.*, 1998a,b; Nagata & Yamazoe, 2002). Alleles *CYP2C9*2*, **3*, **4* and **5* lead to amino acid replacements, and these mutations have been shown to decrease catalytic activities *in vitro* (Rettie *et al.*, 1994; Haining *et al.*, 1996; Sullivan-Klose *et al.*, 1996; Dickmann *et al.*, 2001; Kidd *et al.*, 2001; Takahashi *et al.*, 2004). *CYP2C9*3* is reported to lead to lower activities *in vivo* (Sullivan-Klose *et al.*, 1996; Shintani *et al.*, 2001; Takahashi *et al.*, 2004). It is not known whether these genetic polymorphisms are related to cancer susceptibility in humans (Garcia-Martin *et al.*, 2002a).

(v) *Genetic variability of* CYP3A4

More than 18 genetic polymorphisms of *CYP3A4* have been identified (see http://www.imm.ki.se/CYPalleles) (Nagata & Yamazoe, 2002). However, it is not known to what extent these mutations affect catalytic activities, except that CYP3A4*18 has higher activities and CYP3A4*17 has lower activities *in vitro* (Dai *et al.*, 2001). The significance of these polymorphisms for cancer susceptibility remains unclear.

(vi) *Genetic variability of epoxide hydrolase*

Two genetic polymorphisms of the *mEH* gene that encodes microsomal epoxide hydrolase have been reported to affect its catalytic activities (Omiecinski *et al.*, 2000; Kiyohara *et al.*, 2002a; Gsur *et al.*, 2003; Hosagrahara *et al.*, 2004). The *Tyr113His* variant (*EH3*) was found in exon 3 and caused a decrease in catalytic activities. The other *His139Arg (EH4)* variant in exon 4 is reported to have high catalytic activity compared with that of the wild-type enzyme (Hassett *et al.*, 1994; Kiyohara *et al.*, 2002a; Zhao *et al.*, 2002; Huang *et al.*, 2005; Park, J.Y. *et al.*, 2005). However one study showed that the specific activity of microsomal epoxide hydrolase was similar for each variant (Hassett *et al.*, 1994). The structural differences encoded by the *Tyr113His* and *His139Arg* variants have been reported to exert only a modest impact on the activities of microsomal epoxide hydrolase, using *cis*-stilbene oxide and benzo[*a*]pyrene-4,5-oxide as substrates (Hassett *et al.*, 1994; Omiecinski *et al.*, 2000; Hosagrahara *et al.*, 2004).

(vii) *Genetic variability of glutathione S-transferase*

The levels of individual *GST*s may be influenced by induction and by genetic polymorphism. The roles of *GSTM*, *GSTT1* and *GSTP1* polymorphisms have been reported to be important in determining cancer susceptibilities in humans (Williams & Phillips, 2000; Kiyohara *et al.*, 2002a).

GSTM1 amd *GSTT1* are polymorphic due to large deletions in the structural gene. Meta-analysis of 12 case–control studies showed a significant association between the homozygous deletion and an increased risk for lung cancer (Hengstler *et al.*, 1998).

The *GST-Pi* gene has allelic variants that give rise to two different encoded proteins with isoleucine (GSTP1-1/Ile-105) or valine (GSTP1-1/Val-105) at position 105. The latter was threefold more active in catalysing conjugation reactions with *anti*-diol epoxides of the *R*-absolute configuration at the benzylic oxirane carbon than the variant with isoleucine. Individuals with the Val-105 allele show a higher susceptibility to maligancy, which indicates that this allele cannot account for the increase (Sundberg *et al.*, 1998).

(viii) *Genetic variability of uridine 5′-diphosphate glucuronosyltransferase*

The *UGT* superfamily consists of two families, *UGT1* and *UGT2* (Guillemette *et al.*, 2000a,b; Mackenzie *et al.*, 2005). *UGT1* contains five exons and has a unique gene structure in which there are 13 individual promoters/first exons, from *UGT1A1* to

UGT1A13P, and exons 2–5 are used in common in all mRNAs (Guillemette *et al.*, 2000b; Gong *et al.*, 2001; Jinno *et al.*, 2003). Since all members of this family (*UGT1A1–UGT1A13*) share exons 2–5, the resulting isoforms are identical in their last 245 amino acids. The full-length proteins are 530 amino acids in length and transcripts are formed by the splicing of alternative exons (1.1–1.13). The *UGT2* subfamily consists of six members. UGT1 catalyses the conjugation of many drugs and carcinogens as well as bilirubin and steroids, and genetic polymorphisms in exon 1 of this gene family have been reported (Guillemette *et al.*, 2000b; Vogel *et al.*, 2002; Wells *et al.*, 2004).

The UGTs implicated in forming benzo[*a*]pyrene-glucuronide conjugates belong to the UGT1A family. The *UGT1A* variant allele which resides in the TATA box renders the entire *UGT1A* locus inactive. When this allele is present, the production of benzo-[*a*]pyrene glucuronides is dramatically reduced, which leads to elevated levels of DNA adducts. These data support the role of the UGTs in PAH detoxification (Tukey & Starassburg, 2000).

(ix) *Genetic variability of sulfotransferase*

The two sulfotransferases most implicated in PAH metabolism are SULT1A1 (phenol sulfotransferase) which eliminates hydroxybenzo[*a*]pyrenes and SULT2A which conjugates benzylic alcohols of PAHs (e.g. 5-methylchrysene) and leads to their bio-activation (Glatt *et al.*, 2000; Blanchard *et al.*, 2004). Four allelic variants of *SULT1A1* (*SULT1A1*1*, *SULT1A1*2*, *SULT1A1*3* and *SULT1A1*4*) have been reported. The most common single nucleotide polymorphism is *SULT1A1*2* which has a nucleotide change of G to A that causes a change from arginine to histidine at codon 213. This allozyme has lower enzymic activity and thermostability than the wild-type enzyme (Raftogianis *et al.*, 1997; Engelke *et al.*, 2000). An association between genetic polymorphisms of *SULT1A1* and cancer susceptibility has been reported (Dalhoff *et al.*, 2005).

(x) *Genetic variability of aldo-keto reductase*

AKRs implicated in the metabolic activation of PAHs in humans include AKR1A1, and AKR1C1–AKR1C4 (Burczynski *et al.*, 1998; Palackal *et al.*, 2001a, 2002a). Each gene encoding for the enzyme is polymorphic and contains non-synonymous single nucleotide polymorphisms as follows: *AKR1C1* has *Thr38Ala*, *Thr38Ile* and *Ary47His* allelic variants; *AKR1C2* has *Val38Ala*, *Val38Ile*, *Phe46Tyr*, *His47Ary*, *Val111Ala*, A*ry170Pro*, *Ary170Leu*, *Gly176Gln*, *Lys179Glu* and *Glu185Gln* allelic variants; *AKR1C3* has *Gln5His*, *Met175Ile*, *Arg295Ser*, and *Met293Ile* allelic variants; and *AKR1C4* has *Gly135Glu*, *Ser145Cys*, *Arg250Gln* and *Leu311Val* allelic variants (Hyndman *et al.*, 2003; see also http://www.med.upenn.edu./akr). Of these, the variant examined in a population-based study for increased risk for lung cancer was *AKR1C3*Gln5His* (Lan *et al.*, 2004).

(xi) *Genetic variability of NAD(P)H quinone oxidoreductase 1*

A genetic polymorphism of *NQO1*, a C to T transition at nucleotide 609 in the cDNA, which yields a proline to serine substitution in codon 187, is associated with reduced

NQO1 activity (Saldivar *et al.*, 2005). Some reports have examined the relationship between *NOQ1* polymorphism and cancer susceptibilities (see below) (Chen *et al.*, 1999; Xu *et al.*, 2001).

The single nucleotide polymorphism in *NQO1* of most prominence is the *NQO1*2* allele where a C to T transition at position 609 of cDNA results in a Pro187Ser codon change (Saldivar *et al.*, 2005). The *NQO1*2/*2* allele has only 2–4% of the activity of the wild-type protein because of a diminished ability to bind flavin adenine dinucleotide. This allele also leads to poor protein expression due to accelerated degradation. The frequency of the *NQO1*2/*2* allele across ethnic groups ranges from 4% in Caucasians, 5% in African–Americans, 16% in Mexican Hispanics to 22% in Chinese populations (Ross *et al.*, 2000).

A second polymorphism in *NQO1* (*NQO1*3*) has also been characterized. This is a *C456T* change which changes arginine to tryptophan. The affect of this polymorphism on phenotype is substrate-dependent and the frequency of this polymorphism is low (Ross *et al.*, 2000).

4.2 Mechanisms of carcinogenesis

4.2.1 *Bay- and fjord-region PAH diol epoxide and cyclopenta-ring oxidation*

(a) Mechanisms

(i) *Bay- and fjord-region PAH diol epoxides*

The generalized diol epoxide mechanism was developed from the bay-region theory proposed by Jerina *et al.* (1976), based on the earlier observations of the nature of PAH metabolites (see Boyland & Sims, 1964), and results from a quantum mechanical model. This theory recognized that angular benzo ring fusions on PAHs created a topological indentation on the polycyclic ring structure, called the bay region. In benzo[*a*]pyrene, the bay region encompasses four carbons (carbons 10, 10a, 10b and 11) and three carbon–carbon bonds. In the example of benzo[*a*]pyrene, metabolism by the CYP isozymes at the C7–C8 aromatic double bond creates an arene oxide, benzo[*a*]pyrene-7,8-oxide, that disrupts the aromatic nucleus by saturating that carbon–carbon bond. Benzo[*a*]pyrene-7,8-oxide is hydrated by epoxide hydrolase to a 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 an inherent activity to undergo carbon–oxygen bond scission or ring opening to form a carbonium ion on carbon 10 (i.e. a positively charged carbon atom). 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 the carbonium ion delocalization energies ($\Delta E_{deloc}/\beta$) 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 1 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 AKRs on PAH diols. The phase 2 enzymes, UGT, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) SULTs and GST, conjugate PAH diols, phenols and epoxides to glucuronic acid, sulfate and GSH, respectively (see Section 4.1.2).

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 benzo[*a*]anthracene-3,4-oxides (benzo[*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*-benzo[*a*]anthracene diol epoxide — for a total of four benzo[*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 are dependent on species, strain, sex, organ, tissue, type of CYP and phase 2 enzymes.

One of the original tenets of the mechanism for bay-region or fjord-region diol epoxides is that, as the PAH is metabolically activated in sequence through the diol to the diol epoxide, the process creates intermediates that generally possess greater biological activities than their precursors. This effect has been observed for a number of PAHs. An example is benz[*a*]anthracene. One of the *anti*-diol epoxides of benzo[*a*]anthracene (*anti*-benzo[*a*]anthracene-3,4-diol-1,2-oxide) possesses greater tumour-initiating or carcino-

genic activity in mouse skin or mouse lung than its precursor diol (benzo[*a*]anthracene-3,4-diol), which in turn posseses greater activity than the parent PAH, benzo[*a*]anthracene (Levin *et al.*, 1978; Wislocki *et al.*, 1979). While this effect is observed for some PAHs, it is not universal for all PAHs that are metabolized to diols and diol epoxides due to a number of confounding factors (e.g. reactivity with water and biological constituents or cytotoxicity). Also, the formation of a bay-region PAH diol epoxide by itself does not confer carcinogenicity to that PAH, as is the case for phenanthrene; both phenanthrene and its bay-region diol epoxide (*anti*-phenanthrene-1,2-diol-3,4 oxide) are inactive as carcinogens in newborn mice (Buening *et al.*, 1979a).

Bay-region and fjord-region diol epoxides possess many biological activities; one of the most important of these is their ability to form stable covalent adducts with DNA. The nature and sequence specificity of these DNA adducts are 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.*, 1986). 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, 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 (Chramostova *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.*, 1999a), cigarette smokers (Rojas *et al.*, 1995; Lodovici *et al.*, 1998), chimney sweeps (Pavanello *et al.*, 1999a) 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 lung cancer (Smith *et al.*, 2000).

Interpretation of data on DNA adducts requires a full understanding of the strengths and weaknesses of the methods used to identify and quantitate each adduct. An international workshop on genotoxicity test procedures summarized the strengths and weaknesses of test systems that are commonly used to determine DNA adducts (Phillips *et al*., 2000) and discussed the strengths, limitations and sensitivity of three separate methods as follows.

^{32}P-Postlabelling assay

Strengths. ^{32}P-Postlabelling assay is highly sensitive, requires small quantities of DNA (1–10 μg) and can measure DNA adducts of chemicals with diverse structure in a wide variety of tissues.

Limitations. It does not provide structural information directly, is less useful for low-molecular-weight DNA adducts and requires rigorous internal standards to adjust for labelling efficiency and recovery.

Sensitivity. This varies from one to 100 adducts in 10^9 nucleotides.

Immunoassays

Strengths. They are inexpensive and relatively easy to perform, and can be highly sensitive and specific for classes of adducts.

Limitations. This approach requires the production of antibodies, prior knowledge of the adduct being measured and relatively large amounts of DNA for sensitivity, does not give precise structural information and can be non-specific depending on antibody use.

Sensitivity. One adduct is yielded in approximately 10^8 nucleotides.

Mass spectrometry

Strengths. Mass spectrometry has greatest potential for chemical sensitivity and unequivocal characterization of DNA adducts, analytical backgrounds are very low and it is applicable to many classes of DNA adducts.

Limitations. Stable isotope-labelled internal standards are generally required for quantitation and immuno-affinity pre-purification may be required.

Sensitivity. It yields one adduct in 10^9 nucleotides.

There are several variants on the diol epoxide mechanism. Bis-diol epoxide–DNA adducts were formed from dibenz[*a*,*h*]anthracene (Platt & Schollmeier, 1994) and dibenz[*a*,*j*]anthracene (Vulimiri *et al.*, 1999). A bis-diol epoxide was proposed as a mechanism for the carcinogenesis for dibenz[*a*,*h*]anthracene, while its biological significance for dibenz[*a*,*j*]anthracene is unknown. A phenolic diol epoxide–DNA adduct was formed from benz[*b*]fluoranthene and was proposed to contribute to its biological activity (Weyand *et al.*, 1993a,b). Although not a true diol epoxide, a phenolic oxide–DNA adduct of benzo[*a*]pyrene has been described but its biological significance is unknown (Fang *et al.*, 2001).

(ii) *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 benz[*a*]anthracene is benz[*j*]aceanthrylene and that of pyrene is cyclopenta[*cd*]pyrene. In general, cyclopenta-ring derivatives of PAHs are more mutagenic than their unsubstituted counterparts (e.g. anthracene is non-mutagenic while its cyclopenta-ring counterpart, aceanthrylene, is highly mutagenic) (Kohan *et al.*, 1985). Similarly, cyclopenta-ring derivatives of PAHs are generally more carcinogenic than their unsubstituted counterparts (e.g. pyrene is not carcinogenic while cyclopenta[*cd*]pyrene is highly carcinogenic) (Nesnow *et al.*, 1998a). 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). Rat and mouse liver preparations, 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 (Beach & Gupta, 1994; Hsu *et al.*, 1997, 1999). 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). Cyclopenta-ring oxides are hydrated by epoxide hydrolase to diols. Some cyclopenta-ring diols are conjugated to sulfate esters by PAPS SULT. The cyclopenta-ring oxides are mutagenic and can bind to DNA to form adducts (Surh *et al.*, 1993). As an example, a cyclopenta-PAH, cyclopenta[*cd*]pyrene, induced mutations at the Ki-*Ras* proto-oncogene in lung tumours of treated mice (Nesnow *et al.*, 1994a).

(b) Individual compounds

Benz[*j*]aceanthrylene

(i) *Metabolism and metabolic activation*

The metabolism of benz[*j*]aceanthrylene has been documented in a number studies in rat liver microsomes, rodent and human cells in culture and in rats *in vivo*. Three potential centres exist for biochemical oxidation in benz[*j*]aceanthrylene: the cyclopenta-ring (C1–C2), the bay region (C7–C10) and the K-region (C11–C12) are all found to be involved. Aroclor 1254-induced and phenobarbital-induced rat liver microsomes metabolized benz[*j*]aceanthrylene to the cyclopenta-ring diol, *trans*-1,2-dihydroxy-1,2-dihydrobenz[*j*]-aceanthrylene (benz[*j*]aceanthrylene-1,2-diol), the K-region diol, *trans*-11,12-dihydroxy-11,12-dihydrobenz[*j*]aceanthrylene (benz[*j*]aceanthrylene-11,12-diol), the precursor to the bay-region diol epoxide, *trans*-9,10-dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene (benz-

[*j*]aceanthrylene-9,10-diol) and 10-hydroxy-benz[*j*]aceanthrylene (Nesnow *et al.*, 1984, 1988). The major metabolite was benz[*j*]aceanthrylene-1,2-diol which represented approx 60% of the total metabolites in either type of induced microsomes (Nesnow *et al.*, 1988). Liver microsomes from control and Aroclor 1254-induced rats also produced additional metabolites: benz[*j*]aceanthrylene-1,2,9,10-tetrahydrotetrol, 8-hydroxybenz[*j*]aceanthrylene-1,2-diol and 10-hydroxy-benz[*j*]aceanthrylene-1,2-diol. Induction with Aroclor 1254 increased the overall conversion of benz[*j*]aceanthrylene to metabolites, particularly those formed by oxidation at the cyclopenta-ring (Johnsen *et al.*, 1998b). Lung microsomes from control and Aroclor 1254-induced rats produced benz[*j*]aceanthrylene-1,2-diol as the main metabolite with an increase due to induction (Johnsen *et al.*, 1997). Human liver microsomes from five donors produced only benz[*j*]aceanthrylene-1,2-diol and benz[*j*]-aceanthrylene-2-one (Johnsen *et al.*, 1998a,b). Few data are available that characterize the specific CYP isoforms involved in the biotransformation of benz[*j*]aceanthrylene. A series of primary and conjugated metabolites of benz[*j*]aceanthrylene were identified using human and rodent mammalian cells in culture. Hepatocytes from untreated and Aroclor 1254-induced rats produced a large number of metabolites including benz[*j*]-aceanthrylene-1,2-diol, benz[*j*]aceanthrylene-11,12-diol, benz[*j*]aceanthrylene-9,10-diol, 10-hydroxybenz[*j*]aceanthrylene, benz[*j*]aceanthrylene-1,2,9,10-tetrahydrotetrol, 8-hydroxybenz[*j*]aceanthrylene-1,2-diol and 10-hydroxybenz[*j*]aceanthrylene-1,2-diol and sulfate and glucuronide conjugates of many of the primary metabolites (Nyholm *et al.*, 1996). C3H10T1½Cl8 mouse embryo fibroblasts that are known to contain CYP1B1 metabolized benz[*j*]aceanthrylene to benz[*j*]aceanthrylene-1,2-diol, benz[*j*]aceanthrylene-11,12-diol, benz[*j*]aceanthrylene-9,10-diol, 10-hydroxybenz[*j*]aceanthrylene and to sulfate and glucuronide conjugates; however, the major metabolite formed was benz[*j*]-aceanthrylene-9,10-diol (55%) (Mohapatra *et al.*, 1987). Benz[*j*]aceanthrylene was metabolized solely to benz[*j*]aceanthrylene-1,2-diol by isolated human blood lymphocytes (Johnsen *et al.*, 1998a). Rats treated with benz[*j*]aceanthrylene produced biotransformation products in faeces, urine and bile. The major metabolites in faeces were 8-hydroxybenz[*j*]aceanthrylene-1,2-diol and 10-hydroxybenz[*j*]aceanthrylene-1,2-diol, which were also found as conjugated metabolites in the bile. The glucuronide conjugate of 10-hydroxybenz[*j*]aceanthrylene-1,2-diol was also a major metabolite in urine. Two sulfate conjugates of oxidized benz[*j*]aceanthrylene, a sulfate conjugate of a benz-[*j*]aceanthrylene-diol-phenol and benz[*j*]aceanthrylene-1,2-diol-10-sulfate were also detected in bile. Benz[*j*]aceanthrylene-1,2-diol was detected in urine, faeces and bile (Hegstad *et al.*, 1999).

(ii) *Formation of DNA adducts*

DNA adducts have been identified in calf thymus DNA, mammalian cells in culture and in rodents after exposure to benz[*j*]aceanthrylene. The major benz[*j*]aceanthrylene–calf thymus DNA adduct formed in the presence of liver microsomes from rats (control or treated with Aroclor 1254) or from humans was a benz[*j*]aceanthrylene-1,2-oxide adduct (Johnsen *et al.*, 1998b). CYP1A2 was found to be a major CYP involved in the covalent

binding of benz[*j*]aceanthrylene to DNA in the presence of Aroclor 1254-induced rat liver microsomes (Johnsen *et al.*, 1998b). Two major groups of adducts were identified in C3H10T½Cl8 mouse embryo cells exposed to benz[*j*]aceanthrylene: one group was the result of the interaction of benz[*j*]aceanthrylene-1,2-oxide with deoxyguanosine and deoxyadenosine; the second group was a result of the interaction of *anti-trans*-9,10-dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene-7,8-oxide (*anti*-benz[*j*]aceanthrylene-9,10-diol-7,8-oxide) with deoxyguanosine and deoxyadenosine. Qualitative and quantitative analysis of the postlabelling data suggest that benz[*j*]aceanthrylene is metabolically activated by two distinct routes: the bay-region diol epoxide and the cyclopenta-ring oxide; the former was the most significant (Nesnow *et al.*, 1991). In isolated rat hepatocytes and rabbit lung cells, benz[*j*]aceanthrylene-1,2-oxide adducts of both deoxyadenosine and deoxyguanosine were observed (Holme *et al.*, 1993; Johnsen *et al.*, 1998b). Rat Clara cells, type 2 cells and macrophages exposed to benz[*j*]aceanthrylene produced only benz[*j*]aceanthrylene-1,2-oxide–DNA adducts (Johnsen *et al.*, 1997). Human peripheral lymphocytes and HL-60 cells exposed to benz[*j*]aceanthrylene also produced only benz[*j*]aceanthrylene-1,2-oxide adducts (Johnsen *et al.*, 1998a). Addition of Aroclor 1254-induced rat liver microsomes to human lymphocytes increased the levels of DNA adducts (Johnsen *et al.*, 1998a). *In vivo*, only benz[*j*]aceanthrylene-1,2-oxide–DNA adducts were detected in the lymphocytes and lungs of Wistar rats (Johnsen *et al.*, 1998a). Strain A mice exposed intraperitoneally to benz[*j*]aceanthrylene formed a number of adducts in the lung; benz[*j*]aceanthrylene-1,2-oxide adducts of deoxyadenosine and deoxyguanosine and benz[*j*]aceanthrylene-9,10-diol-7,8-oxide adducts of deoxyadenosine and deoxyguanosine were identified (Mass *et al.*, 1993).

(iii) *Genotoxicity of benz[j]aceanthrylene*

Benz[*j*]aceanthrylene was mutagenic in *S. typhimurium* TA98 in the presence of metabolic activation by Aroclor 1254-induced rat liver (Nesnow *et al.*, 1984), control rat liver or human liver microsomes (Johnsen *et al.*, 1998b). It was mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) in the presence of Aroclor 1254-induced rat liver metabolic activation (Nesnow *et al.*, 1984) and induced morphological cell transformation in C3H10T½Cl8 cells in culture (Mohapatra *et al.*, 1987; Nesnow *et al.*, 1991). Only a small increase in single-strand breaks was observed in benz[*j*]-aceanthrylene-treated isolated human lymphocytes in the presence of cytosine-^{1}-β-D-arabinofuranoside and hydroxy urea (added 1 h prior to analysis to prevent strand breaks rejoining), whereas large increases were observed in lymphocytes co-incubated with Aroclor 1254-induced rat liver microsomes (Johnsen *et al.*, 1998b).

(iv) *Benz[j]aceanthrylene-induced mutations in proto-oncogenes*

Benz[*j*]aceanthrylene induced two major classes of Ki-*ras* codon 12 mutations in lung adenomas from benz[*j*]aceanthrylene-treated strain A mice (GGT → CGT (65%) and GGT → GTT (30.4%)), indicating that guanine was a primary target for this PAH (Mass *et al.*, 1993).

(v) *Other effects of benz[j]aceanthrylene*

Benz[*j*]aceanthrylene induced apoptosis and formation of the active form of caspase-3, cleavage of poly(ADP-ribose)polymerase (PARP), DNA fragmentation and an accumulation of the tumour-suppressor protein p53 in mouse hepatoma Hepa1c1c7 cells. However, it did not trigger apoptosis in primary cultures of rat lung cells (Clara cells, type 2 cells or lung alveolar macrophages) (Solhaug *et al.*, 2004a).

(vi) *Evidence for a mechanism of cyclopenta-ring oxide metabolic activation*

Genotoxicity of benz[*j*]aceanthrylene-1,2-oxide

Benz[*j*]aceanthrylene-1,2-oxide was mutagenic in *S. typhimurium* TA98 in the absence and presence of Aroclor 1254-induced rat liver metabolic activation (Bartczak *et al.*, 1987). It induced morphological cell transformation and DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Conclusion

Benz[*j*]aceanthrylene is metabolically activated by cyclopenta-ring oxidation in rat and human liver microsomes, in mouse, rat and human cells in culture and in rats *in vivo*. In addition to the unconjugated metabolites, sulfate, glucuronide and possibly GSH conjugates have been reported. The key evidence for a mechanism of cyclopenta-ring oxide metabolic activation is based on the measurement of the stable cyclopenta-ring dihydrodiol metabolite, benz[*j*]aceanthrylene-1,2-diol, in rodent liver and lung tissues, hepatocytes and fibroblasts and human liver microsomes *in vitro* and in rodents *in vivo*. Benz[*j*]aceanthrylene-1,2-oxide is genotoxic in bacteria and mammalian cells and its adducted form with deoxyadenosine and deoxyguanosine were produced in mouse lung. Benz[*j*]aceanthrylene-induced tumours expressed Ki-*ras* codon 12 guanine mutations.

(vii) *Evidence for a mechanism of diol epoxide metabolic activation*

Genotoxicity of benz[*j*]aceanthrylene-9,10-oxide

Benz[*j*]aceanthrylene-9,10-oxide was mutagenic in *S. typhimurium* TA98 in the presence of Aroclor 1254-induced rat liver metabolic activation (Newcomb *et al.*, 1993). It induced morphological cell transformation and formed DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Genotoxicity of benz[*j*]aceanthrylene-9,10-diol

Benz[*j*]aceanthrylene-9,10-diol was mutagenic in *S. typhimurium* TA98 in the presence of Aroclor 1254-induced rat liver metabolic activation (Newcomb *et al.*, 1993). It induced morphological cell transformation and formed *anti*-benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Genotoxicity of benz[*j*]aceanthrylene-9,10-diol-7,8-oxide

Benz[*j*]aceanthrylene-9,10-diol-7,8-oxide was mutagenic in *S. typhimurium* TA98 in the presence and absence of Aroclor 1254-induced rat liver metabolic activation (Newcomb *et al.*, 1993). It induced morphological cell transformation and formed *anti*-benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Conclusion

Benz[*j*]aceanthrylene is metabolically activated by the diol epoxide pathway in mammalian cells based on the measurement of the diol metabolite benz[*j*]aceanthrylene-9,10-diol in rat liver microsomes and hepatocytes and mouse embryo fibroblasts and the genotoxicity in bacteria and mammalian cells of benz[*j*]aceanthrylene-9,10-diol and benz[*j*]aceanthrylene-9,10-diol-7,8-oxide. *anti*-Benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts were observed in the lungs of strain A mice that are susceptible to benz[*j*]aceanthrylene-induced lung tumorigenesis.

Benz[*l*]aceanthrylene

(i) *Metabolism and metabolic activation*

The metabolism of benz[*l*]aceanthrylene has been documented in a number studies in rat liver microsomes and rodent cells in culture. Aroclor 1254-induced and phenobarbital-induced rat liver microsomes metabolized benz[*l*]aceanthrylene to the cyclopenta-ring diol, *trans*-1,2-dihydroxy-1,2-dihydrobenz[*l*]aceanthrylene (benz[*l*]aceanthrylene-1,2-diol), the K-region diol, *trans*-7,8-dihydroxy-7,8-dihydrobenz[*l*]aceanthrylene (benz[*l*]aceanthrylene-7,8-diol) and a naphtho-ring diol, *trans*-4,5-dihydroxy-4,5-dihydrobenz[*l*]aceanthrylene (benz[*l*]aceanthrylene-4,5-diol) (Gold *et al.*, 1980; Nesnow *et al.*, 1988). The major metabolite was benz[*l*]aceanthrylene-7,8-diol which represented approximately 28–40% of the total metabolites depending on the type of induced microsomes. The cyclopenta-ring diol benz[*l*]aceanthrylene-1,2-diol was the second most abundant metabolite representing 16–20% of the total metabolites formed depending on the inducer type (Nesnow *et al.*, 1988). C3H10T½CL8 mouse embryo fibroblasts that are known to contain CYP1B1 metabolized benz[*l*]aceanthrylene to benz[*l*]aceanthrylene-7,8-diol (45%), benz[*l*]aceanthrylene-4,5-diol (6%), benz[*l*]aceanthrylene-1,2-diol (5%) and unidentified metabolites (44%) as well as sulfate and glucuronide conjugates (Mohapatra *et al.*, 1987).

(ii) *Formation of DNA adducts*

DNA adducts have been identified in calf thymus DNA and in mammalian cells in culture after exposure to benz[*l*]aceanthrylene. Benz[*l*]aceanthrylene formed four unidentified DNA adducts in human peripheral blood lymphocytes and in the human promyelocytic leukaemia HL 60 cell line (Johnsen *et al.*, 1998a). Addition of Aroclor 1254-induced rat liver microsomes to human lymphocytes increased the levels of DNA adducts (Johnsen *et al.*, 1998a). These adducts were also observed in isolated rat

hepatocytes from untreated and Aroclor 1254-treated rats, and in calf thymus DNA incubated with control, Aroclor 1254-induced rat liver microsomes or human microsomes (Johnsen *et al.*, 1998b). Unidentified DNA adducts were observed in rat lung cells and uninduced Clara cells, type 2 cells and alveolar macrophages exposed to benz[*l*]-aceanthrylene. Aroclor 1254-induced rat Clara cells and type 2 cells produced slightly higher levels of DNA adducts (Johnsen *et al.*, 1997). Unidentified adducts were observed in rabbit Clara cells exposed to benz[*l*]aceanthrylene (Holme *et al.*, 1993).

(iii) *Genotoxicity of benz[l]aceanthrylene*

Benz[*l*]aceanthrylene was mutagenic in *S. typhimurium* TA98, in the presence of Aroclor 1254-induced rat liver microsomes, control rat liver microsomes or human liver microsomes (Nesnow *et al.*, 1984; Johnsen *et al.*, 1997, 1998b) and in *S. typhimurium* TA98 co-incubated with rat hepatocytes (Holme *et al.*, 1993). It induced unscheduled DNA synthesis and DNA damage (by alkaline elution) in primary rat hepatocytes and in human lymphocytes co-incubated with Aroclor 1254-induced rat liver microsomes (Holme *et al.*, 1993). Benz[*l*]aceanthrylene was mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) in the presence of Aroclor 1254-induced rat liver metabolic activation, and in V79 cells co-cultured with irradiated primary Syrian hamster embryo cells (Nesnow *et al.*, 1984). It induced morphological cell transformation in C3H10T½C8 cells in culture (Mohapatra *et al.*, 1987) and induced anchorage-independent growth in normal human diploid fibroblasts (Nesnow *et al.*, 1990). Benz[*l*]aceanthrylene was both a gene mutagen and a chromosomal mutagen in L5178Y/TK$^{+/-}$ mouse lymphoma cells in the presence of an Aroclor 1254-induced rat liver metabolic activation. It induced sister chromatid exchange in the peripheral blood lymphocytes of mice (Kligerman *et al.*, 1986).

(iv) *Evidence for a cyclopenta-ring metabolic activation as mechanism of carcinogenesis*

Genotoxicity of benz[*l*]aceanthrylene-1,2-oxide

Benz[*l*]aceanthrylene-1,2-oxide was mutagenic in *S. typhimurium* TA98 in the absence and presence of Aroclor 1254-induced rat liver metabolic activation (Nesnow *et al.*, 1984; Bartczak *et al.*, 1987) and induced 6-thioguanine resistant mutations in Chinese hamster V79 cells (Nesnow *et al.*, 1984).

Conclusion

Benz[*l*]aceanthrylene is metabolically activated by cyclopenta-ring oxidation; epoxidation at the cyclopenta ring is one biotransformation pathway for benz[*l*]aceanthrylene in rat liver microsomes and mouse cells in culture. The cyclopenta-ring oxide, benz[*l*]aceanthrylene-1,2-oxide, is genotoxic in bacteria and mammalian cells. The stable cyclopenta-ring dihydrodiol metabolite, benz[*l*]aceanthrylene-1,2-diol, is formed by microsomal and cell-based systems. However, no benz[*l*]aceanthrylene–DNA adducts

have been identified in bioassay systems that confirm the intermediacy of the cyclopenta-ring oxide.

Benz[*a*]anthracene

(i) *Metabolism and metabolic activation*

The metabolism of benz[*a*]anthracene has been documented in an number of studies in rat liver microsomes and mouse skin explants. There are three centres of metabolism: the 1–2 and 3–4 bonds that lead to bay-region diol epoxides, the K-region (5–6 bond) and the terminal benzo region (8–9 and 10–11 bonds). Benz[*a*]anthracene is metabolized by control and 3-methylcholanthrene-induced rat liver microsomal fractions and a reconstituted purified cytochrome P-448 system to the major metabolites: the K-region diol, 5,6-dihydroxy-5,6-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-5,6-diol) and the terminal benzo ring diol, 8,9-dihydroxy-8,9-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-8,9-diol) with minor amounts of 3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene (benz[*a*]-anthracene-3,4-diol), 10,11-dihydroxy-10,11-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-10,11-diol) and 1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-1,2-diol) (Thakker *et al.*, 1979a). Benz[*a*]anthracene is metabolized by mouse skin explants to a series of diols: benz[*a*]anthracene-3,4-diol, benz[*a*]anthracene-8,9-diol, benz[*a*]anthracene-5,6-diol and benz[*a*]anthracene-10,11-diol (MacNicoll *et al.*, 1980). It was metabolized by 3-phenobarbital-induced rat liver microsomes to (–)-benz[*a*]-anthracene-3*R*,4*R*-diol as the predominant stereochemical form (Thakker *et al.*, 1979a,b).

(ii) *Formation of DNA adducts*

Two major DNA adducts were formed after topical administration of benz[*a*]-anthracene to mouse skin: an *anti*-3,4-dihydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene-1,2-oxide (*anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide)–DNA adduct and an *anti*-8,9-dihydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene-10,11-oxide (*anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide)–DNA adduct (Cooper *et al.*, 1980a). These adducts were further characterized in hamster embryo cells as *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine and *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide–deoxyguanosine. The non-'bay-region' diol epoxide *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide reacts also with guanosine and adenosine in RNA (Cooper *et al.*, 1980b).

(iii) *Genotoxicity of benz[*a*]anthracene*

In a previous monograph, benz[*a*]anthracene was reported to induce DNA damage and mutation in bacteria, mutation in *Drosphila melanogaster*, DNA damage, mutation, chromosomal effects and morphological cell transformation in mammalian cells, and chromosomal effects *in vivo* in mammals (IARC, 1983). Benz[*a*]anthracene was mutagenic in human B lymphoblastoid cells h1A1v2 (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of benz[*a*]anthracene-3,4-diol

Groups of 28–29 female CD-1 mice, 51–65 days of age, received a single dermal application of 2 μmol [525 μg] racemic benz[*a*]anthracene-3,4-diol in acetone followed 7 days later by 16.2 nmol [10 μg] 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in acetone twice a week for 26 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 27 weeks after treatment was 85% (4.7) compared with 6% (0.1) in mice treated with TPA alone (Slaga *et al.*, 1978a).

Groups of 29–30 female CD-1 mice, 60 days of age, received a single dermal application of 0.4, 1 or 2 μmol [105, 262.3 or 525 μg] racemic benz[*a*]anthracene-3,4-diol in acetone dimethyl sulfoxide (DMSO) (95:5) followed 18 days later by 16 nmol [10 μg] TPA in acetone twice a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 22.5 weeks after treatment was 77% (2.4), 79% (3.6) or 80% (4.8) compared with 3% (0.03) in mice treated with TPA alone (Wood *et al.*, 1977a).

Groups of 30 female CD-1 mice, 52–66 days of age, received a single dermal application of 400 nmol [105 μg] racemic benz[*a*]anthracene-3,4-diol, (+)-benz[*a*]-anthracene-3,4-diol or (–)-benz[*a*]anthracene-3,4-diol in acetone:ammonium hydroxide (1000:1) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 45% (1.3), 20% (0.4) or 50% (1.8) compared with 3% (0.03) in mice treated with TPA alone (Levin *et al.*, 1978).

Groups of newborn Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benz[*a*]anthracene-3,4-diol (total dose, 2.8 μmol) [734.5 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 22 weeks. In 28 male and female mice, pulmonary adenomas developed in 96% of the surviving mice (54.3 tumours/mouse) and 24% of the surviving mice had malignant lymphomas. In 20 male and female mice treated with DMSO alone, pulmonary adenomas developed in 5% of the surviving mice (0.05 tumours/mouse) but no malignant lymphoma. Under the same treatment conditions, benz[*a*]anthracene-1,2-diol, benz[*a*]-anthracene-5,6-diol, benz[*a*]anthracene-8,9-diol or benz[*a*]anthracene-10,11-diol did not induce statistically significant numbers of pulmonary tumours (Wislocki *et al.*, 1978a).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intra-peritoneally on days 1, 8 and 15 of life with racemic benz[*a*]anthracene-3,4-diol, (–)-benz[*a*]anthracene-3*R*,4*R*-diol or (+)-benz[*a*]anthracene-3*S*,4*S*-diol (total dose, 280 nmol) [735 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 26 weeks. In 68, 58 or 67 male and female mice, pulmonary tumours were observed in 35, 72 or 9% of the surviving mice (0.37, 1.88 or 0.09 tumours/mouse). In 67 male and female mice treated with DMSO alone, pulmonary tumours developed in 13% of the surviving mice (0.15 tumours/mouse) (Wislocki *et al.*, 1979).

Metabolism of benz[*a*]anthracene-3,4-diol

(–)-Benz[*a*]anthracene-3*R*,4*R*-diol was metabolized by phenobarbital- or 3-methylcholanthrene-induced rat liver microsomes to (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, benz[*a*]anthracene-3,4-quinone and bis-diols. (+)-Benz[*a*]anthracene-3*S*,4*S*-diol was not metabolized to a diol epoxide but was metabolized to benz[*a*]anthracene-3,4-quinone and bis-diols (Thakker *et al.*, 1982).

Other benz[*a*]anthracene diol metabolites (e.g. benz[*a*]anthracene-8,9-diol and benz-[*a*]anthracene-10,11-diol) have been reported to form non-bay-region diol epoxides. The non-bay-region diol epoxide, *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide, was formed (measured as a DNA adduct) in hamster embryo cells and in mouse skin treated with benz[*a*]anthracene (Cooper *et al.*, 1980c). *syn*- and *anti*-Benz[*a*]anthracene-10,11-diol-8,9-oxides were formed from benz[*a*]anthracene-10,11-diol by 3-methylcholanthrene-induced rat liver microsomes, but these diol epoxides did not contribute to the covalent binding of benz[*a*]anthracene to DNA (Cooper *et al.*, 1980d). Benz[*a*]anthracene-8,9-diol and benz[*a*]anthracene-10,11-diol and their diol epoxides are not described further in this monograph because both the diols and diol epoxides were inactive as tumour initiators in mouse skin, possessed either no or low mutagenic activity in mammalian cells and did not induce tumours in newborn mice (Slaga *et al.*, 1978a; Wislocki *et al.*, 1978a).

Genotoxicity of benz[*a*]anthracene-3,4-diol

Benz[*a*]anthracene-3,4-diol was mutagenic to Chinese hamster V79 cells (ouabain resistance) co-cultured with irradiated golden hamsters, and was the most mutagenic of all four metabolic benz[*a*]anthracene diols evaluated (Slaga *et al.*, 1978a).

Carcinogenicity studies of benz[*a*]anthracene-3,4-diol-1,2-oxide

Groups of 27–29 female CD-1 mice, 51–65 days of age, received a single dermal application of 2 μmol [556.6 μg] racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide in acetone followed 7 days later by 16.2 nmol [10 μg] TPA in acetone twice a week for 26 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 27 weeks after treatment was 100% (5) compared with 6% (0.1) in mice treated with TPA alone (Slaga *et al.*, 1978a).

Groups of 30 female CD-1 mice, 52–66 days of age, received a single dermal application of 400 nmol [111.5 μg] racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide or *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide in acetone:ammonium hydroxide (1000:1) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 70% (1.9) or 43% (0.6), respectively, compared with 3% (0.03) in mice treated with TPA alone (Levin *et al.*, 1978).

Groups of 27–30 female CD-1 mice, 58–65 days of age, received a single dermal application of 400 nmol [111.5 μg] racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide, (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide or (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide in acetone followed 14 days later by 16 nmol [10 μg] TPA in acetone

twice a week for 25 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 50% (1.63), 90% (3.35) or 14% (0.14), respectively, compared with 7% (0.07) in mice treated with TPA alone (Levin *et al.*, 1984).

Groups of 27–30 female CD-1 mice, 58–65 days of age, received a single dermal application of 400 nmol [111.5 μg] racemic *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide, (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide or (–)-*syn*-benz[*a*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide in acetone followed 14 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 27 weeks after treatment was 19% (0.36), 47% (0.73) or 17% (0.17), respectively, compared with 7% (0.07) in mice treated with TPA alone (Levin *et al.*, 1984).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide or racemic *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide (total dose, 280 nmol) [78 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 26 weeks. In 38 or 57 male and female mice, pulmonary tumours developed in 100 or 42% of the surviving mice (13.34 or 0.56 tumours/mouse). In 67 male and female mice treated with DMSO alone, 13% of the surviving mice (0.15 tumours/mouse) had pulmonary tumours (Wislocki *et al.*, 1979).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide, (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide or (–)-*syn*-benz[*a*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide (total dose, 140 nmol) [39 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 26–32 weeks. Only (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide and (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide induced statistically significant responses compared with control mice. In 38 or 48 male and female mice, pulmonary tumours developed in 100 or 31% of the surviving mice (23.11 or 0.38 tumours/mouse). In 65 male and female mice treated with DMSO alone, 8% of the surviving mice (0.08 tumours/mouse) had pulmonary tumours (Levin *et al.*, 1984).

DNA adducts of benz[*a*]anthracene-3,4-diol-1,2-oxide

anti-Benz[*a*]anthracene-3,4-diol-1,2-oxide produced a single unidentified DNA adduct in calf thymus DNA and isolated DNA (Cooper *et al.*, 1980a; King *et al.*, 1994), which was probably *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine (Cooper *et al.*, 1980b).

Genotoxicity of benz[*a*]anthracene-3,4-diol-1,2-oxide

Racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide was mutagenic in *S. typhimurium* TA100 and in Chinese hamster V79 cells (8-azaguanine resistance) in the absence of a metabolic activation system. This diol epoxide was 15–35 times more mutagenic in bacteria and 65–125 times more mutagenic in mammalian cells than the isomeric racemic *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide or *anti*-benz[*a*]anthracene-10,11-diol-8,9-

oxide (Wood *et al.*, 1977b). Racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide, racemic *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide, (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide, (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, (–)-*syn*-benz[*a*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide and (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide were all mutagenic in *S. typhimurium* TA98 and TA100 and in Chinese hamster V79 cells (8-azaguanine resistance) in the absence of exogenous metabolic activation. In strain TA98, the (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide isomer was the most active while in strain TA100 and in V79 cells the (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide was the most active (Wood *et al.*, 1983).

Conclusion

Benz[*a*]anthracene is activated metabolically by the diol epoxide mechanism at the bay region (i.e. C1–C4) in mouse skin and mouse lung. Benz[*a*]anthracene was metabolized by control and induced rat liver microsomes, reconstituted CYP systems and mouse skin explants to benz[*a*]anthracene-3,4-diol. In rat liver microsomes, the major enantiomeric form was (–)-benz[*a*]anthracene-3*R*,4*R*-diol. Racemic benz[*a*]anthracene-3,4-diol was genotoxic in mammalian cells and the (–)-benz[*a*]anthracene-3*R*,4*R*-diol enantiomer had the highest activity as a tumour initiator in mouse skin and as a pulmonary carcinogen in newborn mice. (–)-Benz[*a*]anthracene-3*R*,4*R*-diol was metabolized by rat liver microsomes to (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide which had the highest mutagenic activity of all of the isomers in mammalian cells. In mouse skin, benz[*a*]anthracene was metabolized to the diol epoxide, *anti*-benz[*a*]-anthracene-3,4-diol-1,2-oxide, which formed a single DNA adduct, probably *anti*-benz-[*a*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine. (+)-*anti*-Benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide was the most active isomer as a pulmonary carcinogen in mice and racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide was the most active isomer as a tumour initiator in mouse skin.

Benzo[*g*]chrysene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*g*]chrysene has been documented in rat liver microsomes. There is one major centre for biochemical oxidation in benzo[*g*]chrysene, the fjord region (C11–C14). Benzo[*g*]chrysene forms two diols after application to mouse skin *in vivo*: (–)-*trans*-benzo[*g*]chrysene-11*R*,12*S*-diol and (+)-*trans*-benzo[*g*]chrysene-11*S*,12*R*-diol. This conclusion was based on the structure of DNA adducts formed, not by direct measurement of the diols (Giles *et al.*, 1996).

(ii) *Formation of DNA adducts*

Benzo[*g*]chrysene is metabolically activated in human mammary carcinoma MCF-7 cells to form DNA adducts through both *syn*-11,12-dihydroxy-11,12-dihydrobenzo[*g*]-chrysene-13,14-oxide (*syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide) and *anti*-11,12-di-hydroxy-11,12-dihydrobenzo[*g*]chrysene-13,14-epoxide (*anti*-benzo[*g*]chrysene-11,12-

diol-13,14-oxide). Several adducts were identified: an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyguanosine adduct, an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyadenosine adduct, a *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyguanosine adduct and a *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyadenosine adduct (Agarwal *et al.*, 1997). Benzo[*g*]chrysene forms DNA adducts after application to mouse skin *in vivo*. These adducts were derived through the formation of (–)-*anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide and (+)-*syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide. Five adducts were (–)-*anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide-derived, two with deoxyadenosine and three with deoxyguanosine, and two adducts were (+)-*syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide-derived, one with deoxyadenosine and one with deoxyguanosine. The adenine adducts accounted for 64% of the total major adducts formed in benzo[*g*]chrysene-treated mouse skin (Giles *et al.*, 1996). Benzo[*g*]chrysene binds to different extents to the DNA in the epidermis of Cyp1a2$^{-/-}$, Cyp1b1$^{-/-}$ and Ahr$^{-/-}$ knockout mice *in vivo*, with the least binding in Ahr$^{-/-}$ mice, suggesting that CYP1A1 is involved in the bioactivation of benzo[*g*]chrysene (Kleiner *et al.*, 2004).

(iii) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Metabolism of benzo[*g*]chrysene-11,12-diol

Benzo[*g*]chrysene-11,12-diol is metabolized by Aroclor 1254-induced rat liver microsomes to a series of diastereomeric tetraols formed through the intermediary *syn*- and *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides, predominantly in the *anti* form (86:14) (Luch *et al.*, 1998a).

DNA adducts of benzo[*g*]chrysene-11,12-diol

Benzo[*g*]chrysene-11,12-diol produces DNA adducts in human mammary carcinoma MCF-7 cells consistent with the metabolic intermediate, *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide. One adduct was identified as an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyguanosine adduct and the other as an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyadenosine adduct; the latter adduct was the major one (Agarwal *et al.*, 1997). Both (–)-*trans*-benzo[*g*]chrysene-11*R*,12*S*-diol and (+)-*trans*-benzo[*g*]chrysene-11*S*,12*R*-diol formed DNA adducts on mouse skin consistent with the adducts formed by application of benzo[*g*]chrysene (Giles *et al.*, 1996).

Genotoxicity of benzo[*g*]chrysene-11,12-diol

CYP1A1, CYP1A2 and CYP1B1 enzymes activate benzo[*g*]chrysene-11,12-diol metabolically to DNA-damaging forms in cDNA-based recombinant (*Escherichia coli* or *Trichoplusia ni*) systems that express these isoforms of human CYP (Shimada *et al.*, 2001a).

Carcinogenicity studies of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Groups of newborn Hsd:ICR mice were injected intraperitoneally on days 1, 7 and 15 of life with racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide (total dose, 25 nmol) in DMSO. Mice were weaned at age 21 days, separated by sex and killed at age 35 weeks. In treated mice, pulmonary tumours developed in 96.4% of tumour-bearing females (24 animals; 31.6 tumours/mouse) and 100% of the tumour-bearing males (25 animals; 24.4 tumours/mouse); liver tumours developed in 21.4% of the tumour-bearing females (0.54 tumours/mouse) and in 66.7% of tumour-bearing males (eight tumours/mouse). In mice treated with DMSO alone, pulmonary tumours occurred in 14.8% of the tumour-bearing females (26 animals; 0.19 tumours/mouse) and 7.4% of the tumour-bearing males (27 animals; 0.074 tumours/mouse); liver tumours developed in 11.1% of females (0.11 tumours/mouse) and 3.7% of males (0.037 tumours/mouse). The difference between the treated and control groups in males and females for pulmonary tumours, and that in males for hepatic tumours were statistically significant (Amin *et al.*, 1995a).

Groups of 30-day-old female Charles River CD rats were injected twice with a total dose of 1.2 μmol *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide dissolved in DMSO under the three nipples on the left side. The DMSO control was injected under the nipples on the right side. At 41 weeks, 95% of 20 rats had mammary tumours (adenomas, adenocarcinomas, sarcomas), with a mean latent period of 26.5 weeks. In the DMSO controls, 11% of 19 rats had mammary tumours (adenomas, adenocarcinomas), with a mean latent period of 39 weeks. The difference between the treated and control group was statistically significant (Amin *et al.*, 1995b).

DNA adducts of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide forms several DNA adducts in human mammary MCF-7 cells, many of which were the same as those obtained in mice treated with benzo[*g*]chrysene, benzo[*g*]chrysene-11,12-diol and *anti*-benzo[*g*]-chrysene-11,12-diol-13,14-oxide (Agarwal *et al.*, 1997). The major adduct formed between calf thymus DNA and racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide was a *trans-anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide–deoxyadenosine adduct (Szeliga *et al.*, 1995). Several major adducts were formed with calf thymus DNA and racemic *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide, a *trans-syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide–deoxyadenosine adduct and a possible mixture of a *trans-syn*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*R*,14*S*-oxide–deoxyguanosine and *trans-syn*-benzo[*g*]-chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide–deoxyguanosine (Szeliga *et al.*, 1994). Both *R*,*S*,*S*,*R* and *S*,*R*,*R*,*S* enantiomers of *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides formed deoxyguanosine and deoxyadenosine DNA adducts in human breast carcinoma MCF-7 cells (Khan *et al.*, 1998).

Genotoxicity of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Both *syn*- and *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides were mutagenic in *S. typhimurium* TA97, TA98, TA100 and TA104, induced SOS response in *E. coli* (SOS

chromotest in strain PQ37) and were mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) (Glatt *et al.*, 1991). Racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide was mutagenic in the shuttle vector pSP189 with 39% of the mutations at AT pairs which suggested a relationship between adduct formation at deoxyadenosine and mutation (Szeliga *et al.*, 1995). Racemic *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide was mutagenic in the shuttle vector pSP189 system giving A→T and G→T mutations (Szeliga *et al.*, 1994) and in the dihydrofolate reductase gene in Chinese hamster ovary hemizygous UA21 cells giving A→T transversion base substitution in 59% of the total induced changes (Yuan *et al.*, 1995). Both *syn*- and *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides were mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) and formed four unidentified DNA adducts in these cells. The potent mutagenicity of these fjord-region diol epoxides appears to be due to the high frequency with which they form DNA adducts in V79 cells, rather than to formation of adducts with greater mutagenic potential (Phillips *et al.*, 1991). *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide gave base-specific mutations of GC→TA (41%), GC→CG (13%), GC→AT (7%), AT→TA (23%), AT→CG (5%) and AT→GC (11%) in supF DNA using a SV40-based shuttle vector system. *syn*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide gave base-specific mutations of GC→TA (28%), GC→CG (12%), GC→AT (12%), AT→TA (40%), AT→CG (5%) and AT→GC (3%) (Bigger *et al.*, 2000). *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide formed deoxyguanosine adducts particularly within codon 158 of exon 5, codons 237 and 248 of exon 7 and codons 273 and 290 of exon 8 in the human *TP53* tumour-suppressor gene. These codons were also hotspots for mutations in the *TP53* gene of lung cancer patients (Smith *et al.*, 2000).

Other effects of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Human O41 TR cells exposed to 1.2 μM *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide formed four major deoxyadenosine and deoxyguanosine adducts. Similar adduct levels were detected in both p53-proficient and p53-deficient cells, and removal of adducts was not observed in either case. At lower concentrations, p53-proficient cells had fewer adducts than p53-deficient cells. p53 Appeared to minimize the appearance of benzo[*g*]chrysene adducts in human cells by up-regulating global nucleotide excision repair and reducing the maximum adduct levels (Lloyd & Hanawalt, 2002). Racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide and each of the *R*,*S*,*S*,*R* and *S*,*R*,*R*,*S* enantiomers of *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides delayed human breast carcinoma MCF-7 cells in the S phase by delaying DNA synthesis; both enantiomers induced p53 (Khan *et al.*, 1997, 1998). Benzo[*g*]chrysene-11,12-diol-13,14-oxide was found to bind preferentially to methylated CpG sequences at mutational hotspots in the *p53* gene (Chen *et al.*, 1998). *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide is a relatively poor substrate for murine GST 9.5, mGSTP1-1, mGSTM1-1 and mGSTA3-3 (Hu & Singh, 1997).

Conclusion

Benzo[*g*]chrysene can be activated metabolically by a fjord-region diol epoxide mechanism. On mouse skin, benzo[*g*]chrysene was metabolized to DNA adducts through both the *R,R* and *S,S* enantiomers of benzo[*g*]chrysene-11,12-diol. Racemic benzo[*g*]-chrysene-11,12-diols were genotoxic in bacteria and were bioactivated by human CYP1A1, CYP1A2 and CYP1B1. On mouse skin, both diols were further metabolized to the diol epoxides (–)-*anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide and (+)-*syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide, both of which formed deoxyadenosine and deoxyguanosine adducts — the same adducts as those found after treatment with benzo[*g*]chrysene. CYP1A1 is involved in this biotransformation process. *anti*-Benzo[*g*]-chrysene-11,12-diol-13,14-oxide was genotoxic in bacteria and mammalian cells and induced mammary cancer in rats. It was tumorigenic when injected into newborn mice, inducing pulmonary tumours. Both enantiomers of *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide formed deoxyguanosine and deoxyadenosine adducts in human mammary carcinoma cells, and the same pattern of DNA adducts was observed in mouse skin. In human cells, *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide adducts were repaired by nucleotide excision repair. *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide induced p53 in human mammary carcinoma cells and mutated the *p53* tumour-suppressor gene.

Benzo[*b*]fluoranthene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*b*]fluoranthene has been documented in a number of studies in rat liver microsomes and in mouse epidermis *in vivo*. Benzo[*b*]fluoranthene is metabolized by Aroclor 1254-induced rat liver microsomes to 4-hydroxy-, 5-hydroxy-, 6-hydroxy- or 7-hydroxybenzo[*b*]fluoranthene. The major diol metabolite was *trans*-11,12-dihydro-11,12-dihydroxybenzo[*b*]fluoranthene (benzo[*b*]fluoranthene-11,12-diol), and the minor diol metabolite was 1,2-dihydro-1,2-dihydroxybenzo[*b*]fluoranthene (benzo[*b*]-fluoranthene-1,2-diol). No evidence was obtained for the formation of 7b,8-dihydro-7b,8-dihydroxybenzo[*b*]fluoranthene or *trans*-9,10-dihydro-9,10-dihydroxybenzo[*b*]fluor-anthene (benzo[*b*]fluoranthene-9,10-diol) (Amin *et al.*, 1982). Benzo[*b*]fluoranthene is metabolized in mouse skin epidermis *in vivo* to 4-, 5- and 6-hydroxybenzo[*b*]fluoranthene as well as to sulfate and glucuronide conjugates. Minor metabolites included 12-hydroxy-benzo[*b*]fluoranthene, benzo[*b*]fluoranthene-1,2-diol and benzo[*b*]fluoranthene-11,12-diol. Phenolic gluronides and sulfate conjugates of benzo[*b*]fluoranthene were also observed. Benzo[*b*]fluoranthene-9,10-diol was not detected (Geddie *et al.*, 1987).

(ii) *Formation of DNA adducts*

Benzo[*b*]fluoranthene forms two unidentified DNA adducts in mouse skin epidermis *in vivo* (Weyand *et al.*, 1987). Benzo[*b*]fluoranthene administered to male Sprague-Dawley rats produced DNA adducts in the lung, liver and peripheral blood lymphocytes. Only one minor adduct was identified that derived from *anti-trans*-9,10-dihydro-9,10-dihydroxy-benzo[*b*]fluoranthene-11,12-oxide (*anti*-benzo[*b*]fluoranthene-9,10-diol 11,12-

oxide) (Ross *et al.*, 1992). Treatment with benzo[*b*]fluoranthene of mouse epidermis *in vivo*, of human skin maintained in short-term organ culture and single-stranded DNA incubated with Aroclor 1254-induced rat liver microsomes gave two adducts — a major adduct suggested to be a bay-region triol epoxide (containing a phenolic OH-group on carbon 5 or 6 of the peninsula ring) and a minor adduct from *anti*-benzo[*b*]fluoranthene-9,10-diol-11,12-oxide (Pfau *et al.*, 1992). Benzo[*b*]fluoranthene forms a single major adduct with four additional minor DNA adducts in mouse epidermis *in vivo*. The DNA adducts formed with 5-hydroxybenzo[*b*]fluoranthene-9,10-diol were identical to the major and one of the minor adducts observed for benzo[*b*]fluoranthene. *anti*-5-Hydroxybenzo[*b*]fluoranthene-9,10-diol–DNA is the major adduct formed and is a deoxyguanosine adduct (Weyand *et al.*, 1993b). The major benzo[*b*]fluoranthene–DNA adduct in the lungs of benzo[*b*]fluoranthene-treated strain A mice was identified as an *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide–deoxyguanosine adduct (Mass *et al.*, 1996).

(iii) *Genotoxic effects of benzo[*b*]fluoranthene*

In a previous monograph, benzo[*b*]fluoranthene was reported to induce mutations in *S. typhimurium* TA98 and TA100 and to induce chromosomal effects in Chinese hamster bone-marrow cells *in vivo* (IARC, 1983). Benzo[*b*]fluoranthene was mutagenic in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Amin *et al.*, 1985a) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996). Benzo[*b*]fluoranthene administered to male Sprague-Dawley rats produced sister chromatid exchange in peripheral blood lymphocytes (Ross *et al.*, 1992).

(iv) *Benzo[*b*]fluoranthene-induced mutations in proto-oncogenes*

Benzo[*b*]fluoranthene induced two major classes of Ki-*ras* codon 12 mutations in lung adenomas from treated strain A mice — GGT→TGT (56%), GGT→GTT (36%), GGT→GAT (4%) and GGT→CGT (4%) — indicating that deoxyguanosine was a primary target for this PAH (Mass *et al.*, 1996).

(v) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of benzo[*b*]fluoranthene-9,10-diol

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal applications of 10 subdoses every other day for a total initiating application of 10, 30 or 100 μg [35, 105 or 350 nmol] racemic benzo[*b*]fluoranthene-9,10-diol in acetone followed 10 days later by 2.5 μg [4 nmol] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the treated groups 21 weeks after treatment was 95% (8.4), 63% (3.8) and 26% (1.0), respectively. No tumours were observed in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal applications of 10 subdoses every other day for a total initiating application of 100 nmol [29 μg] racemic benzo[*b*]fluoranthene-9,10-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 85% (4.0). The response in control mice treated with TPA alone was not stated (Geddie *et al.*, 1987).

Genotoxicity of benzo[*b*]fluoranthene-9,10-diol

CYP1A1 and CYP1B1 enzymes metabolized benzo[*b*]fluoranthene-9,10-diol to DNA-damaging forms using cDNA-based recombinant (*E. coli* or *T. ni*) systems that express these forms of human CYP (Shimada *et al.*, 2001a). The further metabolism of synthetic benzo[*b*]fluoranthene-9,10-diol by Aroclor 1254-induced rat liver microsomes gave the major metabolites 5- and 6-hydroxybenzo[*b*]fluoranthene-9,10-diol. Little if any benzo[*b*]fluoranthene-9,10,11,12-tetraol was detected (Geddie *et al.*, 1987).

Genotoxicity of benzo[*b*]fluoranthene-9,10-diol-11,12-oxide

anti-Benzo[*b*]fluoranthene-9,10-diol-11,12-oxide induced DNA lesions in human cell-free extracts. This damage was repaired by both nucleotide excision repair and base excision repair of induced apurinic/apyrimidinic sites (Braithwaite *et al.*, 1998).

Conclusion

There is some evidence for two possible routes for the diol epoxide metabolic activation mechanism for benzo[*b*]fluoranthene — a phenolic diol epoxide, *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide, and a diol epoxide, *anti*-benzo[*b*]-fluoranthene-9,10-diol-11,12-oxide. In mouse skin, benzo[*b*]fluoranthene was metabolized to the 5-hydroxybenzo[*b*]fluoranthene metabolite and formed *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide–deoxyguanosine as the major DNA adduct *in vivo*. Benzo[*b*]fluoranthene-9,10-diol was mutagenic in bacteria and initiated mouse skin tumours. *anti*-Benzo[*b*]fluoranthene-9,10-diol-11,12-oxide damaged DNA; in mouse lung *in vivo*, the major DNA adduct was *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide–deoxyguanosine. Lung tumours from benzo[*b*]fluoranthene-treated mice exhibited Ki-*ras* mutations at deoxyguanosine residues.

Benzo[*j*]fluoranthene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*j*]fluoranthene has been documented in a number studies in rat liver microsomes and in mouse epidermis *in vivo*. Benzo[*j*]fluoranthene is metabolized by Aroclor 1254-induced rat liver microsomes to two diol metabolites, one of which was identified as *trans*-9,10-dihydro-9,10-dihydroxybenzo[*j*]fluoranthene (benzo[*j*]fluoranthene-9,10-diol) (LaVoie *et al.*, 1980). Administration of benzo[*j*]fluoranthene to mouse epidermis produced benzo[*j*]fluoranthene-9,10-diol and 4,5-dihydro-4,5-dihydroxybenzo[*j*]fluoranthene (benzo[*j*]fluoranthene-4,5-diol) as major metabolites. 4-Hydroxy-

and 10-hydroxybenzo[*j*]fluoranthene and benzo[*j*]fluoranthene-4,5-dione were also tentatively identified as metabolites (Rice *et al.*, 1987).

(ii) *Formation of DNA adducts*

The major benzo[*j*]fluoranthene–DNA adduct produced in *S. typhimurium* TA97a and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation was an *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine adduct (Marshall *et al.*, 1993), which was also the major DNA adduct formed in mouse epidermis treated with benzo[*j*]fluoranthene (Weyand *et al.*, 1987, 1993a).

(iii) *Genotoxic effects of benzo[j]fluoranthene*

In a previous monograph, benzo[*j*]fluoranthene was reported to induce mutations in *S. typhimurium* TA100 in the presence of an exogenous metabolic activation system (IARC, 1983). Benzo[*j*]fluoranthene was also mutagenic in *S. typhimurium* TA97a, TA98 and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of benzo[*j*]fluoranthene-4,5-diol

Groups of 18–19 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses every other day for a total initiating application of 1 or 3 μmol [287 or 859 μg] benzo[*j*]fluoranthene-4,5-diol in acetone followed 10 days later by 2.5 μg [4 nmol] TPA in acetone three times a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 100% (5.0) and 78% (4.3), respectively. The response in control mice treated with TPA alone was 10% (0.1) (Rice *et al.*, 1987).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with benzo[*j*]fluoranthene-4,5-diol at total doses of 275 or 1100 nmol [79 or 31.5 μg] in DMSO. Mice were weaned at age 28 days, separated by sex and killed at week 52 of the bioassay. Alveolar/bronchiolar carcinomas were observed in 41.2% of 34 and 89.5% of 19 surviving low- and high-dose female mice (0.44 and 3.63 tumours/mouse), respectively, and in 38.5% of 26 and 91.9% of 37 surviving low- and high-dose male mice (0.50 and 4.22 tumours/mouse), respectively, at 52 weeks. Hepatic tumours were observed in 30.8 and 56.8% of the surviving low- and high-dose males (0.5 and 1.43 tumours/mouse), respectively, but not in female mice, and 5.4% of the males had hepatic carcinomas. In 33 females and 33 males treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 and 18.2% of the surviving mice (0.24 and 0.18 tumours/mouse) and hepatic tumours developed in 0 and 9.1% (0 and 0.18 tumours/mouse). No hepatic carcinomas were observed in the DMSO controls (LaVoie *et al.*, 1994).

DNA adducts of benzo[*j*]fluoranthene-4,5-diol

Benzo[*j*]fluoranthene-4,5-diol formed a number of DNA adducts in mouse epidermis, and was determined to be the major proximate diol produced in mouse epidermis after treatment with benzo[*j*]fluoranthene by DNA adduct analyses. The major DNA adduct formed was *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine (Weyand *et al.*, 1993a).

Genotoxicity of benzo[*j*]fluoranthene-4,5-diol

Benzo[*j*]fluoranthene-4,5-diol induced mutations in *S. typhimurium* TA97a, TA98 and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993).

Carcinogenicity studies of benzo[*j*]fluoranthene-4,5-diol-6,6a-oxides

Groups of newborn Charles River CD-1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide at total doses of 110 or 275 nmol [79 or 31.5 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. Alveolar/bronchiolar carcinomas occurred in 88.9% of 36 and 95.8% of 24 surviving low- and high-dose females (2.14 and 8.63 tumours/mouse), respectively, and in 97% of 33 and 100% of 30 surviving low- and high-dose males (2.94 and 5.03 tumours/mouse), respectively. Hepatic tumours developed in 42.4 and 83.3% of the surviving males at 52 weeks (0.79 and 4.2 tumours/mouse), respectively, but not in female mice; hepatic carcinomas were observed in 12.1 and 33.3% of the surviving males, respectively. In mice treated with DMSO alone, alveolar/bronchiolar carcinomas in were observed in 21.2% of 33 female and 18.2% of 33 male surviving mice (0.24 and 0.18 tumours/mouse) and hepatic tumours developed in 0 and 9.1% of the surviving female and male mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed in DMSO controls (LaVoie *et al.*, 1994).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *syn*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide at total doses of 110, 275 or 1100 nmol [31.5, 79 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 29, 37 or 26 female mice, alveolar/bronchiolar carcinomas developed in 41.4, 21.6 or 11.5% of the surviving mice (0.52, 0.50 or 0.12 tumours/mouse), respectively. In 31, 28 or 34 male mice, alveolar/bronchiolar carcinomas developed in 64.5, 39.3 or 17.6% of the surviving mice (0.9, 0.5 or 0.2 tumours/mouse), hepatic tumours in 74.4, 28.6 or 23.5% of the surviving mice (3.27, 0.5 or 0.35 tumours/mouse) and hepatic carcinomas in 16.1, 14.3 and 0% of the surviving mice, respectively. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

Groups of female Charles River CD rats, 30 days of age, were maintained on a high-fat diet and administered *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide (total dose, 1.2 μmol) [343 μg] in DMSO by six direct applications to the tissue underlying each of the thoracic nipples beneath the mammary glands. The experiment was terminated after 44 weeks. In 20 rats, fibroadenomas/adenocarcinomas/dysplastic fibroadenomas incidence was 55% with a tumour latency of 36.2 weeks. In the DMSO-treated rats, mammary tumour incidence was 15% with a tumour latency of 39.7 weeks (Hecht *et al.*, 1996).

DNA adducts of benzo[*j*]fluoranthene-4,5-diol-6,6a-oxides

anti-Benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide formed a number of DNA adducts with calf thymus DNA (King *et al.*, 1994). The major DNA adduct produced by *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide in *S. typhimurium* TA97a and TA100 in the absence of an exogenous metabolic activation system was *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine (Marshall *et al.*, 1993). Both *anti*- and *syn*-benzo[*j*]-fluoranthene-4,5-diol-6,6a-oxides formed DNA adducts in mouse epidermis and the *anti* isomer had a higher adduct level. The major DNA adduct formed from the *anti* isomer was *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine (Weyand *et al.*, 1993a).

Genotoxicity of benzo[*j*]fluoranthene-4,5-diol-6,6a-oxides

Both *anti*- and *syn*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide were mutagenic in *S. typhimurium* TA97a, TA98 and TA100 in the absence of an exogenous metabolic activation system (Marshall *et al.*, 1993).

Carcinogenicity studies of benzo[*j*]fluoranthene-9,10-diol

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses every other day for a total initiating application of 30, 100 or 1000 μg [105, 349 or 3496 nmol] benzo[*j*]fluoranthene-9,10-diol in acetone followed 10 days later by 2.5 μg [4 nmol] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 5% (0.1), 20% (0.3) and 84% (4.5), respectively. No skin tumours were observed in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses every other day for a total initiating application of 3 μmol [859 μg] benzo[*j*]fluoranthene-9,10-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 60% (1.7). The responses in control mice treated with TPA alone was 10% (0.1) (Rice *et al.*, 1987).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with benzo[*j*]fluoranthene-9,10-diol at total doses of 275 or 1100 nmol [31.5 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 39 or 22 female mice, alveolar/bronchiolar carcinomas developed in 43.6 or 22.7% of the surviving mice (0.82 or 0.27 tumours/mouse)

and hepatic tumours in 7.9 or 4.5% of the surviving mice (0.21 or 0.05 tumours/mouse), respectively. In 22 or 40 male mice, alveolar/bronchiolar carcinomas developed in 63.6 or 27.5% of the surviving mice (1.0 or 0.53 tumours/mouse) and hepatic tumours in 81.8 or 25% of the surviving mice (2.82 or 0.32 tumours/mouse), respectively. The incidence of hepatic carcinomas was 27.3 or 5%. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

DNA adducts of benzo[*j*]fluoranthene-9,10-diol

Benzo[*j*]fluoranthene-9,10-diol formed three major and seven minor DNA adducts in mouse epidermis. The major DNA adducts seem to be related to both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide based on application of these diol epoxides to mouse epidermis (Weyand *et al.*, 1993b).

Genotoxicity of benzo[*j*]fluoranthene-9,10-diol

Benzo[*j*]fluoranthene-9,10-diol induced mutations in *S. typhimurium* TA97a, TA98 and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993).

Carcinogenicity studies of benzo[*j*]fluoranthene-9,10-diol-11,12-oxides

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide at total doses of 110, 275 or 1100 nmol [31.5, 79 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 21, 34 or 33 female mice, alveolar/bronchiolar carcinomas developed in 100, 55.9 or 30.3% of the surviving mice (15.1, 1.0 or 0.3 tumours/mouse) and hepatic tumours in 0, 8.8 or 0% of the surviving mice (0, 0.08 or 0 tumours/mouse), respectively. In 22, 22 or 33 male mice, alveolar/bronchiolar carcinomas developed in 100, 95.4 or 27.3% of the surviving mice (11.1, 2.82 or 0.27 tumours/mouse), hepatic tumours in 95.5, 77.3 or 18.2% of the surviving mice (5.51, 2.82 or 0.21 tumours/mouse) and hepatic carcinomas in 18.2, 13.6 or 0% of the surviving mice, respectively. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

Groups of female Charles River CD rats, 30 days of age, were maintained on a high-fat diet and administered *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide (total dose, 1.2 μmol) in DMSO by six direct applications to the tissue underlying each of the thoracic nipples beneath the mammary glands. The experiment was terminated after 44 weeks. In 20 rats, multiple tumour histologies were observed, the major type being fibroadenomas in 70% of the surviving rats, with a tumour latency of 21 weeks. In the DMSO-treated

rats, mammary tumour incidence was 15%, with a tumour latency of 39.7 weeks (Hecht *et al.*, 1996).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide at total doses of 110, 275 or 1100 nmol [31.5, 79 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 25, 25 or 28 female mice, alveolar/bronchiolar carcinomas developed in 68, 24 or 14.3% of the surviving mice (0.88, 0.36 or 0.14 tumours/mouse), respectively, but no hepatic tumours. In 38, 23, or 35 male mice, alveolar/bronchiolar carcinomas developed in 55.2, 26 or 37.1% of the surviving mice (0.78, 0.26 or 0.43 tumours/mouse), hepatic tumours in 68.4, 34.8 or 25.7% of the surviving mice and hepatic carcinomas in 26.3, 4.34 or 0% of the surviving mice, respectively. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

DNA adducts of benzo[*j*]fluoranthene-9,10-diol-11,12-oxides

anti-Benzo[*j*]fluoranthene-9,10-diol-11,12-oxide formed several adducts with calf thymus DNA (King *et al.*, 1994). One major DNA adduct was produced by *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide in *S. typhimurium* TA97a and TA100 in the absence of an exogenous metabolic activation system but not after treatment with benzo[*j*]fluoranthene in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993). Both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxides formed DNA adducts after application to mouse epidermis. However, these adducts were not observed in the epidermis of mice treated with benzo[*j*]fluoranthene (Weyand *et al.*, 1993a).

Genotoxicity of benzo[*j*]fluoranthene-9,10-diol-11,12-oxides

Both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide were mutagenic in *S. typhimurium* TA97a, TA98 and TA100 in the absence of an exogenous metabolic activation system (Marshall *et al.*, 1993).

Conclusion

Benzo[*j*]fluoranthene is metabolically activated by a diol epoxide mechanism.

In mouse skin, benzo[*j*]fluoranthene was metabolized to two diols, benzo[*j*]-fluoranthene-4,5-diol and benzo[*j*]fluoranthene-9,10-diol. Benzo[*j*]fluoranthene-4,5-diol was mutagenic to bacteria, initiated mouse skin tumours and induced both pulmonary and hepatic tumours in newborn mice. Both benzo[*j*]fluoranthene and benzo[*j*]fluoranthene-4,5-diol formed *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine DNA adducts in mouse skin. Both *anti*- and *syn*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide were mutagenic to bacteria, and induced pulmonary and hepatic tumours in newborn mice and DNA adducts on mouse skin. Benzo[*j*]fluoranthene-9,10-diol was mutagenic to bacteria,

initiated tumours and induced pulmonary and hepatic tumours in newborn mice. Benzo[*j*]fluoranthene-9,10-diol formed DNA adducts in mouse skin which are related to both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide. Both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide induced pulmonary and hepatic tumours in newborn mice and DNA adducts in mouse skin; however, these DNA adducts were not observed in mouse skin after treatment with benzo[*j*]fluoranthene. In the rat mammary model, both *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide and *anti*-benzo[*j*]-fluoranthene-4,5-diol-6,6a-oxide induced mammary tumours, although the former produced a higher tumour incidence. In summary, for mouse skin tumorigenesis, there is evidence for a diol epoxide mechanism for benzo[*j*]fluoranthene through benzo[*j*]fluoranthene-4,5-diol.

Benzo[*k*]fluoranthene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*k*]fluoranthene has been documented in several studies in rat liver microsomes. Benzo[*k*]fluoranthene is metabolized by Aroclor 1254-induced rat liver microsomes to the major metabolite, *trans*-8,9-dihydro-8,9-dihydroxybenzo[*k*]fluoranthene (benzo[*k*]fluoranthene-8,9-diol), benzo[*k*]fluoranthene-2,3-quinone and 3-, 8- and 9- hydroxybenzo[*k*]fluoranthene (LaVoie *et al.*, 1980; Weyand *et al.*, 1988).

(ii) *Formation of DNA adducts*

Benzo[*k*]fluoranthene forms a single unidentified DNA adduct in mouse skin epidermis *in vivo* (Weyand *et al.*, 1987).

(iii) *Genotoxic effects of benzo[k]fluoranthene*

In a previous monograph, benzo[*k*]fluoranthene was reported to induce mutations in *S. typhimurium* in the presence of an exogenous metabolic activation system (IARC, 1983). It is mutagenic in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Weyand *et al.*, 1988) and in human B lymphoblastoid h1A1v1 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

DNA adducts of benzo[*k*]fluoranthene-8,9-oxide

Benzo[*k*]fluoranthene-8,9-oxide formed one major DNA adduct with calf thymus DNA (King *et al.*, 1994).

Carcinogenicity study of benzo[*k*]fluoranthene-8,9-diol

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal application of 10 subdoses every other day for total initiating applications of 105, 349, or 3496 nmol [30, 100 or 1000 μg] benzo[*k*]fluoranthene-8,9-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment

was 15% (0.1), 10% (0.1) and 10% (0.4), respectively. No skin tumours were found in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Genotoxicity of benzo[*k*]fluoranthene-8,9-diol

Benzo[*k*]fluoranthene-8,9-diol is mutagenic in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (LaVoie *et al.*, 1980; Weyand *et al.*, 1988).

DNA adducts of benzo[*k*]fluoranthene-8,9-diol-10,11-epoxide

Benzo[*k*]fluoranthene-8,9-diol-10,11-epoxide formed one major DNA adduct with calf thymus DNA (King *et al.*, 1994).

Conclusion

There are insufficient data on benzo[*k*]fluoranthene to propose a route of metabolic activation. Benzo[*k*]fluoranthene is metabolized to benzo[*k*]fluoranthene-8,9-diol by rat liver preparations. Benzo[*k*]fluoranthene-8,9-diol is mutagenic to bacteria but is inactive as a tumour initiator in mouse skin.

Benzo[*c*]phenanthrene

The Working Group did not review all the mechanistic data on benzo[*c*]phenanthrene. However, there is evidence that benzo[*c*]phenanthrene can be activated metabolically by the diol epoxide mechanism. Benzo[*c*]phenanthrene was metabolized to benzo[*c*]phenanthrene-3,4-diol and benzo[*c*]phenanthrene-5,6-diol and unidentified monohydroxy derivatives in the presence of rat liver preparations (Ittah *et al*., 1983). The proximate fjord-region diol, benzo[*c*]phenanthrene-3,4-diol, was metabolized by rat liver microsomes to diastereomeric fjord-region diol epoxides that were mutagenic to bacterial and mammalian cells. One of both diastereomeric diol epoxides was considered to be an ultimate carcinogen (Wood *et al*., 1980). Benzo[*c*]phenanthrene-3,4-diol-1,2-oxide was a tumour initiator in mouse skin (Levin *et al*., 1980, 1986) and it induced mammary tumours in rats (Hecht *et al*., 1994; Amin *et al*., 1995a) and pulmonary tumours in newborn mice (Amin *et al*., 1995b).

Benzo[*a*]pyrene

It is universally accepted that most PAHs are biologically inert and require metabolism to reactive electrophilic metabolites that bind to DNA in the target tissue to initiate carcinogenesis. Using cell culture systems and isolated organelles from rodent and human tissues, PAHs have been shown to be metabolized by various pathways. Three major pathways are known by which PAHs are metabolized. These are: (i) metabolism by CYP enzymes and epoxide hydrolases to diol epoxides; (ii) metabolism through radical cation formation; and (iii) metabolism via quinones to yield reactive oxygen species. These pathways lead to active metabolites that are capable of binding to macromolecules. DNA lesions, if not repaired, may lead to mutations that may constitute the basis of the carcinogenic process. Other cellular pathways affected by benzo[*a*]pyrene may be

important in carcinogenesis, such as effects on cell proliferation, apoptosis and gap-junctional intercellular communication.

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*a*]pyrene has been documented in an extremely large number of in-vitro and in-vivo studies that encompass many phyla. The majority of these studies have used mouse, rat and human tissues. While there are many sites of metabolism on the benzo[*a*]pyrene ring system, there is one primary centre of metabolism and metabolic activation where formation of the bay-region diol epoxide takes place on the benzo ring, at the C7–C8 and C9–C10 bonds. The enzymes primarily responsible for the metabolism of benzo[*a*]pyrene are CYP1A1 and CYP1B1, which convert it to several different arene oxides that are enzymatically converted to diols by epoxide hydrolase.

In general, the most commonly identified metabolites of benzo[*a*]pyrene are three diols: the proximate bay-region diol, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (benzo[*a*]pyrene-7,8-diol), 9,10-dihydroxy-9,10-dihydrobenzo[*a*]pyrene (benzo[*a*]pyrene-9,10-diol) and the K-region diol, 4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene (benzo[*a*]-pyrene-4,5-diol); three quinones: benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone and benzo[*a*]pyrene-6,12-quinone; and several phenols: benzo[*a*]pyrene-3-phenol and benzo[*a*]pyrene-9-phenol. Under some conditions, benzo[*a*]pyrene-4,5-oxide may be observed, while benzo[*a*]pyrene-7,8-oxide and benzo[*a*]pyrene-9,10-oxide are too unstable to be isolated. Other minor metabolites that have been detected are 11,12-dihydroxy-11,12-dihydrobenzo[*a*]pyrene and 6-hydroxymethylbenzo[*a*]pyrene. Benzo-[*a*]pyrene was metabolized by liver microsomes or cultured hepatocytes from control, 5,6-benzoflavone-induced and phenobarbital-induced rats to the following metabolites: benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-9,10-diol, benzo[*a*]pyrene-4,5-diol, benzo[*a*]-pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone, benzo[*a*]pyrene-6,12-quinone, benzo[*a*]-pyrene-3-phenol and benzo[*a*]pyrene-9-phenol (Nesnow *et al.*, 1980).

In studies with cultured rodent cells, benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-9,10-diol, benzo[*a*]pyrene-4,5-diol, benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone, benzo[*a*]pyrene-6,12-quinone, benzo[*a*]pyrene-3-phenol and benzo[*a*]pyrene-9-phenol were identified after incubation of benzo[*a*]pyrene with primary rat hepatocytes (Shen *et al.*, 1980), mouse embryo cells (Nesnow *et al.*, 1981), primary mouse epidermal cells (excluding benzo[*a*]pyrene-4,5-diol) (DiGiovanni *et al.*, 1982a), hamster embryo cells (excluding benzo[*a*]pyrene-4,5-diol) (Nemoto *et al.*, 1979) and short-term organ cultures of rat and hamster trachea (Mass & Kaufman, 1978; Moore & Cohen, 1978).

In studies with cultured human cells, benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-9,10-diol, benzo[*a*]pyrene-4,5-diol, benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone, benzo[*a*]pyrene-6,12-quinone, benzo[*a*]pyrene-3-phenol and benzo[*a*]pyrene-9-phenol were identified after incubation of benzo[*a*]pyrene with cultured human bronchial explants (Hsu *et al.*, 1978), human bronchial epithelial cells (excluding benzo[*a*]pyrene-4,5-diol) (Siegfried *et al.*, 1986), human epidermal keratinocytes (excluding benzo[*a*]-pyrene-4,5-diol) (Kuroki *et al.*, 1980) and human skin fibroblasts (excluding

benzo[*a*]pyrene-4,5-diol and quinones) (Cunningham *et al.*, 1989). Benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-4,5-diol and quinones and 3- and 9-hydroxybenzo[*a*]pyrene were measured in lung and liver tissues from newborn mice after intraperitoneal administration of benzo[*a*]pyrene (Melikian *et al.*, 1989).

Human recombinant liver CYP1B1 and CYP1A1 in combination with epoxide hydrolase metabolized benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol; CYP1A1 was 10 times less active than CYP1B1 (Shimada *et al.*, 1999b). Benzo[*a*]pyrene binds at different levels to DNA in the epidermis of Cyp1a2$^{-/-}$, Cyp1b1$^{-/-}$ and Ahr$^{-/-}$ knockout mice *in vivo*, with the least binding in the Ahr$^{-/-}$ mice, which suggests that CYP1A1 is involved in the bioactivation of benzo[*a*]pyrene (Kleiner *et al.*, 2004). The metabolism of benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol was stereospecific. Uninduced, 3-methylcholanthrene-induced and phenobarbital-induced rat liver microsomes metabolized benzo[*a*]pyrene to benzo[*a*]pyrene-7*R*,8*R*-diol which was a result of the in-situ formation of benzo[*a*]pyrene-7*R*,8*S*-oxide (Yang, 1988).

(ii) *Formation of DNA adducts*

The most ubiquitous adduct detected in isolated mammalian DNA after metabolic activation of benzo[*a*]pyrene, in metabolically competent mammalian cells in culture or in mammals is the N^2-deoxyguanosine adduct, (+)-N^2-10*S*-(7*R*,8*S*,9*R*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene)-yl)–2′-deoxyguanosine, derived from 7*R*,8*S*-dihydroxy-9*R*,10*R*-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide). It was first fully identified from benzo[*a*]pyrene-treated human and bovine bronchial explants (Jeffrey *et al.*, 1977), and was actually visualized by electron microscopy on mouse embryo cell DNA as an antibody complex (Paules *et al.*, 1985).

This *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct has been detected in incubations of benzo[*a*]pyrene with calf thymus DNA or with Chinese hamster lung V79 cells or V79 cell nuclei in the presence of an Aroclor 1254-induced rat liver preparation (Sebti & Baird, 1984; Bodell *et al.*, 1989), in mouse, rat and hamster embryo cells exposed to benzo[*a*]pyrene in culture (Sebti *et al.*, 1985), in mouse skin DNA after topical treatment with benzo[*a*]pyrene (Ashurst *et al.*, 1983; Bodell *et al.*, 1989; Chen *et al.*, 1996), in epidermal and dermal skin of mice treated topically with benzo[*a*]pyrene (Huckle *et al.*, 1986), in mouse lung and liver after treatment with benzo[*a*]pyrene by gavage (Kulkarni & Anderson, 1984), in rat liver, lung and peripheral blood lymphocytes after intraperitoneal treatment with benzo[*a*]pyrene (Garner *et al.*, 1985; Ross *et al.*, 1990), in mouse skin explant cultures exposed to benzo[*a*]pyrene (Huckle *et al.*, 1986), in human bronchus and peripheral lung explants exposed to benzo[*a*]pyrene (Shinohara & Cerutti, 1977; Garner *et al.*, 1985), in human mammary epithelial cells exposed to benzo[*a*]pyrene (Moore *et al.*, 1987), in human MRC5CV1 fibroblast cells exposed to benzo[*a*]pyrene in the presence of an Aroclor 1254-induced rat liver fraction (Hanelt *et al.*, 1997) and in human epithelial lung BEAS-2B cells exposed to benzo[*a*]pyrene without an activation system (van Agen *et al.*, 1997).

In humans, the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts have been detected in autopsy samples from human lungs of smokers and nonsmokers (Lodovici *et al.*, 1998), in white blood cells of coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999a), cigarette smokers (Rojas *et al.*, 1995) and chimney sweeps (Pavanello *et al.*, 1999a).

(iii) *Genotoxicity of benzo[*a*]pyrene*

In a previous monograph, benzo[*a*]pyrene was reported to induce DNA repair, bacteriophages and mutations in bacteria, mutations in *Drosphila melanogaster*, DNA binding, DNA repair, sister chromatid exchange, chromosomal aberrations, point mutations and morphological cell transformation in mammalian cells; and point mutations, sister chromatid exchange, chromosomal aberrations, sperm abnormality and somatic mutations in bioassays in mammals *in vivo* (IARC, 1983). Benzo[*a*]pyrene induced DNA strand breaks and hypoxanthine(guanine)phosphoribosyltransferase (*HPRT*) gene mutation (6-thioguanine resistance) in human MRC5CV1 fibroblast cells in the presence of an Aroclor 1254-induced rat liver fraction (Hanelt *et al.*, 1997). It induced micronuclei and anchorage-independent growth (cell transformation) in human epithelial lung BEAS-2B cells (van Agen *et al.*, 1997).

(iv) *Benzo[*a*]pyrene-induced mutations in proto-oncogenes and tumour-suppressor genes*

Benzo[*a*]pyrene induced three types of Ki-*ras* codon 12 mutations in lung adenomas from treated strain A/J mice: GGT→TGT (56.3%), GGT→GTT (25%) and GGT→GAT (19%), indicating that deoxyguanosine was a primary target for this PAH in mouse lung (Mass *et al.*, 1993). Codon 13 c-Ha-*ras* mutations (GGC→GTC and GGC→CGC) (DiGiovanni *et al.*, 1993) and codon 61 mutations (CAA→CTA) were detected in Sencar mouse skin papillomas induced by benzo[*a*]pyrene (Chakravarti *et al.*, 1995).

Liu *et al.* (2005) have shown that, in murine embryonic fibroblasts from human *TP53* knock-in (Hupki) mice, benzo[*a*]pyrene induced mutations similar to those found in smoking-related lung cancer: a predominance of G→T mutations, unequivocal strand bias of the transversions and mutational hotspots at codons 157 and 158 (see below (viii)).

(v) *Evidence for the diol epoxide pathway as a mechanism of carcinogenesis*

Benzo[*a*]pyrene-7,8-oxide

*Carcinogenicity studies of benzo[*a*]pyrene-7,8-oxide*

Groups of 30–39 female C57BL/6J mice, 63 days of age, received dermal applications of 100 or 400 nmol [27 or 108 μg] racemic benzo[*a*]pyrene-7,8-oxide in acetone:ammonium hydroxide (1000:1) every two weeks for 60 weeks. In three or 25 mice autopsied at 60 weeks, two or 28 had squamous-cell carcinomas of the skin. There were no skin tumours in mice treated with acetone:ammonium hydroxide (1000:1) alone (Levin *et al.*, 1976).

A group of 29 female CD-1 mice, 51–65 days of age, received a single dermal application of 200 nmol [53 μg] racemic benzo[*a*]pyrene-7,8-oxide in acetone:-ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 30 weeks. The incidence of tumours in the dosed group 30 weeks after promotion was 89% (2.52 tumours/mouse). No data were reported on skin tumours in mice treated with TPA alone (Slaga *et al.*, 1976).

Groups of 30 female CD-1 mice, 52–59 days of age, received a single dermal application of 100 or 400 nmol [27 or 108 μg] racemic benzo[*a*]pyrene-7,8-oxide in acetone:ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of skin tumours in the two dose groups 26 weeks after promotion was 50 or 60% (0.83 or 1.67 tumours/mouse), respectively. Mice treated with TPA alone had a skin tumour incidence of 7% (0.07 tumours/mouse) (Levin *et al.*, 1980).

Groups of 30 female CD-1 mice, 52–59 days of age, received a single dermal application of 100 or 400 nmol [27 or 108 μg] (+)-benzo[*a*]pyrene-7,8-oxide or (–)-benzo[*a*]pyrene-7,8-oxide in acetone:ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of skin tumours in the two dose groups 26 weeks after promotion was 18 or 55% (0.54 or 1.03 tumours/mouse) or 11 or 36% (0.11 or 0.43 tumours/mouse), respectively. Mice treated with TPA alone had a tumour incidence of 7% (0.07 tumours/mouse) (Levin *et al.*, 1980).

Groups of newborn Swiss Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-oxide (total dose, 1.4 μmol) [375 μg] in DMSO:ammonium hydroxide (1000:1). Mice were weaned at 25 days of age, separated by sex and killed at 24 weeks of age. In 53 male and female mice, pulmonary tumours developed in 72% of the surviving mice (2.1 tumours/mouse). In 40 male and female mice treated with DMSO:ammonium hydroxide (1000:1) alone, pulmonary tumours developed in 8% of the surviving mice (0.08 tumours/mouse) (Wislocki *et al.*, 1978b).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-oxide, (+)-benzo[*a*]pyrene-7,8-oxide or (–)-benzo[*a*]pyrene-7,8-oxide (total dose, 700 nmol) [188 μg] in DMSO:ammonium hydroxide (1000:1). Mice were weaned at 23 days of age, separated by sex and killed at 31–35 weeks of age. In 63, 74 or 59 male and female mice, pulmonary tumours were observed in 89, 84 or 20% of the surviving mice (3.60, 2.28 or 0.25 tumours/mouse), respectively. In 74 male and female mice treated with DMSO:ammonium hydroxide (1000:1) alone, pulmonary tumours developed in 13% of the surviving mice (0.14 tumours/mouse) (Levin *et al.*, 1980).

Genotoxicity of benzo[a]pyrene-7,8-oxide

Benzo[*a*]pyrene-7,8-oxide was weakly mutagenic in *S. typhimurium* TA1538 (Wood *et al.*, 1975) and induced sister chromatid exchange in Chinese hamster ovary cells (Pal *et al.*, 1980).

Benzo[*a*]pyrene-7,8-diol

Carcinogenicity studies of benzo[a]pyrene-7,8-diol

A group of 29 female CD-1 mice, 51–65 days of age, received a single dermal application of 200 nmol [57 μg] racemic benzo[*a*]pyrene-7,8-diol in acetone:ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 30 weeks. The incidence of skin tumours in the dosed group 30 weeks after promotion was 86% (5.0 tumours/mouse). No data were reported for skin tumours in mice treated with TPA alone (Slaga *et al.*, 1977b).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal applications of 10 subdoses every other day for a total initiating application of 30 μg [136 nmol] racemic benzo[*a*]pyrene-7,8-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of skin tumours in the dosed groups 21 weeks after treatment was 90% (nine tumours/mouse). No skin tumours occurred in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Groups of 29–30 female CD-1 mice, 51–65 days of age, received a single dermal application of 100 nmol [29.6 μg] (–)-benzo[*a*]pyrene-7,8-diol or (+)-benzo[*a*]pyrene-7,8-diol in acetone:ammonium hydroxide (1000:1) followed 11 days later by 16 nmol [10 μg] TPA in acetone twice a week for 21 weeks. The incidence of skin tumours in the dosed groups 22 weeks after treatment was 77% (3.8 tumours/mouse) or 23% (0.43 tumours/mouse), respectively. The incidence of skin tumours in mice treated with TPA alone was 7% (0.7 tumours/mouse) (Levin *et al.*, 1977a).

Groups of 29 female C57BL/6J mice, 56–63 days of age, received dermal applications of 25, 50 or 100 nmol [7.2, 14.5 or 29 μg] racemic benzo[*a*]pyrene-7,8-diol in acetone:ammonium hydroxide (1000:1) every two weeks for 60 weeks. The incidence of squamous-cell carcinomas in the dosed groups at 60 weeks after treatment was 22% (seven tumours), 76% (24 tumours) or 92% (28 tumours), respectively. No skin tumours occurred in mice treated with acetone:ammonium hydroxide (1000:1) alone (Levin *et al.*, 1977b).

Groups of newborn Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-diol (total dose, 28 nmol) [8 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 28 weeks of age. In 62 male and female mice, pulmonary adenomas were observed in 66% of the surviving mice (1.77 tumours/mouse). In 67 male and female mice treated with DMSO alone, pulmonary tumours were observed in 12% of the surviving mice (0.13 tumours/mouse) (Kapitulnik *et al.*, 1978a).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-diol (total dose, 1.4 μmol) [400 μg] in

DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 17 weeks of age. In 18 male and female mice, pulmonary tumours developed in 100% of the surviving mice (59.1 tumours/mouse) and malignant lymphomas in 78% of the surviving mice. Under the same experimental conditions, benzo[*a*]pyrene (1.4 μmol) [353 μg] produced pulmonary tumours in 74% of the surviving mice (4.13 tumours/mouse). In 48 male and female mice treated with DMSO alone, pulmonary tumours were observed in 10% of the surviving mice (0.1 tumours/mouse) and no mice had malignant lymphomas (Kapitulnik *et al.*, 1978b).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (–)-benzo[*a*]pyrene-7,8-diol (total dose, 140, 700 or 1400 nmol) [40, 200 or 400 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 17 weeks of age. In 46, 29 or two male and female mice, pulmonary tumours developed in 98, 100 or 100% of the surviving mice (9.28, 32.2 or 5 tumours/mouse) and malignant lymphomas in 4, 70 or 83% of the surviving mice, respectively. In 48 male and female mice treated with DMSO alone, pulmonary tumours developed in 10% of the surviving mice (0.1 tumours/mouse) and no mice had malignant lymphomas (Kapitulnik *et al.*, 1978b).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (+)-benzo[*a*]pyrene-7,8-diol (total dose, 140, 700 or 1400 nmol) [40, 200 or 400 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 17 weeks of age. In 38, 35 or 47 male and female mice, pulmonary tumours developed in 16, 54 or 94% of the surviving mice (0.16, 2.34 or 18.5 tumours/mouse) and malignant lymphomas in 0, 6 or 0% of the surviving mice, respectively. In 48 male and female mice treated with DMSO alone, pulmonary tumours developed in 10% of the surviving mice (0.1 tumours/mouse) and no mice had malignant lymphoma (Kapitulnik *et al.*, 1978b).

Groups of 20 female Ha:ICR mice, 87 days of age, received oral intubations of racemic benzo[*a*]pyrene-7,8-diol in tricaprylin three times a week for 6 weeks (total dose, 18 μmol) [5153 μg] and were killed at 41 weeks of age. In 14 surviving mice at 32 weeks, tumour incidence was 100% for forestomach papilloma tumours (4.6 tumours/mouse), 30.6 pulmonary adenomas/mouse and 50% for lymphomas (almost all thymic in origin). In 19 surviving control mice that received vehicle alone, tumour incidence was 11% for forestomach papilloma tumours (0.1 tumours/mouse), 0.7 pulmonary adenomas/mouse and no lymphomas (Wattenberg *et al.*, 1979).

Groups of 18–19 male C3H/fCum mice, 28–35 days of age, received a single subcutaneous injection of 900 nmol [258 μg] racemic benzo[*a*]pyrene-7,8-diol in DMSO. The mice were killed after 18 months. The incidence of fibrosarcomas in the dosed group at 18 months after treatment was 42%. There were no tumours in mice treated with DMSO alone (Kouri *et al.*, 1980).

Metabolism of benzo[a]pyrene-7,8-diol

Racemic benzo[*a*]pyrene-7,8-diol was metabolized by control, 3-methylcholanthrene- or phenobarbital-induced rat liver microsomes to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (Thakker *et al.*, 1976). (–)-Benzo[*a*]pyrene-7,8-diol was metabolized by purified induced rabbit liver cytochromes to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide; the *anti* isomer predominated with 5,6-benzoflavone- and phenobarbital-induced forms (Deutsch *et al.*, 1978). Racemic benzo[*a*]pyrene-7,8-diol was metabolized to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide in epidermis after topical administration to mouse skin (Melikian *et al.*, 1987). (+)-Benzo[*a*]pyrene-7,8-diol was metabolized in mouse skin by two pathways: by CYP to (+)-*syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide and by peroxy radicals to (–)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (Pruess-Schwartz *et al.*, 1989). Hamster tracheal and human bronchus explants metabolized racemic benzo[*a*]pyrene-7,8-diol to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide; the *anti* isomer predominated. Prostaglandin H synthetase co-oxygenation in the human bronchus explants produced the *anti* isomer (Reed *et al.*, 1984).

DNA adducts of benzo[a]pyrene-7,8-diol

Racemic benzo[*a*]pyrene-7,8-diol forms four DNA adducts following topical treatment of mouse skin: two *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts and two *syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts (Rojas & Alexandrov, 1986).

Genotoxicity of benzo[a]pyrene-7,8-diol

Racemic benzo[*a*]pyrene-7,8-diol induced mutations in *S. typhimurium* strain TA100 in the presence of intact hepatocytes, homogenized hepatocytes or homogenized hepatocytes with an NADPH-generating system from rats pretreated with Aroclor 1254 (Glatt *et al.*, 1981) and in TA98 in the presence of a reconstituted cytochrome P448 system (Wood *et al.*, 1977c). Racemic benzo[*a*]pyrene-7,8-diol induced mutations in Chinese hamster V79 cells (8-azaguanine and ouabain resistance) in the presence of 3-methylcholanthrene-induced rat liver preparations (Kuroki *et al.*, 1979), in V79 cells (6-thioguanine resistance) that express rat CYP1A1 (Dogra *et al.*, 1990) and in V79 cells (ouabain resistance) co-cultured with human bronchus explants (Hsu *et al.*, 1978). Racemic benzo[*a*]pyrene-7,8-diol induced mutations in Chinese hamster ovary cells (6-thioguanine resistance) supplemented with an Aroclor 1254-induced rat liver preparation (Recio & Hsie, 1987). It induced morphological cell transformation in C3H10T½Cl8 cells (Gehly *et al.*, 1982). Each enantiomer of benzo[*a*]pyrene-7,8-diol induced malignant transformation of cultured rat hepatocytes (Heintz *et al.*, 1980).

Benzo[*a*]pyrene-7,8-diol-9,10-oxide in animals

Carcinogenicity studies of benzo[a]pyrene-7,8-diol-9,10-oxide in animals

Groups of 29 female CD-1 mice, 49–63 days of age, received a single dermal application of 200 nmol [60 μg] racemic *anti*-benzo[*a*]pyrene-7β,8α-diol-9α,10α-oxide or *syn*-benzo[*a*]pyrene-7β,8α-diol-9β,10β-oxide in anhydrous DMSO:acetone (1:3), follo-

wed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 30 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 30 weeks after promotion was 69 or 7% (1.5 or 0.07), respectively. No data were reported on skin tumours in mice treated with TPA alone (Slaga *et al.*, 1977b).

Groups of 29–30 female Sencar mice, 51–65 days of age, received a single dermal application of 100 nmol [30 μg] (+)-*anti*-benzo[*a*]pyrene-7β,8α-diol-9α,10α-oxide, (–)-*syn*-benzo[*a*]pyrene-7β,8α-diol-9β,10β-oxide, (–)-*anti*-benzo[*a*]pyrene-7α,8β-diol-9β,10β-oxide or (+)-*syn*-benzo[*a*]pyrene-7α,8β-diol-9α,10α-oxide in anhydrous tetrahydrofuran, followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 24 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 24 weeks after promotion were 75% (2.0), 13% (0.13), 3% (0.03) or 10% (0.01), respectively. The incidence of skin tumours (tumours/mouse) in mice treated with TPA alone was 10% (0.1). In comparison, the incidence of skin tumours (tumours/mouse) in mice treated with benzo[*a*]pyrene (100 nmol) [25 μg] was 86% (2.8) (Slaga *et al.*, 1979).

Groups of newborn Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide or racemic *syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (total dose, 28 nmol) [8.5 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 28 weeks of age. In 64 or 21 male and female mice, pulmonary adenomas developed in 86 or 10% of the surviving mice (4.42 or 0.14 tumours/mouse), respectively. In 67 male and female mice treated with DMSO alone, pulmonary tumours occurred in 12% of the surviving mice (0.13 tumours/mouse) (Kapitulnik *et al.*, 1978a).

Groups of Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, (–)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, (+)-*syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide or (–)-*syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (total dose, 7 or 14 nmol) [2 or 4 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 34–37 weeks of age. Only mice treated with (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide had a significant incidence of tumours. In 54 or 79 male and female mice treated with (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, pulmonary tumours (adenomas and adenocarcinomas) developed in 100 or 71% of the surviving mice (7.67 or 1.72 tumours/mouse), respectively. In 98 male and female mice treated with DMSO alone, pulmonary tumours (adenomas and adenocarcinomas) occurred in 11% of the surviving mice (0.12 tumours/mouse) (Buening *et al.*, 1978).

*DNA adducts of benzo[*a*]pyrene-7,8-diol-9,10-oxide*

Benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts have been measured in various mammalian cells and tissues exposed to benzo[*a*]pyrene-7,8-diol-9,10-oxide. An *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct was detected in Chinese hamster V79 cells (Sundberg *et al.*, 2002), in mouse skin DNA after topical treatment with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (Chen *et al.*, 1996), in human A549

epithelial lung carcinoma cells (Dreij *et al.*, 2005), in human lymphoblasts *in vitro* (Vähäkangas *et al.*, 1985) and in human fibroblasts (Hanelt *et al.*, 1997).

Genotoxicity of benzo[a]pyrene-7,8-diol-9,10-oxide

Both racemic *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide were mutagenic in *S. typhimurium* TA97, TA98, TA100 and TA104, induced SOS response in *E. coli* (SOS chromotest in strain PQ37) and were mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) (Glatt *et al.*, 1991). Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induced chromosomal aberrations (predominantly single chromatid breaks) in cultures of lymphocytes from normal individuals *in vitro* (Wei *et al.*, 1996). Both racemic *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induced sister chromatid exchange in Chinese hamster ovary cells; the *anti* isomer was 10-fold more active than the *syn* isomer (Pal *et al.*, 1980). Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induced DNA strand breaks and *HPRT* gene mutation (6-thioguanine resistance) in human fibroblast MRC5CV1 cells (Hanelt *et al.*, 1997).

(+)-Benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide induced mutations in the coding region of the *HPRT* gene in Chinese hamster V79 cells. Mutations at GC base pairs exceeded those at AT base pairs (Wei *et al.*, 1993). Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide morphologically transformed C3H/10T½ cells in culture (Krolewski & Little, 1985) and transformed human fibroblast MSU-1.1 cells into cell strains that formed colonies in agarose and formed sarcomas when injected into athymic mice (Yang *et al.*, 1992). (+)-Benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide also induced mutations, mainly at G:C base pairs (GC→TA), frameshift mutations and large deletion mutations in the *supF* gene of pUB3 in an *E. coli* plasmid (Rodriguez & Loechler, 1993).

DNA repair of adducts: quantitative repair

Benzo[*a*]pyrene-diol epoxide–DNA adducts in C57BL/6 mouse skin were repaired within 1 week after a single dose of benzo[*a*]pyrene (Bjelogrlic *et al.*, 1994).

In human A549 lung carcinoma cells, about 45% of the benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts formed by incubation with 50 nM (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide for 2 h were repaired within 8 h after treatment (Schwerdtle *et al.*, 2003).

Conclusion

There is strong evidence that benzo[*a*]pyrene is metabolically activated to the diol epoxide at the bay region in mouse skin and mouse lung carcinogenesis. Benzo[*a*]pyrene was metabolized by mouse and rat liver microsomes, rat hepatocytes, mouse epidermal tissues, hamster and mouse embryo cells, rat and hamster trachea, mouse skin and mouse lung tissues, human liver microsomes, human bronchial explants and epithelial cells, and human epidermal keratinocytes and fibroblasts to benzo[*a*]pyrene-7,8-diol. This metabolism proceeded through to two oxides: the major form, (+)-benzo[*a*]pyrene-7*R*,8*S*-oxide, and the minor form, (–)-benzo[*a*]pyrene-7*S*,8*R*-oxide, which, upon the action of epoxide hydrolase, gave (–)-benzo[*a*]pyrene-7*R*,8*R*-diol and (+)-benzo[*a*]pyrene-7*S*,8*S*-

diol, respectively. Upon further oxidation, (–)-benzo[*a*]pyrene-7*R*,8*R*-diol was metabolized to the major *anti* diol epoxide form, *anti*-(+)-benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide, and the minor *syn* diol epoxide form, *syn*-(–)-benzo[*a*]pyrene-7*R*,8*S*-diol-9*R*,10*S*-oxide; (+)-benzo[*a*]pyrene-7*S*,8*S*-diol was metabolized to the minor *anti* diol epoxide form, *anti*-(–)-benzo[*a*]pyrene-7*S*,8*R*-diol-9*R*,10*S*-oxide, and the major *syn* diol epoxide form, *syn*-(+)-benzo[*a*]pyrene-7*S*,8*R*-diol-9*S*,10*R*-oxide. Racemic benzo[*a*]pyrene-7,8-oxide was genotoxic in bacteria and mammalian cells. (+)-Benzo[*a*]pyrene-7*R*,8*S*-oxide was more active than (–)-benzo[*a*]pyrene-7*S*,8*R*-oxide as a tumour initiator in mouse skin and as a pulmonary carcinogen in newborn mice. Racemic benzo[*a*]pyrene-7,8-diol was genotoxic in bacteria and in mammalian cells in the presence of various metabolic activation systems. (–)-Benzo[*a*]pyrene-7*R*,8*R*-diol was more active than (+)-benzo[*a*]pyrene-7*S*,8*S*-diol as a tumour initiator in mouse skin, as a pulmonary carcinogen in newborn mice and in the induction of malignant lymphomas in newborn mice. In mice, racemic benzo[*a*]pyrene-7,8-diol induced forestomach papillomas after treatment by gavage, malignant skin tumours after repeated treatment of the skin and fibrosarcomas after subcutaneous treatment.

Both racemic *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide were genotoxic in bacteria and mammalian cells. Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide was more active that the *syn* isomer as a tumour initiator in mouse skin and as a pulmonary carcinogen in mice and (+)-*anti*-benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide had the greatest activity in both bioassay systems. Quantitatively, (+)-*anti*-benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide was more active as a tumour initiator in mouse skin or pulmonary carcinogen in newborn mice than (–)-benzo[*a*]pyrene-7*R*,8*R*-diol which was more carcinogenic than benzo[*a*]pyrene. The most common benzo[*a*]pyrene diol epoxide–DNA adduct detected in multiple bioassay systems was the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct. This adduct was formed *in vitro* and *in vivo* after treatment with benzo[*a*]pyrene, benzo[*a*]pyrene-7,8-diol or *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, and is consistent with proto-oncogene mutations in tumours induced by benzo[*a*]pyrene. In addition, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts are preferentially formed at lung cancer mutational hotspots in human p53 (see below (viii)).

(vi) *Evidence for other metabolic activation mechanisms*

9-Hydroxybenzo[*a*]pyrene was metabolized by an Aroclor 1254-induced rat liver preparation to an intermediate that formed a single DNA adduct in calf thymus DNA and in Chinese hamster V79 lung cell DNA (Sebti & Baird, 1984). *In vivo*, intraperitoneal administration of 9-hydroxybenzo[*a*]pyrene to rats produced this adduct in lung and peripheral blood lymphocytes (Ross *et al.*, 1990). It was identified as a 9-hydroxybenzo[*a*]pyrene-4,5-oxide adduct of deoxyguanosine (Vigny *et al.*, 1980; Fang *et al.*, 2001), and was detected *in vivo* after intraperitoneal exposure to benzo[*a*]pyrene of mouse lung (Mass *et al.*, 1993) and of rat lung and peripheral blood lymphocytes (Ross *et al.*, 1990). The significance of this adduct is questionable since 9-hydroxybenzo[*a*]pyrene

was not carcinogenic on mouse skin (Kapitulnik *et al.*, 1976), and was a weak tumour initiator (Slaga *et al.*, 1978b).

(vii) *Epigenetic effects of benzo[a]pyrene and its metabolites*

Effects on cell proliferation

Benzo[*a*]pyrene increased cell proliferation in primary human mammary epithelial cells (Tannheimer *et al.*, 1997) and in the spontaneously immortalized, non-tumorigenic growth factor-dependent human mammary epithelial cell line, MCF-10A (Tannheimer *et al.*, 1998). In human mammary epithelial cells, benzo[*a*]pyrene increased the level of intracellular Ca^{2+} after 2 h, but the effect was maximal at 18 h (Tannheimer *et al.*, 1997). Under the same conditions, no increase in calcium was seen with benzo[*e*]pyrene or anthracene. When benzo[*a*]pyrene was given at concentrations of 1, 3 and 10 μM the increase in Ca^{2+} after 18 h was dose-dependent (Tannheimer *et al.*, 1999) and dependent on metabolism, because benzo[*a*]pyrene-7,8-diol and benzo[*a*]pyrene diol epoxide were more effective than the parent compound and the increase in Ca^{2+} was inhibited by α-naphthoflavone, a CYP1A and CYP1B inhibitor. *N*-Acetylcysteine, an antioxidant, did not inhibit the increase in Ca^{2+}, which indicates that oxidative damage by benzo[*a*]pyrene is not responsible. The increase in Ca^{2+} seemed to be due to the influx of extracellular Ca^{2+}, which may be caused by the effects of benzo[*a*]pyrene on cell membranes where it can perturb the physical organization of phosphatidylcholine membranes (Jiménez *et al.*, 2002). Free intracellular Ca^{2+} is important for the activation of protein kinase C (PKC) pathways, which again is associated with tumour promotion (Rasmussen *et al.*, 1995).

1,6- and 3,6-Benzo[*a*]pyrene quinones produce superoxide anion and hydrogen peroxide in MCF-10A cells. They also increased epidermal growth factor receptor (EGFR), serine/threonine kinase Akt and extracellular signal-regulated kinase (ERK) activity, which led to increased cell numbers in the absence of epidermal growth factor. The benzo[*a*]pyrene quinone-induced EGFR activity and associated cell proliferation were attenuated by the EGFR inhibitor AG1478, as well as by the antioxidant *N*-acetylcysteine (Burdick *et al.*, 2003).

Low concentrations of benzo[*a*]pyrene (1, 3 or 10 μM) [0.25, 0.75 or 2.5 μg/mL] induce proliferation of cultured rat osteoblasts, the human osteosarcoma cell line, MG-63, as well as the human breast carcinoma cell line, MCF-7 (Tsai *et al.*, 2004). This effect could be inhibited by antagonists of estrogen receptors and inhibitors of ERK/mitogen-activated PK (MAPK) and phosphatidylinositol-3-kinase (PI3K), but not by α-naphthoflavone (AhR antagonist) or the p38 MAPK inhibitor. Benzo[*a*]pyrene induced phosphorylation of ERK1/2 and protein kinase B (Akt) (PI3K downstream effector). Other proteins that were increased in benzo[*a*]pyrene-treated osteoblasts were proliferating cell nuclear antigen, cyclooxygenase 2 (COX-2) (but not COX-1) and prostaglandin E_2. COX-2 inhibitors also inhibited benzo[*a*]pyrene-induced osteoblast proliferation. Proliferation at lower concentrations (up to 1 μM [0.25 μg/mL]), but cell death at higher concentrations of benzo[*a*]pyrene (10 μM) [2.5 μg/mL] were reported in

MCF-7 cells. Benzo[*a*]pyrene also induced p53 protein and a partial S-phase arrest (Pliskova *et al.*, 2005).

Benzo[*a*]pyrene diol epoxide increased Cdc25B (which regulates cell cycle progression and genetic stability) mRNA and protein levels in terminal squamous differentiated human bronchial epithelial cells and lung cancer cells but not in undifferentiated bronchial cells. With chronic exposure, the growth rate of lung cancer cells was increased significantly (Oguri *et al.*, 2003).

In the human trophoblastic JEG-3 cell line, benzo[*a*]pyrene inhibits proliferation in a dose-dependent manner by causing cell-cycle arrest at the G2/M phase, with a marked increase in p53 phosphorylation at serine 15 which is known to activate p53. However, no evidence of apoptosis was noted (Drukteinis *et al.*, 2005).

Effects on apoptosis

Benzo[*a*]pyrene has been shown to induce apoptosis in many types of murine and human cells, e.g. rat Sertoli cells (Raychoudhury & Kubinski, 2003), mouse hepatoma Hepa1c1c7 cells (Ko *et al.*, 2004) and human HepG2 cells (Chen *et al.*, 2003). There are many ways to apoptosis and benzo[*a*]pyrene may affect different apoptotic pathways in different cell types. For instance, benzo[*a*]pyrene-induced apoptosis has been reported to be Jun N-terminal kinase (JNK)-dependent in HeLa cells, but independent in Hepa1c1c7 cells (Yoshii *et al.*, 2001; Solhaug *et al.*, 2005).

Using mouse Hepa1c1c7 cells, Chen, S. *et al.* (2003) showed that the effect of benzo[*a*]pyrene-7,8-diol is AhR-dependent, but that of benzo[*a*]pyrene diol epoxide is not. Mouse embryo fibroblasts null for p38 were resistant to the apoptotic effect of the latter. In Hepa1c1c7 cells, Ko *et al.* (2004) showed that catalytic activation of caspases 3 and 9 by benzo[*a*]pyrene was associated with cytosolic release of cytochrome c, a decrease in B-cell lymphoma protein 2 (Bcl-2) to Bcl-2-antagonist X protein (Bax) ratio and ser-15 phosphorylation of p53. Benzo[*a*]pyrene and its 7,8-dihydrodiol and diol epoxide metabolites induced apoptosis and phosphorylation of p53 in mouse hepatoma Hepa1c1c7 cells, while benzo[*a*]pyrene-4,5-dihydrodiol did not. While Bcl-xl, Bad and Bid proteins were down-regulated, the anti-apoptotic phosphor-βad was up-regulated (Solhaug *et al.*, 2005). In rat liver cells, Na^+/H^+ exchange has been implicated as an early target of benzo[*a*]pyrene-induced apoptosis (Huc *et al.*, 2004).

At high concentrations (10, 50 or 100 μg/mL, equivalent to about 50, 200 or 400 μM), benzo[*a*]pyrene inhibits growth and is apoptotic in human mammary carcinoma MCF-7 cells (Ogba *et al.*, 2005). In A549 cells, BAX expression was increased at 24 h by a 25-μM [6.3 μg/mL] concentration of benzo[*a*]pyrene (Zhu *et al.*, 2005). Benzo[*a*]pyrene induced apoptosis in the human lung fibroblast cell line, MRC-5, via JNK1/FasL and JNK/p53 pathways (Chen *et al.*, 2005). According to studies in human embryonal kidney 293T cells and HeLa cells, p21 PK interacting factor accelerates benzo[*a*]pyrene-induced apoptosis through activation of JNK1 pathway kinases (Yoshii *et al.*, 2001). In human HepG2 cells, both benzo[*a*]pyrene-7,8-diol and benzo[*a*]pyrene diol

epoxide induced apoptosis and increased the phosphorylation of both MAPK p38 and ERK1/2 (Chen *et al.*, 2003).

In primary human haematopoietic CD34+ stem cells, benzo[*a*]pyrene induced the expression of the cleaved forms of caspases 3 and 9 and reduced mitochondrial membrane potential, and increased annexin V-positive cells indicative of apoptosis (van Grevenynghe *et al.*, 2005).

Effects on cell cycle

Exposure of A549 cells to a 25-μM concentration of benzo[*a*]pyrene for up to 72 h did not result in substantial phase-specific cell-cycle effects, but induced a distinctive pattern of gene expression: CYP1B1 was up-regulated at 6–24 h and many cell-cycle regulatory genes were down-regulated at 48–72 h (Zhu *et al.*, 2005).

A lower concentration (1 μM) [0.25 μg/mL] of benzo[*a*]pyrene induced an increase in p53 protein in MCF-7 and A549 cells that activate benzo[*a*]pyrene to benzo[*a*]pyrene diol epoxide–DNA adducts (Rämet *et al.*, 1995). A similar effect was seen in the skin of C57BL/6 mice *in vivo* (Bjelogrlic *et al.*, 1994; Tapiainen *et al.*, 1996). There is a positive correlation between the amount of induced p53 and benzo[*a*]pyrene diol epoxide–DNA adducts as measured by synchronous fluorescence spectrophotometry. Since these original studies, benzo[*a*]pyrene-induced, benzo[*a*]pyrene-diol epoxide–DNA-associated induction of p53 protein has been described in many experimental systems (e.g. in human lymphocytes exposed to benzo[*a*]pyrene *in vitro*) (Godschalk *et al*., 2001).

Gap-junctional intercellular communication

Gap-junctional communication is important in cell proliferation, differentiation and apoptosis and has been suggested to be important for the promotion of carcinogenesis (see Blaha *et al.*, 2002). Of 35 PAHs tested for inhibition of gap-junctional communication in WB-F344 rat liver epithelial cells, 12, including benzo[*a*]pyrene, were found to be strong but transient inhibitors (Blaha *et al.*, 2002).

Upham *et al.* (1994) compared the effect of a selection of methylated and non-methylated PAHs on gap-junction intercellular communication of rat liver epithelial cells with a scrape-loading/dye transfer assay. Methylated PAHs inhibited cell–cell communication to a greater extent than their non-methylated counterparts, and three-ringed PAHs to a greater extent than four- or five-ringed PAHs. Benzo[*a*]pyrene reduces the attachment of cultured human endometrial cells to Matrigel-coated membranes in culture, which is also relevant to the effect of benzo[*a*]pyrene on cell–cell communication (McGarry *et al.*, 2002). This was associated with decreased localization of EGFR protein along the cell membrane. A similar effect on EGFRs has been caused by benzo[*a*]pyrene in cell lines of placental origin and by cigarette smoking in the placenta (Zhang *et al*., 1995). *In vitro*, the physical organisation of phosphatidylcholine membranes was disturbed by benzo[*a*]pyrene (Jiménez *et al.*, 2002). Benane *et al.* (1999) demonstrated that multiple concentrations of benzo[*a*]pyrene and dibenzo[*a,l*]pyrene failed to induce any alterations in gap-junction intercellular communication in clones of normal rat

hepatocytes. However, DNA from the exposed cells was shown to contain PAH–DNA adducts, demonstrating that the cells were able to take up and metabolize both PAHs.

(viii) *Relevance of the diol epoxide mechanism for benzo[*a*]pyrene to human cancer*

General

Pathways of benzo[*a*]pyrene metabolism that possibly induce mutations include the formation of bulky benzo[*a*]pyrene-diol epoxide–DNA adducts, or the formation of radical cations, reactive oxygen species and *ortho*-quinones (see below). Both experimental and human data link bulky benzo[*a*]pyrene diol epoxide–DNA adducts with mutations in oncogenes and tumour-suppressor genes in human lung cancer.

The processes that metabolize benzo[*a*]pyrene to its active diol epoxides are present in human tissues, and humans exposed to benzo[*a*]pyrene metabolically activate it to benzo[*a*]pyrene diol epoxides that form DNA adducts. The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts have been measured in populations exposed to benzo[*a*]pyrene in complex mixtures including coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999a) and chimney sweeps (Pavanello *et al.*, 1999a) (as mentioned above in (ii)).

Studies on the ras gene

Experimental studies

anti-Benzo[*a*]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells. The mutations (G→T transversions) in the K-*ras* proto-oncogene in lung tumours from benzo[*a*]pyrene-treated mice are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts (Mass *et al.*, 1993). Although this is not a direct correlation, similar mutations in the Ki-*ras* proto-oncogene were found in lung tumours from nonsmokers exposed to PAH-rich coal combustions (known to contain benzo[*a*]pyrene as well as many other PAHs) (DeMarini *et al.*, 2001).

Low doses of benzo[*a*]pyrene (8 nmol) [2 μg/mL] or benzo[*a*]pyrene combined with ultraviolet A (UVA) radiation induced G→A transitions in H-*ras* codon 12 or 13 in SKH-1 mouse skin, while no *TP53* mutations were found. Only the second guanosine of the codon was changed, and the *ras* mutations were already found in non-tumorous skin of all the mice after 10 weeks of treatment with benzo[*a*]pyrene (Wang *et al.*, 2005).

Benzo[*a*]pyrene induces G:C→T:A mutations in the murine *ras* gene (Lehman & Harris, 1994). While in some studies the majority of K-*ras* mutations in smoking-associated human lung cancer have been G→T transversions, discrepant results occur in the literature (Vähäkangas *et al.*, 2001 and references therein). Codons 12 and 14 of K-*ras* in genomic DNA from normal human bronchial epithelial (NHBE) cells are targeted *in vitro* by benzo[*a*]pyrene-7,8-diol-9,10-epoxide (2 μM [0.5 μg/mL], which is a high dose) (Hu *et al.*, 2003). In NHBE cells, benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts are preferentially formed within K-*ras* codon 12 (Feng *et al.*, 2002). Feng *et al.* (2002) also found that the repair of benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts formed at codon 14 was significantly faster than that of adducts at codon 12.

Human studies

There is a clear difference between the exact localization of K-*RAS* codon 12 mutations in colon cancer (not linked to smoking) and lung cancer (associated with smoking) (see Kelley & Littman, 2002). While in colon cancer 80% of K-*RAS* codon 12 mutations occur at position 2, in lung cancer, 50% occur at position 1, which is the same position where benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts are preferentially formed in NHBE cells.

Benzo[*a*]pyrene adducts (7-(benzo[*a*]pyren-6-yl)guanine) have been found in the urine of nonsmoking women exposed to smoky coal (Casale *et al*., 2001). Lung cancers from such women have a high percentage of GC→TA transversions of the *TP53* (76%) and K-*RAS* (86%) mutations (DeMarini *et al*., 2001). Smoky coal emissions, cigarette-smoke condensate and benzo[*a*]pyrene induce similar mutation spectra in *Salmonella* base substitution strain TA100: primarily GC→TA transversions (Granville *et al*., 2003). In a recent study (Le Calvez *et al.*, 2005) based on the analysis of primary lung tumours from 64 (mainly) heavy smokers, 27 former smokers and 40 never smokers, K-*RAS* mutations were more frequent in former smokers than in never or current smokers.

H-*RAS*-Gene mutations correlated with the N7-guanine and N7-adenine depurinating adducts (Colapietro *et al.*, 1993; Chakravarti *et al.*, 1995). Occupational exposures to PAHs, benzo[*a*]pyrene and gasoline have a weak association with K-*RAS* mutations in pancreatic cancer (Alguacil *et al.*, 2003).

Studies on TP53

The importance of p53

p53 Protein is of interest in connection with benzo[*a*]pyrene because it is in the centre of cellular defence against DNA damage (for recent reviews, see e.g. Meek, 2004; Harris & Levine, 2005). p53 Protein is involved in the regulation of cell cycle and apoptosis, as well as DNA repair, mainly by acting as a transcription factor for cellular key proteins involved in these processes. The significance of p53 protein as a tumour suppressor is demonstrated by studies on p53-deficient mice that develop tumours (predominantly lymphomas) spontaneously by the age of 6 months (reviewed by Hoogervorst *et al.*, 2005). $p53^{-/-}$ Mice are viable except for some female embryos that die before birth of neural tube defects. About 50% of $p53^{+/-}$ mice develop predominantly osteosarcomas and soft-tissue sarcomas by the age of 18 months. This phenotype mimics partially human patients with Li-Fraumeni syndrome, who, in addition to sarcomas, also develop breast and brain tumours that are rarely observed in p53-deficient mice. Importantly for chemical carcinogenesis, p53-deficient mice show enhanced tumour response among other tissues (in lung and skin), when treated with known chemical carcinogens.

Experimental studies of TP53 mutations

Experimental findings on benzo[*a*]pyrene-induced lesions should account for the mutations seen in *TP53* in patients with lung cancer, e.g. mutational pattern (G→T transversions), mutational spectrum (mutations at specific codons 157,158, 245, 248,

273), preference for methylated CpG islands and the bias for the non-transcribed strands. Two experimental paradigms could account for these lesions: (i) site-specific adduction of hotspots by *anti*-benzo[*a*]pyrene diol epoxide or (ii) random mutagenesis by reactive oxygen species coupled with biological selection. Most of the existing data support the former hypothesis (Pfeifer & Hainaut, 2003). Metabolic activation can account for both of these paradigms: CYP activation of benzo[*a*]pyrene to *anti*-benzo[*a*]pyrene diol epoxide or AKR activation of benzo[*a*]pyrene-7,8-diol to benzo[*a*]pyrene-dione which is redox active.

Incorporation of a single (+)- or (–)-*trans*-*anti*-benzo[*a*]pyrene diol epoxide–DNA adduct at the second position of codon 273 ($^{5'}$CGT) of the human *TP53* gene in Simian kidney COS-7 cells resulted in predominant mutations by both stereoisomers being G→T transversions, with some G→A transitions (Dong *et al.*, 2004). When the cytosine 5′ to deoxyguanosine-N^2-benzo[*a*]pyrene diol epoxide was replaced by 5-methylcytosine, the mutational frequencies of (+)-*trans*-deoxyguanosine-N^2-benzo[*a*]pyrene diol epoxide and (–)-*trans*-deoxyguanosine-N^2-benzo[*a*]pyrene diol epoxide were reduced, while the mutational specificity remained unchanged. Thus, the mutational hotspot at codon 273 in *TP53* may reflect either sequence-specific reactivity of benzo[*a*]pyrene diol expoxide and/or inefficient repair of its DNA adducts positioned at this site.

Denissenko *et al.* (1996), Pfeifer and Denissenko (1998) and Smith *et al.* (2000) have shown that codons 157, 248 and 273 in the *TP53* gene are most mutated in lung cancer, are also targets for DNA adduct formation by benzo[*a*]pyrene and are more prone to mutations by it. Slow repair of these adducts (Denissenko *et al.*, 1998) and the preferential formation of benzo[*a*]pyrene diol epoxide adduct at methylated CpG sites (Denissenko *et al.*, 1997; Tang *et al.*, 1999) probably contribute to the mutation spectrum in smoking-related lung cancer, where G→T mutations at CpG sites are the most common single type of mutation (Hainaut & Pfeifer, 2001). The same group (Smith *et al.*, 2000) has also studied other PAH compounds and found a similar preference for adduct formation in codons 157, 158, 245, 248 and 273 in human bronchial epithelial BEAS-2B cells. In support for these findings, Hussain *et al.* (2001) demonstrated a high dose-dependent G:C to T:A transversion rate of *TP53* codons 157 and 248 by benzo[*a*]pyrene diol epoxide in the same BEAS-2B cell line.

Smoky coal combustion induced GC→TA transversions in *Salmonella* to a similar extent as cigarette-smoke condensate and benzo[*a*]pyrene (Granville *et al.*, 2003). Liu *et al.* (2005) showed that, in murine embryonic fibroblasts from human *TP53* knock-in (Hupki) mice, benzo[*a*]pyrene induced mutations similar to those found in smoking-related lung cancer: predominance of G→T mutations, unequivocal strand bias of the transversions and mutational hotspots at codons 157 and 158.

Human studies of TP53 mutations

Data from lung cancer patients exposed to both smoky coal and cigarette smoke provide evidence that PAHs, especially benzo[*a*]pyrene, are involved in human lung carcinogenesis caused by these exposures. Benzo[*a*]pyrene depurinating adducts N7-

guanine and N7-adenine have been found in the urine of cigarette smokers and women exposed to household smoke (Casale *et al.*, 2001).

In the *TP53* gene, G→T transversions are more common in lung cancer than in any other human cancer: over 30% versus 10% (except for hepatocellular cancer associated with exposure to aflatoxin) (Hainaut & Pfeifer, 2001; Pfeifer & Hainaut, 2003). The G→T transversions are found in all histological types of lung cancer of smokers and support their common origin as the direct result of DNA damage. In other organs, differences in *TP53* mutation types occur depending on the histology. There is a statistically significant difference in the frequency of G:C→T:A transversions in lung cancer of smokers (higher) compared with nonsmokers (Hainaut & Pfeifer, 2001; Pfeifer & Hainaut, 2003). In smoking-related lung cancer, the frequency of these mutations increases with increasing smoking (dose–response relationship) (Bennett *et al.*, 1999). Codons 157, 248 and 273 in the *TP53* gene that are most mutated in lung cancer are also targets for DNA adduct formation by benzo[*a*]pyrene and are more prone to its mutations (Denissenko *et al.*, 1996; Pfeifer & Denissenko, 1998; Smith *et al.*, 2000).

However, the final proof for a specific *TP53* mutation spectrum related to smoking requires the comparison of the mutation spectrum in lung cancers from smokers with that in nonsmokers. Such studies are difficult to carry out because of the rarity of lung cancer in nonsmokers. A further difficulty is that most of the smokers and smoking-related lung cancers are found in men and most nonsmokers are women. Consequently, the possible gender difference, as well as the possible geographical difference (Bennett *et al.*, 1999) have to be taken into account in such studies. The few existing studies of nonsmokers support the hypothesis that smoking-related *TP53* mutations are more frequent in smokers than in nonsmokers (Vähäkangas *et al.*, 2001 and references therein; Le Calvez *et al.*, 2005). In the study by Le Calvez *et al.* (2005) on never (n = 40), former (n = 27) and current heavy smokers (n = 64), GC→TA transversions and AT→GC transitions were associated with smoking while GC→AT transitions were associated with never smoking.

Nearly 90% of the G→T mutations occur on the non-transcribed (coding) strand of the gene, as indicated by the overrepresentation of G→T compared with C→A. On the transcribed strand, G→T mutation would result in C→A on the coding, non-transcribed strand according to the Watson-Crick rule for base-pairing. A possible reason for the strand bias is slow repair of bulky benzo[*a*]pyrene diol epoxide–DNA adducts along the non-transcribed strand of human *TP53* (Denissenko *et al.*, 1998). A similar preferential repair of benzo[*a*]pyrene diol epoxide in the *HPRT* gene of diploid human fibroblasts has been described (Chen *et al.*, 1992).

An alternative hypothesis for the origin of specific *TP53* mutations in lung cancer has been put forward by Rodin and Rodin (2005), who propose that repetitive exposure to tobacco components drives clonal expansion of cells with a pre-existing endogenous *TP53* mutation. However, no data are available that would support the idea that mutant proteins encoded by genes with a G→T transversion would give a better growth advantage than other types of mutation, which would explain the overrepresentation of these mutants in lung cancer (Pfeifer & Hainaut, 2003).

Epigenetic modification of C in a CpG sequence to 5-methylcytosine makes the C prone to mutations (Rideout *et al*., 1990; Tornaletti & Pfeifer, 1995). All CpGs are methylated in the central region of the *TP53* gene that codes for the DNA-binding region of the protein. Methylated CpGs in human lung cancers are the preferred sites for G→T mutations (over 50% of such mutations in lung cancer) (Yoon *et al*., 2001; Pfeifer & Hainaut, 2003). In cells that have reporter genes that contain the methylated CpG, these are targets of G→T mutations after exposure to benzo[*a*]pyrene diol epoxide (Yoon *et al*., 2001). In contrast, transitions (C→T and G→A at CpG sites) occur in excess in cancers that are associated with inflammatory diseases, where oxidative stress is a more probable cause of *TP53* mutations. The fact that transition mutations rather than transversions are very common in the *TP53* gene in cancers related to inflammatory responses argues against a role of oxidative stress in *TP53* mutations of smoking-associated lung cancers. Furthermore, there is a discrepancy about whether 8-oxo-deoxyguanosine, which is caused by oxidative stress, is repaired by strand-specific transcription-coupled repair (Pfeifer & Hainaut, 2003).

In China, smoky coal is used for cooking and the indoor air levels of benzo[*a*]pyrene can reach levels comparable with those experienced by workers in old-type coking plants (Lan *et al.*, 2000). The high incidence of lung cancer among nonsmoking Chinese women is clearly associated with the use of smoky coal and the increase in risk is dose-dependent. This association was substantially larger and achieved statistical significance among patients with sputum samples that were positive for p53 overexpression (Lan *et al.*, 1993, 2000, 2001). This is in accordance with the higher number of benzo[*a*]pyrene–DNA adducts in the bronchoalveolar-lavage cells of these women than in non-exposed controls (Gallagher *et al.*, 1993). DeMarini *et al.* (2001) found that the *TP53* mutations in the lung cancer tissue of these women clustered in codons 153–158; most of the mutations were G→T and all G→T transversions were found on the non-transcribed strand. A hotspot at the *TP53* codon 154, which is a hotspot for PAH adducts (Smith *et al.*, 2000) but is not found in lung cancers from smokers (Hainaut & Pfeiffer, 2001), was identified.

Individual susceptibility to benzo[*a*]pyrene-induced mutations is well-illustrated by the study of Hussain *et al.* (2001) that shows that non-tumorous tissue of lung cancer patients contained *TP53* codon 157 G:C→T:A mutations while lung tissue from non-cancer patients did not, regardless of the fact that the majority of the controls were also smokers. The whole chain of events from exposure to lung cancer is thus covered by the current evidence. Many of the details, however, still remain to be solved. Studies in silico (computational tools that translate raw data into workable models or simulations) using the genetic algorithm, a molecular dynamics-based protocol have revealed, for instance, how a single adduct ((+)-*anti*-benzo[*a*]pyrene diol epoxide) may give rise to different mutations by adopting different conformations (Kozack & Loechler, 1999 and references within).

Conclusions

The evidence strongly supports benzo[*a*]pyrene in smoky coal and in cigarette smoke as the cause of *TP53* mutations in lung cancer. This is based on the following:

1. Metabolic activation of benzo[*a*]pyrene and the formation of its 7,8-diol-9,10-oxide and benzo[*a*]pyrene diol epoxide–DNA adducts occur in human bronchial cells and tissue explants.
2. Benzo[*a*]pyrene diol epoxide–DNA adducts are found in PAH-exposed people, with a higher level of the adducts in the lung and bronchial tissue from smokers than in those from nonsmokers.
3. G→T transversions in the TP53 gene are more common in lung cancers from smokers than in any other cancer type.
4. A high frequency of G→T transversions is found in lung cancers from women exposed to smoky coal combustion at either the K-*RAS* or *TP53* gene.
5. The formation of DNA adducts by benzo[a]pyrene in vitro (including in cells with a human *TP53* sequence) targets the same hotspots in the *TP53* gene as those where G→T mutations are found in cigarette smoke- and smoky coal-associated lung cancer.
6. Exposure to both cigarette smoke and smoky coal is associated with a dose-dependent increase in G→T transversions in the *TP53* gene.
7. A similar strand bias, with an overrepresentation of G→T mutations, occurs in the cigarette smoke- and smoky coal-associated *TP53* mutation pattern in human lung cancer and in vitro in benzo[*a*]pyrene-treated mouse cells that contain the human *TP53* sequence (in accordance with slow repair of bulky benzo[*a*]pyrene diol epoxide–DNA adducts on the non-transcribed strand).
8. A preference for G→T transversions in the methylated CpG dinucleotides in human lung tumours is in accordance with in-vitro studies that show the same dinucleotide as a target of benzo[*a*]pyrene diol epoxide.

Cyclopenta[*cd*]pyrene

(i) *Metabolism and metabolic activation*

The metabolism of cyclopenta[*cd*]pyrene has been documented in a number of studies in rat liver, human liver and liver microsomes. There are two potential centres for biochemical oxidation of cyclopenta[*cd*]pyrene ─ the cyclopenta-ring (C3–C4) and the K-region (C9–C10) ─ and both are involved in this process. 3-Methylcholanthrene- and phenobarbital-induced rat liver microsomes metabolized cyclopenta[*cd*]pyrene to the cyclopenta-ring diol, *trans*-3,4-dihydroxy-3,4-dihydro-cyclopenta[*cd*]pyrene (cyclopenta-[*cd*]pyrene-3,4-diol) (Gold & Eisenstadt, 1980). 3-Methylcholanthrene- and phenobarbital-induced rat liver microsomes and reconstituted cytochrome P448 and CYP systems metabolized cyclopenta[*cd*]pyrene to the K-region diol, *trans*-9,10-dihydroxy-9,10-dihydro-cyclopenta[*cd*]pyrene (cyclopenta[cd]pyrene-9,10-diol) (Eisenstadt *et al.*, 1981). Uninduced mouse liver and human liver microsomes metabolized cyclopenta-[*cd*]pyrene to cyclopenta[*cd*]pyrene-3,4-diol, *cis*-cyclopenta[*cd*]pyrene-3,4-diol, 4-hydroxy-

3,4-dihydro-cyclopenta[*cd*]pyrene, 4-oxo-3,4-dihydro-cyclopenta[*cd*]pyrene and two diastereomeric *trans,trans*- 3,4,9,10-tetrahydro-cyclopenta[*cd*]pyrene-3,4,9,10-tetrols (Sahali *et al.*, 1992). Human CYP1A1, CYP1A2 and CYP3A4 metabolized cyclopenta[*cd*]pyrene at the cyclopenta double bond to give cyclopenta[*cd*]pyrene-3,4-diol. Human CYP1A2 metabolized cyclopenta[*cd*]pyrene at the K-region to give cyclopenta[*cd*]pyrene-9,10-diol as indirectly demonstrated by the triols and tetrols formed (Kwon *et al.*, 1992). Cyclopenta[*cd*]pyrene is presumed to be converted to cyclopenta[*cd*]pyrene-3,4-oxide by a 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE)/haematin system (Reed *et al.*, 1988). A 15-HPETE/haematin system, a prostaglandin H synthetase system or a sulfite auto-oxidation system converted cyclopenta[*cd*]pyrene to *cis* and *trans* isomers of cyclopenta[*cd*]pyrene-3,4-diol and 4-keto-(3*H*)-cyclopenta[*cd*]pyrene, presumably through cyclopenta[*cd*]pyrene-3,4-oxide (Reed & Ryan, 1990).

(ii) *Formation of DNA adducts*

Cyclopenta[*cd*]pyrene formed DNA adducts in a rat liver microsomal calf thymus DNA system using livers from untreated, Aroclor 1254-treated, β-naphthoflavone-treated and phenobarbital-treated rats. Cyclopenta[*cd*]pyrene formed DNA adducts in the liver, lung, heart and white blood cells of Sprague-Dawley rats. The rat lung adducts were identified as cyclopenta[*cd*]pyrene-3,4-oxide–deoxyguanosine and cyclopenta[*cd*]pyrene-3,4-oxide–deoxyadenosine adducts. These two DNA adducts were formed in the skin of NIH Swiss mice and in the lungs of $B6C3F_1$ mice treated with cyclopenta[*cd*]pyrene (Beach & Gupta, 1994).

(iii) *Genotoxicity of cyclopenta[*cd*]pyrene*

In a previous monograph, cyclopenta[*cd*]pyrene was reported to induce mutations in *S. typhimurium* TA1537, TA98 and TM677, in mouse lymphoma L5178Y cells and in human lymphoblastoid HH-4 cells *in vitro* and to induce morphological cell transformation in C3H10T½CL8 mouse embryo cells (IARC, 1983). It was mutagenic in *S. typhimurium* TA98 after activation by systems that generate peroxyl radicals, by prostaglandin H synthetase and arachidonic acid, by 15-HPETE and haematin or by the autoxidation of the sulfite ion (Reed *et al.*, 1988). Cyclopenta[*cd*]pyrene was mutagenic in Chinese hamster V79 cells (6-thioguanine resistance or ouabain resistance) co-incubated with X-irradiated golden hamster embryo fibroblasts (Raveh *et al.*, 1982), in human cells that express CYP1A1 such as MCL-3 cells (thymidine kinase resistance) (Lafleur *et al.*, 1993), human B lymphoblastoid MCL-5 cells (6-thioguanine resistance) (Keohavong *et al.*, 1995) and in human B lymphoblastoid H1A1v2 cells (thymidine kinase locus) (Durant *et al.*, 1996). Cyclopenta[*cd*]pyrene induced sister chromatid exchange in C3H10T½Cl8 cells in culture (Krolewski *et al.*, 1986).

(iv) *Cyclopenta[*cd*]pyrene-induced mutations in proto-oncogenes*

Cyclopenta[*cd*]pyrene induced two major classes of Ki-*ras* codon 12 mutations in lung adenomas from cyclopenta[*cd*]pyrene-treated strain A mice: GGT→CGT (50%),

GGT→GTT (15%), GGT→TGT (25%) and GGT→GAT (10%) which indicated that guanine was a primary target for this PAH (Nesnow *et al.*, 1994a, 1995, 1998a).

(v) *Other effects of cyclopenta[*cd*]pyrene*

Cyclopenta[*cd*]pyrene induced apoptosis in mouse hepatoma Hepa1c1c7 cells, both apoptotic (DNA-dependent PK, ataxia telangiectasia-mutated (ATM) and/or ATM-related kinase, p53, and p38-MAPK) and anti-apoptotic (Akt and ERK) signals and the formation of the active form of caspase-3, cleavage of PARP and DNA fragmentation (Solhaug *et al.*, 2004a,b).

(vi) *Evidence for cyclopenta-ring oxidation as a mechanism of carcinogenesis*

DNA adducts of cyclopenta[*cd*]pyrene-3,4-oxide

Cyclopenta[*cd*]pyrene-3,4-oxide forms primarily deoxyguanosine DNA adducts in calf thymus DNA (Beach *et al*., 1993; Hsu *et al*., 1997); the major adducts observed were diastereoisomers of *cis*-3-(deoxyguanosin-N^2-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]-pyrene (Hsu *et al.*, 1997). Cyclopenta[*cd*]pyrene-3,4-oxide-2′–deoxyguanosine adducts have been detected in lung DNA from strain A/J mice treated with cyclopenta[*cd*]pyrene (Nesnow *et al.*, 1995).

Genotoxicity of cyclopenta[*cd*]pyrene-3,4-oxide

Cyclopenta[*cd*]pyrene-3,4-oxide was mutagenic in *E. coli* and induced G→T and A→T mutations (Eisenstadt *et al.*, 1982) and in *S. typhimurium* strains TA98, TA100, TA1537 and TA1538 in the absence and presence of a phenobarbital- or a 3-methyl-cholanthrene-induced rat liver preparation (Gold & Eisenstadt, 1980). Cyclopenta-[*cd*]pyrene-3,4-oxide was mutagenic in mouse lymphoma L5178Y cells (thymidine kinase resistance) and induced morphological cell transformation in C3H10T½CL8 mouse embryo cells in culture (Gold *et al.*, 1980).

DNA adducts of cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydro-cyclopenta[*cd*]pyrene

Cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene were bound to calf thymus DNA in the presence of rodent liver cytosol and 3′-phos-phoadenosine-5′-phosphosulfate, suggesting a SULT-mediated activation mechanism (Surh *et al.*, 1993). The adducts formed with cyclopenta[*cd*]pyrene-3,4-diol were iden-tified as *cis*-3-(deoxyguanosine-N^2-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene (Hsu *et al*., 1999). SULT activation of 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene with deoxyguanosine or reaction of 4-sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene with deoxy-guanosine gave the same DNA adduct (Hsu *et al.*, 1999).

Genotoxicity of cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene (4-sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene)

Cyclopenta[*cd*]pyrene-3,4-diol was mutagenic to *S. typhimurium* TM677 in the presence of a rat liver cytosol and 3′-phosphadenosine-5′-phosphosulfate (Surh *et al.*, 1993).

4-Hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene was mutagenic to *S. typhimurium* TM677 in the presence of a rat liver cytosol and 3′-phosphadenosine-5′-phosphosulfate. 4-Sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene was a direct-acting mutagen in *S. typhimurium* TM677 (without metabolic activation) (Surh *et al.*, 1993).

Conclusion

Cyclopenta[*cd*]pyrene is metabolically activated by the cyclopenta-ring mechanism. The evidence for this mechanism is based on the detection of the cyclopenta-ring hydroxylated metabolites, cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*] pyrene, in microsomal systems from rat, mouse and human liver and by peroxy radical-mediated activation systems. The formation of cyclopenta[*cd*]pyrene-3,4-oxide is inferred by the detection of cyclopenta[*cd*]pyrene-3,4-diol as a metabolite. Cyclopenta[*cd*]pyrene-3,4-oxide is genotoxic in bacteria and mammalian cells and forms DNA adducts with deoxyguanosine. 4-Hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene–DNA adducts are formed from SULT-activated 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene. In mouse lung, cyclopenta[*cd*]pyrene forms cyclopenta[*cd*]pyrene-3,4-oxide–deoxyguansine adducts and induces mutations in tumours at guanine in codon 12 of the Ki-*ras* oncogene. Cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene have not been evaluated for tumour initiation in mice.

Dibenz[*a,h*]anthracene

(i) *Metabolism and metabolic activation*

The metabolism of dibenz[*a,h*]anthracene has been documented in a number of studies in rat microsomes, recombinant human liver CYPs and human liver microsomes. Two equivalent potential centres of metabolism and metabolic activation are situated on the two benzo rings (or two bay regions) at the 1–2 and 3–4 bonds and the 10–11 and 12–13 bonds. Dibenz[*a,h*]anthracene was metabolized by 3-methylcholanthrene-induced rat liver preparations to a compound supposed to be 5,6-dihydroxy-5,6-dihydrodibenz-[*a,h*]anthracene (dibenz[*a,h*]anthracene-5,6-diol) (Selkirk *et al.*, 1971) and by Aroclor 1254-induced rat liver microsomes or a reconstituted cytochrome P448 system to 1,2-dihydroxy-1,2-dihydrodibenz[*a,h*]anthracene (dibenz[*a,h*]anthracene-1,2-diol), the major metabolite, 3,4-dihydroxy-3,4-dihydrodibenz[*a,h*]anthracene (dibenz[*a,h*]anthracene-3,4-diol), trace amounts of dibenz[*a,h*]anthracene-5,6-diol and two unidentified phenols (Nordqvist *et al.*, 1979). Dibenz[*a,h*]anthracene formed more than 30 metabolites after incubation with Aroclor 1254-induced rat liver microsomes. Among those identified were dibenz-[*a,h*]anthracene-5,6-oxide, dibenz[*a,h*]anthracene-1-, -2-, -3-, -4-, -5- and -6-phenols, *trans-trans*-3,4:12,13-tetrahydroxy-3,4:12,13-tetrahydrodibenz[*a,h*]anthracene, *r*1,*t*2,*t*3,*c*4-

tetrahydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene, *r*1,*t*2,*c*3,*t*4-tetrahydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene, dibenz[*a*,*h*]anthracene-3,4-catechol and a dibenz[*a*,*h*]-anthracenephenol dihydrodiol derived from the 2-phenol (Platt & Reischmann, 1987). 3-Methylcholanthrene-induced microsomes metabolized dibenz[*a*,*h*]anthracene to a bis-diol, *trans-trans*-3,4:10,11-tetrahydroxy-3,4:10,11-tetrahydrodibenz[*a*,*h*]anthracene (dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol), and the related hexols 1,2,3,4,8,9-hexahydroxy-1,2,3,4,8,9-hexahydrodibenz[*a*,*h*]anthracene, 1,2,3,4,10,11-hexahydroxy-1,2,3,4,10,11-hexahydrodibenz[*a*,*h*]anthracene and the related tetrol, 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene (Lecoq *et al.*, 1989, 1991a). Dibenz[*a*,*h*]anthracene was stereospecifically metabolized by Aroclor 1254-induced rat liver microsomes to the *trans* diols, dibenz[*a*,*h*]anthracene-1,2-diol, dibenz[*a*,*h*]anthracene-3,4-diol and dibenz[*a*,*h*]-anthracene-5,6-diol, each of which is highly enriched in *R*,*R* enantiomers (Platt *et al.*, 1990). Microsomes from untreated, 3-methylcholanthrene-treated, phenobarbital-treated and Aroclor 1254-treated rats metabolized dibenz[*a*,*h*]anthracene to dibenz[*a*,*h*]-anthracene-5,6-oxide highly enriched with the dibenz[*a*,*h*]anthracene-5*S*,6*R*-oxide form (Mushtaq *et al.*, 1989). Dibenz[*a*,*h*]anthracene was metabolized by recombinant human CYP enzymes CYP1A2, 2B6, 2C8, 2C9, 2E1, 3A3, 3A4 and 3A5 expressed in hepatoma G2 cells and by 14 different human liver microsomes to dibenz[*a*,*h*]anthracene-1,2-diol, dibenz[*a*,*h*]anthracene-3,4-diol, dibenz[*a*,*h*]anthracene-5,6-diol and several phenols. CYP1A2 and CYP2C9 were the most active and CYP2B6 was moderately active in the rate of total dibenz[*a*,*h*]anthracene metabolism. Human liver microsomes gave metabolic patterns similar to those generated from CYP1A2 (Shou *et al.*, 1996a). Rat liver microsomal UGT mediated the glucuronidation of the 1-, 2-, 4-, 5-, 6- and 7-phenols of dibenz[*a*,*h*]anthracene and the UGT activities were affected by the inducers 3-methylcholanthrene and phenobarbital (Lilienblum *et al.*, 1987).

(ii) *Formation of DNA adducts*

Dibenz[*a*,*h*]anthracene formed 14 DNA adducts in mouse liver DNA *in vitro* in the presence of 3-methylcholanthrene-induced rat liver microsomes. The same pattern of adducts was obtained in mouse liver DNA after incubation *in vitro* with dibenz[*a*,*h*]anthracene-3,4-diol. One adduct was identified as a *trans* adduct of *anti*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene-1,2-oxide (*anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide) and the other as a *trans* adduct of *syn*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene-1,2-oxide (*syn*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide) (Lecoq *et al.*, 1991b). Dibenz[*a*,*h*]anthracene formed eight DNA adducts with calf thymus DNA incubated with Aroclor 1254-induced rat liver microsomes. The major bay-region diol epoxide adduct was identified as *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine. Other DNA adducts were *anti*-dibenz[*a*,*h*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyguanosine, *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyadenosine, *anti*-dibenz[*a*,*h*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyadenosine, *syn*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide–deoxyadenosine and *syn*-dibenz[*a*,*h*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide–deoxyadenosine. Unidentified, highly polar adducts were also

observed (Mlcoch *et al.*, 1993). The highly polar dibenz[*a*,*h*]anthracene calf thymus DNA adducts originated from the bis-diols, 3*R*,4*R*,10*R*,11*R*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]anthracene and 3*R*,4*R*,10*S*,11*S*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]-anthracene. Quantitatively, the highly polar bis-diol DNA adducts represented 38% of the adducts and the dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide bay-region diol epoxide adducts represented 25% (Fuchs *et al.*, 1993a). Calf thymus DNA adducts of 3*R*,4*R*,10*R*,11*R*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]anthracene and 3*R*,4*R*,10*S*,11*S*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]anthracene were found after incubation of racemic dibenz-[*a*,*h*]anthracene-3,4:10,11-bis-diol with Aroclor 1254-induced rat liver microsomes (Fuchs *et al.*, 1993b). Dibenz[*a*,*h*]anthracene formed DNA adducts in mouse embryo C3H10T½CL8 cells, most of which were also found in C3H10T½CL8 cells treated with dibenz[*a*,*h*]anthracene-3,4-diol or *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide. Two of these were *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts. Several additional polar dibenz[*a*,*h*]anthracene–DNA adducts were derived from racemic dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol (Nesnow *et al.*, 1994b). Dibenz[*a*,*h*]anthracene formed DNA adducts on mouse skin *in vivo*. A similar pattern of adducts was observed after topical treatment of mouse skin with dibenz[*a*,*h*]anthracene-3,4-diol, but only four of these adducts were observed after topical treatment with *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide and none after treatment with *syn*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide. The authors suggested that most of the DNA adducts formed in dibenz[*a*,*h*]-anthracene-treated mouse skin proceeded through the related dibenz[*a*,*h*]anthracene-3,4-diol and that only some were formed following the conversion of this diol to the bay-region *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide (Lecoq *et al.*, 1991c). Further analyses of the polar dibenz[*a*,*h*]anthracene DNA adducts in mouse skin indicated that one of them was from the further metabolism of the intermediate, dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol. A bis-diol epoxide, dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol-1,2-oxide, was proposed as the ultimate DNA-binding intermediate (Carmichael *et al.*, 1993). Human skin or mouse skin treated in short-term organ culture with dibenz[*a*,*h*]anthracene gave an adduct profile that was qualitatively similar to that obtained from treatment of mouse skin *in vivo*. Mouse skin treated with dibenz[*a*,*h*]anthracene-3,4-diol *in vivo* and in short-term organ culture gave similar DNA adduct profiles (Lecoq *et al.*, 1992). Dibenz[*a*,*h*]anthracene binds at different levels to the DNA in the epidermis of Cyp1a2$^{-/-}$, Cyp1b1$^{-/-}$ and Ahr$^{-/-}$ knockout mice *in vivo*, with the least binding in the Ahr$^{-/-}$ mice; this suggests that CYP1A1 is involved in the bioactivation of dibenz[*a*,*h*]anthracene (Kleiner *et al.*, 2004).

Dibenz[*a*,*h*]anthracene induced five DNA adducts in strain A/J mouse lung, three of which resulted from metabolism via dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol and two via dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide (Ross *et al*., 1995).

(iii) *Genotoxicity of dibenz[*a,h*]anthracene*

In a previous monograph, dibenz[*a*,*h*]anthracene was reported to induce DNA damage and mutations in bacteria, DNA damage in rodent and human cells in culture,

mutations and chomosomal damage in rodent cells in culture and morphological cell transformation (IARC, 1983).

Dibenz[*a,h*]anthracene was mutagenic in *S. typhimurium* TA100 in the presence of a phenobarbital-induced mouse liver preparation (Platt *et al.*, 1982), 3-methylcholanthrene-induced rat liver microsomes or a metabolic activation system (Lecoq *et al.*, 1989), in Chinese hamster V79 cells co-cultivated with human hepatoma HepG2 cells (Diamond *et al.*, 1984) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996). Dibenz[*a,h*]anthracene induced sister chromatid exchange and micronucleus formation in lung cells cultured *in vitro* that were taken from rats treated with dibenz[*a,h*]anthracene by intratracheal instillation (Whong *et al.*, 1994). Dibenz[*a,h*]anthracene induced micronucleus formation in bone marrow and spleen polychromatic erythrocytes from rats treated *in vivo* by intratracheal instillation (Zhong *et al.*, 1995). It induced morphological cell transformation in C3H10T½CL8 mouse embryo fibroblasts (Nesnow *et al.*, 1994).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Metabolism of dibenz[*a,h*]anthracene-3,4-diol

Dibenz[*a,h*]anthracene-3,4-diol formed more than 13 metabolites when metabolized by Aroclor 1254-induced rat liver microsomes. The major metabolites were *anti*-dibenz-[*a,h*]anthracene-3,4-diol-1,2-epoxide, *trans,trans*-3,4:8,9-tetrahydroxy-3,4,8,9-tetrahydro-dibenz[*a,h*]anthracene, dibenz[*a,h*]anthracene-3,4:10,11-bis-diol and *trans,trans*-3,4:12,13-tetrahydroxy-3,4,12,13-tetrahydrodibenz[*a,h*]anthracene (Platt & Schollmeier, 1994).

Carcinogenicity studies of dibenz[*a,h*]anthracene-3,4-diol

Groups of 29 or 30 female Sencar mice, 51–65 days of age, received a single dermal application of 100 nmol [31 μg] racemic dibenz[*a,h*]anthracene-3,4-diol followed 7 days later by 3.25 nmol [2 μg] TPA in acetone twice a week for 15 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 16 weeks after treatment was 37% (0.7). Skin tumours developed in 6% (0.1 tumours/mouse) of the mice treated with TPA alone (Slaga *et al.*, 1980).

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 10, 40 or 160 nmol [3.1, 12.4 or 50 μg] racemic dibenz[*a,h*]-anthracene-3,4-diol in acetone:ammonium hydroxide (1000:1) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 31 (0.69), 50 (1.17) and 57% (1.52), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Buening *et al.*, 1979b).

Groups of newborn Swiss Webster BLU:Ha(ICR) mice were injected intra-peritoneally on days 1, 8 and 15 of life with racemic dibenz[*a,h*]anthracene-3,4-diol (total dose, 70 or 420 nmol) [22 or 130 μg] in DMSO. Mice were weaned at 23 days of age, separated by sex and killed at 25–29 weeks of age. In 42 or 40 female mice, pulmonary

tumours developed in 81 or 100% (2.5 or 33.4 tumours/mouse), respectively, but no liver tumours. In 27 or 45 male mice, pulmonary tumours developed in 85 or 98% (2.3 or 23.8 tumours/mouse), and liver tumours in 0 or 19%, respectively. In 40 female mice treated with DMSO alone, pulmonary tumours developed in 12.5% (1.2 tumours/mouse), but no liver tumours. In 36 male mice treated with DMSO alone, pulmonary tumours developed in 11% (1.25 tumours/mouse), but no liver tumours (Buening *et al.*, 1979b).

DNA adducts of dibenz[*a,h*]anthracene-3,4-diol

Dibenz[*a,h*]anthracene-3*R*,4*R*-diol produced four DNA adducts in calf thymus DNA after metabolic activation by Aroclor 1254-induced rat liver microsomes. The same adducts were also observed in calf thymus DNA incubated with dibenz[*a,h*]anthracene after metabolic activation by Aroclor 1254-induced rat liver microsomes. One adduct was identified as *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine and two others were derived from dibenz[*a,h*]anthracene-3,4:10,11-bis-diol. Dibenz[*a,h*]-anthracene-3*S*,4*S*-diol gave two DNA adducts in calf thymus DNA after metabolic activation by Aroclor 1254-induced rat liver microsomes. These adducts were also observed in calf thymus DNA incubated with dibenz[*a,h*]anthracene after metabolic activation by Aroclor 1254-induced rat liver microsomes. Two adducts were derived from dibenz[*a,h*]anthracene-3,4,10,11-bis-diol (Fuchs *et al.*, 1993b).

Dibenz[*a,h*]anthracene-3,4-diol formed 14 DNA adducts in mouse liver DNA *in vitro* in the presence of 3-methylcholanthrene-induced rat liver microsomes. One of these adducts was an adduct of *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide and the other was an adduct of *syn*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide. It was estimated that 50% of the adducts from dibenz[*a,h*]anthracene may be formed though the dibenz[*a,h*]-anthracene-3,4-diol and *syn*- and *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide pathways (Lecoq *et al.*, 1991b). Dibenz[*a,h*]anthracene-3*S*,4*S*-diol and dibenz[*a,h*]anthracene-3*R*,4*R*-diol formed six and seven DNA adducts in calf thymus DNA, respectively, after incubation with Aroclor 1254-induced rat liver microsomes. The major bay-region diol epoxide adduct was identified as *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine (Mlcoch *et al.*, 1993). Dibenz[*a,h*]anthracene-3,4-diol formed a number of DNA adducts in mouse embryo C3H10T½CL8 cells, two of which were *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts; one polar adduct identical to a polar dibenz[*a,h*]anthracene–DNA adduct was derived from racemic dibenz[*a,h*]anthracene-3,4:10,11-bis-diol (Nesnow *et al.*, 1994b).

Genotoxicity of dibenz[*a,h*]anthracene-3,4-diol

Racemic dibenz[*a,h*]anthracene-3,4-diol induced mutations in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver preparation or a purified monoxygenase system (Wood *et al.*, 1978; Platt & Schollmeier, 1994). Both dibenz[*a,h*]-anthracene-3*R*,4*R*-diol and dibenz[*a,h*]anthracene-3*S*,4*S*-diol induced mutations in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver fraction; the *R,R* enantiomer was the stronger mutagen (Platt *et al.*, 1990). Racemic dibenz[*a,h*]anthracene-

3,4-diol induced morphological cell transformation in C3H10T½CL8 mouse embryo fibroblasts (Nesnow *et al.*, 1994b).

Carcinogenicity study of dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide

Groups of 29 or 30 female Sencar mice, 51–65 days of age, received a single dermal application of 100 nmol [33 μg] racemic *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide, followed 7 days later by 3.25 nmol [2 μg] TPA in acetone twice a week for 15 weeks. The incidence of tumours in the dosed groups 16 weeks after treatment was 7% (0.1 tumours/mouse). Skin tumours developed in 6% (0.1 tumours/mouse) of the mice treated with TPA alone (Slaga *et al.*, 1980). [The Working Group noted that it is possible that the diol epoxide was not stable in the solvent used for its application to the backs of mice.]

DNA adducts of dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide

anti-Dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide and *syn*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide each form a number of DNA adducts in mouse liver DNA *in vitro* (Lecoq *et al.*, 1991b). In calf thymus DNA, *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2 oxide formed *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine, *anti*-dibenz[*a,h*]-anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyguanosine, *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyadenosine and *anti*-dibenz[*a,h*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyadenosine adducts. In calf thymus DNA, *syn*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide formed *syn*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide–deoxyadenosine and *syn*-dibenz[*a,h*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide–deoxyadenosine adducts (Mlcoch *et al.*, 1993). *anti*-Dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide formed DNA adducts in mouse embryo C3H10T½CL8 cells, two of which were *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts and one polar adduct identical to a polar dibenz[*a,h*]-anthracene–DNA adduct was derived from racemic dibenz[*a,h*]anthracene-3,4,10,11-bis-diol (Nesnow *et al.*, 1994b).

Genotoxicity of dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide

Racemic dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide induced morphological cell transformation in C3H10T½CL8 mouse embryo fibroblasts (Nesnow *et al.*, 1994b).

Conclusion

Dibenz[*a,h*]anthracene is activated metabolically by the diol epoxide mechanism. It was metabolized to a diol that was further metabolized in mouse and rat tissues to a bay-region diol epoxide that then formed DNA adducts and induced mutations. Dibenz[*a,h*]anthracene was metabolized to the dibenz[*a,h*]anthracene-3,4-diol by rat liver preparations, human liver CYPs, human liver microsomes, mouse skin and human skin in organ culture and mouse skin *in vivo*. The *R,R*-diol enantiomer was the predominant rat liver microsomal form. Dibenz[*a,h*]anthracene-3,4-diol is mutagenic to bacteria, morphologically transforms cells in culture, initiates tumours in mouse skin and is carcinogenic in newborn mouse lung. Dibenz[*a,h*]anthracene-3,4-diol was metabolized to

two bay-region diol epoxides, *syn*- and *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide, by rat liver preparations and mouse skin and to *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide by mammalian cells in culture. There is a consistent pattern of formation of *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide–DNA adducts in calf thymus DNA and in mouse skin by dibenz[*a*,*h*]anthracene, dibenz[*a*,*h*]anthracene-3,4-diol and *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, and an *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine bay-region diol epoxide adduct is common to all in-vivo treatments. In the only tumour initiation study in mouse skin, *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide was inactive; however, this result could have been due to the instability of the diol epoxide in the solvent used to apply the agent.

(v) *Evidence for bis-diol epoxide metabolic activation as a mechanism of carcinogenesis*

DNA adducts of dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol

Racemic dibenz[*a*,*h*]anthracene-3,4,10,11-diol gave two DNA adducts in calf thymus DNA after metabolic activation by Aroclor 1254-induced rat liver microsomes. These adducts were also observed in calf thymus DNA incubated with dibenz[*a*,*h*]anthracene-3*R*,4*R*-diol or dibenz[*a*,*h*]anthracene after metabolic activation by Aroclor 1254-induced rat liver microsomes (Fuchs *et al.*, 1993a,b). Topical application of racemic dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol to the skin of mice gave two adducts which were also observed in dibenz[*a*,*h*]anthracene- or dibenz[*a*,*h*]anthracene-3,4-diol-treated mouse skin (Carmichael *et al.*, 1993). Racemic dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol gave two polar dibenz[*a*,*h*]anthracene–DNA adducts in C3H10T½CL8 cells in culture. One of these DNA adducts was observed in C3H10T½CL8 cells treated with dibenz[*a*,*h*]anthracene or dibenz[*a*,*h*]anthracene-3,4-diol (Nesnow *et al.*, 1994b).

Genotoxicity of dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol

Dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol was mutagenic in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Platt & Schollmeier, 1994).

Conclusion

Dibenz[*a*,*h*]anthracene is metabolically activated to bis-diol epoxides. Dibenz[*a*,*h*]anthracene was metabolized to a diol that was then metabolized to a bis-diol and further converted to intermediates that formed DNA adducts in mouse and rat tissues. Dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol was a rat liver metabolite of both dibenz[*a*,*h*]-anthracene and dibenz[*a*,*h*]anthracene-3,4-diol. It was mutagenic in bacteria and formed highly polar calf thymus DNA adducts when activated by rat liver preparations, and highly polar DNA adducts in mouse embryo cells in culture and in mouse skin. The same highly polar DNA adducts were also observed in calf thymus DNA incubated with dibenz[*a*,*h*]anthracene-3,4-diol and rat liver preparations and in mouse skin and human skin treated topically with dibenz[*a*,*h*]anthracene or dibenz[*a*,*h*]anthracene-3,4-diol. Human skin treated in short-term organ culture with dibenz[*a*,*h*]anthracene gave an

adduct profile that was qualitatively similar to the profiles obtained from the treatment of mouse skin with dibenz[*a,h*]anthracene *in vivo*. Dibenz[*a,h*]anthracene-3,4:10,11-bis-diol has not been evaluated for tumour initiation in mice.

Dibenz[*a,j*]anthracene

(i) *Metabolism and metabolic activation*

The metabolism of dibenz[*a,j*]anthracene has been documented in a number studies in rat microsomes and mouse keratinocyes in culture. Two equivalent potential centres of metabolism and metabolic activation are situated on the two benzo rings (or two bay regions) at the 1–2 and 3–4 bonds or the 10–11 and 12–13 bonds. 3-Methylcholanthrene-induced microsomes metabolized dibenz[*a,j*]anthracene to the bis-diol, *trans-trans*-3,4:8,9-tetrahydroxy-3,4:8,9-tetrahydrodibenz[*a,j*]anthracene (Lecoq *et al.*, 1989, 1991a). Dibenz[*a,j*]anthracene is metabolized in primary cultures of mouse keratinocytes to *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]anthracene (dibenz[*a,j*]anthracene-3,4-diol), *trans*-5,6-dihydroxy-5,6-dihydrodibenz[*a,j*]anthracene (dibenz[*a,j*]anthracene-5,6-diol) and several other unidentified metabolites. Both diols were also conjugated to glucuronic acid (Nair *et al.*, 1992).

(ii) *Formation of DNA adducts*

Dibenz[*a,j*]anthracene forms DNA adducts in mouse epidermis after topical application. Eleven adducts were detected and seven were identified as deoxyguanosine and deoxyadenosine adducts formed from the *anti*- and *syn*-bay-region diol epoxides of dibenz[*a,j*]anthracene, *anti-trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]anthracene-1,2-oxide (*anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide) and *syn-trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]anthracene-1,2-oxide (*syn*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide). The major bay-region diol epoxide–DNA adduct formed was *anti-trans*-dibenz[*a,j*]-anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine and substantial amounts of an *anti-trans*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyadenosine adduct were produced. In addition, a K-region adduct, dibenz[*a,j*]anthracene-5,6-oxide–deoxy-adenosine, was identified. Unidentified, more polar covalent DNA adducts in epidermal DNA samples from dibenz[*a,j*]anthracene-treated mice were also detected (Baer-Dubowska *et al.*, 1995). Topical application of dibenz[*a,j*]anthracene and its metabolites to mouse skin gave at least 23 DNA adducts, including four less polar (derived from *syn*- and *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide; 23% of total adducts) and 19 highly polar (derived primarily from the dibenz[*a,j*]anthracene-3,4:10,11-bis-diol; 77% of total adducts) DNA adducts (Vulimiri *et al.*, 1999). Dibenz[*a,j*]anthracene forms DNA adducts in primary cultures of mouse keratinocytes that were identified as (+)-*anti*-dibenz[*a,j*]-anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts, (+)-*anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyadenosine adducts (the deoxyadenosine adducts were predominant), *trans* and *cis* adducts with deoxyguanosine and deoxyadenosine of (+)-*syn*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide and the major adduct, dibenz[*a,j*]anthracene-5,6-oxide–deoxyadenosine (Nair *et al.*, 1991).

(iii) *Genotoxicity of dibenz[*a,j*]anthracene*

In a previous monograph, dibenz[*a,j*]anthracene was reported to induce mutations in *S. typhimurium* TA100 (IARC, 1983). Dibenz[*a,j*]anthracene was mutagenic in *S. typhimurium* TA100 in the presence of 3-methylcholanthrene-induced rat liver microsomes or preparations (Lecoq *et al.*, 1989) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Dibenz[a,j]anthracene-induced mutations in proto-oncogenes*

Dibenz[*a,j*]anthracene induced $A^{182} \rightarrow T$ tranversion mutations in Ha-*ras* codon 61 in mouse skin papillomas obtained from a mouse skin initiation–promotion study of dibenz[*a,j*]anthracene (Gill *et al.*, 1992).

(v) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenz[*a,j*]anthracene-3,4-diol

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 or 800 nmol [124 or 248 μg] racemic dibenz[*a,j*]anthracene-3,4-diol in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 20 weeks. The incidence of tumours in the dosed groups 22 weeks after treatment was 97 (2.38 tumours/mouse) and 90% (3.17 tumours/mouse), respectively. Skin tumours developed in 16% (0.16 tumours/mouse) of the mice treated with TPA alone. Similar results were obtained in a repeat study in which dibenz[*a,j*]anthracene-3,4-diol was administered in tetrahydrofuran (Sawyer *et al.*, 1988).

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 nmol [124 μg] racemic dibenz[*a,j*]anthracene-3,4-diol in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 16 weeks. In the dosed group, 3.48 tumours/mouse were observed 18 weeks after treatment. No data were given on the control mice treated with TPA alone (Vulimiri *et al.*, 1999).

DNA adducts of dibenz[*a,j*]anthracene-3,4-diol

Dibenz[*a,j*]anthracene-3,4-diol formed DNA adducts in mouse epidermis after topical application. Eleven adducts were formed and five were identified as (–)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine, (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine (major adduct), (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyadenosine and two *syn*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide adducts (Baer-Dubowska *et al.*, 1995).

Carcinogenicity study of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 or 600 nmol [124 or 248 μg] racemic *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 22 weeks after treatment was 86 (3.55) and 80% (3.7), respectively. Skin tumours developed in 16%

(0.16 tumours/mouse) of the mice treated with TPA alone. Similar results were obtained in a repeat study in which dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide was administered in tetrahydrofuran (Sawyer *et al.*, 1988).

DNA adducts of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide

(+)-*anti*-Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide formed three DNA adducts in mouse epidermis after topical application: (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine, (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–adenosine (major adduct) and (+)-*anti-cis*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine (Baer-Dubowska *et al.*, 1995). Racemic *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide formed DNA adducts with calf thymus DNA. The major adducts were (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine and (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyadenosine (major adduct) (Nair *et al.*, 1989, 1991).

Genotoxicity of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide

(+)-*anti*-Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide primarily induced point mutations in the *supF* gene in SOS-induced mutants of *E. coli* ES87. The specific mutations were: GC→AT (37%), GC→TA (21%) and GC→CG (8.6%) (Gill *et al.*, 1993).

Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide-induced mutations in proto-oncogenes

Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide induced A^{182}→T tranversion mutations in Ha-*ras* codon 61 in mouse skin papillomas from a tumour initiation–promotion study of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide (Gill *et al.*, 1992).

Conclusion

Dibenz[*a,j*]anthracene is activated metabolically by a diol epoxide mechanism. Dibenz[*a,j*]anthracene is metabolized to a diol that is further metabolized to a bay-region diol epoxide in mouse tissues and then forms DNA adducts and induces mutations. In primary cultures of mouse skin keratinocytes, dibenz[*a,j*]anthracene is metabolized to the bay-region diol, dibenz[*a,j*]anthracene-3,4-diol. In mouse skin and in mouse skin kerotinocytes, dibenz[*a,j*]anthracene forms DNA adducts primarily from the bay-region diol epoxide, (+)-*anti*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide with both deoxyguanosine and deoxyadenosine. Dibenz[*a,j*]anthracene-3,4-diol is a tumour initiator in mouse skin and forms (+)-*anti*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–DNA adducts with both deoxyguanosine and deoxyadenosine in mouse skin. *anti*-Dibenz[*a,j*]-anthracene-3*S*,4*R*-diol-1,2-oxide is mutagenic in bacteria and initiates tumours in mouse skin. In mouse skin, it forms (+)-*anti*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–DNA adducts with both deoxyguanosine and deoxyadenosine. Tumours induced by both dibenz[*a,j*]anthracene and *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide have A^{182}→T tranversion mutations in Ha-*ras* codon 61 which suggests that deoxyadenosine is a major target.

(vi) *Evidence for bis-diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity study of dibenz[*a,j*]anthracene-3,4:10,11-bis-diol

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 nmol [124 μg] racemic dibenz[*a,j*]anthracene-3,4:10,11-bis-diol in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 16 weeks. No tumours were observed in the dosed group 18 weeks after treatment. No data were given on the control mice treated with TPA alone (Vulimiri *et al.*, 1999).

DNA adducts of dibenz[*a,j*]anthracene-3,4:10,11-bis-diol

Topical application of dibenz[*a,j*]anthracene-3,4:10,11-bis-diol to mouse skin *in vivo* produced nine adducts, seven of which were found in the skin of mice treated with dibenz[*a,j*]anthracene and five of which were detected in the skin of mice treated with dibenz[*a,j*]anthracene-3,4-diol (Vulimiri *et al.*, 1999).

Conclusion

The data are insufficient to demonstrate a mechanism of bis-diol epoxide metabolic activation. Bis-diols are mouse skin metabolites of dibenz[*a,j*]anthracene, the major one of which is dibenz[*a,j*]anthracene-3,4:10,11-bis-diol. Dibenz[*a,j*]anthracene-3,4:10,11-bis-diol forms DNA adducts in mouse skin; however, the contribution of this metabolite to the tumour-initiating activity of dibenz[*a,j*]anthracene appears to be small on the basis of its inability to initiate tumours in mouse skin.

Dibenzo[*a,e*]pyrene

(i) *Metabolism and metabolic activation*

Dibenzo[*a,e*]pyrene was metabolized by 3-methylcholanthrene-induced rat liver microsomes. The major metabolites were the proximate bay-region diol, 3,4-dihydroxy-3,4-dihydrodibenzo[*a,e*]pyrene, and 3-, 7- and 9-hydroxydibenzo[*a,e*]pyrene (Devanesan *et al.*, 1990).

(ii) *Formation of DNA adducts*

Dibenzo[*a,e*]pyrene applied topically to the skin of male mice formed unidentified DNA adducts in both skin and lungs (Hughes & Phillips, 1990).

(iii) *Genotoxic effects of dibenzo[*a,e*]pyrene*

In a previous monograph, dibenzo[*a,e*]pyrene was reported to induce mutations in *S. typhimurium* TA100 (IARC, 1983). Dibenzo[*a,e*]pyrene is mutagenic in *S. typhimurium* TA98, TA100 and TM677 (8-azaguanine resistance) in the presence of an Aroclor 1254-induced rat liver preparation (Devanesan *et al.*, 1990; Busby *et al.*, 1995). It was mutagenic in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996) and in human B-lymphoblastoid MCL-5 cells that contain five human liver CYPs and microsomal epoxide hydrolase (Busby *et al.*, 1995).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

No data were available to the Working Group.

Dibenzo[*a,h*]pyrene

(i) *Metabolism and metabolic activation*

Dibenzo[*a,h*]pyrene was metabolized to a bay-region diol, *trans*-1,2-dihydroxy-1,2-dihydrodibenzo[*a,h*]pyrene (dibenzo[*a,h*]pyrene-1,2-diol), and *trans*-3,4-dihydroxy-3,4-dihydrodibenzo[*a,h*]pyrene by an Aroclor 1254-induced rat liver preparation (Hecht *et al.*, 1981).

(ii) *Formation of DNA adducts*

Dibenzo[*a,h*]pyrene formed two major DNA adducts in the skin of topically treated mice (Hughes & Phillips, 1990). Laser-excited fluorescence analyses suggested that the adducts resulted from metabolism on either or both terminal benzo rings (Marsch *et al.*, 1992).

(iii) *Genotoxic effects of dibenzo[a,h]pyrene*

In a previous monograph, dibenzo[*a,h*]pyrene was reported to induce mutations in *S. typhimurium* TA98 and TA100 (IARC, 1983). Dibenzo[*a,h*]pyrene was very weakly mutagenic in human B-lymphoblastoid MCL-5 cells that contain activity for five human liver CYPs and microsomal epoxide hydrolase (Busby *et al.*, 1995). It was mutagenic in human B-lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1999) and in Chinese hamster V79 cells (ouabain and 6-thioguanine resistance) co-cultivated with golden hamster embryo cells (Hass *et al.*, 1982).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenzo[*a,h*]pyrene-1,2-diol

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [17, 67 or 202 μg] racemic dibenzo[*a,h*]pyrene-1,2-diol in DMSO:tetrahydrofuran (1:10) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with 50 nmol. The incidence of tumours (tumours/mouse) in the dosed group 17 weeks after treatment was 39 (0.96), 57 (2.73) and 80% (4.4), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a,h*]-1,2-diol was 79% (2.96), and no skin tumours developed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intra-peritoneally on days 1, 8 and 15 of life with racemic dibenzo[*a,h*]pyrene-1,2-diol (total dose, 87.5 nmol) [30 μg] in DMSO. Mice were weaned at 25 days of age, separated by

sex and killed at 49–54 weeks of age. In 28 female mice, at 54 weeks pulmonary tumours developed in 96% of the surviving mice (15.82 tumours/mouse) and liver tumours in 7% (0.07 tumours/mouse). In 17 male mice, pulmonary tumours developed in 100% of the surviving mice (19 tumours/mouse) and liver tumours in 41% (3.76 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,h*]pyrene-1,2-diol

Dibenzo[*a*,*h*]pyrene-1,2-diol was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Wood *et al.*, 1981).

Carcinogenicity studies of dibenzo[*a,h*]pyrene-1,2-diol-3,4-oxide

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [18, 71 or 212 μg] racemic *anti-trans*-1,2-dihydroxy-1,2,3,4-tetrahydrodibenzo[*a*,*h*]pyrene-3,4-oxide (*anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide) in DMSO:tetrahydrofuran (1:10), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with of 50 nmol. The incidence of tumours (tumours/mouse) in the dosed groups 17 weeks after treatment was 33 (0.43), 70 (1.87) and 50% (1.83), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a*,*h*]-1,2-diol-3,4-oxide was 60% (one tumour/mouse), and no skin tumours developed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide (total dose, 87.5 nmol) [31 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 49–54 weeks of age. In 18 female mice, pulmonary tumours developed in 94% of the surviving mice (5.72 tumours/mouse) and liver tumours in 6% (0.06 tumours/mouse). In 19 male mice, pulmonary tumours developed in 95% of the surviving mice (5.37 tumours/mouse) and liver tumours in 26% (1.37 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,h*]pyrene-1,2-diol-3,4-oxide

anti-Dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide was mutagenic in *S. typhimurium* TA98 and TA100 in the absence of exogenous metabolic activation and in Chinese hamster V79 cells in culture (8-azaguanine resistance) (Wood *et al.*, 1981)**.**

Conclusion

Dibenzo[*a*,*h*]pyrene can be metabolically activated by the diol epoxide pathway. Dibenzo[*a*,*h*]pyrene is metabolized to the proximate bay-region diol, dibenzo[*a*,*h*]pyrene-1,2-diol, by rat liver preparations. Dibenzo[*a*,*h*]pyrene-1,2-diol was mutagenic to bacteria and was a tumour initiator in mouse skin. Dibenzo[*a*,*h*]pyrene-1,2-diol induced pulmonary and hepatic tumours in newborn mice. Although *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide–DNA adducts have not been characterized directly, synthetic *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide is genotoxic in bacteria and mammalian cells in culture. Synthetic *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide is a tumour initiator in mouse skin and induces pulmonary and hepatic tumours in newborn mice.

Dibenzo[*a*,*i*]pyrene

(i) *Metabolism and metabolic activation*

Dibenzo[*a*,*i*]pyrene was metabolized to a proximate bay-region diol, *trans*-3,4-dihydroxy-3,4-dihydrodibenzo[*a*,*i*]pyrene (dibenzo[*a*,*i*]pyrene-3,4-diol), and *trans*-1,2-dihydroxy-1,2-dihydrodibenzo[*a*,*i*]pyrene by an Aroclor 1254-induced rat liver preparation (Hecht *et al.*, 1981).

(ii) *Formation of DNA adducts*

Dibenzo[*a*,*i*]pyrene formed two unidentified DNA adducts in the lungs of rats dosed by intratracheal instillation (Whong *et al.*, 1994) and in the skin of mice treated topically (Hughes & Phillips, 1990).

(iii) *Genotoxic effects of dibenzo[*a,i*]pyrene*

In a previous monograph, dibenzo[*a*,*i*]pyrene was reported to induce DNA damage in bacteria and mutations in *S. typhimurium* TA98 and TA100 but not unscheduled DNA synthesis in primary rat hepatocytes (IARC, 1983). Dibenzo[*a*,*i*]pyrene was mutagenic in *S. typhimurium* TM677 in the presence of an Aroclor 1254-induced rat liver preparation (Busby *et al.*, 1995), in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1999) and in Chinese hamster V79 cells (ouabain and 6-thioguanine resistance) co-cultivated with golden hamster embryo cells (Hass *et al.*, 1982). It induced cell transformation of rat epithelial cells *in vivo* after intracheal instillation and in treated isolated cultures of rat epithelial cells *in vitro* (Ensell *et al.*, 1998). The rat epithelial cells transformed *in vivo* were shown to exhibit anchorage-independent growth and to produce squamous-cell carcinomas when injected into nude mice (Ensell *et al.*, 1999). Dibenzo[*a*,*i*]pyrene induced micronucleus formation in bone marrow and spleen polychromatic erythrocytes in rats treated *in vivo* (Zhong *et al.*, 1995). It induced sister chromatid exchange and micronucleus formation in lung cells cultured *in vitro* taken from rats dosed by intratracheal instillation (Whong *et al.*, 1994).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenzo[*a,i*]pyrene-3,4-diol

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [17, 67 or 202 μg] racemic dibenzo[*a,i*]pyrene-3,4-diol in DMSO:tetrahydrofuran (1:10), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with 50 nmol only. The incidence of tumours (tumours/mouse) in the dosed groups 17 weeks after treatment was 37 (0.6), 66 (3.03) and 81% (5.0), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a,i*]-3,4-diol was 60% (2.14), and no skin tumours developed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic dibenzo[*a,i*]pyrene-3,4-diol (total dose, 87.5 nmol) [31 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 49–54 weeks of age. In 30 female mice, pulmonary tumours developed in 100% of the surviving mice (32.2 tumours/mouse) and liver tumours in 6% (0.1 tumours/mouse). In 21 male mice, pulmonary tumours developed in 100% of the surviving mice (35 tumours/mouse) and liver tumours in 67% (4.48 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,i*]pyrene-3,4-diol

Dibenzo[*a,i*]pyrene-3,4-diol was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Wood *et al.*, 1981)**.**

Carcinogenicity studies of dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [18, 71 or 212 μg] racemic *anti-trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenzo[*a,i*]pyrene-1,2-oxide (*anti*-dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide) in DMSO:tetrahydrofuran (1:10), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with 50 nmol only. The incidence of tumours (tumours/mouse) in the dosed groups 17 weeks after treatment was 20 (0.2), 43 (0.9) and 67% (2.03), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide was 53% (0.9) and no skin tumours were observed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-dibenzo[*a*,*i*]pyrene-3,4-diol-1,2-oxide (total dose, 87.5 nmol) [31 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 49–54 weeks of age. In seven female mice, pulmonary tumours developed in 100% of the surviving mice (1.57 tumours/mouse) but no liver tumours. In eight male mice, pulmonary tumours developed in 62% of the surviving mice (3.13 tumours/mouse) and liver tumours in 25% (0.25 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide

anti-Dibenzo[*a*,*i*]pyrene-3,4-diol-1,2-oxide was mutagenic in *S. typhimurium* TA98 and TA100 in the absence of exogenous metabolic activation and in Chinese hamster V79 cells in culture (8-azaguanine resistance) (Wood *et al.*, 1981).

Conclusion

There is some evidence that dibenzo[*a*,*i*]pyrene can be activated metabolically by the diol epoxide mechanism. Dibenzo[*a*,*i*]pyrene was metabolized to the proximate bay-region diol, dibenzo[*a*,*i*]pyrene-3,4-diol, by rat liver preparations. Dibenzo[*a*,*i*]pyrene-3,4-diol was mutagenic to bacteria and was a tumour initiator in mouse skin. It induced pulmonary and hepatic tumours in newborn mice. Although DNA adducts from *anti*-dibenzo[*a*,*i*]pyrene-3,4-diol-1,2-oxide have not been identified, synthetic *anti*-dibenzo-[*a*,*i*]pyrene-3,4-diol-1,2-oxide was genotoxic in bacteria and mammalian cells in culture, was a tumour initiator in mouse skin and induced pulmonary and hepatic tumours in newborn mice.

Dibenzo[*a,l*]pyrene

(i) *Metabolism and metabolic activation*

The metabolism of dibenzo[*a*,*l*]pyrene has been documented in a number of studies in rat liver microsomes, recombinant human CYPs, cells that express human CYPs and human liver and lung microsomes. One primary potential centre of metabolism and metabolic activation of dibenzo[*a*,*l*]pyrene leads to the fjord-region diol epoxide at the 11–12 and 13–14 bonds. Dibenzo[*a*,*l*]pyrene is metabolized by 3-methylcholanthrene-induced rat liver microsomes to 11,12-dihydroxy-11,12-dihydro-dibenzo[*a*,*l*]pyrene (dibenzo[*a*,*l*]pyrene-11,12-diol), 8,9-dihydroxy-8,9-dihydro-dibenzo[*a*,*l*]pyrene (dibenzo-[*a*,*l*]pyrene-8,9-diol), 7-hydroxydibenzo[*a*,*l*]pyrene and a dibenzo[*a*,*l*]pyrene-dione (Devanesan *et al.*, 1990). Dibenzo[*a*,*l*]pyrene was metabolized by human recombinant CYPs and human lung and liver microsomes to 13,14-dihydroxy-13,14-dihydro-dibenzo[*a*,*l*]pyrene, dibenzo[*a*,*l*]pyrene-11,12-diol, dibenzo[*a*,*l*]pyrene-8,9-diol, 7-hydroxy-dibenzo[*a*,*l*]pyrene and a dibenzo[*a*,*l*]pyrene-dione. Of all of the recombinant CYPs

evaluated, CYP1A1 was the most active in the metabolism of dibenzo[*a*,*l*]pyrene. The order of activity in the formation of dibenzo[*a*,*l*]pyrene-11,12-diol was CYP1A1 > CYP2C9 > CYP1A2 > CYP2B6 > CYP3A4 (Shou *et al.*, 1996b). The two major CYPs involved in the metabolism of dibenzo[*a*,*l*]pyrene are CYP1A1 and CYP1B1. In human lung, both CYP1A1 (in smokers) and CYP1B1 proteins are expressed (Hukkanen *et al.*, 2002). Dibenzo[a,l]pyrene was metabolized by Chinese hamster V79 cells that express human CYP1A1 or CYP1B1 to dibenzo[a,l]pyrene-11,12-diol, 7-hydroxydibenzo-[a,l]pyrene, dibenzo[a,l]pyrene-11,12-diol-phenol and 11r,12t,13t,14c-tetrahydroxy-11,12,13,14,-tetrahydrodibenzo[a,l]pyrene. Chinese hamster V79 cells that express human CYP1B1 also formed *cis*-dibenzo[*a*,*l*]pyrene-11,12-diol. Those that express either of the human CYP enzymes stereospecifically catalysed the formation of (–)-dibenzo-[*a*,*l*]pyrene-11*R*,12*R*-diol from dibenzo[*a*,*l*]pyrene (Luch *et al.*, 1999a).

(ii) *Formation of DNA adducts*

The formation of dibenzo[*a*,*l*]pyrene–DNA adducts has been studied in calf thymus DNA, in rodent and human mammalian cells in culture and in rodents. Dibenzo[*a*,*l*]pyrene forms DNA adducts in calf thymus DNA in the presence of liver microsomes from β-naphthoflavone- or Aroclor 1254-treated rats. These adducts were identified as deoxyadenosine and deoxyguanosine adducts of both *anti*- and *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides in the following order: *anti*-dibenzo[*a*,*l*]-pyrene-11,12-diol-13,14-oxide–deoxyadenosine, *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine >> *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguano-sine, *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine (Arif & Gupta, 1997). Dibenzo[*a*,*l*]pyrene forms adducts in calf thymus DNA in the presence of 3-methylcholanthrene-induced rat liver microsomes. These adducts were identified as a *syn-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adduct, an *anti-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adduct, a *syn-trans*-dibenzo-[*a*,*l*]pyrene-11,12,-diol-13,14-oxide–doxyadenosine adduct, a *syn-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine and an *anti-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adduct. Equal amounts of deoxyguanosine and deoxy-adenosine adducts were formed (Devanesan *et al.*, 1999). Dibenzo[*a*,*l*]pyrene formed DNA adducts in mouse embryo fibroblast C3H10T½Cl8 cells that were derived from the metabolism of dibenzo[*a*,*l*]pyrene to its fjord-region diol epoxides through dibenzo-[*a*,*l*]pyrene-11,12-diol. The predominant adduct was *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine. Other major adducts were *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine and *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine with smaller amounts of *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine (Nesnow *et al.*, 1997).

Chinese hamster V79 cells that express human CYP1B1 produced six DNA adducts exclusively derived from dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide (Luch *et al.*, 1998b). These adducts were similar to those found after the treatment of human mammary carcinoma MCF-7 cells in culture, which were identified as four (–)-*anti*-

dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide adducts and two (+)-*syn*-dibenzo[*a*,*l*]-pyrene-11*S*,12*R*-diol-13*S*,14*R*-oxide adducts. The three major adducts found in MCF-7 cells were identified as a (–)-*anti*-dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide–deoxyadenosine and two (+)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adducts. This indicated that, in these cells, the metabolism of dibenzo[*a*,*l*]pyrene proceeds through dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol (Ralston *et al.*, 1995, 1997). Chinese hamster V79 cells that express human or rat CYP1A1 formed many of the same dibenzo[*a*,*l*]pyrene adducts as cells that express CYP1B1, as well as several unidentified highly polar DNA adducts (Luch *et al.*, 1998b). Embryonic fibroblasts from wild-type mice (mixed genetic background of C57B6 and 129/Sv mice) were exposed to dibenzo[*a*,*l*]pyrene. Those isolated from *CYP1B1*-null mice failed to bind dibenzo[*a*,*l*]pyrene to DNA. In contrast, those from wild-type mice produced a series of five DNA adducts derived from both *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides, the *anti* form of which predominated. The same pattern of dibenzo[*a*,*l*]pyrene–DNA adducts was observed in dibenzo[*a*,*l*]pyrene-exposed Chinese hamster V79 cells that express murine CYP1B1 (Buters *et al.*, 2002).

Female Sprague-Dawley rats injected intramammillary with dibenzo[*a*,*l*]pyrene formed DNA adducts in mammary tissues as well as distal tissues (lung, heart, bladder and pancreas), which were identified as deoxyadenosine and deoxyguanosine adducts of both *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides (Arif *et al.*, 1997, 1999). Dibenzo[*a*,*l*]pyrene binds at different levels to the DNA in the epidermis of *Cyp1a2*$^{-/-}$, *Cyp1b1*$^{-/-}$ and *Ahr*$^{-/-}$ knockout mice *in vivo*; the least binding occurs in the *Cyp1a2*$^{-/-}$ and *Cyp1b1*$^{-/-}$ mice, which suggests that CYP1A2 and CYP1B1 and possibly CYP1A1 are involved in the bioactivation of dibenzo[*a*,*l*]pyrene (Kleiner *et al.*, 2004).

Dibenzo[*a*,*l*]pyrene forms three major and four minor DNA adducts after topical application to mouse skin. One of the major adducts is (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine (with some possible contribution of a (±)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine). A second major adduct is primarily (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine (with some possible contribution of (+)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–DNA). The third major adduct is (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine. The minor adducts are (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine, (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine and deoxyadenosine adducts of (±)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide. Quantitatively, the majority of dibenzo[*a*,*l*]pyrene–DNA adducts in mouse skin are (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosines (Jankowiak *et al.*, 1998).

Strain A/J mice injected intraperitoneally with dibenzo[*a*,*l*]pyrene produced dibenzo[*a*,*l*]pyrene–DNA adducts in the lung which were deoxyadenosines and deoxyguanosines derived from both *anti*- and *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide; the major adduct was *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine (Prahalad *et al.*, 1997). C57BL/6 mice exposed to dibenzo[*a*,*l*]pyrene by gavage formed a number of DNA adducts in their lungs, the majority of which were

derived from *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide and one from *syn*-dibenzo-[*a*,*l*]pyrene-11,12-diol-13,14-oxide (Mahadevan *et al.*, 2005a).

(iii) *Genotoxicity of dibenzo[*a,l*]pyrene*

Dibenzo[*a*,*l*]pyrene is mutagenic in *S. typhimurium* TA98, TA100 and TM677 (8-azaguanine resistance) in the presence of an Aroclor 1254-induced rat liver preparation (Devanesan *et al.*, 1990; Busby *et al.*, 1995). It was mutagenic in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996) and in human B-lymphoblastoid MCL-5 cells that contain five human liver CYPs and microsomal epoxide hydrolase (Busby *et al.*, 1995). Chinese hamster V79 cells (6-thioguanine resistance) co-cultivated with irradiated human mammary carcinoma MCF-7 cells were mutated by dibenzo[*a*,*l*]pyrene (Ralston *et al.*, 1997). Dibenzo[*a*,*l*]-pyrene induced morphological cell transformation in mouse embryo fibroblast C3H10T½Cl8 cells and was more active than benzo[*a*]pyrene (Nesnow *et al.*, 1997). Dibenzo[*a*,*l*]pyrene induced mutations in the *cII* locus of Big-Blue mouse embryonic fibroblasts (Yoon *et al.*, 2004).

(iv) *Dibenzo[*a,l*]pyrene-induced mutations in ras gene*

Dibenzo[*a*,*l*]pyrene induced Ki-*ras* codon 12 and codon 61 mutations in lung adenomas from treated strain A/J mice. In codon 12, the following mutations were observed: GGT→TGT (28%), GGT→GTT (11%) and GGT→CGT (6%). In codon 61, the following mutations were observed: CAA→CTA (22%), CAA→CGA (17%), CAA→CAT (11%) and CAA→CAC (6%) (Prahalad *et al.*, 1997). Dibenzo[*a*,*l*]pyrene induced Ha-*ras* codon 61 mutations in mouse skin papillomas with CAA→CTA mutations being predominant (Chakravarti *et al.*, 1995; Khan *et al.*, 2005). It induced A→G mutations in the Ha-*ras* proto-oncogene in early preneoplastic skin (Chakravarti *et al.*, 2000).

(v) *DNA damage and repair of dibenzo[*a,l*]pyrene*

In comparison with benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene induces 10–100 times more DNA adducts (Binkova *et al.*, 2000; Melendez-Colon *et al.*, 2000; Binkova & Sram, 2004), which are more resistant to nucleotide excision repair, probably because they distort DNA to a lesser degree (Dreij *et al.*, 2005). Dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide reacts preferably with deoxyadenosine while benzo[*a*]pyrene reacts more readily with deoxyguanosine (Ralston *et al.*, 1997; Jankowiak *et al.*, 1998; Smith *et al.*, 2001). These differences also apply more generally to fjord-region and bay-region diol epoxides (see references in Dreij *et al.*, 2005). However, the preferred adduction of dibenzo[*a*,*l*]-pyrene-11,12,-diol-13,14-oxide to deoxyadenosine was challenged (Todorovic *et al.*, 2005). The repair of DNA adducts by (+)-*syn*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide is significantly quicker than that of adducts by (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide (Yoon *et al.*, 2004). Accordingly, (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide adducts are the major adducts in Chinese hamster cells V79 that express

CYP1B1 (Luch *et al.*, 1998b) and in the lungs of orally dosed mice (Mahadevan *et al.*, 2005a).

(vi) *Apoptosis and cellular pathways of dibenzo[*a,l*]pyrene*

In many ways, benzo[*a*]pyrene and dibenzo[*a*,*l*]pyrene seem to have a similar profile in cellular effects that differ from many other PAH compounds (See Section 4.2.1.(*b*) **Benzo[*a*]pyrene** (vii)). Both are strong mutagens and induce apoptosis and cell-cycle arrest (Chramostova *et al.*, 2004), increase greatly the percentage of S-phase cells and induce phosphorylation of extracellular signal-regulated kinases (Andrysik *et al.*, 2005), and the number of their DNA adducts correlates with the p53 response to both compounds in cells (Rämet *et al.*, 1995; Luch *et al.*, 1999b). (–)-*anti*-Dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide induced DNA adducts and cell-cycle arrest in human diploid fibroblast cultures (Mahadevan *et al.*, 2001).

(vii) *Changes in gene expression induced by dibenzo[*a,l*]pyrene*

Very few data exist on the effect of dibenzo[*a*,*l*]pyrene on gene expression. In colon carcinoma Caco cells, dibenzo[*a*,*l*]pyrene induces CYP1A1, CYP1B1, UGT and multi drug resistance 1 (MDR-1) genes, with no effect on epoxide hydrolase or SULTs (Lampen *et al.*, 2004). In MCF-7 cells, however, benzo[*a*]pyrene, but not dibenzo-[*a*,*l*]pyrene, induces CYP1A1 and CYP1B1 (Mahadevan *et al.*, 2005b).

(viii) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenzo[*a,l*]pyrene-11,12-diol

Groups of 24 female SENCAR mice, 56 days of age, received dermal applications of 4, 20 or 100 nmol [1.3, 7 or 34 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 21 days later by 3.24 nmol [2 μg] TPA in acetone three times a week for 24 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 27 weeks after treatment was 100 (7.08), 96 (6.96) and 87% (2.5), respectively. The numbers of squamous-cell carcinomas/number of tumour bearing mice were 6/3, 1/1 and 1/1, respectively. A group of mice treated with 100 nmol [34 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol only without TPA promotion had a 12% tumour incidence (0.17 tumours/mouse) with two squamous-cell carcinomas in two mice. In an initiation–promotion study, the parent compound dibenzo[*a*,*l*]pyrene (4 nmol) [1.2 μg] induced a skin tumour incidence of 92% with 6.96 tumours/mouse and three squamous-cell carcinomas in two mice. There were no skin tumours in control mice treated with TPA only (Cavalieri *et al.*, 1991).

Groups of 24 female SENCAR mice, 56 days of age, received dermal applications of 0.25 or 1 nmol [0.084 or 0.336 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 7 days later by 2.16 nmol [1.33 μg] TPA in acetone twice a week for 27 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 27 weeks after

treatment was approximately 0 (0) and 18% (0.17), respectively. The responses in control mice treated with TPA alone were not stated (Higginbotham *et al.*, 1993).

Groups of 23–25 female SENCAR mice, 56 days of age, received dermal applications of 1.33, 4 or 12 nmol [0.5, 1.3 or 4 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 1 week later by 1.62 nmol [1 μg] TPA in acetone twice weekly for 28 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 29–30 weeks after treatment was 72 (2.84), 76 (4.36) and 78% (4.65), respectively. The numbers of squamous-cell carcinomas/number of tumour-bearing mice were 2/2, 3/2 and 3/2, respectively. The responses in control mice treated with TPA alone were not given. In the same study, dibenzo[*a*,*l*]pyrene (1.33 nmol) [0.5 μg] gave a 70% tumour incidence and 5.22 tumours/mouse (Gill *et al.*, 1994).

Groups of 16 female outbred NMRI mice, 49 days of age, received a dermal application of 40 nmol [13.5 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 7 days later by 10 nmol [6.2 μg] TPA in acetone twice a week for 18 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 19 weeks after treatment was 100% (5.8). In the same study, dibenzo[*a*,*l*]pyrene (40 nmol) [13 μg] gave a skin tumour incidence of 94% with 6.5 tumours/mouse. No skin tumours were observed in control mice treated with TPA alone (Luch *et al.*, 1999c).

Groups of 16 female outbred NMRI mice, 49 days of age, received dermal applications of 10, 20 or 40 nmol [3.4, 6.8 or 13.6 μg] (+)-dibenzo[*a*,*l*]pyrene-11*S*,12*S*-diol in acetone, followed 7 days later by 10 nmol [6.2 μg] TPA in acetone twice a week for 18 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 19 weeks after treatment was 0 (0), 13 (0.1) or 13% (0.1), respectively. No skin tumours were observed in control mice treated (Luch *et al.*, 1999c).

Groups of 16 female outbred NMRI mice, 49 days of age, received dermal applications of 10, 20 or 40 nmol [3.4, 6.8 or 13.6 μg] (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol in acetone, followed 7 days later by 10 nmol [6.2 μg] TPA in acetone twice a week for 18 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 19 weeks after treatment was 93 (4.7), 88 (3.6) and 80% (1.7), respectively. No skin tumours occurred in control mice treated with TPA alone (Luch *et al.*, 1999c).

Groups of 22–27 female Swiss mice, 56 days of age, received dermal applications of 1 or 4 nmol [0.34 or 1.3 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone twice weekly for 40 weeks and the surviving mice were killed at 48 weeks. In 23 or 22 mice, skin tumours occurred in 9 or 36% of the surviving mice with a mean latency of 45 or 33 weeks. The major tumours identified were squamous papillomas and sebaceous gland tumours in the 4-nmol treatment group and sebaceous gland tumours in the 1-nmol treatment group. In the same study, dibenzo[*a*,*l*]pyrene (4 nmol) [1.2 μg] gave a higher tumour incidence (83%) and the majority of tumours were squamous-cell carcinomas. Mice treated with acetone alone developed no tumours (Higginbotham *et al.*, 1993).

Groups of newborn Charles River Crl:CD®-1(ICR)BR mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic dibenzo[*a*,*l*]pyrene-11,12-diol (total dose, 40 or 100 nmol) [13.6 or 34 μg] in DMSO. Mice were weaned at 3–4 weeks

of age, separated by sex and killed at 26 or 55 weeks of age. In 12 or 13 female mice, pulmonary tumours developed in 83.3 or 84.6% of the surviving mice (5.92 or 5.69 tumours/mouse), liver tumours in 25 or 23.1% (0.42 or 0.23 tumours/mouse) and other tumours in 41.7 or 30.8% (0.5 or 0.38 tumours/mouse), respectively. In 13 or seven male mice, pulmonary tumours developed in 100 or 71.4% of the surviving animals (8.85 or 3.14 tumours/mouse), liver tumours in 92.3 or 28.6% (9.77 or 2.29 tumours/mouse) and other tumours in 23.1 or 42.9% (0.54 or 0.43 tumours/mouse), respectively. In 27 female mice treated with DMSO alone, pulmonary tumours developed in 37% of the surviving mice (0.67 tumours/mouse) but no liver or other tumours. In 35 male mice treated with DMSO alone, pulmonary tumours developed in 31.4% of the surviving mice (0.54 tumours/mouse) but no liver or other tumours (Platt *et al.*, 2004).

Metabolism of dibenzo[*a,l*]pyrene-11,12-diol

Dibenzo[*a,l*]pyrene-11,12-diol is metabolized by Aroclor 1254-induced rat or mouse liver microsomes to a series of diastereomeric tetraols formed through intermediary *syn*- and *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxides; the *anti* isomer predominated (90:10) (Luch *et al.*, 1997, 1998b).

DNA adducts of dibenzo[*a,l*]pyrene-11,12-diol

Racemic and enantiomeric dibenzo[*a,l*]pyrene-11,12-diol formed calf thymus DNA adducts in the presence of Aroclor 1254-induced liver microsomes from Sprague-Dawley rats or CD-1 mice. Larger quantities of these adducts were formed by the (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol compared with the (+)-dibenzo[*a,l*]pyrene-11*S*,12*S*-diol (Luch *et al.*, 1997). Chinese hamster V79 cells that express human recombinant CYP1A1 produced both non-polar and polar DNA adducts from (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol (Luch *et al.*, 1998b). Of the four non-polar DNA adducts, one was identified specifically as (–)-*anti*-dibenzo[*a,l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide–deoxyadenosine (Ralston *et al.*, 1995) and the other three were all (–)-*anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide–DNA adducts (Luch *et al.*, 1998b). These four non-polar adducts were also formed exclusively in V79 cells that express human CYP1B1 after exposure to (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol. Chinese hamster V79 cells that express human recombinant CYP1A1 produced fewer adducts than those that express CYP1B1 (Luch *et al.*, 1998b).

Genotoxicity of dibenzo[*a,l*]pyrene-11,12-diol

Chinese hamster V79 cells (6-thioguanine resistance) co-cultivated with irradiated human mammary carcinoma MCF-7 cells were mutated by racemic dibenzo[*a,l*]pyrene-11,12-diol and (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol, but not by (+)-dibenzo[*a,l*]pyrene-11*S*,12*S*-diol (Ralston *et al.*, 1997). Chinese hamster V79 cells (6-thioguanine resistance) in the presence of an Aroclor 1254-induced rat liver preparation were mutated by racemic dibenzo[*a,l*]pyrene-11,12-diol and each enantiomer. The (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol was more mutagenic than the racemic mixture which was more active than the (+)-dibenzo[*a,l*]pyrene-11*S*,12*S*-diol (Luch *et al.*, 1997).

Carcinogenicity studies of dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide

Groups of 24 female SENCAR mice, 56 days of age, received dermal applications of 1.33, 4 or 12 nmol [0.5, 1.4 or 4.3 μg] racemic *syn*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide in acetone, followed 1 week later by 1.62 nmol [1 μg] TPA in acetone twice a week for 28 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 29–30 weeks after treatment was 54 (1.87), 50 (1.54) and 58% (1.79), respectively. The numbers of squamous-cell carcinomas/number of tumour-bearing mice were 5/3 in the 1.33-nmol [0.5-μg] dose group only. The responses in control mice treated with TPA alone were not given (Gill *et al.*, 1994).

Groups of 23–25 female SENCAR mice, 56 days of age, received dermal applications of 1.33, 4 or 12 nmol [0.5, 1.4 or 4.3 μg] racemic *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide in acetone, followed 1 week later by 1.62 nmol [1 μg] TPA in acetone twice a week for 28 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 29–30 weeks after treatment was 44 (0.68), 44 (0.68) and 68% (1.96), respectively. The responses in control mice treated with TPA alone were not given (Gill *et al.*, 1994).

Groups of female Charles River CD rats, 30 days of age, were injected twice with a total dose of 1200 nmol [425.3 μg] *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide dissolved in DMSO under the three nipples on the left side. The DMSO control was injected under the nipples on the right side. At 41 weeks, 88% of the 16 rats had mammary tumours (adenomas, adenocarcinomas, sarcomas, poorly differentiated tumours and carcinosarcoma) after a mean latent period of 15.1 weeks. In the DMSO groups, 11% of the 19 rats had mammary tumours (adenomas, adenocarcinomas) after a mean latent period of 39 weeks. The differences between the treated and control group were statistically significant (Amin *et al.*, 1995b).

Groups of newborn Hsd:ICR mice were injected intraperitoneally on days 1, 7 and 15 of life with racemic *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide (total dose, 1 nmol) [0.35 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 20 female mice, pulmonary tumours occurred in 95.8% of the surviving mice (3.83 tumours/mouse) and liver tumours in 29.2% (0.83 tumours/mouse). In 27 male mice, pulmonary tumours occurred in 92.8% of the surviving mice (3.39 tumours/mouse) and liver tumours in 71.4% (6.29 tumours/mouse). In 17 female mice treated with DMSO alone, pulmonary tumours occurred in 5.9% of the surviving mice (0.059 tumours/mouse) and liver tumours in 5.9% (0.059 tumours/mouse). In eight male mice treated with DMSO alone, no pulmonary tumours and liver tumours occurred in 25% of the surviving mice (0.25 tumours/mouse). The differences in pulmonary tumours between the treated and control groups of males and females were statistically significant (Amin *et al.*, 1995a).

Groups of newborn Charles River Crl:CD®-1(ICR)BR mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide (total dose, 0.2 or 0.4 nmol) [71 or 142 μg] in DMSO. Mice were weaned at 3–4 weeks of age, separated by sex and killed at 55 weeks of age. In 20 or six female mice, pulmonary tumours were observed in 35 or 50% of the surviving mice (0.45

or 0.50 tumours/mouse), liver tumours in 5 or 16.7% (0.05 or 0.17 tumours/mouse) and other tumours in 10 or 33.3% (0.1 or 0.33 tumours/mouse), respectively. In 22 or nine male mice, pulmonary tumours occurred in 18.2 or 11.1% of the surviving mice (0.23 or 0.33 tumours/mouse), liver tumours in 9.1 or 66.7% (0.77 or 2.33 tumours/mouse) and other tumours in 0 or 11.1% (0 or 0.11 tumours/mouse), respectively. In 27 female mice treated with DMSO alone, pulmonary tumours developed in 37% of the surviving mice (0.67 tumours/mouse) but no liver or other tumours. In 35 male mice treated with DMSO alone, pulmonary tumours developed in 31.4% of the surviving mice (0.54 tumours/mouse) but no liver or other tumours (Platt *et al.*, 2004).

Groups of newborn Charles River Crl:CD®-1(ICR)BR mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide (total dose, 0.2 or 0.4 nmol) [77 or 142 μg] in DMSO. Mice were weaned at 3–4 weeks of age, separated by sex and killed at 55 weeks of age. In 27 or 16 female mice, pulmonary tumours developed in 11.1 or 12.5% of the surviving mice (0.11 or 0.13 tumours/mouse), liver tumours in 7.4 or 12.5% (0.11 or 0.38 tumours/mouse) and other tumours in 7.4 or 12.5% (0.07 or 0.13 tumours/mouse), respectively. In 26 or 26 male mice, pulmonary tumours were observed in 15.4 or 15.4% of the surviving mice (0.15 or 0.19 tumours/mouse), liver tumours in 7.7 or 23.1% (0.12 or 0.35 tumours/mouse) and other tumours in 7.7 or 3.8% (0.08 or 0.04 tumours/mouse), respectively. In 27 female mice treated with DMSO alone, pulmonary tumours developed in 37% of the surviving mice (0.67 tumours/mouse) but no liver or other tumours. In 35 male mice treated with DMSO alone, pulmonary tumours were observed in 31.4% of the surviving mice (0.54 tumours/mouse) but no liver or other tumours (Platt *et al.*, 2004).

Metabolism of dibenzo[*a,l*]pyrene-11,12,-diol-13,14-oxides

syn- and *anti*-Dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide were hydrolysed to diasteromeric tetraols (Jankowiak *et al.*, 1997). Of human recombinant α-class GSTs, GSTA1-1 was highly active for conjugating dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxides and was seven times more active against the (+)-*syn* than the (–)-*anti*-isomer (Dreij *et al.*, 2002).

DNA adducts of dibenzo[*a,l*]pyrene-11,12,-diol-13,14-oxides

Racemic *anti*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide formed adducts with calf thymus DNA that were identified as *anti-cis*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide–deoxyguanosine, *anti-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine and *anti-cis*- dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine. Racemic *syn*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide formed adducts with calf thymus DNA that were identified as two *syn-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adducts, a *syn-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adduct, a *syn-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adduct and two *syn-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adducts. For both *syn-*

and *anti-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides, deoxyguanosine adducts were 1.4-fold more frequent than deoxyadenosine adducts (Devanesan *et al.*, 1999).

Genotoxicity of dibenzo[*a,l*]pyrene-11,12,-diol-13,14-oxides

Racemic *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide were mutagenic in *S. typhimurium* TA97, TA98, TA100 and TA104 and in Chinese hamster V79 cells in the absence of exogenous metabolic activation. In *S. typhimurium* TA104, the *anti* isomer was more active than the *syn* isomer and produced the highest number of revertants/nmol (1 030 000) ever recorded (Luch *et al.*, 1994; Seidel *et al.*, 1994).

Conclusion

Dibenzo[*a*,*l*]pyrene is activated metabolically by a diol epoxide mechanism. Dibenzo[*a*,*l*]pyrene was stereospecifically metabolized by CYP1A1 and CYP1B1 of both rodent and human liver microsomes, by cells that express these CYPs and by mouse skin to give two diols: the major diol, (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol, and the minor diol, (+)-dibenzo[*a*,*l*]pyrene-11*S*,12*S*-diol. (–)-Dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol was then further metabolized by rodent or human CYP1B1 or rodent CYP1A1 to the fjord-region diol epoxide, (–)-*anti*-dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide, while (+)-dibenzo-[*a*,*l*]pyrene-11*S*,12*S*-diol was metabolized to (+)-*syn*-dibenzo[*a*,*l*]pyrene-11*S*,12*R*,-diol-13*S*,14*R*-oxide. Human CYP1A1 metabolizes (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol to dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide adducts and highly polar unidentified DNA adducts. Dibenzo[*a*,*l*]pyrene-11,12-diol was genotoxic in mammalian cells, and (–)-dibenzo-[*a*,*l*]pyrene-11*R*,12*R*-diol was the most active form. Racemic dibenzo[*a*,*l*]pyrene-11,12-diol initiated skin tumours in SENCAR and NMRI mice with an activity approximately equal to that of the parent dibenzo[*a*,*l*]pyrene. In NMRI mice, (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol was slightly less active than dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide adducts and (+)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol was inactive. Racemic dibenzo-[*a*,*l*]pyrene-11,12-diol was carcinogenic and induced benign skin tumours in mice but with lower activity at the same dose than the parent dibenzo[*a*,*l*]pyrene, which induced malignant skin tumours in Swiss mice. These studies on mouse skin are complicated by the concurrent toxicity of all of these agents which confounds the interpretation of the quantitative results. Racemic dibenzo[*a*,*l*]pyrene-11,12-diol also induced lung tumours in newborn mice. Racemic *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide were genotoxic in bacteria, and the *anti* isomer was the most active mutagen ever measured. Racemic *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide was slightly less active than racemic *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide as a skin tumour initiator in SENCAR mice. Racemic *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide induced benign and malignant mammary tumours when injected into rats and lung and liver tumours in newborn mice. The DNA adducts formed by dibenzo[*a*,*l*]pyrene, dibenzo[*a*,*l*]pyrene-11,12-diol and *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide were similar. In mammalian cells, the major adducts were derived from both (–)-*anti*-dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide and (+)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide and

were adducts of deoxyadenosine and deoxyguanosine. These DNA adducts are consistent with the mutations observed in *ras* proto-oncogenes in skin or lung tumours from mice treated with dibenzo[*a*,*l*]pyrene.

(ix) *Relevance of stable dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide adducts*

There are divergent views on the relevance of stable dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide adducts and unstable depurinating dibenzo[*a*,*l*]pyrene adducts in the metabolic activation mechanism of dibenzo[*a*,*l*]pyrene. A number of investigators have studied this in different test systems, such as isolated DNA, mammalian cells in culture and mammals *in vivo*. When the quantity of stable dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–DNA adducts was compared with apurinic site formation in either isolated DNA from Chinese hamster ovary B11 cells or in Chinese hamster ovary B11 cells treated directly with (+)-*syn*- or (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide, stable adducts could be measured (3 or 10 per 14 kb DNA fragment, respectively), while the levels of apurinic sites were below the detection limits (0.1 apurinic site in a 14 kb DNA fragment). When human mammary carcinoma MCF-7 cells were treated with dibenzo-[*a*,*l*]pyrene, only stable adducts and no apurinic sites were detected. These results suggested a predominant role for stable DNA adducts in the carcinogenic activity of dibenzo[*a*,*l*]pyrene (Melendez-Colon *et al.*, 1997). Human leukaemia HL-60 cells (that contain high peroxidase activity and lack CYP enzymes responsible for activation of PAH to diol epoxide) or human mammary carcinoma MCF-7 cells (that contain CYP activity but no peroxidase activity) were exposed to dibenzo[*a*,*l*]pyrene in a effort to discern the relative proportions of stable and depurinating DNA adducts (measured as apurinic sites). Human HL-60 cells produced no detectable levels of either stable DNA adducts or apurinic sites while MCF-7 cells produced stable adducts and low levels of apurinic sites that were not increased by dibenzo[*a*,*l*]pyrene. The levels of stable adduct increased 100-fold after exposure for 24 h, while the levels of apurinic sites remained low. These results indicated that metabolic activation of dibenzo[*a*,*l*]pyrene in human MCF-7 cells by CYP enzymes proceeds through diol epoxides that form stable DNA adducts with little formation of apurinic sites. Human HL-60 cells that contain high levels of peroxidase form neither stable DNA adducts nor apurinic sites. However, in these studies no measurement of unstable adducts was reported (Melendez-Colon *et al.*, 1999a, 2000).

The proportions of stable diol epoxide dibenzo[*a*,*l*]pyrene–DNA adducts and dibenzo[*a*,*l*]pyrene-induced apurinic sites formed in epidermal DNA were compared after topical application of dibenzo[*a*,*l*]pyrene to the skin of female SENCAR mice. Regardless of time after treatment and dose of dibenzo[*a*,*l*]pyrene applied, the levels of stable DNA adducts were significantly (from 32- to 86-fold) higher than those of apurinic sites (Melendez-Colon *et al.*, 1999b). The authors suggested that stable DNA adducts rather than apurinic sites were responsible for tumour initiation by dibenzo[*a*,*l*]pyrene in mouse skin.

(x) *Relevance of the diol epoxide metabolic activation mechanism for dibenzo[*a,l*]pyrene to human cancer*

The metabolic processes that metabolize dibenzo[*a*,*l*]pyrene to its active diol epoxides are present in human tissues (CYP1A1 and CYP1B1) and in human mammary cells in culture, which suggests the strong possibilty that humans exposed to dibenzo[*a*,*l*]pyrene would metabolically activate the compound to dibenzo[*a*,*l*]pyrene diol epoxides that could form DNA adducts and induce mutations and other genotoxic damage.

The mutations (G→T and A→T transversions) in the K-*ras* proto-oncogene codon 12 and 61 in mouse lung tumours from dibenzo[*a*,*l*]pyrene-treated mice are associated with *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–DNA adducts (Prahalad *et al.*, 1997). Although this is not a direct correlation, similar mutations in the Ki-*RAS* proto-oncogene were found in lung tumours from nonsmokers exposed to PAH-rich emissions from coal combustion (known to contain dibenzo[*a*,*l*]pyrene as well as many other PAHs) (DeMarini *et al.*, 2001).

Indeno[1,2,3-*cd*]pyrene

(i) *Metabolism and metabolic activation*

The metabolism of indeno[1,2,3-*cd*]pyrene has been documented in a number studies in rat liver microsomes and in mouse epidermis *in vivo*. Indeno[1,2,3-*cd*]pyrene is metabolized by Aroclor 1254-induced rat liver preparations to the K-region diol, *trans*-1,2-dihydroxy-1,2-dihydro-indeno[1,2,3-*cd*]pyrene (indeno[1,2,3-*cd*]pyrene-1,2-diol), 8-, 9- and 10-hydroxy-indeno[1,2,3-*cd*]pyrene, 8- and 9-hydroxy-*trans*-1,2-dihydroxy-1,2-dihydro-indeno[1,2,3-*cd*]pyrene and indeno[1,2,3-*cd*]pyrene-1,2-quinone (Rice *et al.*, 1985). Topical application of indeno[1,2,3-*cd*]pyrene to mouse epidermis *in vivo* produced 8-hydroxy-indeno[1,2,3-*cd*]pyrene as the primary metabolite, and 9-hydroxy-indeno[1,2,3-*cd*]pyrene and *trans*-1,2-dihydroxy-1,2-dihydro-indeno[1,2,3-*cd*]pyrene as major metabolites. Several minor metabolites were also identified: *trans*-1,2-dihydro-1,2,8-trihydroxy-indeno[1,2,3-*cd*]pyrene, *trans*-1,2-dihydro-1,2,9-trihydroxy-indeno[1,2,3-*cd*]pyrene, indeno[1,2,3-*cd*]pyrene-1,2-quinone and 10-hydroxy-indeno[1,2,3-*cd*]pyrene (Rice *et al.*, 1986).

(ii) *Formation of DNA adducts*

Indeno[1,2,3-*cd*]pyrene formed a single unidentified DNA adduct in mouse skin epidermis *in vivo* (Weyand *et al.*, 1987). Studies of DNA adducts in mouse epidermis *in vivo* using fluorine probes suggested that indeno[1,2,3-*cd*]pyrene undergoes metabolic activation at carbons 7–10 either alone or in conjunction with dihydrodiol formation at the 1,2 carbons (Rice *et al.*, 1990).

(iii) *Genotoxic effects of indeno[1,2,3-*cd*]pyrene*

In a previous monograph, indeno[1,2,3-*cd*]pyrene was reported to induce mutations in *S. typhimurium* in the presence of an exogenous metabolic activation system (IARC, 1983). Indeno[1,2,3-*cd*]pyrene was mutagenic in *S. typhimurium* TA100 in the presence

of an Aroclor 1254-induced rat liver preparation (Rice *et al.*, 1985) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity study of indeno[1,2,3-*cd*]pyrene-1,2-diol

Groups of 25 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses of indeno[1,2,3-*cd*]pyrene-1,2-diol in acetone every other day (total dose, 3226 nmol) [1 mg], followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours in the dose groups 21 weeks after treatment was 80% with 1.68 tumours/mouse. The response in control mice treated with TPA alone was 0.04 tumours/mouse. Quantitatively, indeno[1,2,3-*cd*]pyrene-1,2-diol produced fewer tumours/mouse than the parent indeno[1,2,3-*cd*]pyrene (Rice *et al.*, 1986).

Genotoxicity of indeno[1,2,3-*cd*]pyrene-1,2-diol

Indeno[1,2,3-*cd*]pyrene-1,2-diol did not induce mutations in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Rice *et al.*, 1985).

Carcinogenicity study of indeno[1,2,3-*cd*]pyrene-1,2-oxide

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses of indeno[1,2,3-*cd*]pyrene-1,2-oxide in acetone every other day (total dose, 3425 nmol) [1 mg], followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dose group 21 weeks after treatment was 80% (1.60). The response in control mice treated with TPA alone was 0.04 tumours/mouse. Quantitatively, indeno[1,2,3-*cd*]pyrene-1,2-oxide produced fewer tumours/mouse than the parent indeno[1,2,3-*cd*]pyrene (1.60 versus 2.83) (Rice *et al.*, 1986).

DNA adducts of indeno[1,2,3-*cd*]pyrene-1,2-oxide

Indeno[1,2,3-*cd*]pyrene-1,2-oxide forms one major DNA adduct with calf thymus DNA (King *et al.*, 1994).

Genotoxicity of indeno[1,2,3-*cd*]pyrene-1,2-oxide

Indeno[1,2,3-*cd*]pyrene-1,2-oxide induced mutations in *S. typhimurium* TA100 in the absence of an exogenous metabolic activation system (Rice *et al.*, 1985).

Carcinogenicity study of 8-hydroxy-indeno[1,2,3-*cd*]pyrene

Groups of 25 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses of 8-hydroxy-indeno[1,2,3-*cd*]pyrene in acetone every other day (total dose, 3425 nmol) [1 mg], followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours in the dose group 21

weeks after treatment was not stated; however, there were 0.48 tumours/mouse. The response in control mice treated with TPA alone was 0.04 tumours/mouse (Rice *et al.*, 1986).

Genotoxicity of 8-hydroxy-indeno[1,2,3-*cd*]pyrene

8-Hydroxy-indeno[1,2,3-*cd*]pyrene induced mutations in *S. typhimurium* TA100 in the absence of an exogenous metabolic activation system (Rice *et al.*, 1985).

Conclusion

No data are available that support a diol epoxide mechanism for indeno[1,2,3-*cd*]pyrene. While indeno[1,2,3-*cd*]pyrene-1,2-oxide is an ultimate bacterial mutagenic metabolite of indeno[1,2,3-*cd*]pyrene and forms a single DNA adduct *in vitro*, it is not the ultimate tumorigenic metabolite on mouse skin based on quantitative tumour formation. 8-Hydroxy-indeno[1,2,3-*cd*]pyrene is mutagenic to bacteria but exhibited only weak tumour-initiating activity. These results indicate that the principal metabolic activation pathways associated with the mutagenic activity of indeno[1,2,3-*cd*]pyrene are not related to its tumour-initiating activity in mouse skin. In mouse skin, indeno[1,2,3-*cd*]pyrene appears to undergo metabolic activation at carbons 7–10 either alone or in conjunction with dihydrodiol formation at the 1,2 carbons. The route of metabolic activation of indeno[1,2,3-*cd*]pyrene in mouse skin has yet to be determined.

5-Methylchrysene

(i) *Metabolism and metabolic activation*

The metabolism of 5-methylchrysene has been documented in a number studies in rat and mouse liver microsomes, in mouse epidermis *in vivo* and in human liver and lung microsomes. Two potential centres of metabolism and metabolic activation are situated on the two benzo rings, the pseudo-fjord-region (1–2 and 3–4 bonds) and the bay-region (7–8 and 9–10-bonds). Control, Aroclor-treated and 3-methylcholanthrene-induced rat liver preparations metabolized 5-methylchrysene to 1,2-dihydro-1,2-dihydroxy-5-methylchrysene (5-methylchrysene-1,2-diol), 7,8-dihydro-7,8-dihydroxy-5-methylchrysene (5-methylchrysene-7,8-diol), 9,10-dihydro-9,10-dihydroxy-5-methylchrysene (5-methylchrysene-9,10-diol), 3,4-dihydro-3,4-dihydroxy-5-methylchrysene (5-methylchrysene-3,4-diol), 9-hydroxy-5-methylchrysene, 7-hydroxy-5-methylchrysene, 1-hydroxy-5-methylchrysene and 5-hydroxymethylchrysene (Hecht *et al.*, 1978; Amin *et al.*, 1985b). Phenobarbital-induced rat liver microsomes metabolized 5-methylchrysene to two K-region diols, 5,6-dihydroxy-5,6-dihydro-5-methylchrysene and 11,12-dihydroxy-11,12-dihydro-5-methylchrysene (Bao & Yang, 1986). 5-Methylchrysene was stereoselectively metabolized to the *R*,*R* dihydrodiol enantiomers, 5-methylchrysene-1*R*,2*R*-diol and 5-methylchrysene-7*R*,8*R*-diol, *in vitro* by 3-methylcholanthrene-induced mouse liver preparations and human liver microsomes, and *in vivo* in mouse epidermis (Melikian *et al.*, 1983; Amin *et al.*, 1987; Koehl *et al.*, 1996). Mouse skin epidermis *in vivo* produced the metabolites 5-methylchrysene-1,2-diol, 5-methylchrysene-7,8-diol, 5-methylchrysene-

9,10-diol and 5-hydroxymethylchrysene and their glucuronide and sulfate conjugates (Melikian *et al.*, 1983). Human liver microsomes metabolized 5-methylchrysene to 5-methylchrysene-1,2-diol, 5-methylchrysene-7,8-diol, 5-methylchrysene-9,10-diol (5-methylchrysene-3,4-diol), 9-hydroxy-5-methylchrysene, 7-hydroxy-5-methylchrysene, 1-hydroxy-5-methylchrysene and 5-hydroxymethylchrysene. Human lung microsomes metabolized 5-methylchrysene to 5-methylchrysene-1,2-diol, 5-methylchrysene-7,8-diol, 5-methylchrysene-9,10-diol, 5-methylchrysene-3,4-diol and 1-hydroxy-5-methylchrysene. CYP1A1 was shown to play a major role in the metabolic activation of 5-methylchrysene in human lung microsomes. Purified recombinant human CYP1A1 had high activity for the formation of 5-methylchrysene-1,2-diol and 5-methylchrysene-7,8-diol and CYP3A4 was active for methyl hydroxylation (Koehl *et al.*, 1996).

(ii) *Formation of DNA adducts*

5-Methylchrysene forms a major DNA adduct in mouse skin after dermal application and in the human MCF-7 mammary carcinoma cell line (Shiue *et al.*, 1987; Kuljukka-Rabb *et al.*, 2001). It forms several bay-region diol epoxide–DNA adducts after application to mouse skin *in vivo*, which were identified as *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-5-methylchrysene (*anti*-5-methylchrysene-1,2-diol-3,4-oxide)–DNA and *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-5-methylchrysene–DNA in the ratio of 2.7:1. This indicated that both 5-methylchrysene-1,2-diol and 5-methylchrysene-7,8-diol were metabolized to their corresponding diol epoxides *in vivo* (Melikian *et al.*, 1982). The adducts were further identified as *trans*-N^2-deoxyguanosine adducts at the benzylic carbon of the epoxide ring of each diol epoxide (Melikian *et al.*, 1984). The *anti*-5-methylchrysene-1,2-diol-3,4-oxide–DNA adduct was further characterized as a 1*R*,2*S*,3*S*-trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene adduct (Melikian *et al.*, 1988), which was detected in the lungs of newborn mice injected with 5-methylchrysene (Melikian *et al.*, 1991) and as one of six DNA adducts observed in lung tissues of mice treated with 5-methylchrysene (You *et al.*, 1994).

(iii) *Genotoxicity of 5-methylchrysene*

In a previous monograph, 5-methylchrysene was reported to induce mutations in *S. typhimurium* TA100 and to induce unscheduled DNA synthesis in primary rat hepatocytes (IARC, 1983). 5-Methylchrysene was a gene mutagen in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Cheung *et al.*, 1993). It produced G:C→A:T (31.2%) transitions, G:C→T:A (15.3%) transversions and A:T→T:A (37.2%) transversions in a *lacZ* reversion assay in *E. coli* in the presence of an Aroclor 1254-induced rat liver preparation (Garganta *et al.*, 1999). Human CYP1A1 and CYP1B1 enzymes have higher activity for the metabolism of 5-methylchrysene-1,2-diol than for the parent compound 5-methylchrysene to DNA-damaging forms using cDNA-based recombinant (*E. coli* or *T. ni*) systems that express these forms of CYP (Shimada *et al.*, 2001a).

(iv) *5-Methylchrysene-induced mutations in proto-oncogenes*

5-Methylchrysene induced three major classes of Ki-*ras* codon 12 mutations in lung adenomas from intraperitoneally treated strain A/J mice — GGT→TGT (50%), GGT→GTT (23%) and GGT→CGT (27%) — which indicates that deoxyguanosine was a primary target for this PAH in mouse lung (You *et al.*, 1994; Nesnow *et al.*, 1998b). Tumours from mice treated by skin application with racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide (the ultimate metabolite of 5-methylchrysene) had very low numbers of Ha-*ras* codon 12, 13 or 61 mutations (Hecht *et al.*, 1998).

(v) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of 5-methylchrysene-1,2-diol

Groups of 20 female Ha(ICR) Swiss mice, 50–55 days of age, received dermal applications of 10 subdoses of racemic 5-methylchrysene-1,2-diol [total dose, 109 nmol] (30 μg) in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 95% (7.3). No skin tumours developed in mice treated with TPA alone (Hecht *et al.*, 1980).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [9 μg] racemic 5-methylchrysene-1,2-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 80% (2.5). Skin tumours developed in 5% (0.5 tumours/mouse) of the mice treated with TPA alone. The tumour incidence in the treatment and control groups was statistically different (Amin *et al.*, 1985b).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [9 or 27.5 μg] racemic 5-methylchrysene-1,2-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 85 (9.9) and 100% (12.7), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 10 or 33 nmol [2.75 or 9 μg] 5-methylchrysene-1*S*,2*S*-diol or 5-methylchrysene-1*R*,2*R*-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was: 5-methylchrysene-1*S*,2*S*-diol, 60 (0.9) and 85% (3.0); 5-methylchrysene-1*R*,2*R*-diol, 100 (9.4) and 85% (7.9), respectively. No skin tumours developed in mice treated with TPA alone. These results indicated a higher reactivity of the *R*,*R* diol compared with the *S*,*S* diol (Amin *et al.*, 1987).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic 5-methylchrysene-1,2-diol (total dose, 56 nmol) [15 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In

43 female mice, pulmonary tumours occurred in 12% of the surviving mice (0.14 tumours/mouse) and liver tumours in 7% (0.23 tumours/mouse). In 44 male mice, pulmonary tumours occurred in 11% of the surviving mice (0.18 tumours/mouse) and liver tumours in 25% (0.52 tumours/mouse). In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

DNA adducts of 5-methylchrysene-1,2-diol

1*R*,2*S*,3*S*-Trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene was detected in the DNA of lungs of newborn mice injected with 5-methylchrysene-1*R*,2*R*-diol (Melikian *et al.*, 1991).

Genotoxicity of 5-methylchrysene-1,2-diol

Human CYP1A1 and CYP1B1 enzymes metabolically activated 5-methylchrysene-1,2-diol to DNA-damaging forms in cDNA-based recombinant (*E. coli* or *T. ni*) systems that express different forms of the CYP (Shimada *et al.*, 2001a).

Carcinogenicity studies of 5-methylchrysene-1,2-diol-3,4-oxide

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [10 or 29.5 μg] racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 65 (1.3) and 80% (4.4), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [10 μg] 5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide, *anti*-5-methylchrysene-1*S*,2*R*-diol-3*R*,4*S*-oxide or racemic *syn*-5-methylchrysene-1,2-diol-3,4-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 80 (3.2), 20 (0.4) or 5% (0.1), respectively. No skin tumours developed mice treated with TPA alone (Hecht *et al.*, 1987).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33, 100 or 400 nmol [10, 29.5 or 118 μg] racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone twice a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 90 (3.1), 100 (7.5) and 100% (9.1), respectively. Skin tumours developed in 5% (0.05 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1998).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide or racemic *syn*-5-methyl-

chrysene-1,2-diol-3,4-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 48 or 41 female mice, pulmonary tumours were observed in 81 or 29% of the surviving mice (5.6 or 0.34 tumours/mouse) and liver tumours in 4 or 7% (0.13 or 0.46 tumours/mouse), respectively. In 38 or 49 male mice, pulmonary tumours were observed in 82 or 6% of the surviving mice (3.3 or 0.06 tumours/mouse) and liver tumours in 34 or 14% (2.6 or 0.2 tumours/mouse), respectively. In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with *anti*-5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide or *anti*-5-methylchrysene-1*S*,2*R*-diol-3*R*,4*S*-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 20 or 25 female mice, pulmonary tumours developed in 85 or 56% of the surviving mice (19.4 or 0.84 tumours/mouse), respectively, but no liver tumours. In 24 or 40 male mice, pulmonary tumours developed in 88 or 18% of the surviving mice (8.4 or 0.23 tumours/mouse) and liver tumours in 46 or 8% (1.38 or 0.1 tumours/mouse), respectively. In 28 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) but no liver tumours. In 31 male mice treated with DMSO alone, pulmonary tumours developed in 3% of the surviving mice (0.13 tumours/mouse) but no liver tumours. The numbers of pulmonary tumours/mouse in both male and female mice and hepatic tumours/mouse in male mice were significantly different from those in the controls for *anti*-5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide and those of pulmonary tumours/mouse in female mice for *anti*-5-methylchrysene-1*S*,2*R*-diol-3*R*,4*S*-oxide (Hecht *et al.*, 1987).

DNA adducts of 5-methylchrysene-1,2-diol-3,4-oxide

anti-5-Methylchrysene-1,2-diol-3,4-oxide and *anti*-5-methylchrysene-7,8-diol-9,10-oxide formed DNA adducts with calf thymus DNA (Melikian *et al.*, 1984; King *et al.*, 1994). These adducts result from the addition of the exocyclic amino group of deoxyguanosine to the benzylic carbon of the epoxide ring of the dihydrodiol epoxide (Melikian *et al.*, 1984). One *anti*-5-methylchrysene-1,2-diol-3,4-oxide–calf thymus DNA adduct was further characterized as 1*R*,2*S*,3*S*-trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene (Reardon *et al.*, 1987; Melikian *et al.*, 1988). Other *anti*-5-methylchrysene-1,2-diol-3,4-oxide–calf thymus DNA adducts have been identified as *cis* and *trans* deoxyadenosine (Reardon *et al.*, 1987; Melikian *et al.*, 1988). Both *syn* and *anti* forms of 5-methylchrysene-1,2-diol-3,4-oxide form *cis* and *trans* adducts with calf thymus DNA (Szeliga *et al.*, 1999). 1*R*,2*S*,3*S*-Trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene was detected in the DNA of lungs of newborn mice injected with *anti*-5-methylchrysene-1,2-diol-3,4-oxide (Melikian *et al.*, 1991).

Genotoxicity of 5-methylchrysene-1,2-diol-3,4-oxide

The *R,S,S,R* enantiomer of *anti*-5-methylchrysene-1,2-diol-3,4-oxide was mutagenic in *S. typhimurium* TA100 (Melikian *et al.*, 1988). *anti*-5-Methylchrysene-1,2-diol-3,4-oxide produced the base-specific mutations GC→TA (62%), GC→CG (23%), GC→AT (9%) and AT→TA (4%) and *syn*-5-methylchrysene-1,2-diol-3,4-oxide produced the base-specific mutations GC→TA (43%), GC→CG (22%), GC→AT (14%) and AT→TA (12%) in *supF* DNA using a SV40-based shuttle vector system (Bigger *et al.*, 2000). In the *supF* gene of the pSP189 vector, *syn*-5-methylchrysene-1,2-diol-3,4-oxide gave transversion mutations at GC pairs, predominantly G→T and G→C, which showed the preference for reaction with deoxyguanosine residues in DNA (Page *et al.*, 1996). Both *syn*- and *anti*- 5-methylchrysene-1,2-diol-3,4-oxide were mutagenic at *Hprt* gene of Chinese hamster V79 (6-thioguanine resistance) cells, and the *anti* form was the most mutagenic (Brookes *et al.*, 1986).

In normal human bronchial epithelial cells, *anti*-5-methylchrysene-1,2-diol-3,4-oxide formed significant levels of guanine adducts within codon 158 of exon 5, codons 237, 245 and 248 of exon 7 and codon 273 of exon 8 in the human *TP53* tumour-suppressor gene. These codons were also hotspots for mutations in the *TP53* gene of lung cancer patients (Smith *et al.*, 2000). (+)-*anti*-5-Methylchrysene-1,2-diol-3,4-oxide was conjugated to GSH to a greater extent than (–)-*anti*-5-methylchrysene-1,2-diol-3,4-oxide by human hGSTP1-1 (I104, A113) allele, the most frequent allele in human populations (Hu *et al.*, 1998).

Other effects of 5-methylchrysene-1,2-diol-3,4-oxide

anti-5-Methylchrysene-1,2-diol-3,4-oxide induces activator protein-1 through PI3K- and Akt-dependent and $p70^{SK6}$-independent pathways in mouse epidermal Cl41 cells suggesting that oxidative stress, such reactive oxygen species may be involved (Li *et al.*, 2004a). *anti*-5-Methylchrysene-1,2-diol-3,4-oxide also resulted in the activation of MAPKs as well as the activation of inhibitory subunit kappa-B phosphorylation and degradation involved in the activation of nuclear factor (NF) kappaB. The authors suggested that *anti*-5-methylchrysene-1,2-diol-3,4-oxide may play a role in the tumour promotion effects of its parent compound 5-methylchrysene or of itself (Li *et al.*, 2004b). In human breast carcinoma MCF-7 cells, p53 was stabilized in response to DNA damage by *anti*-5-methylchrysene-1,2-diol-3,4-oxide. However, the protective mechanism of G1 arrest of the cells was not induced. Therefore, this DNA damage escapes the p53-mediated cellular defence mechanism of G1 arrest (Khan *et al.*, 1999).

Carcinogenicity studies of 5-methylchrysene-7,8-diol

Groups of 20 female Ha/ICR Swiss mice, 50–55 days of age, received 10 subdoses by dermal application of racemic 5-methylchrysene-7,8-diol (total dose, 109 nmol) [30 μg] in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks

after treatment was 50% (1.1). No skin tumours developed in mice treated with TPA alone (Hecht *et al.*, 1980).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [9 or 27.5 μg] racemic 5-methylchrysene-7,8-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 30 (0.3) and 75% (1.3), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [9 μg] 5-methylchrysene-7*S*,8*S*-diol or 5-methylchrysene-7*R*,8*R*-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of papillomas (papillomas/mouse) in the dosed groups 21 weeks after treatment was: 5-methylchrysene-7*S*,8*S*-diol, 30% (0.4); 5-methylchrysene-7*R*,8*R*-diol, 75% (2.3). No skin papillomas developed in mice treated with TPA alone. These results indicated a higher reactivity of the *R*,*R* diol compared with the *S*,*S* diol (Amin *et al.*, 1987).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic 5-methylchrysene-7,8-diol (total dose, 56 nmol) [15 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 45 female mice, pulmonary tumours developed in 18% of the surviving mice (0.24 tumours/mouse) and liver tumours in 11% (0.49 tumours/mouse). In 46 male mice, pulmonary tumours developed in 13% of the surviving mice (0.13 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

Carcinogenicity studies of 5-methylchrysene-7,8-diol-9,10-epoxide

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [10 or 29.5 μg] racemic *anti*-5-methylchrysene-7,8-diol-9,10-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 5 (0.1) and 0% (0), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [10 μg] racemic *syn*-5-methylchrysene-7,8-diol-9,10-oxide, *anti*-5-methylchrysene-7*R*,8*S*-diol-9*S*,10*R*-oxide or *anti*-5-methylchrysene-7*S*,8*R*-diol-9*R*,10*S*-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] PA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed

groups 21 weeks after treatment was 21 (0.3), 5 (0.1) or 16% (0.2), respectively. No skin tumours developed in mice treated with TPA alone (Hecht *et al.*, 1987).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-5-methylchrysene-7,8-diol-9,10-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 50 female mice, pulmonary tumours developed in 6% of the surviving mice (0.06 tumours/mouse) and liver tumours in 4% (0.04 tumours/mouse). In 49 male mice, pulmonary tumours developed in 18% of the surviving mice (0.18 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with 5-methylchrysene-7*R*,8*S*-diol-9*S*,10*R*-oxide or 5-methylchrysene-7*S*,8*R*-diol-9*R*,10*S*-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 34 or 23 female mice, pulmonary tumours developed in 38% or 17% of the surviving mice (0.47 or 0.83 tumours/mouse) and liver tumours in 0 or 4% (0 or 0.04 tumours/mouse), respectively. In 26 or 38 male mice, pulmonary tumours developed in 35% or 0% of the surviving mice (0.23 or 0 tumours/mouse) and liver tumours in 19% or 3% (0.23 or 0.03 tumours/mouse), respectively. In 28 female mice treated with DMSO alone, pulmonary tumours occurred in 7% of the surviving mice (0.07 tumours/mouse) and no liver tumours. In 31 male mice treated with DMSO alone, pulmonary tumours occurred in 3% of the surviving mice (0.13 tumours/mouse) and no liver tumours (Hecht *et al.*, 1987).

Genotoxicity of 5-methylchrysene-7,8-diol-9,10-oxide

The *R,S,S,R* enantiomer of *anti*-5-methylchrysene-7,8-diol-9,10-oxide was mutagenic in *S. typhimurium* TA100 (Melikian *et al.*, 1988). Both *syn*- and *anti*-5-methylchrysene-7,8-diol-9,10-oxide were mutagenic in the *Hprt* gene of Chinese hamster V79 cells (6-thioguanine resistance) (Brookes *et al.*, 1986).

Conclusion

5-Methylchrysene is activated metabolically by the diol epoxide mechanism at the pseudo bay region in mouse skin carcinogenesis. 5-Methylchrysene was stereoselectively metabolized to the *R,R* enantiomer, 5-methylchrysene-1*R*,2*R*-diol, in mouse epidermis *in vivo* and with human liver and lung microsomes. 5-Methylchrysene-1*R*,2*R*-diol was a tumour initiator in mouse skin and was more active than the *S,S* enantiomer. *anti*-5-Methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide was the most active 5-methylchrysene diol epoxide in mouse skin. The major diol epoxide adduct from treatment of mouse skin with 5-methylchrysene was *anti*-5-methylchrysene-1,2-diol-3,4-oxide–deoxyguanosine. In the

newborn mouse model, 5-methylchrysene-1,2-diol and *anti*-5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide were pulmonary carcinogens. 1*R*,2*S*,3*S*-Trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene was detected in the DNA of lungs of newborn mice injected with 5-methylchrysene, 5-methylchrysene-1*R*,2*R*-diol and *anti*-5-methylchrysene-1,2-diol-3,4-oxide. This DNA adduct was also observed in the lungs of juvenile mice administered 5-methylchrysene, and its formation was related to the detection of Ki-*ras* mutations in 5-methylchrysene-induced tumours. *anti*-5-Methylchrysene-1,2-diol-3,4-oxide–DNA adducts were formed *in vitro* within human *TP53* codons that were also hotspots for mutation in the *TP53* gene of lung cancer patients.

The bay-region diol, 5-methylchrysene-7*R*,8*R*-diol, was formed in mouse skin where it was a tumour initiator. It was a weak carcinogen in the newborn mouse model. *anti*- and *syn*-5-Methylchrysene-7,8-diol-9,10-oxide were either weak or not active as carcinogens in mouse skin or in newborn mice. The role of the bay-region diol, 5-methylchrysene-7,8-diol, and 5-methylchrysene-7,8-diol-9,10-oxide in chemical carcinogenesis remains to be determined.

4.2.2 *Mechanism via formation of Radical cations*

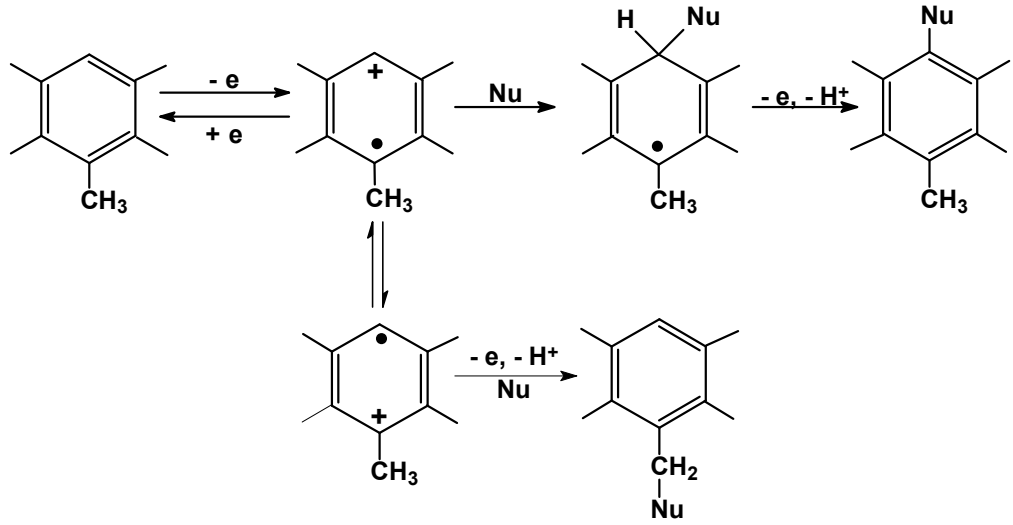
(a) Chemistry and physical chemistry of PAH radical cations

Removal of one electron from the π system of a polycyclic aromatic compound 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. When the charge is localized adjacent to the methyl group, the latter becomes electrophilic and subsequently reacts with a nucleophile (Figure 4.4).

Development of the chemistry of PAH radical cations has provided evidence that these intermediates can play a role in the process of tumour initiation of several potent PAHs (Cavalieri & Rogan, 1985, 1992). Radical cations of unsubstituted and methyl-substituted PAHs have been generated by iodine oxidation (Hanson *et al*., 1998), manganic acetate oxidation (Cremonesi *et al*., 1992) and electrochemical oxidation (RamaKrishna *et al*., 1993a,b), with subsequent binding to nucleophiles. Furthermore, radical cations of benzo[*a*]pyrene and its derivatives have been isolated after oxidation with iodine in the presence of silver perchlorate (Stack *et al*., 1995).

The notion that radical cations play an important role in the metabolic activation of some PAHs derives from certain features that are common to several carcinogenic PAHs such as benzo[*a*]pyrene, dibenzo[*a,l*]pyrene, DMBA and 3-methylcholanthrene. These characteristics are: first, a relatively low ionization potential, which allows the removal of one electron and the formation of a relatively stable radical cation; second, a charge localization in the radical cation that renders this intermediate specifically and efficiently reactive toward nucleophiles; and third, an optimal geometric configuration that allows

Figure 4.4. Nucleophilic trapping in PAH radical cations at unsubstituted and methylsubstituted sites



Adapted from Cavalieri & Rogan (1985)
Nu, Nucleophile; PAH, polycyclic aromatic hydrocarbon

the formation of appropriate intercalating radical-cation complexes with DNA, and thus favours the formation of covalent DNA adducts.

(i) *Ionization potential*

The formation and relative stability of PAH radical cations are dependent on their ionization potential. Above a certain potential, activation by one-electron oxidation becomes improbable due to the more difficult removal of one electron by the oxidant, CYP enzymes or peroxidases. A cut-off ionization potential above which one-electron oxidation will probably not occur was estimated to be about 7.35 eV, based on the level of horseradish peroxidase-catalysed or prostaglandin H synthase-catalysed covalent binding to DNA of a series of PAHs with ionization potentials ranging from 8.19 eV (phenanthrene) to 6.68 eV (6,12-dimethylanthanthrene) (Cavalieri *et al*., 1983; Devanesan *et al*., 1987). Therefore, the ionization potential of a PAH can serve as an indicator to predict whether or not one-electron oxidation can play a role in its metabolic activation.

(ii) *Charge localization*

A relatively low ionization potential is a necessary but not a sufficient prerequisite for the activation of a PAH by one-electron oxidation. Another important factor that must be considered is the localization of the charge in the PAH radical cation. PAHs have positions of unequal charge localization and the extent of this unequal distribution depends on the symmetry of the condensed benzene ring. In benzo[*a*]pyrene, C6 is the

position with the greatest electron density and the highest reactivity with electrophiles in the neutral molecule (Cavalieri & Calvin, 1971). In the radical cation, C6 has the lowest electron density and the highest reactivity with nucleophiles (Cremonesi *et al*., 1989). Therefore, the relatively low ionization potential of 7.23 eV for benzo[*a*]pyrene (Cavalieri *et al*., 1983) and the charge localization at C6 render this molecule receptive to activation by one-electron oxidation.

For dibenzo[*a*,*l*]pyrene, the charge in the radical cation is mainly localized at C10 (Cremonesi *et al*., 1992). Nucleophilic substitution occurs regiospecifically at this position with subsequent formation of an intermediate radical. The intermediate is rapidly oxidized to an arenium ion with loss of a proton to complete the substitution reaction.

(iii) *Optimal geometric configuration*

An important factor that determines the carcinogenic activity of PAHs is related to the geometry of the molecule. In general, activity is found in PAHs that contain three to seven condensed rings (Boström *et al.*, 2002). A more precise requirement related to the geometric characteristics of PAHs is the presence of an angular ring, for example, in the benz[*a*]anthracene series. Optimal geometric configuration of PAHs is essential for the arrangement of the appropriate intercalation complexes with DNA, which are a prerequisite for the formation of a covalent bond with nucleophiles (Lesko *et al*., 1968). Strong evidence for the formation of these intercalation complexes is demonstrated by the fact that unstable adducts that generate apurinic sites upon release from DNA (hereafter called unstable adducts) are formed from the reaction of radical cations of benzo[*a*]pyrene, DMBA or dibenzo[*a*,*l*]pyrene with double-stranded DNA but not with single-stranded DNA or RNA (Devanesan *et al*., 1993; Rogan *et al*., 1993; Li *et al*., 1995).

(b) Enzymology of PAH activation

PAHs are activated by both peroxidases and CYPs to form ultimate carcinogenic metabolites that bind to DNA. One-electron oxidation of PAHs can be catalysed by both types of enzyme (Devanesan *et al*., 1987; Cavalieri *et al*., 1990), but only CYPs can carry out the initial epoxidation of PAHs, which leads to the proximate dihydrodiol metabolites in the diol epoxide pathway of activation (Sims *et al*., 1974; Conney, 1982). CYP is present in both the endoplasmic reticulum and the nuclear membrane of mammalian cells. Enzymes in the nuclear membrane catalyse the formation of radical cation adducts with DNA because radical cations formed in the endoplasmic reticulum react with other cellular nucleophiles before they can reach the nucleus to react with DNA. There is evidence that the CYP isoforms in the nucleus are similar to those in the endoplasmic reticulum (Bresnick *et al.*, 1977; Mukhtar *et al.*, 1979).

(c) Pathways of PAH activation: radical cations and diol epoxides

Based on several lines of evidence discussed in this section, it has been postulated that PAHs are activated via two main pathways: one-electron oxidation to form radical cations

and mono-oxygenation to yield diol epoxides. The two types of reactive intermediate, radical cations and diol epoxides, can bind to DNA to form adducts that presumably initiate the process of tumour formation. Some PAHs are activated exclusively to diol epoxides, for example 5-methylchrysene and benzo[*c*]phenanthrene, whereas several others, such as benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene, DMBA and 3-methylcholanthrene, are activated by the formation of both radical cations and diol epoxides.

Adenine and guanine are the two DNA bases that are most susceptible to the nucleophilic attack of activated PAHs, whereas thymine and cytosine are less reactive. Covalent binding to the endocyclic positions *N*3 and *N*7 of adenine and *N*7, and sometimes C8, of guanine produces unstable adducts that generate apurinic sites upon release from DNA. These adducts are sometimes called 'depurinating adducts' or 'depurination adducts'. In contrast, adduct formation at the exocyclic 2-amino group of guanine and the 6-amino group of adenine results in stable adducts that remain in DNA unless removed by repair.

The main approach to demonstrating that PAHs are indeed activated primarily by these two mechanisms has been the identification and quantification of PAH–DNA adducts formed *in vitro* and *in vivo*. The unstable PAH–DNA adducts formed at endocyclic positions of adenine and guanine have been identified by a combination of high-performance liquid chromatography (HPLC) and fluorescence line-narrowing spectroscopy (Jankowiak & Small, 1998). The mechanism of activation, the types of DNA damage formed and their biological significance in initiating the tumour process are supported by these studies.

(i) *Benzo[*a*]pyrene*

Benzo[*a*]pyrene–DNA adducts were among the first PAH–DNA adducts to be identified. Important evidence for the diol epoxide activation pathway was the identification of a stable adduct formed *in vitro* and *in vivo* by the reaction of benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide with the exocyclic 2-amino group of guanine (Sims *et al*., 1974; Koreeda *et al*., 1978). The formation of adducts by one-electron oxidation was demonstrated by the identification of an unstable benzo[*a*]pyrene–6-*N7*-guanine adduct after in-vitro activation of benzo[*a*]pyrene by horseradish peroxidase or CYP in the presence of DNA (Rogan *et al*., 1988). Subsequently, complete profiles of the two types of benzo[*a*]pyrene–DNA adduct formed after activation of benzo[*a*]pyrene by CYP *in vitro* and in mouse skin *in vivo* were determined (Chen *et al.*, 1996). Qualitative and quantitative comparisons of the adducts were made by treating mouse skin *in vivo* with 200 nmol each of tritiated benzo[*a*]pyrene, benzo[*a*]pyrene-7,8-dihydrodiol (its proximate carcinogenic metabolite) or *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (an ultimate carcinogenic metabolite) in 50 μL acetone. The comparison comprised assessment of the relative amounts of stable and unstable adducts and the relative abundance of unstable adducts at guanine and adenine.

Benzo[*a*]pyrene formed both stable (29%) and unstable (71%) adducts in mouse skin (Chen *et al*., 1996). The unstable adducts were 8-(benzo[*a*]pyrene-6-yl)guanine (34%), 7-

(benzo[*a*]pyren-6-yl)guanine (10%), 7-(benzo[*a*]pyren-6-yl)adenine (22%), 10-(guanin-7-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (2%) and 10-(adenin-7-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (3%), whereas the stable 10-(guanin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene constituted 23% and the remaining 6% were unidentified stable adducts. Most of the adducts found after treatment with benzo[*a*]pyrene were unstable adducts formed by one-electron oxidation (66%), whereas stable adducts were mostly formed by the diol epoxide, the major one of which is 10-(guanin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

Benzo[*a*]pyrene-7,8-dihydrodiol formed more stable adducts (63%) than unstable adducts (37%) in mouse skin (Chen *et al*., 1996), but the absolute amount of unstable adducts (1.2 μmol/mol DNA-Phosphate) was very similar to that formed after treatment with benzo[*a*]pyrene (1.5 μmol/mol DNA-Phosphate). After treatment with *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, fewer unstable adducts (0.6 μmol/mol DNA-Phosphate) were formed than with the precursors benzo[*a*]pyrene and benzo[*a*]-pyrene-7,8-dihydrodiol. Among the stable adducts formed by benzo[*a*]pyrene, benzo[*a*]-pyrene-7,8-dihydrodiol and *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, the main adduct is 10-(guanin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, and the ratio of the amount of stable adducts formed by these three agents is 1:3:80, respectively. The relative tumorigenicity of these three compounds corresponds to the amount of unstable adducts formed: benzo[*a*]pyrene and benzo[*a*]pyrene-7,8-dihydrodiol exhibit similar potency in mouse skin and *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide is less active (Slaga *et al*., 1976; Levin *et al*., 1977b; Slaga *et al*., 1977b). In contrast, carcinogenicity is not related to the amount of stable adducts, since the less carcinogenic *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide forms much higher levels of such adducts (Chen *et al*., 1996).

(ii) *Dibenzo[*a,l*]pyrene*

Dibenzo[*a*,*l*]pyrene is the most potent of the known carcinogenic PAHs (Cavalieri *et al*., 1989; LaVoie *et al*., 1993). Comparative carcinogenicity studies in mouse skin and rat mammary gland indicated that dibenzo[*a*,*l*]pyrene is slightly more potent than its 11,12-dihydrodiol and much more potent than its 11,12-dihydrodiol-13,14-epoxide derivatives (Higginbotham *et al*., 1993; Gill *et al*., 1994). Identification and quantification of the adducts formed in the skin of mice treated with dibenzo[*a*,*l*]pyrene revealed that 99% are unstable (Cavalieri *et al*., 2005; Todorovic *et al*., 2005). Most of these adducts are formed by one-electron oxidation, with 63% at adenine and 12% at guanine. The remainder are formed by the diol epoxide, with 18% at adenine and 6% at guanine. When mouse skin is treated *in vivo* with dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol, unstable adducts comprise 80% of the total and are formed mainly with adenine (69%). Treatment of mouse skin with (±)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol-13,14-epoxide results in 32% unstable adducts, primarily at adenine (25%), whereas treatment with (±)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol-13,14-epoxide produces 97% stable adducts (Cavalieri *et al*., 2005). Comparison of the relative tumorigenicity of dibenzo[*a*,*l*]pyrene and its metabolites with

the amount of DNA adducts formed in mouse skin suggests that the unstable adducts at adenine play a major role in the initiation of tumours by dibenzo[*a*,*l*]pyrene.

(iii) *7,12-Dimethylbenz[*a*]anthracene*

When DMBA is bound to DNA after activation with CYP *in vitro* or in mouse skin *in vivo*, 99% of the adducts are formed by one-electron oxidation. Of these, 79–82% have the 12-methyl group of DMBA linked to *N*7 of adenine, whereas 20–17% have the 12-methyl group linked to the *N*7 of guanine. Stable adducts arising from the bay-region diol epoxide represent less than 1% of the adducts formed in mouse skin (RamaKrishna *et al*., 1992; Devanesan *et al*., 1993).

The 12-methyl group of DMBA plays an important role in radical cation formation and DNA binding. When the two methyl groups are substituted with two ethyl groups, the resulting 7,12-diethylbenz[*a*]anthracene was not carcinogenic when administered to Long-Evans rats by subcutaneous injection (Pataki & Balick, 1972), which is consistent with the absence of nucleophilic substitution at the benzylic methylene group of the radical cation of an ethyl-substituted PAH (Cavalieri & Roth; 1976; Tolbert *et al*., 1990). Furthermore, 7-methyl-12-ethylbenz[*a*]anthracene is a much weaker carcinogen than DMBA, whereas 7-ethyl-12-methylbenz[*a*]anthracene displays a carcinogenic activity similar to that of DMBA (Pataki & Balick, 1972). These data clearly suggest that the 12-methyl group plays a major role in the carcinogenic activity of DMBA.

Although less potent than DMBA, 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene is a strong carcinogen despite being fully saturated in the angular ring and, thus, unable to be activated by the diol epoxide pathway (DiGiovanni *et al*., 1982b). Electrochemical oxidation of 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene in the presence of deoxyguanosine or deoxyadenine yields numerous adducts that include those at the 12-methyl and 7-methyl groups that are similar to those obtained from DMBA (Mulder *et al*., 1996).

In conclusion, based on the several lines of evidence described above, DMBA initiates tumours by forming radical cations at the 12-methyl group that bind specifically to the *N*7 of adenine. Although adducts may also be formed via the diol epoxide mechanism, these adducts are expected to make at most only a minor contribution.

4.2.3 *Mechanism via formation of ortho-quinones and generation of reactive oxygen species*

(a) Description

PAHs with a terminal benzo-ring in a bay region are complete carcinogens in experimental animals (Cook *et al*., 1932, 1933; Bachmann *et al*., 1937). They are metabolically activated by CYP1A1/1B1 enzymes to form arene oxides of defined stereochemistry, which are hydrated by epoxide hydratase to form non-K region *R,R-trans*-dihydrodiols (Gelboin, 1980; Shimada *et al*., 1996). These *trans*-dihydrodiols are proximate carcinogens that undergo further mono-oxygenation by CYP1A1/1B1 to form

predominantly bay-region *anti*-diol epoxides as described in Section 4.2.1. The formation of *trans*-dihydrodiols represents a branch point in PAH metabolism (Figure 4.5).

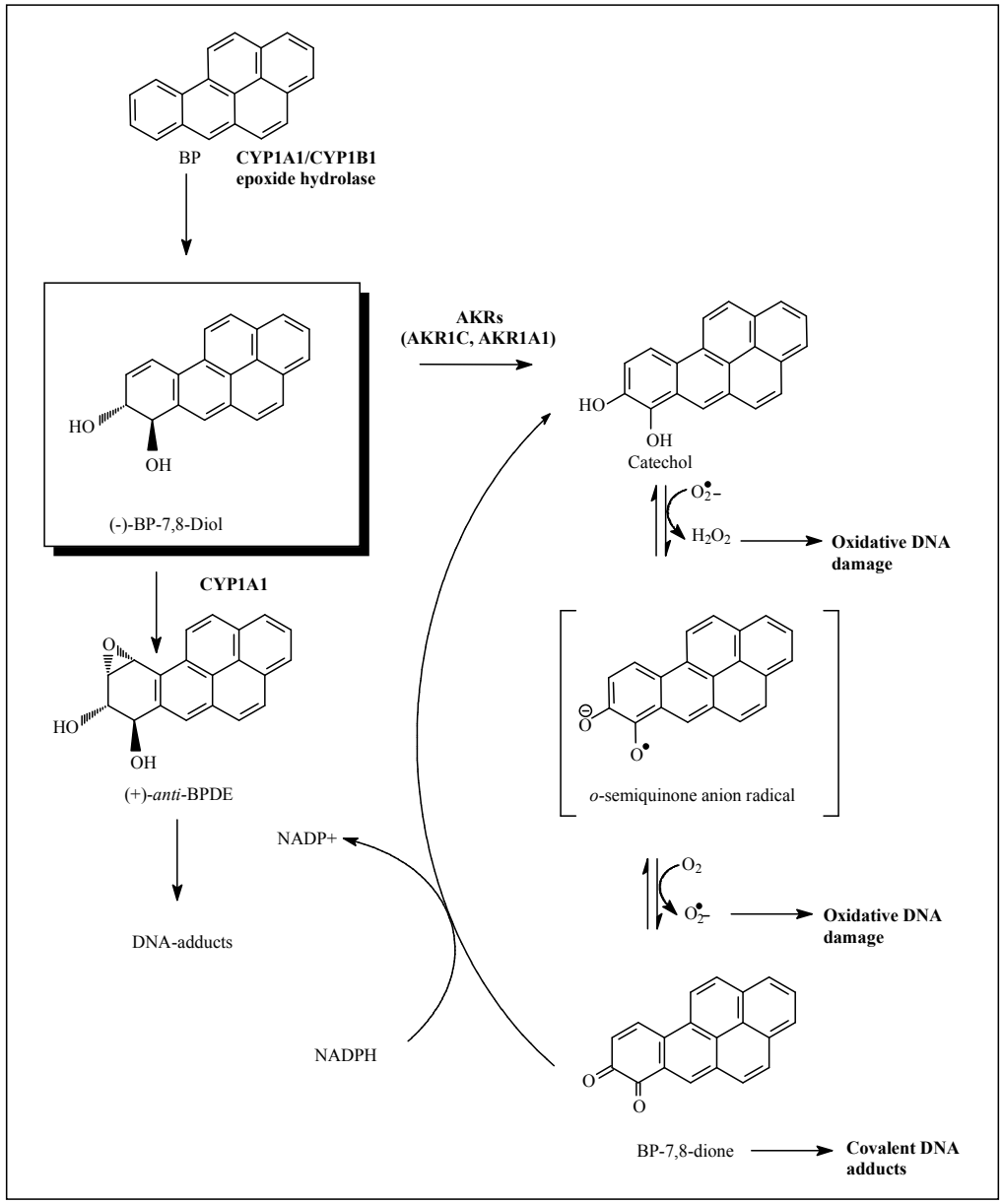
Non-K region *trans*-dihydrodiols also undergo a $NAD(P)^+$-dependent dehydrogenation that is catalysed by monomeric cytosolic oxidoreductases of the AKR superfamily to yield ketols, which spontaneously rearrange to yield catechols. Catechols are extremely air-sensitive and undergo two sequential one-electron auto-oxidation steps to yield the corresponding reactive PAH *ortho*-quinones (Smithgall *et al*., 1986, 1988a). 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). 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 (see Figure 4.5) (Penning *et al*., 1996).

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). PAH *ortho*-quinones can also be reduced back to the catechol, either non-enzymatically by the addition of $2H^+ + 2e^-$ from cellular reducing species (e.g. NADPH) or in two sequential one-electron steps catalysed by NADPH:CYP reductases (Flowers-Geary *et al*., 1992, 1995). Once re-formed, the catechol can undergo further auto-oxidation to create a futile redox-cycle in which each round of auto-oxidation forms reactive oxygen species which continues until the reducing agent is exhausted. This leads to oxidative stress and a pro-oxidant state. 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 (see below). In this manner, the pathway may contribute to the complete carcinogenicity of the parent PAH. An evaluation of this mechanism of PAH carcinogenesis requires an understanding of the human enzymes involved and the genotoxic and non-genotoxic properties of PAH *ortho*-quinones.

(i) *Aldo-keto reductase isoforms*

In humans, five AKR isoforms catalyse the oxidation of non-K region *trans*-dihydrodiols to *ortho*-quinones; these are AKR1C1, AKR1C2, AKR1C3, AKR1C4 and AKR1A1 (Burczynski *et al*., 1998, 1999a,b; Palackal *et al*., 2001a,b, 2002a,b). AKR1C1 isoforms are induced by bi- and monofunctional inducers, e.g. 3-methylcholanthrene and *tert*-butylhydroquinone, respectively, which is consistent with regulation via an anti-oxidant-response element (Burczynski *et al*., 1999a,b). These enzymes have a substrate preference for non-K region *trans*-dihydrodiols of bay-region methylated PAHs, oxidize both the *R,R* and *S,S* stereoisomers and are overexpressed in lung adenocarcinoma (A549) cells (Palackal et al., 2002a,b). Two independent studies have validated the elevated

Figure 4.5. Formation of DNA-reactive metabolites of benzo[*a*]pyrene via formation of 7,8-dihydrodiol and (a) diol epoxide or (b) catechol and *ortho*-quinone



Modified from Penning 2004
AKR, aldo-keto reductase; BP, benzo[a]pyrene; CYP, cytochrome P450; DE, diol epoxide; NAD(P)+/NAD(P)H, nicotinamide adenine dinucleotide (phosphate)

expression of AKR1C1 in lung cancer tissue of patients with non-small-cell lung carcinoma where it was an indicator of poor prognosis (Hsu *et al*., 2001; Fukumoto *et al*., 2005). In contrast, AKR1A1 is ubiquitously and endogenously expressed since it is a major metabolic enzyme; it is stereospecific for non-K region *R,R-trans*-dihydrodiols (Palackal *et al*., 2001a,b).

(ii) *Quinone-mediated DNA lesions and mutation*

PAH *ortho*-quinones produced by AKRs can form stable adducts, e.g., benzo[*a*]-pyrene-7,8-dione–N^2-deoxyguanosine (Shou *et al*., 1993; Balu *et al*., 2004), and unstable adducts, e.g., benzo[*a*]pyrene-7,8-dione–*N*7-guanine (McCoull *et al*., 1999). In addition, the reactive oxygen species generated by PAH *ortho*-quinones can attack DNA bases to produce oxidized purines (8-oxo-7,8-dihydro-2′–deoxyguanosine; 8-oxo-dGuo) (Park, J.-H. *et al*., 2005). Further oxidation can take place to yield highly mutagenic spiroiminodihydantoin or guanidinohydantoin adducts (Luo *et al*., 2001; Henderson *et al*., 2003). Reactive oxygen species can also abstract the C4-hydrogen from deoxyribose to form base propenals, which can lead to formation of a malondialdehyde–deoxyguanosine adduct. Alternatively, they can attack polyunsaturated fatty acids to yield lipid hydroperoxides that decompose to 4-hydroperoxy-2-nonenal, 4-hydroxy-2-nonenal and 4-oxo-2-nonenal, each of which can react with bases in DNA to yield potentially mutagenic lesions (Burcham, 1998; Pollack *et al*., 2003). However, most of the lesions derived from reactive oxygen species are generally not detected by the traditional methods used to analyse covalent PAH–DNA adducts.

In the initiation phase of PAH-induced carcinogenesis, critical proto-oncogenes, e.g., K-*ras*, and tumour-suppressor genes, e.g., *p53*, are mutated to undergo a change in function. One of the most prominent mutations observed are G→T transversions which occur at so-called hotspots, e.g. the K-*ras* codons 12 and 61 and the *p53* codons 157, 248 and 273 (Rodenhuis, 1992; Hainaut & Pfeifer, 2001). Two of the DNA lesions produced by PAH *ortho*-quinones provide straightforward routes to these G→T transversions. First, benzo[*a*]pyrene-7,8-dione–*N*7-guanine adducts are unstable and lead to formation of abasic sites which, if unrepaired, result in the incorporation of A opposite the lesion and, upon replication of the daughter strand lead to, a G→T transversion (Sagher & Strauss, 1983). Second, 8-oxo-dGuo, if unrepaired, undergoes a base-pair mismatch with A and, upon replication of the daughter strand, a G→T transversion would again occur (Breen & Murphy, 1995). Under redox-cycling conditions, individual PAH *ortho*-quinones produce substantial amounts of 8-oxo-dGuo (Park, J.-H. *et al*., 2005) and introduce G→T transversions in the *p53* gene, which results in a loss of transcriptional competency (Yu *et al*., 2002a,b). The pattern of G→T transversions is similar to that seen in patients with lung cancer. The formation of these mutations can be prevented by scavengers of reactive oxygen species, which suggests that 8-oxo-dGuo is the responsible lesion (Yu *et al*., 2002a,b). It is noteworthy that 100% of small-cell lung cancers and 50% of non-small-cell lung cancers have lost one allele of the gene *hOOG1*, which encodes the key *N*-glycosylase/AP lyase in the base-excision repair pathway responsible for the removal of

8-oxo-dGuo (Lu *et al*., 1997). Thus deficient base-excision repair of oxidative lesions is a risk factor in human lung cancer.

For a complete and more detailed review of the mechanism involved in the formation of PAH *ortho*-quinones and reactive oxygen species the reader is referred to Penning *et al*. (1999).

(b) Key relevant data

(i) *Benz[*a*]anthracene*

Enzymatic data

Homogeneous rat liver dihydrodiol dehydrogenase (AKR1C9) and AKR1C1–AKR1C4 oxidize both the (–)-3*R*,4*R*- and (+)-3*S*,4*S*-entantiomers of benz[*a*]anthracene-3,4-dihydrodiol to yield benz[*a*]anthracene-3,4-dione (Smithgall *et al*., 1988a; Palackal *et al*., 2002a). AKR1A1 was found to be stereospecific and to oxidize only the relevant (–)-3*R*,4*R*-benz[*a*]anthracene-3,4-diol to yield benz[*a*]anthracene-3,4-dione (Palackal *et al*., 2001a).

Cell-based data

No studies have been performed to show that benz[*a*]anthracene-3,4-dione is a cellular metabolite. However, the formation of DMBA-3,4-dione has been demonstrated by liquid chromatography/mass spectrometry (LC/MS) in cell lysates from human lung A549 adenocarcinoma cells, which constitutively overexpress AKR1C enzymes. In this instance, DMBA-3,4-dione was trapped as both a mono-thioether (1,6-addition product) and a bis-thioether conjugate (Palackal *et al*., 2002a).

Chemical reactivity

Benz[*a*]anthracene-3,4-dione is highly reactive with cellular thiols including GSH, L-cysteine and *N*-acetyl-L-cysteine, with a bi-molecular rate constant for GSH of 1.8×10^4 min^{-1} M^{-1} (Murty & Penning, 1992a). Reactions with deoxyribonucleosides have not been investigated.

Cytotoxicity

Benz[*a*]anthracene-3,4-dione (20 μM) had no effect on cell viability but reduced cell survival (>50%) of H4-II-e rat hepatoma cells following a single treatment for up to 4 h (Flowers-Geary *et al*., 1996).

Mutagenicity (see Table 4.2)

Benz[*a*]anthracene-3,4-dione was not mutagenic in the absence of redox-cycling in the Ames test using *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA104 (Flowers-Geary *et al*., 1996). In the presence of rat liver metabolic activation and an NADPH-generating system, no mutagenic effect was found either, perhaps due to sequestration of the quinone by protein. Benz[*a*]anthracene-3,4-dione was a poor direct-acting mutagen in a yeast reporter-gene assay that detects p53 mutations. In the same assay,

Table 4.2. Genetic and related effects of polycyclic aromatic hydrocarbon *ortho*-quinones

Test system	Result[a]		Dose[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Benz[*a*]anthracene-3,4-dione				
Salmonella typhimurium TA97a, TA98, TA100, TA102	–	–	–[1]	Flowers-Geary *et al.* (1996)
Saccharomyces cerevisiae, *p53* reporter gene assay	–	+[2]	31 nM	Shen *et al.* (2006)
8-oxo-dGuo formation *in vitro*	–	+[3]	160 nM	Park, J.H. *et al.* (2005)
Benzo[*a*]pyrene-7,8-dione				
Salmonella typhimurium TA97a, TA98, TA100	+	–	70 nmol/plate	Flowers-Geary *et al.* (1996)
Salmonella typhimurium TA102, TA104	+	–	35 nmol/plate	Flowers-Geary *et al.* (1996)
Saccharomyces cerevisiae, *p53* reporter gene assay	–	+[2]	125 nM	Shen *et al.* (2006)
8-oxo-dGuo formation *in vitro*	–	+[3]	160 nM	Park, J.H. *et al.* (2005)
DNA single-strand breaks (plasmid)	–	+[4]	10 μM	Flowers *et al.* (1997)
Gene mutation, A549 cells, *p53* gene, *in vitro*	+	NT	10 μM	Penning *et al.* (2004)
5-Methylchrysene-1,2-dione				
Salmonella typhimurium TA97a	+	–	70 nmol/plate	Flowers-Geary *et al.* (1996)
Salmonella typhimurium TA100	+	–	35 nmol/plate	Flowers-Geary *et al.* (1996)

[a] +, positive; –, negative; NT, not tested
[b] the lowest effective dose (LED) is given
[1] None of the tester strains was responsive (dose range, 0–100 μM)
[2] 1 nM NADPH plus 100 μM $CuCl_2$
[3] 180 μM NADPH plus 10 μM $CuCl_2$
[4] 1 mM NADPH plus 10 μM $CuCl_2$

it was highly mutagenic under redox-cycling conditions in the presence of copper chloride and NADPH: single point mutations were observed, and the mutation pattern showed a high preference (>42%) for the formation of G→T transversions (Field *et al*., 2005; Shen *et al*., 2006).

(ii) *Benzo[a]pyrene*

Enzymatic data

Purified dihydrodiol dehydrogenase of rat liver oxidizes both the (–)-7*R*,8*R*- and (+)-7*S*,8*S*-entantiomers of the benzo[*a*]pyrene-7,8-*trans*-dihydrodiol to yield benzo[*a*]pyrene-7,8-dione (Smithgall *et al*., 1986, 1988b). Benzo[*a*]pyrene-7,8-dione was trapped as a thioether conjugate with 2-mercaptoethanol and the product was identified by electron-impact mass spectrometry and nuclear magnetic resonance (NMR) spectrometry (Smithgall *et al*., 1988b). During this oxidation of benzo[*a*]pyrene-7,8-diol, consumption of molecular oxygen was observed and the formation of a superoxide anion radical was quantified wiht the spin-trapping agent, 5,5-dimethyl-1-pyrroline-*N*-oxide, which provided evidence for the generation of reactive oxygen species (Penning *et al*., 1996). Human recombinant AKR1C1–AKR1C4 each oxidized both stereoisomers of (±)-benzo[*a*]pyrene-7,8-diol in the following rank order AKR1C2 >AKR1C1 >AKR1C4 >AKR1C3. Each AKR1C isoform consumed the entire racemic benzo[*a*]pyrene-diol mixture, which indicated that both the minor (+)-*S,S*- and major (–)-*R,R*-stereoisomers formed *in vivo* are substrates for these enzymes. The quinone products of the reactions were trapped as either glycine or thioether conjugates, which were identified by co-elution with authentic synthetic standards (Burczynski *et al*., 1998, 1999a). AKR1A1 was found to be stereospecific and to oxidize only the metabolically relevant (–)-7*R*,8*R*-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to benzo[*a*]pyrene-7,8-dione, which was identified by use of LC/MS (Palackal *et al*., 2001b).

Cell-based data

Formation of benzo[*a*]pyrene-7,8-dione has been observed in rat hepatocytes (Flowers-Geary *et al*., 1995), in MCF-7 human breast carcinoma cells stably transfected with AKR1C9 (Tsuruda *et al*., 2001) and in human bronchoalveolar H-358 cells stably transfected with AKR1A1 (Jiang *et al*., 2005b). Formation of benzo[*a*]pyrene-7,8-dione and reactive oxygen species in rat hepatocytes was blocked by the AKR1C9 inhibitor, indomethacin, which showed that their formation was AKR1C9-dependent (Flowers-Geary *et al*., 1995).

Chemical reactivity

Benzo[*a*]pyrene-7,8-dione is highly reactive with cellular thiols including GSH, L-cysteine and *N*-acetyl-L-cysteine. For GSH, the bi-molecular rate constant is 1.3×10^3 min^{-1} M^{-1} (Murty & Penning, 1992a). The thioether conjugates of benzo[*a*]pyrene-7,8-dione have been characterized by two-dimensional NMR as the 1,4-Michael addition

products (Murty & Penning, 1992b). Reactions with deoxyribonucleosides are described below.

Cytotoxicity

Benzo[*a*]pyrene-7,8-dione (20 μM) reduced cell viability (by up to 40% after 4 h), had no effect on cell survival and depleted reduced GSH in H4-II-e rat and Hep-G2 human hepatoma cells (Flowers-Geary *et al*., 1993, 1996).

Genotoxicity

Benzo[*a*]pyrene-7,8-dione forms stable deoxyguanine and deoxyadenine adducts in reactions with deoxyribonucleosides and in calf-thymus DNA. Reactions of [^{3}H]benzo[*a*]pyrene-7,8-dione with calf thymus DNA or plasmid DNA followed by digestion led to the isolation of a single nucleoside adduct that co-eluted with a standard that was synthesized by reaction of benzo[*a*]pyrene-7,8-dione with oligo-deoxyguanosine. No adducts were observed upon reaction with oligomers of deoxythymidine, deoxycytidine or deoxyadenosine (Shou *et al.*, 1993). Benzo[*a*]pyrene-7,8-dione–deoxyguanosine and –deoxyadenosine adducts were also obtained by reaction with deoxyribonucleosides. Analysis of the benzo[*a*]pyrene-7,8-dione–N^2-deoxyguanosine adducts shows that they exist as either hydrated 1,4-Michael addition products or as cyclized hydrated 1,6-Michael addition products. The structure of the deoxyadenosine adduct provides evidence for a hydrated cyclized 1,4-Michael addition product involving the *N*1 position of adenine. A number of different stereoisomers of each adduct are possible (Balu *et al.*, 2004).

Upon incubation with deoxyguanosine under acidic conditions, benzo[*a*]pyrene-7,8-dione also forms the unstable adduct benzo[*a*]pyrene-7,8-dione-*N*7–guanine, which has been characterized by LC/MS (McCoull *et al.*, 1999). Under redox-cycling conditions in the presence of copper chloride and NADPH, nanomolar concentrations of benzo[*a*]-pyrene-7,8-dione can produce significant amounts of 8-oxo-dGuo (>60 adducts/10^5dGuo, a 30-fold increase over background) as measured by HPLC with electrochemical detection (ECD). In this case, the oxidant is singlet oxygen (Park, J.-H. *et al*., 2005). Under the same redox-cycling conditions, low micromolar concentrations of benzo[*a*]-pyrene-7,8-dione caused strand scission of plasmid-DNA and oligonucleotides; the damaging species was the hydroxyl radical. The corresponding *ortho*-semiquinone did not cause strand scission (Flowers *et al.*, 1997).

Mutagenicity (see Table 4.2)

Benzo[*a*]pyrene-7,8-dione was found to be a direct-acting mutagen in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA104 at concentrations of 35 and 70 nmol/plate, and was more mutagenic than the positive control used for each tester strain. The majority of the mutations observed were frameshifts. No increase in mutation efficiency was noted in the presence of a metabolic activation system containing rat liver microsomes and an NADPH-generating system when tester strains sensitive to oxidative mutagens (TA102 and TA194) were used (Flowers-Geary *et al*., 1996).

Benzo[*a*]pyrene-7,8-dione was found to be a poor direct-acting mutagen in a yeast reporter-gene assay that can detect *p53* mutations. In the same assay, benzo[*a*]pyrene-7,8-dione was found to be highly mutagenic at concentrations of 125 nM under redox-cycling conditions (copper chloride and NADPH). In contrast, (±)-*anti*-benzo[*a*]pyrene diol epoxide was only mutagenic at concentrations greater than 10 μM. Benzo[*a*]pyrene-7,8-dione produced single point mutations in *p53* and the mutation pattern showed a high preference for G→T transversions, which occurred preferentially at the same hotspots that were mutated in patients with lung cancer. The mutations were not found when the assay was conducted in the presence of scavengers of reactive oxygen species and the enzymes superoxide dismutase and catalase, which suggested that the superoxide anion and the hydroxyl radical were the responsible mutagens (Yu *et al*., 2002b). In human lung adenocarcinoma A549 cells, benzo[*a*]pyrene-7,8-dione was found to mutate *p53*, which led to predominately unstable A→G (T→C) transitions following acute treatment (Penning *et. al*., 2004).

(iii) *5-Methylchrysene*

Enzymatic data

5-Methylchrysene can be metabolically activated in mouse skin to yield 1,2-*trans*-dihydroxy-1,2-dihydro-5-methylchrysene and 7,8-*trans*-dihydroxy-7,8-dihydro-5-methylchrysene and their respective 3,4- and 9,10-epoxides (Melikian *et al*., 1982; Hecht *et. al*., 1985). The former is the more relevant metabolite. With regard to stereospecificity, it is probable that rat liver dihydrodiol dehydrogenase oxidizes (+)-*1S,2S*-dihydroxy-1,2-dihydro-5-methylchrysene and (–)-*7R,8R-trans*-dihydroxy-7,8-dihydro-5-methylchrysene to yield the presumptive quinone (Smithgall *et al*., 1986). Each of the human AKR1C enzymes (AKR1C1–AKR1C4) oxidizes the racemic 5-methylchrysene-7,8-*trans*-dihydrodiol but the stereochemistry of the reaction has not been elucidated. The reaction preference was AKR1C4 >AKR1C2 >AKR1C1 >AKR1C3 (Burczynski *et al*., 1999b; Palackal *et al*., 2002a,b). Human AKR1A1 also oxidizes racemic 5-methylchrysene-7,8-*trans*-dihydrodiol, which is the *trans*-dihydrodiol substrate with the highest V_{max}/K_m utilization ratio identified for this enzyme to date (Palackal *et al*., 2001a,b).

Cytoxicity

5-Methylchrysene-7,8-dione significantly reduced the survival of H4-II-e cells (Flowers-Geary *et al*., 1996).

Mutagenicity (see Table 4.2)

5-Methylchrysene-7,8-dione was found to be a direct-acting mutagen in *S. typhimurium* TA97a and TA100 at concentrations of 70 and 35 nmol/plate, respectively, and was more mutagenic than the positive control used for each tester strain (Flowers-Geary *et. al*., 1996). The mutagenicity of this compound has not been examined in other assays.

Data gaps

There are gaps in our knowledge concerning the possible role of the PAH *ortho*-quinone pathway and reactive oxygen species in carcinogenesis: (i) not all the relevant PAHs have been examined; (ii) human AKRs other than AKR1A1 and AKR1C1–AKR1C4 may be involved in the metabolic activation of PAH *trans*-dihydrodiols, e.g., AKR1B10, which is highly elevated in non-small-cell lung carcinomas (Fukumoto *et al*., 2005); (iii) there is little information to address the competing roles of CYP- versus AKR-mediated activation of PAHs; (iv) covalent DNA adducts (stable or unstable) or oxidative lesions that can be assigned to the AKR pathway have yet to be detected in cells or animals; the successful detection of these adducts requires the development of appropriate analytical chemical methods with sufficient sensitivity; (v) the mutagenicity of PAH *ortho*-quinones in mammalian cells has not been completely addressed; (vi) the transforming potential of the PAH *ortho*-quinones has not been measured; and (vii) the tumorigenicity of PAH *ortho*-quinones as initiators, promoters or both has not been examined systematically.

4.2.4 *Mechanism via meso-region biomethylation and benzylic oxidation*

The role of mechanisms that involve meso-region biomethylation and benzylic oxidation in the carcinogenesis of PAHs is based on the methylation of unsubstituted PAHs and the subsequent metabolic activation of the methyl group to electrophilic moieties. The meso-region of PAHs (also known as the L-region) has been purported to be a region of high reactivity either in an aromatic nucleus or on a side chain (Flesher *et al.*, 2002, 2004). According to this theory, the chemical and biochemical activation pathways of both unsubstituted and meso-substituted PAHs are essentially the same, since unsubstituted PAHs are converted to generally more carcinogenic meso-methyl-substituted PAHs in the metabolic activation process. For example, DMBA is more carcinogenic than 7-methylbenzo[*a*]anthracene which is more carcinogenic than benzo[*a*]anthracene (see review by Dipple *et al.*, 1984). In a series of three biochemical transformation reactions, the first is the aralkylation (methylation) of unsubstituted PAHs at a meso-centre of high reactivity (Myers & Flesher, 1991). This conversion is mediated by the methyl donor, *S*-adenosyl methionine (Flesher *et al.*, 1986). The second step is the hydroxylation of a meso-region methyl group by CYP isozymes (Sims, 1970); more recently, a chemical one-electron oxidation process has also been proposed at this stage (Flesher *et al.*, 2004; Lehner *et al.*, 2004). The third step is the formation of a reactive ester (e.g. sulfuric acid ester) via 3′-phosphoadenosine-5′-phosphosulfate (Chou *et al.*, 1998). Sulfooxymethyl esters generate a highly reactive benzylic carbonium ion that may form DNA adducts (Lehner *et al.*, 2004; Ravi Kumar *et al.*, 2005), and some have been found to be mutagenic (Watabe *et al.*, 1986) and carcinogenic (Surh *et al.*, 1991; Flesher *et al.*, 1997a,b).

*(a) Benzo[*a*]pyrene*

(i) *Biomethylation to 6-methylbenzo[a]pyrene*

Benzo[*a*]pyrene was converted to 6-methylbenzo[*a*]pyrene using rat liver microsomal preparations (Flesher *et al.*, 1990). *S*-Adenosylmethionine was identified as a carbon donor in this biotransformation (Flesher *et al.*, 1982).

(ii) *DNA adducts of benzo[*a*]pyrene-related methylated intermediates*

Weanling female Sprague-Dawley rats injected subcutaneously with benzo[*a*]pyrene produced two groups of adduct profile: one that resulted from alkyl substitution and the other from ring oxidation. One major and two minor aralkyl–DNA adducts were detected in subcutaneous tissues. The total levels of diol epoxide adducts were 15–50 times higher than those of aralkyl adducts. Rats injected with 6-methylbenzo[*a*]pyrene, 6-hydroxymethylbenzo[*a*]pyrene and 6-acetoxymethylbenzo[*a*]pyrene produced the same aralkyl–DNA adducts as those obtained from benzo[*a*]pyrene. The predominant in-vivo aralkyl–DNA adduct was a deoxyguanosine adduct while the second major adduct was a deoxyadenosine adduct (Stansbury *et al.*, 1994). 6-Hydroxymethylbenzo[*a*]pyrene is converted to 6-sulfooxymethylbenzo[*a*]pyrene by 3′-phosphoadenosine-5′-phosphosulfate sulfotransferase *in vitro* (Surh *et al.*, 1990).

(iii) *Genotoxicity of 6-methylbenzo[*a*]pyrene and related metabolites*

6-Methylbenzo[*a*]pyrene and 6-sulfooxymethylbenzo[*a*]pyrene were mutagenic to *S. typhimurium* TA100 (Santella *et al.*, 1982; Rogan *et al.*, 1986) and formed DNA adducts in the liver of infant mice (Surh *et al.*, 1990).

(iv) *Carcinogenicity of 6-methylbenzo[*a*]pyrene, 6-hydroxymethylbenzo[a]pyrene and 6-sulfooxymethylbenzo[a]pyrene*

The carcinogenic activities of 6-substituted benzo[*a*]pyrenes have been reviewed to some extent (Dipple *et al.*, 1984; Harvey, 1991). 6-Methylbenzo[*a*]pyrene and 6-hydroxymethylbenzo[*a*]pyrene were weak initiators of skintumours in CD1 mice, with five and eight fold lower activity, respectively, than that of benzo[*a*]pyrene (Slaga *et al.*, 1978c) and similar results were found for 6-methylbenzo[*a*]pyrene in SENCAR mice (Iyer *et al.*, 1980). Based on studies that involved inhibition of CYP, it was concluded that methylation and hydroxymethylation of benzo[*a*]pyrene were not important pathways in the initiation of mouse skin tumours (Slaga *et al.*, 1978c). However, 6-methyl- and 6-hydroxymethylbenzo[*a*]pyrene induced malignant skin carcinomas after repeated application on mouse skin; 6-methylbenzo[*a*]pyrene was the more active. The sodium salt of 6-sulfooxymethylbenzo[*a*]pyrene was also tumorigenic on mouse skin (Cavalieri *et al.*, 1978). 6-Methyl-, 6-hydroxymethyl- and 6-sulfooxymethylbenzo[*a*]pyrene induced sarcomas at the injection site when given subcutaneously to rats (Flesher *et al.*, 1997a),

and 6-sulfooxymethylbenzo[*a*]pyrene was also tumorigenic in the liver of infant mice (Surh *et al.*, 1990).

In conclusion, there is no direct evidence that this mechanism contributes to the tumorigenic activity of benzo[*a*]pyrene in mouse skin.

*(b) Benzo[*a*]anthracene*

(i) *Biomethylation to methylbenz[*a*]anthracenes*

Qualitative studies of the metabolism of benz[*a*]anthracene *in vitro* with rat liver cytosol preparations fortified with *S*-adenosyl-L-methionine showed the formation of 7-methylbenz[*a*]anthracene and 12-methylbenz[*a*]anthracene, which were further methylated to DMBA (Flesher *et al.*, 1984). Subcutaneous injection of benz[*a*]anthracene into rats confirmed the formation of 7-methylbenz[*a*]anthracene, 12-methylbenz[*a*]anthracene and DMBA as well as that of hydroxymethylated benz[*a*]anthracene metabolites. No bioalkylation was detected after injection of six non-carcinogenic PAHs. (Flesher & Myers, 1990).

(ii) *Benzylic oxidation and formation of reactive intermediates*

In in-vitro studies with rat liver cytosol, 7-methylbenz[*a*]anthracene, 12-methylbenz[*a*]anthracene and DMBA were metabolized to their hydroxymethyl metabolites. No oxidation was detected at the ring positions (Flesher & Myers, 1985). *In vivo*, DMBA was metabolized in rat subcutaneous tissues to the corresponding hydroxyalkyl metabolites, 7 hydroxymethylbenz[*a*]anthracene, 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 7,12-dihydroxymethylbenz[*a*]anthracene (Myers & Flesher, 1989). These hydroxymethylated benz[*a*]anthracenes were further conjugated to sulfate to form reactive and mutagenic sulfooxymethylated benz[*a*]anthracene species (Watabe *et al.*, 1985, 1986). Subcutaneous injection of either 7-hydroxymethyl-12-methylbenz[*a*]anthracene or 7-sulfooxymethyl-12-methylbenz[*a*]anthracene into rats produced adduct patterns in DNA of subcutaneous tissue that were similar to those observed after injection of DMBA, with a 2:1 ratio of DMBA diol epoxide–DNA adducts to benzylic adducts (Ravi Kumar *et al.*, 2005).

(iii) *Carcinogenicity of methylated benz[*a*]anthracene and its metabolites*

There is extensive literature on the carcinogenic activities of methylated benz[*a*]anthracenes. DMBA is carcinogenic and induces lung, skin and liver tumours in mice and tumours of the subcutaneous tissue and mammary gland in rats. 7-Methylbenz[*a*]anthracene and 12-methylbenz[*a*]anthracene are also carcinogenic in mice and rats (see Dipple *et al.*, 1984). 7-Hydroxymethyl-12-methylbenz[*a*]anthracene and 7-sulfooxymethyl-12-methylbenz[*a*]anthracene were tumorigenic when injected subcutaneously into rats (Flesher *et al.*, 1997b). However, it was considered improbable that DMBA would act via metabolic activation to 7-sulfooxymethyl-12-methylbenz[*a*]anthracene to induce hepatomas in male B6C3F_1 mice and lung adenomas in A/J mice,

to initiate mouse skin tumours, to induce injection-site sarcomas in rats or to initiate preneoplastic enzyme-altered foci in rat liver. In all these systems, the sulfate ester was not more carcinogenic than DMBA itself (Surh *et al.*, 1991).

In conclusion, in subcutaneous tissues of rats, benz[*a*]anthracene was biomethylated to DMBA which was metabolized to a series of hydroxymethylated and sulfooxymethylated methylbenz[*a*]anthracenes, some of which formed benzylic DNA adducts. However, for DMBA, there is disagreement in the literature on the role of this biomethylation mechanism in the formation of subcutaneous tumours in rats, and there is no evidence that this mechanism explains the tumorigenic activities of benzo[*a*]anthracene in mouse skin and lung.

*(c) Dibenz[*a,h*]anthracene*

Qualitatively, rat liver cytosol fortified with *S*-adenosyl-L-methionine transformed dibenz[*a*,*h*]anthracene to methylated and hydroxymethylated metabolites (Flesher *et al.*, 1986). No quantitative results were available. Both 7-methyl- and 7,14-dimethyldibenz[*a*,*h*]anthracene were carcinogenic (see review by Dipple *et al.*, 1984), while the biological activities of hydroxymethylated dibenz[*a*,*h*]anthracenes are unknown. There is no evidence of genotoxicity of these intermediates or of DNA adducts of methylated dibenz[*a*,*h*]anthracene in rodent or human tissues.

There are no adequate data to support a role for the biomethylation mechanism in the carcinogenicity of dibenz[*a*,*h*]anthracene.

(d) 5-Methylchrysene

5-Methylchrysene was converted *in vitro* by induced rat liver microsomal preparations and *in vivo* in rat dorsal subcutaneous tissues to the metabolites 5-hydroxymethylchrysene and 4,5-methylenechrysene (Myers & Flesher, 1991). 5-Hydroxymethylchrysene was a metabolite of 5-methylchrysene after incubation with induced rat liver and human liver microsomes. Further metabolism of 5-hydroxymethylchrysene by Aroclor 1254-induced rat-liver preparations produced the 1,2-diol and the 7,8-diol (Amin *et al.*, 1981). 5-Hydroxymethylchrysene, after activation to its sulfate conjugate, formed DNA adducts in calf thymus DNA (Okuda *et al*., 1986). 4,5-Methylenechrysene, a cyclization product of 5-methylchrysine, caused skin tumours in mice (Rice *et al.*, 1988).

5-Methylchrysene administered to strain A/J mice induced six different DNA adducts in the lung, none of which co-migrated with the adduct formed by sulfotransferase-mediated activation of 5-hydroxymethylchrysene (Ross *et al*., 1995).

(i) *Carcinogenicity study of 5-hydroxymethylchrysene*

Groups of Charles River CD-1(ICR)BR mice received 10 daily dermal applications of 5-hydroxymethylchrysene (total dose, 116 or 39 nmol) in acetone followed 10 days later by 4 nmol TPA in acetone three times a week for 20 weeks. At 21 weeks, the incidence of skin tumours (tumours/mouse) in the two dose groups was 90%

(9.5 tumours/mouse) and 45% (2.6), respectively. Skin tumours developed in 5% (0.1 tumours/mouse) of the mice treated with TPA alone (Amin *et al.*, 1981).

(ii) *Genotoxicity of 5-hydroxymethylchrysene*

Rat-liver hydroxysteroid sulfotransferase converted 5-hydroxymethylchrysene to its sulfate ester, 5-sulfooxymethylchrysene (Ogura *et al.*, 1990), which bound *in vitro* to purine bases of calf thymus DNA to form N^6-[(chrysen-5-yl)methyl]adenosine and N^2-[(chrysen-5-yl)methyl]guanosine (Okuda *et al.*, 1989). This 5-hydroxymethylchrysene sulfate is a strong bacterial mutagen in the presence of a 3′-phosphoadenosine 5′-phosphosulfate-generating system (Okuda *et al*, 1986)

In conclusion, 5-methylchrysene was metabolized in mouse skin to 5-hydroxymethylchrysene which acted as a skin carcinogen. *In vitro*, 5-hydroxymethylchrysene was conjugated to sulfate and this conjugate formed DNA adducts. However, 5-hydroxymethylchrysene–DNA adducts have not been detected after treatment of mouse skin with 5-methylchrysene.

4.2.5 *Receptor-mediated mechanism*

Several of the biological effects of PAHs, such as induction of xenobiotic metabolizing enzymes, immunosuppression, teratogenicity and carcinogenicity, are thought to be mediated by activating AhR signalling. This receptor is widely distributed and has been detected in most cells and tissues. 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. In addition, high-affinity ligands for AhR such as benzo[*a*]pyrene and 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) differ in their biological responses. Furthermore, translocation of activated AhR may require threshold concentrations of the ligand and involves a variety of cellular responses. AhR induces phase I and II metabolism; additional responses include lipid peroxidation and the production of arachidonic acid-reactive metabolites, decreased levels of serum thyroxine and vitamin A, persistent activation of thyroid hormone receptor and cross-talk with steroid hormone receptors. Responses to altered AhR signalling may therefore be designated as adaptive or toxic and/or as perturbations of endogenous pathways. Some basic information on AhR-mediated mechanisms in relation to biochemical and toxicological effects is discussed below.

(a) Basic principles of AhR

AhR is a ligand-activated transcription factor that mediates responses to a variety of toxins, including halogenated aromatic toxins such as TCDD, PAHs and combustion products and numerous phytochemicals such as flavonids and indole-3-carbinol (Nebert *et al.*, 2004 ; Chang *et al.*, 2005). 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 the initiation of homeostatic responses (also termed AhR signalling in endogenous pathways) upon exposure to xenobiotics.

Despite many attempts to identify AhR endogenous ligands, the nature of these ligands remains enigmatic. However, Adachi *et al.* (2001) identified the tryptophan analogues, indirubin and indigo in human urine, and these activate AhR.

The greatest source of AhR ligands is food. AhR ligands may activate signalling pathways in a manner similar to that of growth factors, hormones, neurotransmitters and extracellular signals and can therefore act like classical mitogenic factors. Little information is available on receptor binding and AhR activity of individual PAHs. In the unligated state, AhR is complexed with chaperone proteins, i.e. the heat-shock protein 90 dimer. Upon ligand binding, the chaperones dissociate, which enables AhR to translocate into the nucleus (Schmidt & Bradfield, 1996). Therefore, AhR is regulated primarily through receptor occupancy and through de-novo synthesis of the AhR repressor (AhRR), a protein which was first identified in mice (Mimura *et al.*, 1999). AhRR is closely related to AhR in the primary sequence of the basic helix-loop-helix (bHLH) and Period (Per)-AhR nuclear translocator (Arnt)-Single-minded protein (Sim) (PAS)-A domain (N-terminal half of the PAS domain) but is highly divergent from AhR in the PAS-B domain (ligand binding domain). AhRR proteins typically do not bind AhR ligands and inhibit AhR signalling by competing for binding to xenobiotic or dioxin response element (XRE/DRE) recognition sites usually occupied by AhR-Arnt (Mimura *et al.*, 1999).

In addition, PKC is of critical importance for AhR activity. Inhibiton of PKC by staurosporine prevented ligand-induced DNA binding to XRE/DRE recognition sites of targeted genes (Delescluse *et al.*, 2000). Furthermore, phosphorylation of AhR and Arnt threonine residues has been reported, and threonine kinases probably modify the activity of AhR (Puga *et al.*, 2002). Notably, Vaziri *et al.* (1996) demonstrated the induction of AhR expression by the tyrosine kinase v-src (a viral oncoprotein) and by the platelet-derived growth factor receptor basic fibroblast growth factor.

Early observations suggest that the role of AhR was confined to adaptive responses to exposure to PAHs that mediated toxicity upon activation by halogenated dioxins, polychlorinated biphenyls (PCBs) and other halogenated aromatic hydrocarbons. More recently, however, AhR was shown to play a wider role in cell-cycle control (Elferink, 2003) and seems to act as an environmental check-point that senses exposure to environmental toxicants. AhR may inhibit the cell cycle by displacing p300 from early region 2 binding factor-dependent promoters and repressing S phase-specific gene expression (Marlowe *et al.*, 2004). Furthermore, AhR interacts with the retinoblastoma suppressor protein (pRb) by the control of $p27^{kip1}$ expression which in turn inhibits cell-cycle progression by the repression of CDK2 activity which prevents pRb hyperphosphorylation. This enables cell-cycle arrest in the G1 phase (Marlowe et *al.*, 2004).

(b) Control of gene expression by AhR

The nuclear AhR complex consists of a heterodimer of AhR and Arnt. Unligated AhR is almost exclusively located in the cytoplasm whereas Arnt is found in the nucleus. AhR can still translocate to the nucleus in the absence of Arnt as shown in Arnt-deficient mice. Both AhR and Arnt are member of the bHLH-PAS family of transcription factor proteins which have essential roles in development (differentiation, neurogenesis, myogeneisis, B-cell differentiation, sex determination) and in various signalling pathways. The PAS domain is required for binding to DNA and the bHLH motif is localized in the N-terminal region where it codes for two α helices that are separated by a non-helical loop. AhR and Arnt proteins were shown to contact the DRE or XRE sequence of regulated genes. The thymine position within a half-site of an E-box element (5′-GTG-3′) is specifically contacted by Arnt whereas AhR contacts a thymine outside of the E-box element (Bacsi *et al.*, 1995). It is of considerable importance that E-box elements are recognized by many bHLH transcription factors. Thereafter, binding to the DRE or XRE leads to activation of a large number of genes, including cell-cycle genes, and xenobiotic metabolizing enzymes. Furthermore, in-depth studies over the past years have revealed many details of the mechanism of action of the AhR transcription factor and the activation of promotors of targeted genes.

Transcriptional activation of targeted genes in response to AhR are not only species- and tissue-specific, but are also ligand-specific. This is of importance since AhR activation by certain PAHs did not produce the same spectrum of toxic effects as those observed with TCDD. AhR-mediated toxic responses may therefore be ligand-dependent and differences in biological responses may also be linked to the resistance of halogenated hydrocarbons to metabolism and may thereby increase AhR occupancy. Furthermore, there is evidence that the ligand-dependent recruitment of additional transcription factors upon activation of AhR results in combinatorial interactions of networked transcription factors with consequent alterations in signalling that perturb several metabolic pathways. Recently, a genetic algorithm was developed to study promoters of AhR-regulated genes. This method enabled the identification of transcription factor-binding sites of AhR-regulated genes (Kel *et al.*, 2004). Promoters and long-distance regulatory regions of AhR-responsive genes were analysed by the genetic algorithm and this computational approach was applied to currently unknown AhR-regulated genes. In essence, the method was able to predict in silico novel gene candidates, which were confirmed experimentally using cultures of human and rat hepatocytes. Mathematical models are therefore promising for the prediction of novel gene targets of AhR by interrogating promoter and regulatory sequences for further consideration in risk assessment. This includes, among others, the identification of tumour-suppressor and proto-oncogenes regulated by activated AhR.

(c) Genetic models to investigate AhR signalling

Genetic studies and the application of gene targeting technologies provided valuable insight into AhR signalling pathways. Studies with AhR knockout mice provided clear evidence for the need of a functional receptor to obtain a xenobiotic response after exposure to halogenated aromatic hydrocarbons, PAHs and related chemicals (Fernandez-Salguero *et al.*, 1995). Specifically, AhR is required to induce toxicity or carcinogenicity after exposure to TCDD or benzo[*a*]pyrene, respectively (Fernandez-Salguero *et al.*, 1995; Mimura *et al.*, 1997; Peters *et al.*, 1999; Shimizu *et al.*, 2000). Furthermore, Andersson *et al.* (2002) showed that a constitutively active AhR reduced the lifespan of transgenic mice and induced tumours in the stomach. This further demonstrates the oncogenic potential of AhR.

The various AhR knockout strains, however, differ in phenotypes as a result of targeting mutations either in the first exon by deletion of the first methionine and a portion of the basic region (Fernandez-Salguero *et al.*, 1995) or by deletion of exon 2 which encodes bHLH–DNA binding and the dimerization domain (Schmidt *et al.*, 1996). Targeting exon 2 resulted in a frameshift with no synthesis of AhR protein. However, deletion of exon 1 resulted in 50% neonatal mortality and the inflammation of several major organs. Furthermore, surviving mice had decreased liver weights and portal fibrosis similar to that seen in mice with the exon 2 deleted, although the phenotypes appeared to be more severe upon targeting of exon 1. In addition, reduced liver size in AhR-null mice was associated with the incidence of apoptosis by a currently unknown mechanism (Zaher *et al.*, 1998).

(d) Cross-talk of AhR with steroid and other nuclear receptors

AhR plays a much broader role than that initially assumed and there is evidence for inhibitory and additive cross-talk of AhR with other nuclear receptors (Pocar *et al.*, 2005).

TCDD induces hepatocellular carcinogenesis in female but not male Sprague-Dawley rats and the tumorigenic response in females has been reported to be estrogen-dependent (Safe, 2001). Cross-talk of AhR with the steroid receptor has been studied in human breast cancer and endometrial carcinoma cell lines. Inhibitory XRE/DRE were identified in the promotors of the estrogen-inducible *pS2* breast cancer coding genes, in cathepsin D and c-*fos* genes (Safe *et al.*, 2000). AhR–ARNT complexes competitively inhibited the binding of estrogen receptor (ER)-α to imperfect ER element sites. In T47D human breast cancer cells, a rapid proteasome-mediated degradation of AhR and ERα by activated AhR was observed, but TCDD elicited stronger effects than benzo[*a*]pyrene or 6-methyl-1,3,8-trichlorodibenzofuran (Wormke *et al.*, 2000). In MCF-7 cells, the direct interaction of activated AhR with the hypophosphorylated tumour suppressor *RB* was independent of Arnt and did not require an intact AhR–ARNT complex to contact XRE/DRE in order to mediate transcriptional activity (Carlson & Perdew, 2002). In contrast, a strong association of ARNT splice variants with ER-negative breast cancer and poor prognosis was reported. A truncated ARNT may affect AhR signalling and receptor cross-talk (Qin

et al., 2001). AhR–ER cross-talk affects multiple estrogen-dependent pathways with the induction of CYP monooxygenases by activated AhR that results in rapid metabolism and depletion of 17β-estradiol *in vitro*; however, this was not seen *in vivo* (Safe, 2001).

Comparisons of AhR-sensitive and AhR knockout mice revealed thymic athrophy in AhR-sensitive strains as a result of T-cell apopotosis (Fernandez-Salguero *et al.*, 1995; Kamath *et al.*, 1997). Several AhR ligands, including DMBA, benzo[*a*]pyrene and TCDD, have been reported to induce apoptosis in various cell types of non-reproductive tissues (Miller *et al.*, 1996; Lei *et al.*, 1998; Jyonouchi *et al.*, 1999). In human folicular granulosa cells, the AhR ligand TCDD also induced apoptosis in a dose- and time-dependent manner by disturbing steroid metabolism (Heimler *et al.*, 1998). Matikainen *et al.* (2001) studied AhR-dependent activation of the pro-apoptotic *bax* encoding gene. In murine oocytes, DMBA but not TCDD induced bax protein expression and subsequent apopotosis. When, however, guanine or cytosine was exchanged for adenine three bases downstream of the core XRE/DRE recognition site, TCDD also induced the bax protein. Therefore, ligand-dependent and single base-pair discrimination of flanking AhR recognition sites in promotors of targeted genes may be an important mechanism to rationalize selective responses in the control of gene expression. Furthermore, in-utero exposure to the AhR ligand TCDD induced cleft clitoris and vaginal threads of mesenchymal tissue in female rat offspring, which suggested an imbalance of proliferation and apoptosis in the development of female sexual organs. AhR may act as a novel regulator of ovulation, and the ovulatory gonadotropin surge has been shown to induce expression of AhR-regulated genes (Chaffin *et al.*, 1999). In summary, AhR appears to play a prominent role in female reproduction, and complex interactions occur between AhR–Arnt and sex steroid receptors.

Furthermore, pleiotropic responses to AhR signalling are probably tissue- and may be ligand-dependent and induce transcriptional activation of genes, the expression of which is normally restricted or even prevented. Selective and de-novo activation of additional genes, as well as repression of constitutively expressed genes, impacts cellular phenotype. There is clear evidence for tissue-specific regulation of certain genes with the induction for instance of transforming growth factor and plasminogen activator inhibitor-2 in human keratinocytes but not in rat hepatocytes (Vanden Heuvel *et al.*, 1994). Cell type-specific responses may also depend on the ability to recruit co-activator proteins selectively. Taken collectively, this may provide a molecular mechanism for tissue specificity and sensitivity to responses to AhR activation.

(e) Ligand-independent AhR regulation

Other regulatory mechanisms of AhR signalling may be active during inflammation when exposure to cytokines is increased. Indeed, various interleukins (iL) and interferons can provoke the expression of NF-κB, signal transducers and activators of transcription as well as CCAAT enhancer-binding proteins. Networking of AhR with these additional transcription factors may alter its signalling pathways. For instance, AhR and NF-κB interact physically and modulate each other transcriptionally (Tian *et al.*, 1999, 2002).

Divergent signalling of AhR may also be operable through activation by cyclic adenosine monophosphate in the absence of an AhR ligand (Oesch-Bartlomowicz *et al.*, 2005). Regulation of xenobiotic metabolizing enzymes by nuclear receptors other than AhR has been studied extensively. The pregnane X receptor, the retinoid x-receptor and the constitutive androstane receptor have been identified and play pivotal roles in induction of *CYP* monooxygenase gene families 2 and 3 (Xu *et al.*, 2005). Other nuclear receptors involved in the regulation of xenobiotic metabolizing CYP1 forms include the glucocorticoid receptor and ER, both of which potentiate the induction of CYP1 (Honkakoski & Negishi, 2000).

The orphan nuclear receptor hepatic nuclear factor 4 is of critical importance in regulating the expression of CYP monooxygenase families 2 and 3. Cross-talk of AhR with these nuclear receptors remains uncertain and the importance of genetic polymorphism in xenobiotic metabolizing enzymes has been adressed elsewhere (Section 4.1).

(f) Regulation of xenobiotic metabolizing enzymes by antioxidant/electrophile response elements

An electrophile response element has been identified in the promotors of some AhR-regulated genes and provides an AhR-independent means to control gene expression. Examples include CYP1A1 and other members of the AhR gene battery. Specifically, for phase II metabolizing enzymes, a number of inducers have been identified such as butylated hydroxyanisole, *tert*-butylhydroquinone, green tea phenol, (–)-epicatechin-3-gallate and the isothiocyanates. Upon metabolic activation, electrophiles are produced and cause cellular stress. This, in turn, activates MAPK pathways and results in the activation of the basic leucine zipper transcription factor, Nrf2, which dimerizes with the basic zipper transcription factors, Mafs. The heterodimeric complex then binds to an antioxidant/electrophile response element which has been identified in many xenobiotic metabolizing (phase I/II) and other cellular defence enzymes, of which thioredoxins and haemeoxygenase-1 are examples (Kong *et al.*, 2001). Therefore, cellular stress can regulate xenobiotic metabolizing enzymes with the aim of removing and detoxifying harmful intermediates such as reactive oxygen species.

(g) Genetic variability in AhR

Genetic variability in the coding sequences of *AhR* accounts for marked strain and species differences in sensitivity to AhR ligands when responsiveness to AhR ligands depends on different alleles. To date, relatively few polymorphisms have been reported in humans and, in the case of the murine *AhR* gene, approximately 2200 mutations have been identified in 13 inbred strains (Thomas *et al.*, 2002). Although variation in the human *AhR* gene exists, the relevance of this to risk for cancer remains uncertain. Indeed, a >12-fold variation in CYP1A1 activity in 3-methylcholanthrene-treated lymphocytes from 47 unrelated individuals has been reported, but none of the *AhR* gene

polymorphisms could explain the observed variability (Harper *et al.*, 2002). In contrast, 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 II metabolic reactions may be influential factors for risk of toxicity and cancer (see Sections 4.1.2 (b) and 4.3 on genetic variability in PAH-metabolizing enzymes).

4.2.6 *Immunological and haematological mechanisms*

(a) Introduction

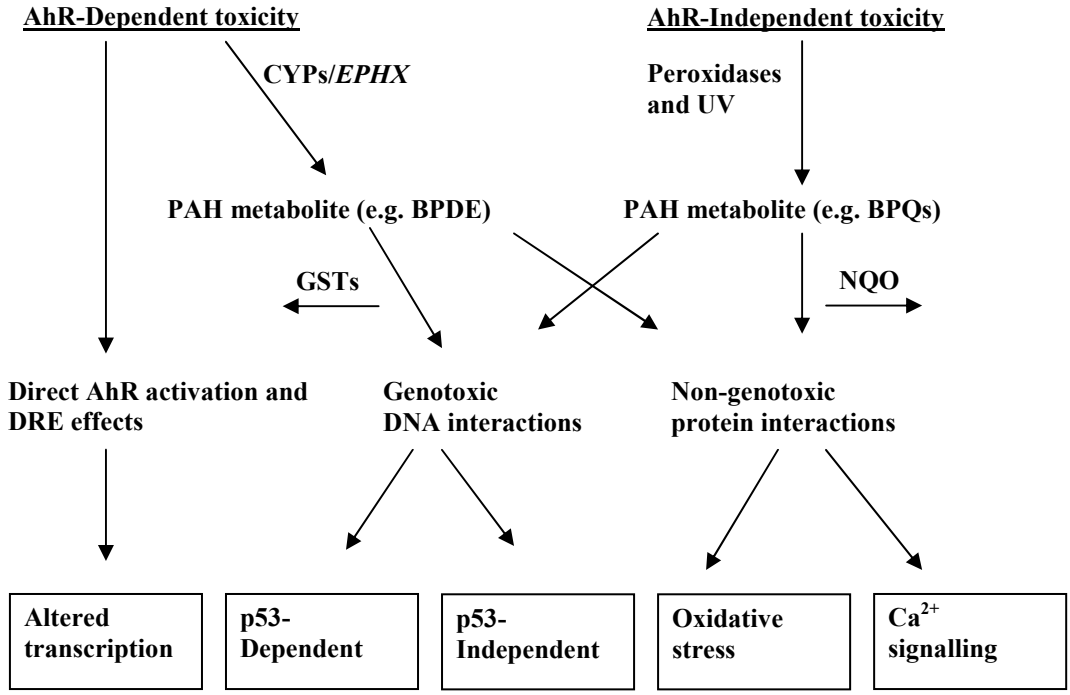
Innate and adaptive immune responses appear to play important roles in the protection of mammalian host organisms from cancer (Dupont, 2002). Therefore, a decrease in immune surveillance by agents such as PAHs might have negative consequences for cancer in humans. A significant number of studies have demonstrated that PAHs are immunosuppressive in animal models and following in-vitro exposure of human leukocytes. In animals, the concentrations of PAHs that are required to produce immunosuppression are generally quite high compared with those required to produce cancer. There are limited human epidemiological data to show that PAHs are immunosuppressive following environmental exposures. There are also extremely limited or no immunotoxicity data on many of the PAHs or complex mixtures that contain PAHs, with the exception of benzo[*a*]pyrene, on the immune system.

The biological and toxicological actions of PAHs on the immune and haematopoietic systems represent a complicated interplay between the ability of a specific PAH to bind to endogenous AhR and induce CYPs in central and peripheral organs which results in the formation of oxidative and electrophilic metabolites and the removal of reactive molecules via secondary metabolic processes. Therefore, as with many tissues, the toxicity of PAHs to the immune system is dependent upon the exposure of cells and tissues to circulating parent compounds and metabolites, their expression of AhR and their propensity to form bioactive versus detoxified metabolites. The dose and route of exposure to PAHs are important determinants of immunotoxicity in animals and humans. In general, the total cumulative dose of exposure to PAHs appears to correlate with immunotoxicity in mice. It should be noted that PAHs have been observed in several studies to produce biphasic dose–response curves, whereby low doses stimulate immune responses and high doses produce inhibition (Burchiel & Luster, 2001; Booker & White, 2005). An explanation for this finding may relate to signalling properties, which are discussed below.

The overall effects of PAHs on the immune and haematopoietic systems result from activation of both genotoxic and non-genotoxic (epigenetic) pathways (Figure 4.6). 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 PAHs have been difficult to assess. Many PAHs clearly exert effects on the developing as well as the mature immune system in many mammalian species, and some correlation exists between the carcinogenicity of PAHs and their ability to produce immunosuppression. An understanding of the mechanisms of action for both carcinogenicity and immunotoxicity may help to evaluate potential risks of untested agents or complex mixtures.

Figure 4.6 Polycyclic aromatic hydrocarbon (PAH) immunotoxicity



AhR, aryl hydrocarbon receptors; BPDE, benzo[*a*]pyrene diol epoxide;BPQ, benzo[*a*]pyrene quinone; CYP, cytochrome P450; DRE, dioxin response element; EPHX, epoxide hydrolase enzyme; GST, glutathione *S*-transferase; NQO, nicotinamide adenine dinucleotide phosphate quinone oxidoreductase; UV, ultraviolet light

(b) Aromatic hydrocarbon receptors

(i) *Immunosuppressive PAHs as AhR ligands*

In general, PAHs are semi-volatile compounds that are quite lipophilic and exert both specific and non-specific effects on membranes. Highly specific structure–activity relationships have been observed for the effects of PAHs on cells and tissues, including the immune system. Specific effects generally relate to the expression of AhR and the ability of 'bay-region' PAHs to bind to AhR and to activate AhR-dependent gene

promoter regions referred to as DREs. AhR appears to play an important role in normal development of the immune system in mice (Fernandez-Salguero *et al.*, 1995). The biological and toxicological activities of PAHs are largely dependent on their ability to interact with AhRs present in many mammalian cells and tissues (Nebert *et al.*, 1993; Hankinson, 1995; Schmidt & Bradfield, 1996; Whitlock, 1999; Nebert *et al.*, 2004).

The mechanism whereby the activation of AhR leads to immunotoxicity is not known for AhR agonists, although certain effects have become better understood in recent years. Because many 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. However, certain AhR ligands that are poorly metabolized, such as TCDD (or dioxin) and some PCBs, have been studied extensively for their immunotoxicity. AhR-dependent processes are activated by TCDD and some PCBs through binding to AhR and accessory molecules (such as Arnt), which leads to immune effects via XRE/XDREs. XRE/XDREs are also activated by many PAH AhR ligands, and it appears that their activation is well correlated with immunotoxicity for halogenated aromatic hydrocarbons and many PAHs. Several lymphoid cell lines have been found to express AhR in mammalian species, including humans, although T lymphocytes may require activation of phytohaemagglutinin before significant levels of CYP1A1 can be induced (Whitlock *et al.*, 1972; Germolec *et al.*, 1996). TCDD suppresses numerous B- and T-cell responses in rodents (Vecchi *et al.*, 1983) and produces thymic atrophy at higher concentrations (Camacho *et al.*, 2005; Nohara *et al.*, 2005). It may also induce immunosuppressive factors or prevent growth factors from being released from cells (Jensen *et al.*, 2003; Boverhof *et al.*, 2004). Current evidence suggests that direct activation of AhR in T cells may play a major role in the immunosuppressive effects of TCDD on cell-mediated and perhaps humoral immunity (Kerkvliet *et al.*, 2002; Funatake *et al.*, 2004; Temchura *et al.*, 2005). The affinity of AhR in various mouse strains is correlated with the potency of immunosuppression and thymic atrophy (Nebert *et al.*, 1993).

(ii) *PAH metabolism in the immunotoxicity of benzo[*a*]pyrene and DMBA*

CYP metabolism occurs in central (liver, lung) and peripheral lymphoid tissues (lymph nodes, spleen and bone marrow) and is very important in the immunotoxicity of PAHs. The local metabolism of PAHs is generally much lower than that in the liver and is modified by the expression of AhR and constitutive levels of CYPs such as CYP1B1. CYP1A1 is highly inducible in some white blood cells. In murine spleen cells, the highest levels of CYP activity have been detected in monocytes and macrophages, and low levels have been detected in B and T cells (Kawabata & White, 1989; Ladics *et al.*, 1992a,b,c). Most human and murine B- and T-cell lines used for in-vitro modelling studies have little CYP activity. Human peripheral blood T cells demonstrate an increase in levels of CYP1A1 following treatment with phytohaemagglutinin and PAHs (Whitlock *et al.*,

1972). PAHs are metabolized by CYP-dependent and -independent pathways, which are referred to as phase I metabolic pathways. The CYP-dependent pathways, most notably CYP1A1, CYP1A2 (liver only) and CYP1B1, are induced following binding of AhR and activation of DREs.

In general, benzo[*a*]pyrene and many other bay-region PAHs, including DMBA, are metabolized by CYP and other enzymes to form oxidative metabolites, such as epoxides. Although DMBA is not a naturally occurring compound, knowledge of its mechanism of action on the immune system has helped investigators to understand PAH pathways more generally. In the presence of microsomal epoxide hydrolase, epoxides are converted to dihydrodiols, which, in the presence of CYP1A1 or CYP1B1, can then undergo further metabolism to form diol epoxides, including benzo[*a*]pyrene-7,8-diol-9,10-epoxide and DMBA-3,4-diol-1,2-epoxide. These diol epoxides and other metabolites of benzo[*a*]-pyrene (such as benzo[*a*]pyrene quinones) and DMBA (such as DMBA quinones) are strong electrophiles that trigger the induction of secondary phase 2 metabolizing enzymes through the Keap-1/Nrf-2 redox sensing system and the anti-oxidant/electrophilic response element signalling systems (Primiano *et al*., 1997; Nguyen *et al.*, 2005). Phase II enzymes such as GSTs, NQO1, UGTs, epoxide hydrolase, aldehyde dehydrogenases and others are mainly expressed in the liver and lung, although low levels of GSTs are detected in human lymphocytes. Because lymphoid cells have limited expression of phase II detoxification enzymes, they may be somewhat sensitive to the formation of the phase I metabolites. Electrophilic metabolites of benzo[*a*]pyrene, most notably benzo[*a*]pyrene-7,8-diol-9,10-epoxide and perhaps 7,8-benzo[*a*]pyrene quinone, bind covalently to DNA which leads to genetic mutations that are responsible for tumour initiation. In addition, the formation of benzo[*a*]pyrene diol epoxide–DNA adducts leads to the induction of the p53 pathway, which may trigger cell-cycle arrest and apoptotic pathways in target cells. Therefore, the same general genotoxic mechanisms that lead to mutagenicity may also play a role in immunosuppression for PAHs that can be bioactivated.

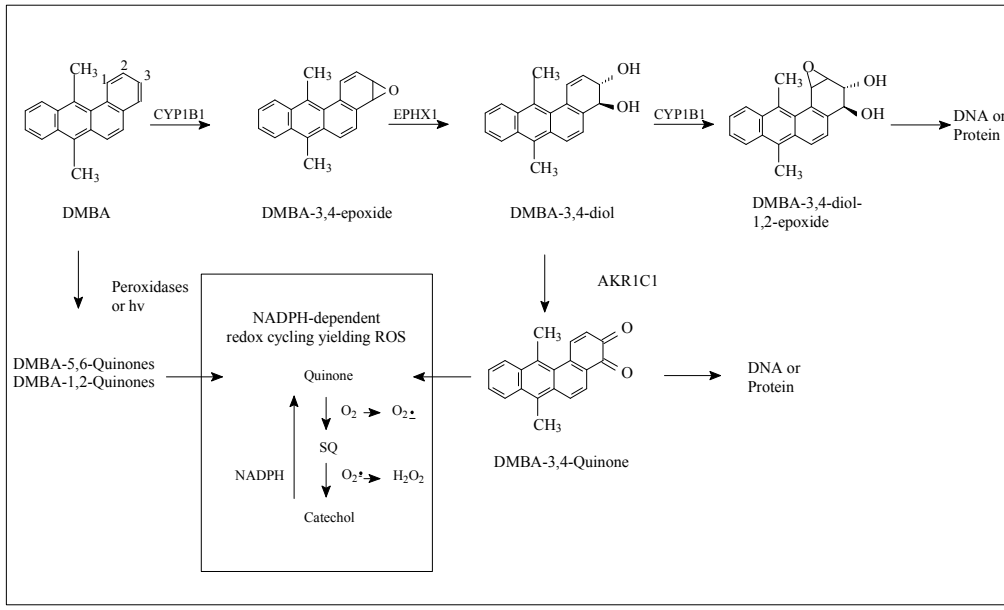
Several oxidative metabolites of PAHs, such as benzo[*a*]pyrene quinones, are known to redox-cycle and lead to the production of reactive oxygen species (Zhu *et al*., 1995). PAH quinones are formed via CYP-dependent and -independent reactions, such as by peroxidases or by ultraviolet light (Reed *et al*., 2003). PAH quinones redox-cycle and thereby form reactive oxygen species, including superoxide anion and hydrogen peroxide. Through reactive oxygen species, PAH quinones may exert both genotoxic and non-genotoxic effects, as shown by their ability to form 8-hydroxydeoxyguanosine on DNA as well as to alter signalling pathways in cells. Redox-cycling requires the reduction of equivalents generally supplied by NADPH, and thus benzo[*a*]pyrene quinones and related agents interact with the mitochondrial electron transport chain that leads to oxidative stress and ATP depletion. The immunotoxicological effects of benzo[*a*]pyrene quinones have not been fully evaluated.

Support for an important role of CYP metabolism in the immunotoxicity of PAHs has been obtained from studies that demonstrate that the AhR antagonist and CYP1A1/CYP1B1, inhibitor, α-naphthoflavone, prevents the immunotoxicity of benzo-

[*a*]pyrene and DMBA in murine spleens cells (Kawabata & White, 1987; Ladics *et al*., 1991), human peripheral blood T cells (Davila *et al*., 1996) and murine bone marrow (Dertinger *et al*., 2001). The putative CYP1A1 and CYP1B1 metabolite responsible for murine spleen cell and human T-cell immunotoxicity due to benzo[*a*]pyrene is probably its diol epoxide (Davila *et al.*, 1996). In addition, in these studies, the rank order of PAH immunotoxicity was found to be benzo[*a*]pyrene >DMBA >dibenz[*a*,*c*]anthracene >dibenz[*a*,*h*]anthracene >dimethylanthracene, benzo[*e*]pyrene >benz[*a*]anthracene >anthracene. The latter three PAHs had minimal immunotoxicity.

An important role of metabolism in the toxicity of DMBA in the bone marrow of mice has been established through the use of CYP1B1-null (knockout) mice. DMBA-induced pre-B cell toxicity was nearly totally abolished in mice that did not express CYP1B1 (Heidel *et al*., 2000). The splenic immunotoxicity of DMBA has been shown to be dependent on the expression and activity of CYP1B1 (Gao *et al*., 2005a), and it has recently been reported that microsomal epoxide hydrolase is also required for this spleen cell immunotoxicity. Thus, it is probable that DMBA-3,4-diol-1,2-epoxide is responsible for the immunotoxicity of DMBA in mice (Figure 4.7).

Figure 4.7. 7,12-dimethylbenz[*a*]anthracene (DMBA) metabolism that yields genotoxic and non-genotoxic metabolites



CYP, cytochrome P450; EPHX1, epoxide hydrolase enzyme; NADPH, nicotinamide adenine dinucleotide phosphate

(c) Correlation between carcinogenicity and immunotoxicity for some PAHs

Activation of AhR may be linked to tumour promotion in various cells, since TCDD has been shown to increase the proliferation of epithelial cells through the stimulation of cell proliferation, inhibition of apoptosis and production of growth factors (Davis *et al*., 2000, 2003). TCDD is a suspected tumour promoter in several tissues. The role of TCDD in promoting human leukaemia and lymphomas is controversial, but studies suggest that continual activation of AhR in human T cells leads to adult T-cell leukaemia (Hayashibara *et al*., 2003). Many PAHs are complete carcinogens and have the ability both to initiate and promote cancers. Tumour initiation pathways are associated with DNA binding and mutagenesis.

Carcinogenic PAHs have been found to suppress the immune system of animals (White & Holsapple, 1984; Wojdani & Alfred, 1984; Wojdani *et al*., 1984). Initial studies showed that benzo[*a*]pyrene, DMBA and 3-methylcholanthrene suppressed humoral immunity, and later studies showed that many immune cells are targets of PAH. The humoral immune response to T-dependent antigens is considered to be a sensitive indicator of immune suppression. Suppression of T- and B-cell proliferation has been observed at similar exposure levels, but proliferation is considered to be a somewhat less sensitive indicator of immunotoxicity than T-dependent antibody responses. In general, there is some correlation between the carcinogenicity of a PAH and its immunotoxicity, which is probably due to requirements for AhR-binding activity and metabolic activation. (White *et al*., 1985). In a detailed analysis of more than 50 selected chemicals, many of which were PAHs, Luster *et al.* (1992) found that immunotoxicity in rodents was probably correlated with carcinogenicity; however, examples of immunotoxic chemicals that had unknown carcinogenicity status were found, and therefore the converse relationship might not be true.

Mechanistically, the immunosuppression produced by benzo[*a*]pyrene and DMBA has both similarities and differences. Benzo[*a*]pyrene is a moderate to strong AhR ligand, whereas DMBA is a weak AhR ligand. In addition, as discussed above, much of the immunotoxicity of benzo[*a*]pyrene is largely due to CYP1A1-dependent metabolism. CYP1A1 is expressed only at low levels in lymphocytes until AhRs are activated. In the case of DMBA, the major metabolizing enzyme is CYP1B1, which is expressed in many tissues constitutively, but can also be induced through AhR and antioxidant response element mechanisms.

(d) Targets of PAH toxicity in the immune system

(i) *Bone marrow*

Mammalian bone-marrow cells, and especially bone-marrow stromal cells, express AhR. PAHs that bind to and/or are activated by CYP are toxic to mouse and human stem cells (Fine *et al*., 1989; Murante & Gasiewicz, 2000; van Grevenynghe *et al*., 2005), and myeloid (Luster *et al*., 1985; Laupeze *et al*., 2002; van Grevenynghe *et al*., 2003) and

lymphoid progenitors (Luster *et al*., 1988; Yamaguchi *et al*., 1997a,b; Near *et al*., 1999; Thurmond *et al*., 2000). Both DMBA and benzo[*a*]pyrene exert important effects on bone marrow that alter the formation of B cells. The mechanism of pre-B cell bone-marrow suppression appears to be CYP1B1-dependent and may be caused by pre-B cell apoptosis (Heidel *et al*., 1999; Mann *et al*., 1999; Heidel *et al*., 2000; Allan *et al*., 2003; Galvan *et al*., 2003, 2005).

(ii) *Thymus*

Overactivation by TCDD of AhR in the thymus causes thymic atrophy (Nohara *et al*., 2005). Most PAHs do not produce significant thymic atrophy until very high exposure levels are reached; however, DMBA is an exception and thymic atrophy is observed at low doses (Burchiel *et al.,* 1992). The effects of DMBA on the thymus have not been found to correlate with AhR phenotype (Thurmond *et al*., 1987; Holladay & Smith, 1995).

(iii) *Spleen*

Many studies have been performed with spleens obtained from PAH-treated mice, in which both DMBA and benzo[*a*]pyrene have been found to be immunosuppressive for humoral (Ward *et al*., 1984; White & Holsapple, 1984; Ward *et al*., 1986; Thurmond *et al*., 1987; Burchiel *et al*., 1988) and cell-mediated (Dean *et al*., 1986; House *et al*., 1989) immunity. DMBA was also found to be immunosuppressive following exposure *in vitro* (Thurmond *et al*., 1988). In general, the most sensitive target cells of PAHs appear to be B- and T-helper cells for humoral immunity and cytotoxic T cells for cell-mediated immunity, although macrophages and antigen-presenting cells have also been implicated (Myers *et al.*, 1987; Blanton *et al.*, 1988; Myers *et al.*, 1988). Splenic natural killer cells have also been shown to be suppressed by DMBA (Dean *et al*., 1985, 1986).

(iv) *Peripheral lymphoid tissues*

A few peripheral lymphoid tissues have been examined for the effects of PAHs following intragastric administration of benzo[*a*]pyrene to rats; lymphoid organ weights were decreased at high doses (90 mg/kg) of benzo[*a*]pyrene (De Jong *et al*., 1999). It was found that the antibody response of lung-draining lymph nodes was suppressed in rats exposed intratracheally to benzo[*a*]pyrene (Bice *et al*., 1979). In mice, DMBA suppressed the response of murine mesenteric lymph nodes and Peyer's patches following intragastric administration (Burchiel *et al*., 1990; Davis *et al*., 1991). The concentrations of PAHs required to suppress humoral and cell-mediated immunity in mice are extremely high, typically in the range of 10–50 mg/kg benzo[*a*]pyrene. In general, DMBA produced more cytotoxicity in peripheral lymphoid organs than benzo[*a*]pyrene.

(v) *Skin*

The skin is not considered to be a primary or secondary lymphoid tissue; however, there are significant immune responses that occur in this tissue, many of which fall into the category of hypersensitivity responses. PAHs, such as DMBA, have been shown to

enhance cell-mediated hypersensitivity reactions in the skin of mice that may play a role in immune surveillance (Casale *et al.*, 2000).

(e) Potential mechanisms of immunosuppression by PAHs

(i) *Genotoxic mechanisms of PAH metabolite-induced immunotoxicity*

The mechanisms by which PAHs produce immunotoxicity are divided into two general categories: genotoxic (DNA targets) and non-genotoxic (protein targets). Genotoxic and epigenetic effects are seen for many complete carcinogens, such as PAHs that both initiate and promote tumours. Many PAHs are bioactivated to reactive metabolites that bind to DNA and exert mutagenicity and genotoxicity. The mechanism by which genotoxic chemicals produce immunosuppression is probably p53-dependent and may result from stable adduct formation in lymphoid cells. Bulky PAH–DNA adducts induce p53 which in turn inhibits cell cycling and induces apoptosis in many cells (Vogelstein & Kinzler, 1992).

DMBA has been found to produce immunotoxicity in bone marrow through the induction of apoptosis (Page *et al*., 2002), and recent studies showed that p53 knockout mice are resistant to the suppressive effects of DMBA on bone marrow (Heidel *et al*., 2000; Page *et al*., 2003). Many genotoxic chemicals that induce p53 are also immunosuppressive. Therefore, p53 is probably an important pathway for the immunotoxicity of numerous agents. In some studies, there is good agreement between the functional immunotoxicity observed and changes in immunophenotypic cell-surface markers (Burchiel *et al*., 1988), while in others, there has been a lack of agreement (House *et al*., 1987). These differences probably relate to the degree of apoptosis and cell death produced by PAHs, as it is improbable that functional changes would be detected simply by immunophenotypic analysis of spleen cells. Thus, immunophenotyping is probably not a sensitive marker for immunotoxicity at non-cytotoxic concentrations of xenobiotic compounds.

(ii) *Non-genotoxic (epigenetic) mechanisms of PAH immunotoxicity*

Several studies have shown that PAHs can activate or interfere with lymphocyte signalling pathways in both murine and human B and T cells. The ability of PAHs to signal through various receptor and oxidative stress pathways may correlate with tumour promotion. Many xenobiotic compounds produce biphasic effects on immune responses: low concentrations stimulate and high doses inhibit responses. Agents that mimic or alter signalling pathways may manifest these characteristics.

AhR ligands

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).

Direct AhR-dependent immunosuppression has been reported in both T (Kerkvliet *et al*., 2002) and B cells (Sulentic *et al*., 2000) for the pure AhR agonist, TCDD. TCDD has been shown to produce persistent changes in immunosuppressive cytokine production in monkeys (Rier *et al*., 2001), although the prevention of tumour necrosis factor -α activity has not been found to restore the T-dependent antibody responses in mice (Moos & Kerkvliet, 1995). Nevertheless, AhR-dependent altered cytokine production is a potential mechanism for PAH-induced immunosuppression.

Calcium signalling

The antigen receptor signalling pathways of lymphocytes are linked to changes in intracellular Ca^{2+} (reviewed in Davila *et al*., 1995; Burchiel & Luster, 2001). The structure–activity relationships for elevated of Ca^{2+} have largely been determined *in vitro*, and there appear to be PAH metabolism-dependent and -independent mechanisms that rapidly increase levels of Ca^{2+}. DMBA produces a rapid increase in intracellular Ca^{2+} (Burchiel *et al*., 1991). Elevation of Ca^{2+} appears to be an inositol-1,4,5-triphosphate (IP_3)-dependent process (Archuleta *et al*., 1993). However, further studies revealed that many PAHs also produce a rapid increase in intracellular Ca^{2+} that did not follow any structure–activity relationships relating to AhR binding (Davila *et al*., 1999). Sustained elevations in intracellular Ca^{2+} appeared to correlate with human T-cell signalling (Krieger *et al*., 1994). Benzo[*a*]pyrene increased intracellular Ca^{2+} in human peripheral blood mononuclear B and T cells and monocytes (Mounho *et al*., 1997). Benzo[*a*]pyrene diol epoxide appeared to be responsible for this elevation of Ca^{2+}, perhaps due to the activation of protein tyrosine kinase (PTK) detected in a human B-cell line (Mounho *et al*., 1997). Thus, there appear to be several potential mechanisms of elevation of Ca^{2+} by PAHs in murine and human lymphocytes. The significance of altered Ca^{2+} signalling in PAH-induced immunosuppression may be increased in the presence of cyclosporine A, which is a potent immunosuppressive drug that is used in the prevention of transplant rejection and autoimmunity, and which interferes with Ca^{2+} signalling (Ruggenenti *et al*., 1993).

Inhibition of sarcoplasmic–endoplasmic reticulum calcium–ATPase

Initial studies showed that the ability of PAHs to increase intracellular Ca^{2+} in human T cells correlated with an inhibition of sarcoplasmic–endoplasmic reticulum Ca^{2+}–ATPase (SERCA) activity (Krieger *et al*., 1995). However, PAHs failed to inhibit cloned rat SERCA enzymes (transiently expressed in human embryonic kidney cells), which suggested that metabolism might be required for this activity (Zhao *et al*., 1996). A well known SERCA inhibitor, thapsigargin, has been classified as a tumour promoter (Thastrup *et al*., 1990), which suggests that inhibition of SERCA by PAHs may be a potential mechanism of tumour promotion.

Increased PTK activity

PAHs have been found to increase PTK activity in lymphocytes leading to Ca^{2+}-dependent signalling in B and T cells in mice and humans (for a review, see Davila *et al*.,

1995; Burchiel & Luster, 2001). DMBA activates human T cells directly (Burchiel *et al*., 1991; Archuleta *et al*., 1993; Davis & Burchiel, 1992), whereas benzo[*a*]pyrene requires metabolism to its diol epoxide to activate human B cells (Mounho & Burchiel, 1998). In-vitro studies with PAHs have shown that non-specific components are involved in PTK activation (Davila *et al*., 1999). The precise mechanism whereby PAHs activate PTK appears to be through the inhibition of protein tyrosine phosphatases. There is evidence that UV light increases PTK activity via this mechanism, as well as numerous oxidant chemicals and some metals (Schieven *et al*., 1994; Rhee *et al*., 2005; Tonks, 2005). Therefore, the mechanism of inhibition of protein tyrosine phosphatases by PAHs may be due to oxidative changes in the protein. Overactivation of PTK pathways and receptor tyrosine kinase pathways is associated with cancer in many cell types.

Activation of Ca^{2+} channels: IP_3 and ryanodine receptors

Phospholipase Cγ (Cγ1 in T cells and Cγ2 in B cells) is activated by PAHs in human and murine T and B cells by PTKs. Phospholipase Cγ is responsible for the cleavage of membrane phosphatidylinositol-4,5-biphosphate and the release of IP_3 and diacylglycerol. IP_3 diffuses into the cytoplasm and binds to IP_3 receptors that are located on the endoplasmic reticulum. IP_3 receptors control release of Ca^{2+} into the endoplasmic reticulum and their activation leads to a rapid increase in intracellular Ca^{2+}. Benzo[*a*]pyrene diol epoxide has been shown to activate B cell PTKs that are associated with the formation of IP_3 (Mounho *et al*., 1989), and this activation may be dependent on protein tyrosine phosphatase. Therefore, one mechanism of PAH-induced elevation of Ca^{2+} is via the activation of phospholipase Cγ.

A novel mechanism, ryanodine receptors that control Ca^{2+} refilling of endoplasmic reticulum stores, was discovered in the endoplasmic reticulum of lymphocytes (Sei *et al*., 1999; Schwarzmann *et al*., 2002). Gao *et al*. (2005b) found that a novel metabolite, 7,8-benzo[*a*]pyrene-quinone, produced a rapid increase in intracellular Ca^{2+} via the ryanodine receptor in human peripheral blood leukocytes and murine splenic B and T cells.

Consequences of increased Ca^{2+} signalling in lymphocytes

PAHs have the potential to activate numerous isoforms of PKC in lymphocytes and other immune cells. During the activation of PTKs, diacylglycerol and Ca^{2+} are produced and activate these isoforms of PKC, which are defined on the basis of their sensitivities to these two substances (Parker & Murray-Rust, 2004). Activation of PKC has been associated with tumour promotion by phorbol esters in many cells and tissues (Weinstein, 1991). It is unclear which of the isoforms of PKC have immunological consequences. Because of altered B- and T-cell Ca^{2+} signalling, inappropriate activation may lead to such phenomena as persistent immunosuppression and tolerance (Ward *et al*., 1986; Burchiel *et al*., 1988). In T and B cells, excess Ca^{2+} signalling has been associated with tolerance (Schwartz, 2003). One of the consequences of helper T-cell tolerance would be the lack of production of IL-2, which is a key cytokine for both humoral and cell-mediated immunity. DMBA prevents the formation of IL-2 and exogenously added IL-2 can

partially overcome the immunosuppression produced by this agent (House *et al*., 1987; Pallardy *et al*., 1989).

Oxidative stress

In many cells, PAHs are known to cause oxidative stress (Nebert *et al*., 2000), which is associated with the activation of the AhR (Senft *et al*., 2002). Although few studies have been carried out on oxidative stress produced by benzo[*a*]pyrene quinones in lymphocytes, previous studies in bone-marrow cells demonstrated that these redox-cycling agents may also lead to GSH depletion (Zhu *et al*., 1995; Romero *et al*., 1997). Direct exposure of murine and human lymphocytes to 7,8-benzo[*a*]pyrene quinone (but not 1,6-, 3,6- or 6,12-benzo[*a*]pyrene quinone) led to rapid elevation of Ca^{2+} (Gao *et al*., 2005b), whereas treatment with 1,6-, 3,6-, and 6,12-benzo[*a*]pyrene quinone increased free intracellular Ca^{2+} only after prolonged (12–18 h) exposures *in vitro*, presumably due to mitochondrial redox-cycling, depletion of ATP and Ca^{2+} overload. Other PAH quinones have also been shown to produce immunotoxicity. For example, treatment of murine spleen cells *in vitro* with 1,4-naphthaquinone produced immunotoxicity in mice, whereas exposure to naphthalene produced no adverse immune effects (Kawabata & White, 1990). In-vitro exposure of murine spleen cells to benzo[*a*]pyrene quinones produced a strong proliferative signal in T cells, but not B cells (Burchiel *et al*., 2004). Thus, the effects of PAHs that induce oxidant stress on murine spleen cells appear to be mixed.

(f) Complex mixtures

Humans are exposed to complex mixtures of PAHs via the diet, air and skin contact (Rothman *et al*., 1993; Schoket, 1999; Scherer *et al*., 2000; Arrieta *et al*., 2003; Oh *et al*., 2005). Low concentrations of high-molecular-weight PAHs, such as benzo[*a*]pyrene, are found in diesel exhaust and higher levels are found in woodsmoke (Burchiel *et al*., 2005). Occasional dermal exposure may also occur and is usually associated with occupational exposures to tars, soots and vapours. Exposures to PAHs can be monitored using major urinary metabolites (Wu *et al*., 2002). Some epidemiological evidence indicates that complex mixtures containing PAHs produce immunosuppression under conditions of environmental and industrial exposures in humans (Szczeklik *et al.*, 1994; Winker *et al.*, 1997; Karakaya *et al*., 1999; Biro *et al.*, 2002; Karakaya *et al.*, 2004; Oh *et al*., 2005).

In animal models, recent data demonstrate that diesel exhaust, which contains an abundance of low-molecular-weight PAHs (such as naphthalene, anthracene and phenanthrene), and woodsmoke (which contains measurable concentrations of benzo[*a*]pyrene) are immunosuppressive to murine spleen cells following chronic (6 months) inhalation (Burchiel *et al*., 2004, 2005). It is unclear which components of diesel exhaust and woodsmoke are responsible for this suppression, but PAHs may play a role. The particulate fraction of diesel exhaust contains many potential immuno-suppressive chemicals, including PAHs and metals, and important interactions may occur between oxidant-generating PAHs and metals. In addition, because it is well known that

diesel exhaust causes inflammation in the lung and generates significant inflammatory and chemo-attractant cytokine production, it is possible that immune modulation may be caused secondary to altered cytokine production.

(g) Summary

PAHs exert many important effects on the immune system of many species. The dose and route of exposure determine the nature of the effect of 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 diol epoxide mechanisms which lead to DNA interactions that cause genotoxicity and suppress immunity by p53-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 PAH quinones may exert oxidative stress in lymphoid cells. Human exposures to PAHs are usually in the form of complex mixtures, and it is difficult to attribute the relative contributions of individual PAHs to the overall immunotoxic effects. Some evidence suggests that environmental exposures to PAH may produce immunotoxicity, but further epidemiological studies are needed.

4.2.7 *Genotoxic and epigenetic effects of mixtures*

(a) Aluminium production

Filter extracts of airborne particles from a Söderberg pot-room and an anode paste plant were mutagenic in *S. typhimurium* TA98 and TA100 after metabolic activation, and positive results were obtained without metabolic activation in strain TA98 (Krokje *et al.*, 1985). Several studies examined the levels of aromatic DNA adducts in peripheral blood lymphocytes of aluminium workers and showed mixed results. In 172 Hungarian aluminium plant workers, increased levels of aromatic DNA adducts were measured compared with controls (Schoket *et al.*, 1999). Other studies have observed aromatic DNA adducts in peripheral blood lymphocytes of Hungarian aluminium workers at different factories, at different times of the year and in different job categories (Schoket *et al.*, 1993a,b, 1995). A significant linear correlation was observed between total aromatic DNA adducts in white blood cells and urinary 1-hydroxypyrene levels in Hungarian pot-room workers with the GSTM1-nul genotype (Schoket *et al.*, 2001).

Other populations of aluminium plant workers had detectable levels of aromatic DNA adducts (Kriek *et al.*, 1993; Ovrebo *et al.*, 1995; van Schooten *et al.*, 1995). Ninety-eight Swedish pot-room workers were examined for aromatic DNA adducts and polymorphisms. No significant differences were observed in the levels of total or individual DNA adducts between pot-room workers and controls (Tuominen *et al.*, 2002). Only one sample from the lymphocytes of 30 aluminium plant workers was found to contain benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts (Vähäkangas *et al.*, 1985).

Analysis of levels in a group of 36 aluminium anode plant workers did not show a significant percentage of subjects with DNA adduct levels that exceeded the 95th percentile of the control value (Pavanello *et al.*, 1999a,b). Serum antibodies to benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA were detected in 13.3% of 105 aluminium plant workers (Galati *et al.*, 2001). Urine samples from pot workers and anode paste workers in a Swedish aluminium plant were not mutagenic in *S. typhimurium* (Krokje *et al.*, 1988). The lymphocytes of 42 Italian aluminium plant workers were examined for micronuclei and DNA damage (single-cell gel electrophoresis assay). While none of the workers showed significant changes in micronucleus formation, significant increases in DNA damage were noted (Crebelli *et al.*, 2002). Ninety-eight Swedish pot-room workers and 55 controls were examined for the effect of genetic polymorphisms of biotransformation enzymes on gene mutations, DNA strand breaks and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine. No correlations were found between any of the genotoxicity biomarkers and any of the exposure measures, length of employment in the pot-room, 1-hydroxypyrene in urine or PAH–DNA adducts in peripheral lymphocytes, even when genotypes for biotransformation enzymes were considered (Carstensen *et al.*, 1999).

(b) Chimney sweeps

The frequencies of micronuclei in peripheral B or T lymphocytes and whether genetic polymorphisms in metabolic activating enzymes could explain some of the variation in micronucleus formation was studied in 71 Swedish chimney sweeps. The sweeps did not have higher frequencies of micronuclei in either cell type when the results were adjusted for age and tobacco smoking and there was no association between duration of employment and micronuclei formation (Carstensen *et al.*, 1993). The same cohort was further studied for the presence of aromatic DNA adducts and micronuclei and was genotyped for *CYP1A1* and *GST1*. While no specific DNA adducts were identified, the sweeps had higher but not significantly increased total DNA adduct levels in white blood cells. There were no systematic differences in DNA adduct patterns between sweeps and controls. DNA adducts in sweeps were moderately but statistically significantly correlated with micronuclei in both T and B lymphocytes. This correlation between adduct levels and micronuclei was most marked in the T lymphocytes of individuals who lacked the *GST1* gene (Ichiba *et al.*, 1994).

The lymphocytes of 45 five Swedish chimney sweeps and 49 controls were investigated for micronucleus formation after stimulation with phytohaemagglutinin and pokeweed mitogen and analysis of lymphocyte subgroups and neutrophilic leukocytes. There was a statistically significant effect among sweeps with respect to both micronucleus variables and neutrophilic leukocytes. The effect on lymphocyte micronuclei was more significant in pokeweed mitogen-stimulated cells, which may imply that T4 lymphocytes were preferentially damaged by the occupational exposure (Holmen *et al.*, 1994). Analysis of benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels in a group of 19 chimney sweeps showed that the percentage of subjects with adduct levels that

exceeded the 95th percentile of the control value was significantly high in chimney sweeps (Pavanello *et al.*, 1999a), and these higher levels were associated with the lack of GSTM1 activity (Pavanello *et al.*, 1999b).

(c) Creosote

Creosote induced mutations in *S. typhimurium* TA1537, TA1538, TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver metabolic activation system (Bos *et al.*, 1983, 1985). A portion of the mutagenicity was attributed to benzo[*a*]pyrene, benz[*a*]anthracene and fluoranthene in the complex mixture (Bos *et al.*, 1984a, 1987). Four creosotes used in Finland were mutagenic in *S. typhimurium* TA98 , TA100, YG1021 and YG1024 in the presence of an Aroclor 1254-induced rat liver metabolic activation system (Nylund *et al.*, 1992). The urine from rats treated with creosote by intraperitoneal administration was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver homogenate supplemented with β-glucuronidase (Bos *et al.*, 1984b). Using the same bioassay method, urine samples of three workers in the creosote wood-preserving industry were not mutagenic although organic extracts of wipe-test samples from the surfaces of their work environments were (Bos *et al.*, 1984a). Several creosote samples induced sister chromatid exchange in Chinese hamster ovary cells in the presence of an Aroclor 1254-induced rat liver metabolic activation system (Nylund *et al.*, 1992). Creosote applied topically to mouse skin *in vivo* or human skin in short-term organ culture produced a complex pattern of aromatic DNA adducts with similar levels in both systems (Schoket *et al.*, 1988a,b). Multiple topical treatments of mice with creosote resulted in accumulation of DNA adducts in lung tissues (Schoket *et al.*, 1988a). Extracts of soil samples from a wood-preserving waste site known to contain creosote and pentachlorophenol were topically applied to mouse skin. Aromatic DNA adducts were detected in distal organs (lung, liver, kidney and heart) as well as the skin. The *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct was detected in all organs (Randerath *et al.*, 1996, 1997).

(d) Coal tar

(i) *Human data*

Urine samples from some nonsmoking psoriasis patients treated with coal tar and UV light were mutagenic in *S. typhimurium* TA98 in the presence of an Aroclor 1254-induced rat liver metabolic system (Wheeler *et al.*, 1981). The urine of all 15 nonsmoking patients who were treated with a 2% coal-tar ointment and who had avoided a high-temperature cooked meat diet was mutagenic in *S. typhimurium* YG1024 with exogenous metabolic activation. *GSTM1*-nul patients had higher levels of mutagens in their urine than *GSTM1*-positive patients (Gabbani *et al.*, 1999). The skin and white blood cells (monocytes, lymphocytes and granulocytes) of a group of eczema patients treated topically with coal-tar ointments showed the presence of aromatic DNA adducts by ^{32}P-postlabelling analysis. One of the adducts co-migrated with the benzo[*a*]pyrene-7,8-diol-9,10-oxide–

DNA adduct (Godschalk *et al.*, 1998). Analysis of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels by an HPLC/fluorescence method in a group of 26 psoriasis patients showed that the percentage of subjects with adduct levels that exceeded the 95th percentile of the control value was not significant (Pavanello *et al.*, 1999a). The white blood cells of 23 psoriasis patients who were undergoing clinical coal-tar therapy were examined for benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts by an enzyme-linked immunsorbent (ELISA) method. Although these adducts were detected and their levels decreased with time after treatment, no relationship could be ascertained between the level of exposure and the amount of adducts. Also, no difference in the level of DNA adducts was found between smoking and nonsmoking patients (Paleologo *et al.*, 1992). PAH diol epoxide–DNA adducts and *GSTM1* genotype in the white blood cells of 57 psoriasis patients and 53 controls were determined by ELISA methods and polymerase chain reaction respectively. PAH diol epoxide–DNA adducts were slightly elevated in patients compared with controls, but there was no relationship between the presence of the *GSTM1* gene and DNA adducts (Santella *et al.*, 1995). Skin biopsy samples from 12 psoriasis patients who received therapy with coal-tar ointment contained aromatic DNA adducts as measured by ^{32}P-postlabelling analysis (Schoket *et al.*, 1990). No significant effect of coal-tar treatment of psoriasis patients on the levels of benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts was detected by ^{32}P-postlabelling analyses in peripheral blood lymphocytes (Pavanello & Levis, 1994). In a study of 111 Korean coal tar-based paint workers, the levels of aromatic DNA adducts measured by ^{32}P-postlabelling analysis were slightly higher than those of 27 on-site control workers (Lee *et al.*, 2003). The lymphocytes of 49 coal-tar workers exhibited a significant increase in the frequency of chromosomal aberrations, sister chromatid exchange and satellite associations compared with controls (Yadav & Seth, 1998). Increased levels of p53 were found in skin biopsies of atopic eczema patients treated topically with coal tar. A correlation was also observed between p53 and levels of aromatic DNA adducts measured in the same tissue by ^{32}P-postlabelling analysis (Godschalk *et al.*, 2001).

(ii) *Studies in experimental systems*

In a previous monograph, coal-tar pitch and roofing-tar emissions were found to be mutagenic in bacteria (in the presence of an Aroclor 1254-induced rat liver metabolic system) and mammalian cells (in the presence and absence of an Aroclor 1254-induced rat liver metabolic system), to induce sister chromatid exchange in Chinese hamster ovary CHO cells and to enhance viral transformation in Syrian hamster embryo cells (both in the absence of an Aroclor 1254-induced rat liver metabolic system) (IARC, 1985). Coal tar applied topically to the skin of male Parkes mice *in vivo* produced a complex pattern of DNA adducts in skin and lung tissues (Schoket *et al.*, 1988a). In the skin, several groups of adducts were attributed to groups of PAHs. Coal-tar adduct spot 2 was the major adduct formed by benzo[*ghi*]perylene (Hughes *et al.*, 1993). Male B6C3F1 mice fed a diet of coal tar from manufactured gas plant residue produced a complex pattern of aromatic adducts in the liver, lung and forestomach DNA which increased with dose and

time of treatment (Culp & Beland, 1994). In lung DNA, one adduct was tentatively identified as the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct (Culp & Beland, 1994). The identity of this adduct was confirmed by analysis of the lung DNA of female B6C3F1 mice fed a diet of coal tar from manufactured gas plant residue; however, based on the levels of this adduct, it was suggested that benzo[*a*]pyrene may contribute only a small fraction to the DNA adducts formed in the lung tissue of mice administered coal tar (Beland *et al.*, 2005). Female B6C3F1 mice were fed diets of coal tar, and DNA adduct formation, cell proliferation and mutations in tumours in the K-*ras*, H-*ras* and *p53* genes were determined. The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct was identified in forestomach DNA. Cell proliferation was increased in the small intestines by dietary coal tar. Of the K-*ras*, H-*ras* and *p53* mutations observed in coal tar-induced tumours, the most abundant were K-*ras* mutations in forestomach and lung tumours (Culp *et al.*, 2000). B6C3F1 mice fed diets containing coal tar from manufactured gas plant residue produced a complex pattern of aromatic DNA adducts in the lung, forestomach and spleen tissues. Benzo[*a*]pyrene content alone in the coal tar could not account for the levels of aromatic DNA adducts (Weyand *et al.*, 1991). Strain A/J mice formed aromatic DNA adducts in the lungs when fed coal tar (from manufactured gas plant residue) in the diet. Three major DNA adducts were identified as being derived from benzo[*b*]fluoranthene, benzo[*a*]pyrene and benzo[*c*]-fluorene (Koganti *et al.*, 2001). Female ICR mice received topical applications of manufactured gas plant residue. Of the complex pattern of lung adducts, one was identified as being derived from 7*H*-benzo[*c*]fluorene. The quantitative results suggested that components other than 7*H*-benzo[*c*]fluorene played an important role in lung DNA adduct formation (Cizmas *et al.*, 2004). Epidermal cells but not hepatocytes isolated from C3H/Tif/hr hairless mice that received topical applications of coal tar had higher levels of DNA strand breaks as measured by the alkaline comet assay. Coal tar applied topically to lambda lacZ transgenic mice (MutaMouse) strongly increased the mutation frequency in epidermal cells but not in hepatocytes (Thein *et al.*, 2000). A retrospective comparison of tumour induction and DNA adduct formation by benzo[*a*]pyrene and coal tars in several experimental protocols indicated that tumour outcomes were not predicted by either quantitation of total DNA adducts or by DNA adducts formed by benzo[*a*]pyrene. These data suggested that benzo[*a*]pyrene content alone may not accurately predict tumour outcomes (Goldstein *et al.*, 1998).

(e) Coke ovens

As reported in a previous monograph, coke-oven materials were found to be mutagenic in bacteria in the presence or absence of an Aroclor 1254-induced rat liver metabolic system, to be mutagenic in several mammalian cell lines, to induce DNA strand breaks in Syrian hamster embryo cells, to induce sister chromatid exchange in Chinese hamster ovary CHO cells (in the presence or absence of an Aroclor 1254-induced rat liver metabolic system) and to enhance viral transformation in Syrian hamster embryo cells and morphological cell transformation of BALB/c 3T3 cells (in the absence of an Aroclor

1254-induced rat liver metabolic system) (IARC, 1984). In two early studies (Harris *et al*., 1985; Haugen *et al*.,1986), two methods were used to confirm the presence of benzo[*a*]-pyrene diol epoxide–DNA adducts in the lymphocytes of coke-oven workers. Urine samples from 31 male nonsmoking coke-oven workers were mutagenic in *S. typhimurium* YG1024 in the presence of an exogenous source of metabolic activation but not two samples from 31 male nonsmoking controls (Simioli *et al.*, 2004). A higher percentage (51%) of 39 French coke-oven workers had *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts in their lymphocytes and monocytes as measured by an HPLC/fluorescence method compared with 18% in 39 unexposed persons. Smokers in the exposed group had 3.5 times more DNA adducts than nonsmokers (Rojas *et al.*, 1995). Analysis of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels by an HPLC/fluorescence method in a group of 15 male coke-oven workers showed that the percentage of subjects with adduct levels that exceeded the 95th percentile of the control value was significantly higher (Pavanello *et al.*, 1999a), and these higher levels were associated with a lack of *GSTM1* activity (Pavanello *et al.*, 1999b). In a study of 95 male Polish coke-oven workers, those with the *GSTM1*-nul genotype had a significantly higher risk of having high levels of *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide–DNA adduct as measured by an HPLC/fluorescence method than individuals with the active *GSTM1* genotype (Pavanello *et al.*, 2004). Eighty-nine male Taiwanese coke-oven workers in three exposure groups (topside workers, cokeside workers and plant office staff) were compared with 63 referents. All groups contained smokers and nonsmokers. Levels of aromatic DNA adducts in white blood cells were measured by ^{32}P-postlabelling analysis and were highest in the topside workers; smoking contributed only a small effect (Chen, M.L. *et al.*, 2003). Thirty-five Dutch coke-oven workers were compared with 37 controls for genotoxic effects and genetic polymorphisms in *GSTM1* and *GSTT1*. Occupational exposure did not cause a significant induction of sister chromatid exchange, high-frequency sister chromatid exchange, DNA strand breaks or aromatic DNA adducts (as measured by ^{32}P-postlabelling analysis) in lymphocytes or micronuclei in exfoliated urothelial cells. Smoking caused a significant increase in the incidence of sister chromatid exchange, high-frequency sister chromatid exchange and DNA adducts, but not of micronuclei or DNA strand breaks. *GSTM1* and *GSTT1* polymorphisms had no effect on any biomarker (van Delft *et al.*, 2001). Twenty-nine coke-oven workers and a control group were studied for frequencies of DNA single-strand breakage, DNA protein cross-links (alkaline filter elution assay), sister chromatid exchange and DNA adducts (measured by ^{32}P-postlabelling analyses) in lymphocytes. While the frequency of DNA strand breaks in the lymphocytes of coke-oven workers was significantly higher than that in controls, the DNA adduct rate was not significantly increased in workers (Popp *et al.*, 1997). Eighty-nine French coke-oven workers were compared with 44 power plant workers for *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels and genetic polymorphisms to *CYP1A1*, *GSTM1* and *GSTT1* genes. Higher levels of DNA adducts were detected in individuals with the combined *CYP1A1*(*1/*2 or *2A/*2A)-*GSTM1*-nul genotype (Rojas *et al.*, 2000). Non-tumorous lung tissues from 20 lung cancer patients and white blood

cells from 20 coke-oven workers were examined for levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts by HPLC/fluorescence analyses and for genetic polymorphisms. All subjects were current smokers. Subjects with the combination of homozygous mutated *CYP1A1* (MspI/MspI) and *GSTM1*0/*0* genotypes had higher levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels than those with *CYP1A1* and *GSTM1* wild-types (Rojas *et al.*, 1998). Twenty coke-oven workers, 30 graphite electrode-producing plant workers and 47 control subjects were compared for 8-oxo-deoxyguanosine levels, DNA damage by the alkaline single-cell comet assay and genetic polymorphisms in their white blood cells. Levels of 8-oxo-deoxyguanosine in white blood cells and DNA damage in lymphocytes were higher in coke-oven workers and graphite electrode-producing plant workers than in control subjects. The polymorphisms of the genes *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* showed no association with the biomarkers of effect (Marczynski *et al.*, 2002). Urinary 8-oxo-deoxyguanosine levels were higher in 55 Taiwanese topside coke-oven workers than in 162 sideoven coke-oven workers (Wu *et al.*, 2003). The influence of four polymorphisms of nucleotide excision-repair genes and of *GSTT1* on levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts was studied in lymphocyte and monocyte fractions of white blood cells from 67 Polish coke-oven workers by HPLC/fluorescence analyses. The increase in *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels was significantly related to lack of *GSTM1* activity and to the low nucleotide DNA excision-repair capacity of the *XPC-PAT*$^{+/+}$ genotype (Pavanello *et al.*, 2005). The peripheral blood lymphocytes of coke-oven workers (143) and non-coke-oven workers (50) were studied for DNA damage by the alkaline comet assay and for genetic polymorphisms of *XRCC1* and *ERCC2* DNA excision-repair genes. DNA damage was significantly greater in coke-oven workers than in non-coke-oven workers and greater in coke-oven workers with the GA genotype of the G27466A polymorphism of *XRCC1* than those with the GG genotype (Leng *et al.*, 2004a). Peripheral blood lymphocytes from 141 coke-oven workers and 66 non-coke-oven worker controls were studied for cytokinesis-block micronucleus frequencies and the effects of genetic polymorphisms. The micronucleus frequency was significantly higher in coke-oven workers than in control workers. Among the coke-oven workers, the *mEH His*^{113} variant genotype exhibited a significantly lower frequency of micronuclei than the *Tyr*^{113}*/Tyr*^{113} genotype; the low-microsomal epoxide hydrolase activity phenotype exhibited a lower frequency than the high-microsomal epoxide hydrolase activity phenotype; the *GSTP1 Val*^{105}*/Val*^{105} genotype exhibited a higher frequency than the *GSTP1 Ile*^{105}*/Ile*^{105} or *Ile*^{105}*/Val*^{105} genotypes; and a joint effect of high –microsomal epoxide hydrolase activity phenotype and *GSTM1*-null genotype on frequencies of micronuclei was also found (Leng *et al.*, 2004b). The frequency of mutants at the *HPRT* locus (6-thioguanine resistance) in lymphocytes from a population of 43 coke-oven workers was compared with that of a group of 26 non-exposed workers. A non-significant increase in frequency was observed in the exposed group compared with the non-exposed group. The percentages of the different types of gene alteration were similar in exposed and non-exposed subjects based on an analysis of mutations in 161

HPRT clones derived from exposed and non-exposed workers. Only the frequency of splice mutations in mutant clones derived from coke-oven workers was higher than that in controls (Zanesi *et al.*, 1998). No difference in the plasma levels of either p53 (mutated or wild-type) or $p21^{WAF1}$ protein was found between 66 exposed Czech coke-oven workers and 49 controls; however, significantly higher levels of p53 and $p21^{WAF1}$ protein were found in the group exposed to higher levels of carcinogenic PAHs compared with the group exposed to lower levels. Overall, a negative correlation between the levels of p53 protein and personal exposure to carcinogenic PAHs was found (Rossner *et al.*, 2003). Cytogenetic markers (chromosomal aberrations, sister chromatid exchange, cells with a high frequency of sister chromatid exchange, the heterogeneity index of sister chromatid exchange and genetic polymorphism of genotypes *GSTM1* and *NAT2*) were evaluated in the peripheral lymphocytes of 64 coke-oven workers and 34 machine worker controls from the same plant. All the cytogenetic markers and sister chromatid exchange were significantly increased in the exposed compared with the control group even when smoking status was taken into account. No effects of *GSTM1* or N-*acetyltransferase* (*NAT2*) genotypes (individually or in combination) on the cytogenetic markers were observed (Kalina *et al.*, 1998).

4.3 Potentially susceptible subpopulations

4.3.1 *Polymorphisms*

(a) Introduction

Individuals who are deficient in enzymes that activate PAHs to reactive metabolites may be at lower risk for chemical carcinogenesis (Nebert *et al.*, 1999; Bouchardy *et al.*, 2001; Kiyohara *et al.*, 2002a; Daly, 2003; Nebert, 2005b), whereas those who are deficient in enzymes that detoxicate reactive metabolites of PAHs may have a higher risk (Bartsch *et al.*, 2000; Williams, 2001; see Table 4.3).

A large number of epidemiological studies have been conducted to ascertain whether genetic polymorphisms of drug-metabolizing enzymes are related to cancer susceptibility in humans (Bartsch *et al.*, 2000; Williams & Phillips, 2000; Kiyohara *et al.*, 2002b; Daly, 2003). Some showed positive relationships between genetic polymorphisms of particular enzymes and the occurrence of cancers in selected organs (Bartsch *et al.*, 2000; Williams & Phillips, 2000), but others suggested that none of the roles of these genetic polymorphisms can be determined with respect to cancer susceptibility, even when the same combination of enzymes and sites of cancers are compared (Kiyohara *et al.*, 2002b). There are many race-related differences in genetic polymorphisms of drug-metabolizing enzymes in humans, and these phenomena may explain in part why such different results were obtained (Bartsch *et al.*, 2000; Inoue *et al.*, 2000; Williams & Phillips, 2000; Kiyohara

Table 4.3. Genetic polymorphisms in polycyclic aromatic hydrocarbon-metabolizing enzymes and susceptible subpopulations

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
AKR1C3	Aldo-keto reductase 1C3; dihydrodiol dehydrogenase	*AKR1C3*Gln5His*	Residents in Xuan Wei, China, exposed to smoky coal (113 lung cancer cases/119 controls)	*AKR1C3*Gln/Gln* genotype [1.84-fold risk for lung cancer; 95% CI, 0.98–3.45]	Lan *et al.* (2004)
SULT1A1[a]	Phenol sulfotransferase	*SULT1A1*2*	Ethnically homogeneous Han Chinese exposed to indoor cooking and tobacco smoke (805 lung cancer cases/809 controls)	*SULT1A1*Arg/His*+ *SULT1A1*His/His*, genotypes [1.85-fold risk for lung cancer; 95% CI, 1.44–2.37]	Liang *et al.* (2004)
		*SULT1A1*2*	Caucasians with lung cancer versus matched controls exposed to tobacco smoke (463 lung cancer cases/485 controls)	*SULT1A1*Arg/His*+ *SULT1A1*His/His*, genotypes [1.41-fold risk for lung cancer; 95% CI, 1.04–1.91]	Wang *et al.* (2002)
		*SULT1A1*2*	Population in Brescia, Italy, exposed to tobacco smoke (201 bladder cancer cases/214 controls)	*SULT1A1*Arg/His*+ *SULT1A1*His/His*, genotypes [0.67-fold risk for bladder cancer; 95% CI, 0.45–1.03]	Hung *et al.* (2004)
		*SULT1A1*1* (fast sulfonation)	Patients from 8 Dutch hospitals exposed to tobacco smoke (431 colorectal cancer cases/432 controls)	*SULT1A1(*1/*1)* (synonymous) combination within smoking for more than 25 years doubled risk for colorectal adenomas [4.32; 95% CI, 1.59–11.77 for more than 25 years smoking compared with [1.64; 95% CI, 0.69–3.90 for never smokers]	Tiemersa *et al.* (2004)
UGT1A1	UDP-glucuronosyltransferase	*UGT1A1*28* (non-coding)	Population in Shanghaî, China (1047 breast cancer cases/1083 controls)	*UGT1A1*28* [1.7; 95% CI, 1.0–2.7]	Adegoke *et al*. (2004)

Table 4.3 (contd)

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
NQO1	NAD(P)H quinone oxido-reductase 1	*NQO1*2*	Patients from bronchoscopy clinics in the UK (82 lung cancer cases/145 controls)	*NQO1**2 (heterozygote); one variant allele gave 4-fold increase of small-cell lung cancer [3.80; 95% CI, 1.19–12.1]	Lewis *et al.* (2001)
		*NQO1*2/*2*	Lung cancer patients in Nanjing, China, and Chinese controls in Sweden (84 lung cancer cases/84 controls)	No association with lung cancer	Yin *et al.* (2001)
		*NQO1*2/*2*	Non-Hispanic white colorectal cancer patients (725 colorectal cancer cases/729 controls)	*NQO1*2/**2 with variant *CYP1A1* increased risk for adenoma in smokers [17.4; 95% CI, 7.8–79.8]	Hou *et al.* (2005)
		*NQO1*2/*2*	Lung cancer patients and controls from Denmark (265 lung cancer patients/272 controls)	No association with lung cancer	Sorensen *et al.* (2005)
		*NQO1*2*	Lung cancer patients and controls from Sweden (524 lung cancer patients/530 controls)	With variant *CYP1A1*, in smokers, increased risk of squamous cell carcinoma [3.54; 95% CI, 0.88–14.3]	Alexandrie *et al.* (2004)
CYP1A1	Cytochrome P450 1A1	*CYP1A1*2A* *CYP1A1*2C*	Monozygous rare allele in Asians	Increased risk for lung cancer for patients with genotype C [odds ratio, 7.31]	Kawajri & Fujii-Kuriyama (1991)
		*CYP1A1*3*	African–Americans; 15–20% frequency	Lung adenocarcinoma	Taîolì *et al.* (1995a)
CYP1B1	Cytochrome P450 1B1	*CYP1B1*2*	Japanese and Caucasian populations (336 breast cancer cases/330 lung cancer cases/324 controls)	Increased susceptibility to breast and lung carcinoma with a higher penetrance in Caucasians	Watanabe *et al.* (2000)

Table 4.3 (contd)

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
EH	Epoxide hydrolase	*EH3* *EH4*	French Caucasian population (150 lung cancer cases/172 controls)	*EH3 His/His* genotype; decreased risk for lung cancer	Benhamou *et al*. (1998)
			Meta-analysis (2078 lung cancer cases/3081 controls)	*EH3 His/His* genotype; decreased risk for lung cancer [0.98; 95% CI, 0.72–1.35] *EH4 Arg/Arg* genotype; no risk for lung cancer [1.00; 95% CI, 0.71–1.41]	Lee *et al*. (2002)
			Pooled analysis (986 lung cancer cases/1633 controls)	*EH3 His/His* genotype; decreased risk for lung cancer [0.70; 95% CI, 0.51–0.96]	
			Lung cancer patients and controls from Austria (277 lung cancer patients/496 controls)	*EH3 His/His* genotype; decreased risk for lung cancer [0.38; 95% CI, 0.20–0.75]	Gsur *et al*. (2003)
			Caucasian with breast cancer versus matched controls (267 breast cancer cases/293 controls	No association with breast cancer	de Assis *et al*. (2002)
			Caucasian patients with lung cancer (182 lung cancer patients/365 controls)	Increased risk for lung cancer [2.3; 95% CI, 1.2–4.3]	Park, J.Y. *et al*. (2005)
			Patients with advanced adenoma in the distal colon (772 colon cancer patients/777 controls)	Increased risk for colorectal adenoma $_{113}$*Tyr/Tyr* genotype [1.5; 95% CI, 1.0–2.2]; $_{139}$*Arg/Arg* genotype [1.4; 95% CI, 0.8–2.5]	Huang *et al*. (2005)

Table 4.3 (contd)

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
GST	Glutathione *S*-transferase	*GSTM1* *GSTP1* *GSTT1*	Patients with breast cancer (2048 breast cancer cases/1969 controls)	No risk for breast cancer: [0.98; 95% CI, 0.86–1.12] with the *GSTM1* null; [1.01; 95% CI, 0.79–1.28] with *GSTP1* heterozygous mutants; [0.93; 95% CI, 0.62–1.38] with *GSTP1* homozygous mutants	Vogl *et al*. (2004)
		GSTM1	Patients with epithelial ovarian cancer (285 ovarian cancer cases/299 controls)	*GSTM1* null genotype; increased risk of ovarian cancer [2.04; 95% CI, 1.01–4.09]	Spurdle *et al*. (2001)
			Patients with prostate cancer (206 prostate cancer cases/194 controls)	*GSTM1* null genotype; decreased risk of prostate cancer [0.64; 95% CI, 0.43–0.95]	Kidd *et al*. (2003)

[a]*SULT1A1*1*, name of gene for which the nucleotide in original coding sequence is synonymous; *SULT1A1*2*, name of gene for which the nucleotide in the original sequence is non-synonymous; *SULT1A1*2/*2*, name of gene for which the nucleotide in the original sequence and its allele are non-synonymous.
Note: Susceptibilities due to multi-gene polymorphisms were not considered.
CI, confidence interval; NAD(P)H, nicotinamide adenine dinucleotide phosphaste; UDP, uridine 5′-diphosphate

et al., 2002b). Other factors such as age, sex, race, tobacco smoking, alcohol drinking and several environmental factors that modulate (induce or inhibit) drug-metabolizing activities affect the differences in individual susceptibilities towards cancer (Daly, 2003) and indicate that complex interactions occur between multiple genes and the environment.

Individuals have different and unique combinations of drug-metabolizing enzymes in their organs (Nebert *et al.*, 1999; Daly, 2003). For example, some people have enzymes that are defective for the activation of PAHs and normal enzymes that detoxicate PAHs, whereas others have normal enzymes that activate PAHs and enzymes that are defective for the detoxication of reactive PAH metabolites (Garte *et al.*, 2001; Murata *et al.*, 2001; Yin *et al.*, 2001; Kiyohara *et al.*, 2002a; Hung *et al.*, 2004; Lodovici *et al.*, 2004; Masson *et al.*, 2005). In these cases, it is difficult to interpret from epidemiological studies which polymorphisms of drug-metabolizing enzymes are involved in susceptibility to human cancer. In many cases, individuals have different combinations of polymorphic enzymes that activate and/or detoxicate PAHs and metabolites (Le Marchand *et al.*, 1998; Quiñones *et al.*, 1999; Grzybowska *et al.*, 2000; Nerurkar *et al.*, 2000; Olshan *et al.*, 2000; Kiyohara *et al.*, 2002b; Hashibe *et al.*, 2003; Sarmanova *et al.*, 2004; Masson *et al.*, 2005).

(b) Polymorphism of CYP1A1

CYP1A1 is mainly expressed in extrahepatic organs and participates in the activation of carcinogenic PAHs including benzo[*a*]pyrene (Pelkonen & Nebert, 1982; Kawajiri & Fujii-Kuriyama, 1991; Shimada *et al.*, 1992).

Of 11 alleles of *CYP1A1* genetic polymorphisms reported to date (see http://www.imm.ki.se/CYPalleles), four types of genetic polymorphism of *CYP1A1* have been reported to be related to susceptibility to cancer (Kawajiri & Fujii-Kuriyama, 1991). Two genetically linked polymorphisms of the *CYP1A1* gene — the *Msp*I polymorphism (*CYP1A1*2A*) located in the 3'-flanking region and the *Ile-Val* polymorphism (*CYP1A1*2C*) at amino acid residue 462 in the haeme-binding region — were first reported to be associated with susceptibility to tobacco smoking-associated squamous-cell carcinoma of the lung in Japanese populations (Hayashi *et al.*, 1991; Nakachi *et al.*, 1993; Kawajiri *et al.*, 1996). Three genotypes of the *Msp*I polymorphism were found, namely genotype A (dominant homozygous allele), genotype B (heterozygote) and genotype C (homozygous rare allele). Type C was found at high levels among lung cancer patients in Japanese and Asian populations, but not among those in non-Asian populations (Kawajiri & Fujii-Kuriyama, 1991; Kiyohara *et al.*, 2002b). A lower incidence of *CYP1A1* variants in the latter populations has been reported (London *et al.*, 1995; Ishibe *et al.*, 1998; London *et al.*, 2000).

A *CYP1A1*3* polymorphism has been reported, with a single base change (adenine to guanine) in the 3′-noncoding region (Crofts *et al.*, 1994; London *et al.*, 1995; Taioli *et al.*, 1995a; Kiyohara *et al.*, 2002a). No linkage between *CYP1A1*3* and either *CYP1A1*2A* (*Msp*I) or *CYP1A1*2C* (*Ile-Val*) polymorphisms was observed (London *et al.*, 2000). This race-specific polymorphism in African–Americans, with frequencies of 15–20% for

*CYP1A1*2*, is associated with an increased risk for adenocarcinoma (Taioli *et al.*, 1995a; Kiyohara *et al.*, 2002b), although contrasting results have been obtained (Taioli *et al.*, 1995a; Kiyohara *et al.*, 2002b; Nagata & Yamazoe, 2002; Daly, 2003). Rare cases of this polymorphism have been reported in Japanese and Caucasians (Inoue *et al.*, 2000; Kiyohara *et al.*, 2002b). The *CYP1A1*5* polymorphism (*Thr461Asn*) is reported to be unrelated to susceptibility to lung cancer (Cascorbi *et al.*, 1996).

Race-related differences in several types of polymorphism in the *CYP1A1* gene have been reported (Hayashi *et al.*, 1991; Kelsey *et al.*, 1994; Taioli *et al.*, 1995b; Inoue *et al.*, 2000). Inoue *et al*. (2000) showed that the occurrence of *Msp*I (*CYP1A1*1* polymorphism) and *Ile-Val* (*CYP1A1*2* polymorphism) types of *CYP1A1* genetic polymorphism was more frequent in Japanese populations than in Caucasians. No cases of *CYP1A1*4* polymorphism were found in Japanese populations, whereas two of 45 Caucasians had a heterozygous m4-mutant (C4887A at exon 7) (Inoue *et al.*, 2000). The *CYP1A1*3*-type polymorphism, a type specific for black Africans, was not detected in either Japanese or Caucasian populations (Crofts *et al.*, 1993; Cascorbi *et al.*, 1996; Aynacioglu *et al.*, 1998; Inoue *et al.*, 2000).

(c) Polymorphism of CYP1B1

Among 26 CYP1B1 alleles determined (see http://www.imm.ki.se/CYPalleles), different *CYP1B1* polymorphisms with amino acid changes at *Arg48Gly*, *Ala119Ser*, *Leu432Val*, *Ala443Gly* and *Asn453Ser* have been studied in relation to susceptibility to cancer (Stoilov *et al.*, 1997; Bailey *et al.*, 1998; Fritsche *et al.*, 1999; Bejjani *et al.*, 2000; Watanabe *et al.*, 2000; Ko *et al.*, 2001; Aklillu *et al.*, 2002; Sørensen *et al.*, 2005). Several combinations of these polymorphisms lead to various halotypes — *CYP1B1*2* (*Arg48Gly*, *Ala119Ser*), **3* (*Leu432Val*), **4* (*Asn453Ser*), **5* (*Arg48Gly*, *Leu432Val*), **6* (*Arg48Gly, Ala119Ser, Leu432Val*) and **7* (*Arg48Gly, Ala119Ser, Leu432Val*, *Ala443Gly*) (Aklillu *et al.*, 2002; Nagata & Yamazoe, 2002).

Watanabe *et al*. (2000) studied the frequency of *CYP1B1*2* (*Ala-Ser*) and *CYP1B1*3* (*Leu-Val*) polymorphisms in 336 breast and 330 lung cancer patients and 324 randomly selected healthy controls in a Japanese population. Their results showed that the former polymorphism is associated with genetically determined susceptibility to cancer of the breast or squamous-cell carcinoma of the lung but that the *Leu-Val* polymorphism did not show any association with the cancers.

Several studies have reported the relationship between *CYP1B1* polymorphisms and risk for breast cancer (Zheng *et al.*, 2000; Rylander-Rudqvist *et al.*, 2003), squamous-cell neck cancer (Ko *et al.*, 2001) and lung cancer (in combination with *GST1*, *NQO1* and *NAT2*) (Inoue *et al.*, 2000; Kiyohara *et al.*, 2002b; Sørensen *et al.*, 2005) in various ethnic populations. Rylander-Rudqvist *et al*. (2003) found no association between *CYP1B1*1*, **2*, **3* or **4* alleles and risk for breast cancer in a study of 1521 cases and 1498 controls. No association of polymorphisms of *CYP1B1* or other genes (including *GST*, *NQO1* and *NAT 2*) and risk for lung cancer was reported (Sørensen *et al.*, 2005).

Inoue *et al*. (2000) showed that the incidence of the genetic polymorphism in the *CYP1B1* gene is more frequent in Caucasians than in Japanese populations. The frequency of the *CYP1B1*2*- and *CYP1B1*3*-type polymorphisms was expressed by 28.9% and 37.5%, respectively, in Caucasian and by 14.1% and 21.8%, respectively, in Japanese. No *CYP1B1*4*-type polymorphisms were detected in the Japanese population, whereas 24% of Caucasians had this allele mutant (Inoue *et al*., 2000).

(d) CYP1A2, CYP2C9 and 3A4

CYP1A2 is mainly expressed in the liver (Guengerich & Shimada, 1991; Shimada *et al.*, 1994). This is a major enzyme involved in the activation of aryl- and heterocyclic amines to carcinogenic products (Shimada *et al.*, 1989a,b; Shimada & Guengerich, 1991; Lang *et al.*, 1994). CYP1A2 also catalyses the oxidation of PAHs, although usually at much slower rates than CYP1A1 and 1B1 (Shimada *et al.*, 1989b; Shimada & Guengerich, 1991; Shimada *et al.*, 2001a).

Little is known about the relationships between *CYP1A2* polymorphisms and susceptibility to cancer (Chida *et al.*, 1999; Huang *et al.*, 1999; Aklillu *et al.*, 2003; Murayama *et al.*, 2004; Li *et al.*, 2006).

Other CYP enzymes, including CYP2C9 and 3A4, have been shown to play a role in the metabolism of PAHs, particularly in the liver where these enzymes are expressed at high levels (Yun *et al.*, 1992; Shimada *et al.*, 2001a). The implications of polymorphisms of these CYPs in susceptibility to cancer in humans are not clear at present (Garcia-Martin *et al.*, 2002a,b; Plummer *et al.*, 2003).

(e) Epoxide hydrolase

Microsomal epoxide hydrolase (mEH) plays two roles in the metabolism of PAHs: one is detoxication of various reactive PAH metabolites and the other is activation of PAH epoxides to form proximate PAH diols that are finally activated to ultimate carcinogenic metabolites by CYP (Jerina & Dansette, 1977; Lu & West, 1979; Pelkonen & Nebert, 1982; Hassett *et al.*, 1994; Hecht, 2002b).

Two genetic polymorphisms, a *Tyr113His* substitution in exon 3 (*EH3*) and a *His139Arg* substitution in exon 4 (*EH4*), have been reported in relation to susceptibility to cancer (Omiecinski *et al.*, 2000; Kiyohara *et al.*, 2002b; Lee *et al.*, 2002; Park, J.Y. *et al.*, 2005). The exon 3 polymorphism of the *mEH* gene has been reported to be associated with a significantly decreased risk for lung cancer (Benhamou *et al.*, 1998; Lee *et al.*, 2002; Gsur *et al.*, 2003). However, there was no significant association of *mEH* polymorphisms with susceptibility to breast cancer in Caucasian populations (de Assis *et al.*, 2002). A significant increase in risk for lung cancer has been reported with the exon 4 genotype (Park, J.Y. *et al.*, 2005). Reports also indicate that the polymorphisms of *mEH* at exon 3 and 4 are associated with colorectal adenoma (Huang *et al.*, 2005).

(f) Glutathione S-*transferase*

The role of genetic polymorphisms of *GST* genes in influencing susceptibility to cancer is complicated (Strange *et al.*, 2001). GST enzymes participate in the detoxication of reactive metabolites of PAHs and other xenobiotics (Strange *et al.*, 2001). Three types of genetic polymorphism of *GSTM1*, *T1* and *P1* have been examined in relation to susceptibility to cancer in humans (Strange *et al.*, 2001; Wang *et al.*, 2003). A large study with 1969 controls and 2048 cases of breast cancer showed that none of the single gene *GST* polymorphisms (e.g. *GSTM1*, *T1* or *P1*) confer a substantial risk for breast cancer to its carriers (Vogl *et al.*, 2004), although several studies have reported that one or two alleles of *GST* polymorphisms are related to susceptibility to cancer at other sites (Spurdle *et al.*, 2001). Deletion of *GSTM1* may result in an increase in the incidence of ovarian cancer (Spurdle *et al.*, 2001), a decrease in the incidence of prostate cancer (Kidd *et al.*, 2003) and enhance susceptibility to cancer induced by air pollution from indoor coal combustion emissions (Lan *et al.*, 2000).

(g) Sulfotransferase

SULTs play an important role in the conjugation of many endogenous and exogenous substrates (Raftogianis *et al.*, 1999; Wang *et al.*, 2002; Daly, 2003). Relationships between the *SULT1A1* polymorphism (*Arg213His*) and susceptibility to cancer have been reported (Raftogianis *et al.*, 1999; Moreno *et al.*, 2005). The association between this single-nucleotide polymorphism (G→A) and risk for lung cancer was assessed in 805 patients with lung cancer and 809 controls in a hospital-based case–control study in the Chinese Han population. Compared with the *GG* genotype, the variant *SULT1A1* genotype (*638GA* or *AA*) was associated with a significantly increased overall risk for lung cancer [odds ratio, 1.85; 95% CI, 1.44–2.37] (Liang *et al*., 2004). Stratification analysis showed that the increased risk for lung cancer related to the variant *SULT1A1* genotypes was more pronounced in younger subjects and limited to smokers rather than nonsmokers. The risk for lung cancer for the variant genotypes was increased significantly with cumulative dose of smoking (Liang *et al*., 2004). In a second study, the association between the variant A-allele and lung cancer was examined in 463 Caucasian lung cancer cases and 485 frequency-matched Caucasian controls (Wang *et al*., 2002). It was found that there was an overall significant difference between cases and controls when adjusted by sex and smoking status (adjusted odds ratio, 1.41; 95% CI, 1.04–1.91) and was higher in women (adjusted odds ratio, 1.64; 95% CI, 1.06–2.56) than in men (adjusted odds ratio, 1.23; 95% CI, 0.80–1.88). In both these studies, the *SULT1A1*2* (low sulfonation phenotype) was associated with an increased risk which indicated the role of this enzyme in detoxication. In a third study, the *SULT1A1*1* synonymous single-nucleotide polymorphism (fast sulfonation phenotype) was modestly associated with an increased risk for colorectal carcinoma in smokers, which was increased in the presence of the *NAT2* slow-acetylator phenotype which indicated that this enzyme can also lead to carcinogen activation (Tiemersma *et al*., 2004). In another study, the association of

polymorphisms in *GSTM1, GSTT1, NAT, SULT1A1* and *CYP1B1*with tobacco smoking and the incidence of urinary bladder cancer in Bresica, northern Italy, was examined. The *SULT1A1*2* (low sulfonation phenotype) showed a modest protective effect consistent with a role in aromatic amine activation (Hung *et al*., 2004) .

(h) UDP-Glucuronosyltransferases

Of the hepatic UGTs, only UGT1A1 and UGT1A9 exhibit activity against the 7*R*,8*R-trans*-dihydrodiol of benzo[*a*]pyrene, the precursor to the highly mutagenic (+)-*anti*-benzo[*a*]pyrene diol epoxide (Fang *et al*., 2002; Fang & Lazarus, 2004). The *UGT1A1*28* allelic variant contains an additional (TA) dinucleotide repeat in the 'TATTA' box [(TA)6 >(TA)7] of the UGT1A1 promoter which has been linked to decreased expression of the *UGT1A1* gene. Significant decreases in UGT1A1 protein and bilirubin-conjugating enzyme activity were observed in normal liver microsomes from subjects with the homozygous polymorphic *UGT1A1(*28/*28)* genotype variant compared with subjects homozygous for the wild-type *UGT1A1*1* allele. Significant decreases in the glucuronidation of the 7*R*,8*R-trans*-dihydrodiol of benzo[*a*]pyrene were observed in subjects who had the *UGT1A1 (*28/*28*) genotype compared with subjects who had a wild-type *UGT1A1 (*1/*1)* genotype (Fang & Lazarus, 2004). An association of this genotype with an increased risk for cancer induced by benzo[*a*]pyrene was suggested.

(i) *Aldo-keto* reductase

The genotic variability of AKR had been presented previously (see section 4.1.2b). The association between *AKR1C3*Gln5H* is single-nucleotide polymorphism and risk for lung cancer was assessed in a population-based case–control study of 119 cases and 113 controls in Xuan Wei, China, an area that has one of the highest rates of lung cancer in China due to the use of smoky coal for heating and cooking. The *AKR1C3*Gln/Gln* genotype was associated with a 1.84 fold [95% CI, 0.98–3.45] increased risk for lung cancer (Lan *et al*., 2004).

(ii) *NAD(P)H quinone oxidoreductase 1*

The homozygous *NQO1*2/*2* allele is essentially a null phenotype and provides a tool to assess the potential chemoprotective role of the enzyme against xenobiotics (Ross *et al*., 2000). Only one study has been performed on the association of the *NQO1*2/*2* allele with the incidence of lung cancer (Lewis *et al*., 2001). The *NQO1* genotype was related to risk for lung cancer in patients who attended a Manchester (United Kingdom) bronchoscopy clinic; those who carried at least one variant allele were found to have an almost fourfold increased risk for developing small-cell lung cancer (adjusted odds ratio, 3.80; 95% CI, 1.91–12.1). No association between *NQO1* genotypes and risk for non-small-cell lung cancer was found. Furthermore, the excess risk for small-cell lung cancer associated with non-wild-type *NQO1* genotypes was only apparent in heavy smokers who had a >10-fold increased risk (adjusted odds ratio, 12.5; 95% CI, 2.1–75.5). These results

suggest that *NQO1* is involved in the detoxication of those carcinogens that are associated with the development of small-cell lung carcinoma (Lewis *et al*., 2001).

In other studies aimed at identifying susceptible populations, genetic polymorphisms in *NQO1* were scored together with those in other enzymes that affect PAH metabolism/activation. In one study, the association of the *NQO1*2/*2*, *CYP1A1*2A*, *HYL1*2* (epoxide hydrolase) and *HYL1*3* alleles were not associated with the risk for lung cancer in Najing Province in China (Yin *et al*., 2001). However, when the data were stratified, it was found that smokers carrying the *HYL1** allele had a higher relative risk for lung cancer. In a second study, the association of cigarette smoking with the risk for advanced colorectal adenoma in relation to *CYP1A1 Val462* and *NQO1*2/*2* polymorphic variants was investigated in 725 non-Hispanic Caucasian cases with advanced colorectal adenoma of the distal colon and 729 gender- and ethnicity-matched controls. Subjects who carried either allele had a weakly associated risk for colorectal adenoma; however, those who carried both alleles had an increased risk versus nonsmokers, particularly among recent and current smokers (odds ratio, 17.4; 95% CI, 3.8–79.8; p for interaction = 0.02) (Hou *et al*., 2005). A population-based cohort study investigated the occurrence of lung cancer in relation to polymorphisms in *CYP1B1*, *GSTA1*, *NQO1* and *NAT2*. Among 54 200 Scandinavian cohort members, 265 lung cancer cases were identified and a subcohort that comprised 272 individuals was used for comparison. No overall associations were found between the polymorphisms and risk for lung cancer. The *NAT2* fast-acetylator genotype seemed to be protective against lung cancer in light smokers but not in heavy smokers (>20 cigarettes/day) (Sørenson *et al*., 2005). In a fourth study, the modifying influence of *CYP1A1*, *GSTM1*, *GSST1* and *NQO1* polymorphisms on risk for lung cancer was studied in 524 lung cancer cases and 530 control subjects. No evidence of an influence of genetic polymorphisms on risk for lung cancer was found. In smokers, however, there was a suggestion that the variant *CYP1A1 Val462* and *NQO1**2 genotypes may confer an increased risk for squamous-cell carcinoma (Alexandrie *et al*., 2004).

4.3.2 *Age-related susceptibility*

No data were available in humans on age-dependent susceptibility to cancer that results from exposure to PAHs.

Studies in animals indicate that exposure early in life to individual PAHs, including benzo[*a*]pyrene and DMBA, may lead to higher rates of tumour formation later in life. Vesselinovitch *et al*. (1975) showed that a significantly higher incidence of liver hepatomas was observed in male $B6C3F_1$ mice that were treated intraperitoneally with 75 and 150 μg/g bw benzo[*a*]pyrene on postnatal days 1, 15 or 42. Lung tumours were more prevalent in male and female $B6C3F_1$ mice treated with benzo[*a*]pyrene at birth than in animals treated at 15 or 42 days of age. Similar results were found for the incidence of liver tumours in $C3AF_1$ mice under the same experimental conditions. Walters (1966) observed a significantly higher incidence of lung tumours when 15 μg

DMBA were administered subcutaneously to newborn (1-day-old) BALB/c mice compared with suckling (2–3 weeks of age) and adult mice. Pietra *et al*. (1961) observed an increased incidence of lymphomas and lung tumours when 30–900 μg DMBA were administered subcutaneously to newborn mice compared with adult (8-week-old) mice.

The age-dependent susceptibility to benzo[*a*]pyrene and DMBA may be dependent on their ability to act as mutagens. An analysis by the Environmental Protection Agency (2005) indicated that there can be greater susceptibility for the development of tumours as a result of exposures to mutagenic chemicals early in life compared with later stages of life (see also Barton *et al*., 2005). In general, there are limited data to elucidate the mode(s) of action that leads to differences in tumour incidence following exposure early in life or later. Differences in the capacity to metabolize and clear chemicals at different ages can result in larger or smaller internal doses of the active agent(s), which either increases or decreases risk (Ginsberg *et al*., 2002). Several studies have shown increased susceptibility of weanling animals to the formation of DNA adducts following exposure to vinyl chloride (Laib *et al*., 1989; Morinello *et al*., 2002a,b), and in-vivo transplacental micronucleus assays have indicated that fetal tissues are more sensitive than maternal tissues to the induction of micronuclei by mutagenic chemicals (Hayashi *et al*., 2000).

As outlined by the Environmental Protection Agency (2005), some generalized aspects that potentially lead to childhood susceptibility may apply to benzo[*a*]pyrene and other PAHs that act through a mutagenic mechanism. (*i*) More frequent cell division during development can result in enhanced fixation of mutations due to the reduced time available for repair of DNA lesions; clonal expansion of mutant cells produces a larger population of mutants (Slikker *et al*., 2004). (*ii*) Some embryonic cells, such as brain cells, lack key DNA repair enzymes. (*iii*) Some components of the immune system are not fully functional during development (Holladay & Smialowicz, 2000; Holsapple *et al*., 2003). (*iv*) Hormonal systems operate at different levels during different stages of life (Finch & Rose, 1995). (*v*) Induction of developmental abnormalities can result in a predisposition to carcinogenic effects later in life (Birnbaum & Fenton, 2003).

Studies on the carcinogenicity of DMBA indicate a potential period of increased susceptibility during pubertal periods of tissue development. An increased incidence of mammary tumours was found in 5–8-week-old rats treated with DMBA compared with older or younger rats (Meranze *et al*., 1969; Russo *et al*., 1979). This observation corresponds with pubertal development of the mammary tissue, ovarian function that begins between 3 and 4 weeks of age and mammary ductal growth and branching that occur by approximately week 5 of age, all of which are consistent with the 5–8-week susceptible period (Silberstein, 2001).

Several carcinogenicity studies have been conducted on benzo[*a*]pyrene or coal-tar mixtures following neonatal exposure without an adult comparison or in in-utero mouse models. Strain A and C57BL mice were administered single subcutaneous injections of 4 or 6 mg benzo[*a*]pyrene on gestational days 18 and 19 (Nikonova, 1977). The offspring of strain A mice developed lung tumours at a much higher incidence than those of C57BL mice. The incidence of liver tumours was increased above controls only in C57BL

offspring. Direct intraperitoneal administration of 560 nmol benzo[*a*]pyrene in DMSO to neonatal CD-1 mice within 24 h of birth and at 8 and 15 days of age resulted in an increased incidence of liver tumours in males (Wislocki *et al*., 1986), but no liver tumour formation in female mice. This treatment induced a significantly increased incidence of lung tumours in male and female mice.

Groups of 15-day-old male and female B6C3F$_1$ mice received a single intraperitoneal injection of 0.125, 0.25 or 0.375 μg benzo[*a*]pyrene or 7980 μg manufactured gas plant residue in corn oil. Twenty-six weeks after exposure, benzo[*a*]pyrene induced liver tumours in male mice in a dose- and time-dependent manner in the high-dose group. Manufactured gas plant residue induced liver tumours in male mice to a much greater extent than benzo[*a*]pyrene. There was little or no tumour formation in the lung or forestomach with either treatment (Rodriguez *et al*., 1997), using ^{32}P-postlabelling, positive responses for DNA adduct formation were observed in the liver, lung and forestomach of both sexes of mice. However, differences in the time course of adduct accumulation and decline occurred. For example, liver adducts peaked at 24 h and declined over the next 14 days, while forestomach and lung adducts peaked at higher levels at 2–3 days after exposure, then also declined over the next 14 days. Despite the difference in tumorigenicity, adduct levels were similar in male and female mice in all three tissues examined. They indicated that the inability of benzo[*a*]pyrene to induce tumour formation in the lung and forestomach of this strain of mice and in the liver of female animals could not be accounted for in terms of the lack of DNA damage as measured by adduct formation. The authors indicated that the exposure of 15-day-old B6C3F$_1$ mice to low levels of benzo[*a*]pyrene induced well-defined premalignant changes in the liver and hepatocellular carcinomas by 40 weeks after exposure, while single doses of the same carcinogen at 42 days of age did not induce lesions over 2 years after exposure.

Numerous animal studies have been conducted that indicate effects on reproductive and developmental end-points following exposure to mixtures of PAHs and individual PAHs (ATSDR, 1995; IPCS, 1998; see also Section 4.4). These studies suggest susceptibility to the toxicity of PAHs during the prenatal period, a critical time for phase I and II enzyme development. Lack of phase I enzymes may result in protection against the toxicity of benzo[*a*]pyrene, while the lack of phase II enzymes in the presence of phase I enzymes could exacerbate toxicity (see Mukhtar & Bresnick, 1976). Animals as young as 1 day of age can metabolize benzo[*a*]pyrene to a broad spectrum of metabolites (Melikian *et al*., 1989), which allows the possible conclusion that their overall metabolism might not differ significantly from that of adults. These animals have metabolic competence to enable phase I and phase II metabolism (Melikian *et al.*, 1989), although activities may differ between newborn and adult animals.

Lyte and Bick (1985) evaluated the effects of benzo[*a*]pyrene on the generation of antibody-producing cells in young (3–6 months), middle-aged (13–16 months) and old (23–26 months) male mice. Old animals were particularly susceptible to the immunosuppressive effects of benzo[*a*]pyrene.

4.4 Other toxicity

4.4.1 *Phototoxicity*

Sunlight is a complete carcinogen (IARC, 1992) and is responsible for the induction of cutaneous squamous-cell and basal-cell carcinomas in humans. Since PAHs are ubiquitous in the environment and concomitant exposure of humans to PAHs and light is inevitable, the photomutagenicity of these compounds is of considerable importance to human health. Human exposure to PAHs occurs mainly through skin absorption, inhalation or food consumption (IARC, 1983). PAH-contaminated skin may be exposed to sunlight, which is of concern for people who work outdoors and handle products that contain PAHs, such as roofers, tanners and road-construction workers. Because of the multiple aromatic ring systems in PAHs, these compounds can absorb light energy in the UVA region (320–400 nm) and, for some PAHs, also near the visible region (400–700 nm) (Dabestani & Ivanov, 1999) to form reactive species that cause damage to human cellular components. It has been shown that concomitant exposure to PAHs and light can cause DNA single-strand breaks, oxidation of DNA bases and formation of DNA adducts (Yu, 2002). In general, for those PAHs or mixtures of PAHs that exhibit carcinogenic activity in skin, these effects were found to be enhanced when the compounds were tested in combination with UV radiation under certain experimental conditions (Santamaria *et al*., 1966).

From a mechanistic point of view, two pathways result in phototoxicity: (i) dynamic phototoxicity, which involves damage to cells during phototransformation of chemical species. This process includes excited-state energy transfer to biological macromolecules, which results in electron transfer that may convert both the PAH and the biological molecule into free radicals. It also leads to the production of short-lived reactive intermediates such as singlet oxygen, the superoxide free radical and other chemically reactive species (Yu, 2002); and (ii) formation of toxic photoproducts: during photolysis, some relatively light-stable compounds such as quinones and nitro-PAHs are produced, which may be toxic both in the presence and absence of metabolic activation or light-induced activation (Sinha & Chignell, 1983).

Irradiation with UV light (>295 nm) of a mixture of the PAH anthracene and human serum albumin induced covalent binding of anthracene to the protein, which then resulted in the formation of protein–protein cross-links and cell-membrane damage via lipid peroxidation (Sinha & Chignell, 1983). Evidence of damage to cell membranes following irradiation (near UV, 290–400 nm) of benzo[*a*]pyrene, anthracene or 1-nitropyrene mixed with cells has also been reported (Kagan *et al.*, 1989; Tuveson *et al*., 1990; Zeng *et al.*, 2002, 2004). Possible DNA damage induced by the combination of light and a photoreactive chemical includes: (i) single-strand breaks, (ii) double-strand breaks, (iii) release of DNAbases (depurination/depyrimidination), (iv) oxidation of guanine to 8-hydroxy- or 8-oxoguanine, (v) induction of covalent DNA adducts, (vi) induction of DNA–DNA cross-links and (vii) induction of DNA–protein cross-links (Yu, 2002). Most of these forms of damage can be repaired without leading to mutation. To date, several

types of DNA damage caused by the combination of PAHs and light have been studied: the formation of PAH–DNA covalent adducts, DNA single-strand breaks, depurination/depyrimidination and the formation of the oxidative product, 8-hydroxyguanine.

Studies on the photomutagenicity of PAHs (see Table 4.4) indicate that these compounds can be activated by light in the absence of metabolizing enzymes. In particular, six of 16 PAHs tested, including those that are most abundant in the environment — acenaphthene, acenaphthylene, anthracene, benzo[*ghi*]perylene, fluorene and pyrene — were not mutagenic through metabolic activation but were photomutagenic in *S. typhimurium* TA102 (see references cited in Table 4.4). This result may influence the methods of risk assessment of PAHs. At present, the data are insufficient to interpret the results of in-vitro photomutagenicity studies in terms of human health. However, the possible adverse health effects due to skin contamination with PAHs and concomitant exposure to sunlight deserve further study.

Yan *et al*. (2004) determined the photomutagenicity of 16 PAHs on the Environmental Protection Agency priority-pollutant list in *S. typhimurium* TA102 with concomitant irradiation with UVA and visible light (300–800 nm). Positive photomutagenicity (+) was scored (see Table 4.4) when the number of revertant colonies per plate due to combined exposure to light and a PAH exceeded twice that of the control with light only. Strong photomutagenicity (++) was scored when the number of revertant colonies per nanomole of PAH was higher than 2000. As shown in Table 4.4, 11 of the 16 PAHs tested were found to be photomutagenic. Based on the number of revertants per nanomole of test chemical, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*ghi*]-perylene, indeno[1,2,3-*cd*]pyrene and pyrene were identified as strongly photomutagenic, with a number of revertant colonies per nanomole of PAH in the range of 2000–20 000. The data indicated a fair correlation between observed photomutagenicity and reported carcinogenicity.

Phototoxicity, including photomutagenicity, is closely related to the photochemical reactions that generate reactive PAH intermediates and reactive oxygen species during photolysis (Yu, 2002). Certain PAHs with extended aromatic ring systems can absorb light in the UVA (320–400 nm) and visible (>400 nm) range. Usually, PAHs with three or four aromatic rings absorb UVA light and those with five or more aromatic rings as well as the hydroxyl-, amino- and nitro-substituted PAHs with three or four aromatic rings absorb visible light (Dabestani & Ivanov, 1999). Upon absorption of light energy, PAHs are excited to higher energy states (singlet or triplet) that undergo electron or energy transfer to molecular oxygen, solvents or biological molecules in the cell and generate reactive species. These reactive species or intermediates damage cellular constituents such as the cell membrane, nucleic acids or proteins. This activation pathway is usually similar to the enzymatic activation pathway, in that it converts relatively inert PAHs to reactive species. There is evidence that PAH mixtures and individual PAHs can be phototoxic towards microorganisms, plants, cells and animals. In addition, PAHs are

Table 4.4. Photomutagenicity of 16 polycyclic aromatic hydrocarbons (PAHs) on the priority list of the Environmental Protection Agency and their reported carcinogenicity and mutagenicity

PAH	Photomutagenicity[a]	Carcinogenicity[b]	Mutagenicity[c]
Acenaphthene	+	0	–[d]
Acenaphthylene	+	I	–[d]
Anthracene	++	0	–
Benz[*a*]anthracene	++	++	+
Benzo[*a*]pyrene	++	++	+
Benzo[*b*]fluoranthene	–	++	+[d]
Benzo[*ghi*]perylene	++	I	–[d]
Benzo[*k*]fluoranthene	+	++	+[d]
Chrysene	+	+	+
Dibenz[*a,h*]anthracene	–	++	+
Fluoranthene	–	0	+[d]
Fluorene	+	I	–
Indeno[1,2,3-*cd*]pyrene	++	++[e]	+[d]
Naphthalene	–	I	–
Phenanthrene	–	I	–
Pyrene	++	0	–

Adapted from Yan *et al*. (2004)

[a] PAH is defined as photomutagenic (+ or ++) when the number of revertant colonies due to concomitant exposure to light and the PAH is greater than twice that found in the light-only control. A PAH is defined as strongly photomutagenic (++) if the number of revertant colonies per nanomole of the PAH is more than 2000.

[b] Data summarized from IARC (1983), National Toxicology Program (1993), White (2002) and the Environmental Protection Agency web-site pages for acenaphthylene, naphthalene and fluorene. Carcinogenicity symbols are: (0) no evidence of carcinogenicity; (I) inadequate evidence for evaluation; (+) limited evidence of carcinogenicity in experimental animals; (++) sufficient evidence of carcinogenicity in experimental animals.

[c] Based on McCann *et al*. (1975) using *S. typhimurium* TA98 or TA100 with metabolic activation

[d] Based on Nagai *et al*. (2002) using *S. typhimurium* TA98 with metabolic activation

[e] From the Environmental Protection Agency web site for animal carcinogenicity studies (http://www.epa.gov/NCEA/iris/subst/0457.htm)

generally more toxic when they are exposed to UV light than if they are kept in the dark. For certain PAHs, the increase in toxicity can be six- to sevenfold (Swartz *et al*., 1997).*In vivo*, the formation of covalent PAH–DNA adducts through irradiation with light may compete with enzymatic activation. Indeed, irradiation of benzo[*a*]pyrene or DMBA inside the cells tends to lower the amount of covalent DNA adducts formed by enzymatic activation (Prodi *et al*., 1984), an effect that was mainly attributed to photodegradation of the PAHs.

Photo-oxidation of the unsubstituted PAHs — two-ring naphthalene (Vialaton *et al*., 1999); three-ring anthracene (Mallakin *et al*., 2000) and phenanthrene (Wen *et al*., 2002);

four-ring benz[*a*]anthracene (Dong *et al*., 2002) and pyrene (Sigman *et al*., 1998); and five-ring benzo[*a*]pyrene (Lee-Ruff *et al*., 1988) — can produce respective quinones, ring-open products or hydroxy-substituted products. In addition to quinones, hydroxy- (Koizumi *et al*., 1994), carboxy- (Zeng *et al*., 2002), hydroxymethyl- and formyl-PAHs or methyl-substituted PAHs (Wood *et al*., 1979) are also detected among the photoproducts. Quinones are known sensitizers of reactive oxygen species, but are relatively stable against photo-oxidation. There is no appreciable degradation when 7,12-benz[*a*]-anthracene-quinone is irradiated with UVA light (170 J/cm^2/h) for 60 min. However, during the irradiation, 7,12-benz[*a*]anthracene-quinone can cause DNA strand breaks (Dong *et al*., 2002). Therefore, these quinones are potentially more phototoxic.

Some commercial medicines also contain PAHs. For example, coal tar, a complex mixture of PAHs, is widely used in creams, ointments, lotions and shampoos for the treatment of psoriasis, a common skin disease (Comaish, 1987). The formation of DNA adducts in exposed skin and of chromosomal aberrations in peripheral blood lymphocytes have been shown to occur in psoriasis patients treated with coal tar (Sarto *et al.*, 1989; Schoket *et al.*, 1990; Godschalk *et al*., 1998). Topical application of coal tar to the skin followed by UV radiation, known as the Goeckerman therapy for psoriasis, has been reported to increase the risk for developing skin cancer (Stern *et al.*, 1980), although other studies have found no appreciable increase (Pittelkow *et al*., 1981). UVB radiation and coal tar have an additive effect on the induction of metabolizing enzymes and on the formation of DNA adducts in mouse skin when coal tar is applied before, but not after, irradiation (Mukhtar *et al*., 1986). These effects are difficult to interpret because both UVB and PAHs can activate metabolizing enzymes individually. Since human skin is exposed to light, it is of particular importance and significance to investigate human health risks posed by exposure to the combination of PAHs and light.

4.4.2 *Reproductive and developmental toxicity*

The available studies of human exposure to PAHs suggest that these compounds may affect the developing fetus. These studies have generally focused on the exposure of pregnant women in situations in which PAHs are a known or perceived component of diverse complex mixtures, such as tobacco smoke or ambient air pollution, e.g. in areas of the world that are highly industrialized or burn large amounts of coal (Perera *et al*., 1998, 1999; Šrám *et al*., 1999; Landrigan *et al*., 2004; Perera *et al*., 2004; Wormley *et al*., 2004; Perera *et al*., 2005; Šrám *et al*., 2005; Wolff *et al*., 2005). A lack of information on exposure concentrations, time of exposure during pregnancy and co-exposure to other chemicals potentially confounds the results of these studies. Nevertheless, a pattern of effects has emerged that shows reduced fetal birth weight and length, impaired intrauterine growth and reduced length of gestation.

Studies in experimental animals are available that indicate the reproductive and developmental toxicity of individual PAHs, including anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, chrysene, dibenz[*a*,*h*]anthracene and naphthalene (for reviews, see

ATSDR, 1995; IPCS, 1998). The majority focus on the reproductive and developmental toxicity of benzo[*a*]pyrene. Benzo[*a*]pyrene has been shown to impair male and female reproductive performance and to decrease pup weight in laboratory animals exposed by the oral and inhalation routes. Exposure of pregnant female mice to benzo[*a*]pyrene affected the fertility and the histopathology of the ovaries and testes of their offspring (MacKenzie & Angevine, 1981; Kristensen *et al*., 1995). No standard developmental or two-generation reproductive studies are available on exposure to benzo[*a*]pyrene via the oral route, but several studies have evaluated the reproductive effects of in-utero exposure. The available data show that exposure of pregnant females to benzo[*a*]pyrene affects the fertility of male and female mice exposed *in utero*. Teratogenicity has not been reported based on gross examination, and none of the studies conducted detailed skeletal or visceral examinations of pups. Inhalation studies with benzo[*a*]pyrene in pregnant rats have demonstrated decreased fetal survival, which resulted from a reduction of luteotropic hormone synthesis and release (Archibong *et al*., 2002). Similar studies in male rats showed reduced reproductive capacity due to changes in sperm parameters, impaired epididymal function and disturbed hormonal regulation in the testes (Inyang *et al*., 2003).

4.4.3 *Hepatic and renal toxicity*

The available data on the effects of PAHs other than cancer are sparse except for immunological and reproductive/developmental studies (see Sections 4.2.6 and 4.4.2). No studies were found that examined hepatic or renal toxicity following exposure to PAHs in humans. Numerous animal studies (as reviewed in ATSDR, 1995) have been conducted with individual PAHs, such as benzo[*a*]pyrene, benz[*a*]anthracene, fluoranthene, acenaphthene and fluorene. These studies have shown increases in liver weight but no accompanying overt toxicity. In addition, limited data are available concerning the potential renal toxicity of PAHs (see ATSDR, 1995). Some recent studies indicate that benzo[*a*]pyrene may exhibit limited hepato- and nephrotoxic effects at high doses (De Jong *et al*., 1999; Knuckles *et al*., 2001; Kroese *et al*., 2001).

4.5 References

Adachi, J., Mori, Y., Matsui, S., Takigami, H., Fujino, J., Kitagawa, H., Miller, C.A., III, Kato, T., Saeki, K. & Matsuda, T. (2001) Indirubin and indigo are potent aryl hydrocarbon receptor ligands present in human urine. *J. biol. Chem*., **276**, 31475–31478

Adams, J.D., Jr, Yagi, H., Levin, W. & Jerina, D.M. (1995) Stereo-selectivity and regio-selectivity in the metabolism of 7,8-dihydrobenzo[a]pyrene by cytochrome P450, epoxide hydrolase and hepatic microsomes from 3-methylcholanthrene-treated rats. *Chem.-biol. Interact*., **95**, 57–77

Adegoke, O.J., Shu, X.O., Gao Y.-T., Cai, Q., Breyer, J., Smith, J. & Zheng, W. (2004) Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. *Breast Cancer Res. Treat*., **85**, 239–245

Agarwal, R., Coffing, S.L., Baird, W.M., Kiselyov, A.S., Harvey, R.G. & Dipple, A. (1997) Metabolic activation of benzo[g]chrysene in the human mammary carcinoma cell line MCF-7. *Cancer Res*., **57**, 415–419

van Agen, B., Maas, L.M., Zwingmann, I.H., Van Schooten, F.-J. & Kleinjans, J.C.S. (1997) B[a]P–DNA adduct formation and induction of human epithelial lung cell transformation. *Environ. mol. Mutag*., **30**, 287–292

Aklillu, E., Oscarson, M., Hidestrand, M., Leidvik, B., Otter, C. & Ingelman-Sundberg, M. (2002) Functional analysis of six different polymorphic CYP1B1 enzyme variants found in an Ethiopian population. *Mol. Pharmacol*., **61**, 586–594

Aklillu, E., Carrillo, J.A., Makonnen, E., Hellman, K., Pitarque, M., Bertilsson, L. & Ingelman-Sundberg, M. (2003) Genetic polymorphism of CYP1A2 in Ethiopians affecting induction and expression: Characterization of novel haplotypes with single-nucleotide polymorphisms in intron 1. *Mol. Pharmacol*., **64**, 659–669

Alexandrie, A.K., Nyberg, F., Warholm, M. & Rannug, A. (2004) Influence of CYP1A1, GSTM1, GSTT1, and NQO1 genotypes and cumulative smoking dose on lung cancer risk in a Swedish population. *Cancer Epidemiol. Biomarkers Prev*., **13**, 908–914

Alguacil, J., Porta, M., Kauppinen, T., Malats, N., Kogevinas, M. & Carrato, A. (2003) Occupational exposure to dyes, metals, polycyclic aromatic hydrocarbons and other agents and K-*ras* activation in human exocrine pancreatic cancer. *Int. J. Cancer*, **107**, 635–641

Allan, L.L., Mann, K.K., Matulka, R.A., Ryu, H.Y., Schlezinger, J.J. & Sherr, D.H. (2003) Bone marrow stromal-B cell interactions in polycyclic aromatic hydrocarbon-induced pro/pre-B cell apoptosis. *Toxicol. Sci*., **76**, 357–365

Allorge, D., Chevalier, D., Lo-Guidice, J.M., Cauffiez, C., Suard, F., Baumann, P., Eap, C.B. & Broly, F. (2003) Identification of a novel splice-site mutation in the CYP1A2 gene. *Br. J. clin. Pharmacol*., **56**, 341–344

Alworth, W.L., Viaje, A., Sandoval, A., Warren, B.S. & Slaga, T.J. (1991) Potent inhibitory effects of suicide inhibitors of P450 isozymes on 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene initiated skin tumors. *Carcinogenesis*, **12**, 1209–1215

Amin, S., Juchatz, A., Furuya, K. & Hecht, S.S. (1981) Effects of fluorine substitution on the tumor initiating activity and metabolism of 5-hydroxymethylchrysene, a tumorigenic metabolite of 5-methylchrysene. *Carcinogenesis*, **2**, 1027–1032

Amin, S., LaVoie, E.J. & Hecht, S.S. (1982) Identification of metabolites of benzo[b]fluoranthene. *Carcinogenesis*, **3**, 171–174

Amin, S., Huie, K. & Hecht, S.S. (1985a) Mutagenicity and tumour initiating activity of methylated benzo[*b*]fluoranthenes. *Carcinogenesis*, **6**, 1023–1025

Amin, S., Huie, K., Melikian, A.A., Leszczynska, J.M. & Hecht, S.S. (1985b) Comparative metabolic activation in mouse skin of the weak carcinogen 6-methylchrysene and the strong carcinogen 5-methylchrysene. *Cancer Res*., **45**, 6406–6442

Amin, S., Huie, K., Balanikas, G., Hecht, S.S., Pataki, J. & Harvey, R.G. (1987) High stereoselectivity in mouse skin metabolic activation of methylchrysenes to tumorigenic dihydrodiols. *Cancer Res*., **47**, 3613–3617

Amin, S., Krzeminski, J., Rivenson, A., Kurtzke, C., Hecht, S.S. & el-Bayoumy, K. (1995a) Mammary carcinogenicity in female CD rats of fjord region diol epoxides of benzo[*c*]phenanthrene, benzo[*g*]chrysene and dibenzo[*a*,*l*]pyrene. *Carcinogenesis*, **16**, 1971–1974

Amin, S., Desai, D., Dai, W., Harvey, R.G. & Hecht, S.S. (1995b) Tumorigenicity in newborn mice of fjord region and other sterically hindered diol epoxides of benzo[*g*]chrysene, dibenzo[*a*,*l*]pyrene (dibenzo[*def*,*p*]chrysene), 4*H*-cyclopenta[*def*]chrysene and fluoranthene. *Carcinogenesis*, **16**, 2813–2817

Andersson, P., McGuire, J., Rubio, C., Gradin, K., Whitelaw, M.L., Pettersson, S., Hanberg, A. & Poellinger, L. (2002) A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. *Proc. natl Acad. Sci. USA*, **99**, 9990–9995

Andrysik, Z., Machala, M., Chramostova, K., Hofmanova, J., Kozubik, A. & Vondracek, J. (2005) Activation of ERK1/2 and p38 kinases by polycyclic aromatic hydrocarbons in rat liver epithelial cells is associated with induction of apoptosis. *Toxicol. appl. Pharmacol*., **211**, 198–208

Archibong, A.E., Inyang, F., Ramesh, A., Greenwood, M., Nayyar, T., Kopsombut, P., Hood, D.B. & Nyanda, A.M. (2002) Alteration of pregnancy related hormones and fetal survival in F-344 rats exposed by inhalation to benzo(*a*)pyrene. *Reprod. Toxicol*., **16**, 801–808

Archuleta, M.M., Schieven, G.L., Ledbetter, J.A., Deanin, G.G. & Burchiel, S.W. (1993) 7,12-Dimethylbenz[a]anthracene activates protein-tyrosine kinases Fyn and Lck in the HPB-ALL human T-cell line and increases tyrosine phosphorylation of phospholipase C-gamma 1, formation of inositol 1,4,5-trisphosphate, and mobilization of intracellular calcium. *Proc. natl Acad. Sci. USA*, **90**, 6105–6109

Arif, J.M. & Gupta, R.C. (1997) Microsome-mediated bioactivation of dibenzo[*a*,*l*]pyrene and identification of DNA adducts by ^{32}P-postlabeling. *Carcinogenesis*, **18**, 1999–2007

Arif, J.M., Smith, W.A. & Gupta, R.C. (1997) Tissue distribution of DNA adducts in rats treated by intramammillary injection with dibenzo[*a*,*l*]pyrene, 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene. *Mutat. Res*., **378**, 31–39

Arif, J.M., Smith, W.A. & Gupta, R.C. (1999) DNA adduct formation and persistence in rat tissues following exposure to the mammary carcinogen dibenzo[*a*,*l*]pyrene. *Carcinogenesis*, **20**, 1147–1150

Arrieta, D.E., Ontiveros, C.C., Li, W.W., Garcia, J.H., Denison, M.S., McDonald, J.D., Burchiel, S.W. & Washburn, B.S. (2003) Aryl hydrocarbon receptor-mediated activity of particulate organic matter from the Paso del Norte airshed along the U.S.-Mexico border. *Environ. Health Perspect*., **111**, 1299–1305

Ashurst, S.W., Cohen, G.M., Nesnow, S., DiGiovanni, J. & Slaga, T.J. (1983) Formation of benzo(*a*)pyrene/DNA adducts and their relationship to tumor initiation in mouse epidermis. *Cancer Res*., **43**, 1024–1029

de Assis, S., Ambrosone, C.B., Wustrack, S., Krishnan, S., Freudenheim, J.L. & Shields, P.G. (2002) Microsomal epoxide hydrolase variants are not associated with risk of breast cancer. *Cancer Epidemiol Biomarkers Prev.*, **11**, 1697–1698

ATSDR (1995) *Toxicological Profile for Polycyclic Aromatic Hydrocarbons*, Atlanta, GA, Agency for Toxic Substances and Disease Registry, Department of Health Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp69.pdf

Aynacioglu, A.S., Cascorbi, I., Mrozikiewicz, P.M. & Roots, I. (1998) High frequency of CYP1A1 mutations in a Turkish population. *Arch. Toxicol.*, **72**, 215–218

Bachmann, W.E., Cook, J.W., Dansi, A., de Worms, C.G.M., Haslewood, G.A.D., Hewett, C.L. & Robinson, A.M. (1937) The production of cancer by pure hydrocarbons. Part IV. *Proc. roy. Soc. London*, **Ser. B**, 343–367

Bacsi, S.G., Reisz-Porszasz, S. & Hankinson, O. (1995) Orientation of the heterodimeric aryl hydrocarbon (dioxin) receptor complex on its asymmetric DNA recognition sequence. *Mol. Pharmacol*., **47**, 432–438

Baer-Dubowska, W., Nair, R.V., Cortez, C., Harvey, R.G. & DiGiovanni, J. (1995) Covalent DNA adducts formed in mouse epidermis from dibenz[*a,j*]anthracene: Evidence for the formation of polar adducts. *Chem. Res. Toxicol*., **8**, 292–301

Bailey, L.R., Roodi, N., Dupont, W.D. & Parl, F.F. (1998) Association of cytochrome P450 *1B1 (CYP1B1)* polymorphism with steroid receptor status in breast cancer. *Cancer Res*., **58**, 5038–5041

Balu, N., Padgett, W.T., Lambert, G.R., Swank, A.E., Richard, A.M. & Nesnow, S. (2004) Identification and characterization of novel stable deoxyguanosine and deoxyadenosine adducts of benzo[*a*]pyrene-7,8-quinone from reactions at physiological pH. *Chem. Res. Toxicol*., **17**, 827–838

Bao, Z. & Yang, S.K. (1986) Two K-regions of 5-methylchrysene are sites of oxidative metabolism. *Biochem. biophys. Res. Commun*., **141**, 734–740

Baron, J.M., Zwadlo-Klarwasser, G., Jugert, F., Hamann, W., Rübben, A., Mukhtar, H. & Merk, H.F. (1998) Cytochrome P450 1B1: A major P450 isoenzyme in human blood monocytes and macrophage subsets. *Biochem. Pharmacol*., **56**, 1105–1110

Bartczak, A.W., Sangaiah, R., Ball, L.M., Warren, S.H. & Gold, A. (1987) Synthesis and bacterial mutagenicity of the cyclopenta oxides of the four cyclopenta-fused isomers of benzanthracene. *Mutagenesis*, **2**, 101–105

Barton, H.A., Cogliano, V.J., Flowers, L., Valcovic, L., Setzer, R.W. & Woodruff, T.J. (2005) Assessing susceptibility from early-life exposure to carcinogens. *Environ. Health Perspect*., **113**, 1125–1133

Bartsch, H., Nair, U., Risch, A., Rojas, M., Wikman, H. & Alexandrov, K. (2000) Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 3–28

Beach, A.C. & Gupta, R.C. (1994) DNA adducts of the ubiquitous environmental contaminant cyclopenta[*cd*]pyrene. *Carcinogenesis*, **15**, 1065–1072

Beach, A.C., Agarwal, S C., Lambert, G.R., Nesnow, S. & Gupta, R.C. (1993) Reaction of cyclopenta[*c,d*]pyrene-3,4-epoxide with DNA and deoxynucleotides. *Carcinogenesis*, **14**, 767–771

Beall, H.D., Liu, Y., Siegel, D., Bolton, E.M., Gibson, N.W. & Ross, D. (1996) Role of NAD(P)H:quinone oxidoreductase (DT-diaphorase) in cytotoxicity and induction of DNA damage by streptonigrin. *Biochem. Pharmacol.,* **51**, 645–652

Bejjani, B.A., Stockton, D.W., Lewis, R.A., Tomey, K.F., Dueker, D.K., Jabak, M., Astle, W.F. & Lupski, J.R. (2000) Multiple *CYP1B1* mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent *de novo* events and a dominant modifier locus. *Hum. mol. Genet*., **9**, 367–374

Beland, F.A., Churchwell, M.I., Von Tungeln, L.S., Chen, S., Fu, P.P., Culp, S.J., Schoket, B., Gyorffy, E., Minarovits, J., Poirier, M.C., Bowman, E.D., Weston, A. & Doerge, D.R. (2005) High-performance liquid chromatography electrospray ionization tandem mass spectrometry for the detection and quantitation of benzo[a]pyrene–DNA adducts. *Chem. Res. Toxicol.*, **18**, 1306–1315

Benane, S.G., Nelson, G.B., Ross, J.A. & Blackman, C.F. (1999) Benzo[*a*]pyrene and dibenzo[*a*,*l*]pyrene do not alter gap junction communication in rat liver epithelial cells. *Polycyc. arom. Comp*., **17**, 53–62

Benhamou, S., Reinikainen, M., Bouchardy, C., Dayer, P. & Hirvonen, A. (1998) Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Res*., **58**, 5291–5293

Bennett, W.P., Hussain, S.P., Vahakangas, K.H., Khan, M.A., Shields, P.G. & Harris, C.C. (1999) Molecular epidemiology of human cancer risk: Gene–environment interactions and *p53* mutation spectrum in human lung cancer. *J. Pathol*., **187**, 8–18

Bice, D.E., Harris, D.L., Schnizlein, C.T. & Mauderly, J.L. (1979) Methods to evaluate the effects of toxic materials deposited in the lung on immunity in lung-associated lymph nodes. *Drug chem. Toxicol*., **2**, 35–47.

Bigger, C.A.H., Ponten, I., Page, J.E. & Dipple, A. (2000) Mutational spectra for polycyclic aromatic hydrocarbons in the *supF* target gene. *Mutat. Res*., **450**, 75–93

Binkova, B. & Šrám, R.J. (2004) The genotoxic effect of carcinogenic PAHs, their artificial and environmental mixtures (EOM) on human diploid lung fibroblasts. *Mutat. Res*., **547**, 109–121

Binkova, B., Giguère, Y., Rössner, P., Jr, Dostal, M. & Šrám, R.J. (2000) The effect of dibenzo[*a*,*l*]pyrene and benzo[*a*]pyrene on human diploid lung fibroblasts: The induction of DNA adducts, expression of p53 and $p21^{WAF1}$ proteins and cell cycle distribution. *Mutat. Res*., **471**, 57–70

Birnbaum, L.S. & Fenton, S.E. (2003) Cancer and developmental exposure to endocrine disruptors. *Environ. Health Perspect*., **111**, 389–394

Biro, A., Pallinger, E., Major, J., Jakab, M.G., Klupp, T., Falus, A. & Tompa, A. (2002) Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed solvents. *Immunol. Lett.*, **81**, 133–140

Bischoff, K.B. & Dedrick, R.L. (1970) Generalized solution to linear, two-compartment, open model for drug distribution. *J. theor. Biol*., **29**, 63–68

Bjelogrlic, N.M., Mäkinen, M., Stenbäck, F. & Vähäkangas, K. (1994) Benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts and increased p53 protein in mouse skin. *Carcinogenesis*, **15**, 771–774

Blaha, L., Kapplova, P., Vondracek, J., Upham, B. & Machala, M. (2002) Inhibition of gap-junctional intercellular communication by environmentally occurring polycyclic aromatic hydrocarbons. *Toxicol. Sci*., **65**, 43–51

Blanchard, R.L., Freimuth, R.R., Buck, J., Weinshilboum, R.M. & Coughtrie, M.W.H. (2004) A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics*, **14** , 199–211

Blanton, R.H., Myers, M.J. & Bick, P.H. (1988) Modulation of immunocompetent cell populations by benzo[a]pyrene. *Toxicol. appl. Pharmacol*., **93**, 267–274

Bodell, W.J., Devanesan, P.D., Rogan, E.G. & Cavalieri, E.L. (1989) ^{32}P-Postlabeling analysis of benzo[*a*]pyrene–DNA adducts formed in vitro and in vivo. *Chem. Res. Toxicol*., **2**, 312–315

Booker, C.D. & White, K.L., Jr (2005) Benzo(a)pyrene-induced anemia and splenomegaly in NZB/WF1 mice. *Food chem. Toxicol.*, **43**, 1423–1431

Bos, R.P., Hulshof, C.T., Theuws, J.L. & Henderson, P.T. (1983) Mutagenicity of creosote in the Salmonella/microsome assay. *Mutat. Res*., **119**, 21–25

Bos, R.P., Theuws, J.L., Leijdekkers, C.M. & Henderson, P.T. (1984a) The presence of the mutagenic polycyclic aromatic hydrocarbons benzo[a]pyrene and benz[a]anthracene in creosote P1. *Mutat. Res*., **130**, 153–158

Bos, R.P., Hulshof, C.T., Theuws, J.L. & Henderson, P.T. (1984b) Genotoxic exposure of workers creosoting wood. *Br. J. ind. Med*., **41**, 260–262

Bos, R.P., Jongeneelen, F.J., Theuws, J.L. & Henderson, P.T. (1985) Detection of volatile mutagens in creosote and coal tar. *Mutat. Res*., **156**, 195–198

Bos, R.P., Prinsen, W.J.C., van Rooy, J.G.M., Jongeneelen, F.J., Theuws, J.L.G. & Henderson, P.T. (1987) Fluoranthene, a volatile mutagenic compound, present in creosote and coal tar. *Mutat. Res.,* **187**, 119–125

Boström, C.-E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Rannug, A., Törnqvist, M., Victorin, K. & Westerholm, R. (2002) Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ. Health Perspect.*, **110**, 451–489

Bouchardy, C., Benhamou, S., Jourenkova, N., Dayer, P. & Hirvonen, A. (2001) Metabolic genetic polymorphisms and susceptibility to lung cancer. *Lung Cancer*, **32**, 109–112

Boverhof, D.R., Tam, E., Harney, A.S., Crawford, R.B., Kaminski, N.E. & Zacharewski, T.R. (2004) 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces suppressor of cytokine signaling 2 in murine B cells. *Mol. Pharmacol*., **66**, 1662–1670

Boyland, E. & Sims, P. (1964) Metabolism of polycyclic compounds. 24. The metabolism of benz[*a*]anthracene. *Biochem. J.,* **91**, 493–506

Braithwaite, E., Wu, X. & Wang, Z. (1998) Repair of DNA lesions induced by polycyclic aromatic hydrocarbons in human cell-free extracts: Involvement of two excision repair mechanisms in vitro. *Carcinogenesis*, **19**, 1239–1246

Breen, A.P. & Murphy, J.A. (1995) Reactions of oxyl radicals with DNA. *Free Rad. Biol. Med*., **18**, 1033–1077

Bresnick, E., Vaught, J.B., Chuang, A.H.L., Stoming, T.A., Bockman, D. & Mukhtar, H. (1977) Nuclear aryl hydrocarbon hydroxylase and interaction of polycyclic hydrocarbons with nuclear components. *Arch. Biochem. Biophys.*, **181**, 257–269

Brookes, P., Ellis, M.V., Pataki, J. & Harvey, R.G. (1986) Mutation in mammalian cells by isomers of 5-methylchrysene diolepoxide. *Carcinogenesis*, **7**, 463–466

Buening, M.K., Wislocki, P.G., Levin, W., Yagi, H., Thakker, D.R., Akagi, H., Koreeda, M., Jerina, D.M. & Conney, A.H. (1978) Tumorigenicity of the optical enantiomers of the diastereomeric benzo[*a*]pyrene-7,8-diol-9,10-epoxides in newborn mice: Exceptional activity of (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Proc. natl Acad. Sci. USA*, **75**, 5358–5361

Buening, M.K., Levin, W., Karle, J.M., Yagi, H., Jerina, D.M. & Conney, A.H. (1979a) Tumorigenicity of bay-region epoxides and other derivatives of chrysene and phenanthrene in newborn mice. *Cancer Res*., **39**, 5063–5068

Buening, M.K., Levin, W., Wood, A.W., Chang, R.L., Yagi, H., Karle, J.M., Jerina, D.M. & Conney, A.H. (1979b) Tumorigenicity of the dihydrodiols of dibenzo(*a*,*h*)anthracene on mouse skin and in newborn mice. *Cancer Res*., **39**, 1310–1314

Burcham, P.C. (1998) Genotoxic lipid peroxidation products: Their DNA damaging properties and role in formation of endogenous DNA adducts. *Mutagenesis*, **13**, 287–305

Burchiel, S.W. & Luster, M.I. (2001) Signaling by environmental polycyclic aromatic hydrocarbons in human lymphocytes. *Clin. Immunol*., **98**, 2–10

Burchiel, S.W., Hadley, W.M., Barton, S.L., Fincher, R.H., Lauer, L.D. & Dean, J.H. (1988) Persistent suppression of humoral immunity produced by 7,12-dimethylbenz(a)anthracene (DMBA) in B6C3F1 mice: Correlation with changes in spleen cell surface markers detected by flow cytometry. *Int. J. Immunopharmacol*., **10**, 369–376

Burchiel, S.W., Davis, D.A., Gomez, M.P., Montano, R.M., Barton, S.L. & Seamer, L.C. (1990) Inhibition of lymphocyte activation in splenic and gut-associated lymphoid tissues following oral exposure of mice to 7,12-dimethylbenz[a]anthracene. *Toxicol. appl. Pharmacol*., **105**, 434–442

Burchiel, S.W., Thompson, T.A. & Davis, D.A. (1991) Alterations in mitogen-induced calcium mobilization and intracellular free calcium produced by 7,12-dimethylbenz(a)anthracene in the Jurkat human T cell line. *Int. J. Immunopharmacol*., **13**, 109–115

Burchiel, S.W., Davis, D.A., Ray, S.D., Archuleta, M.M., Thilsted, J.P. & Corcoran, G.B. (1992) DMBA-induced cytotoxicity in lymphoid and nonlymphoid organs of B6C3F1 mice: Relation of cell death to target cell intracellular calcium and DNA damage. *Toxicol. appl. Pharmacol*., **113**, 126–132

Burchiel, S.W., Lauer, F.T., McDonald, J.D. & Reed, M.D. (2004) Systemic immunotoxicity in AJ mice following 6-month whole body inhalation exposure to diesel exhaust. *Toxicol. appl. Pharmacol*., **196**, 337–345

Burchiel, S.W., Lauer, F.T., Dunaway, S.L., Zawadzki, J., McDonald, J.D. & Reed, M.D. (2005) Hardwood smoke alters murine splenic T cell responses to mitogens following a 6-month whole body inhalation exposure. *Toxicol. appl. Pharmacol*., **202**, 229–236

Burczynski, M.E. & Penning, T.M. (2000) Genotoxic polycyclic aromatic hydrocarbon *ortho*-quinones generated by aldo-keto reductases induce CYP1A1 via nuclear translocation of the aryl hydrocarbon receptor. *Cancer Res.*, **60**, 908–915

Burczynski, M.E., Harvey, R.G. & Penning, T.M. (1998) Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoforms: Oxidation of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to the activated *o*-quinone metabolite benzo[*a*]pyrene-7,8-dione. *Biochemistry*, **37**, 6781–6790

Burczynski, M.E., Lin, H.-K. & Penning, T.M. (1999a) Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress: Implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. *Cancer Res*., **59**, 607–614

Burczynski, M.E., Palackal, N.T., Harvey, R.G. & Penning, T.M. (1999b) Polycyclic aromatic hydrocarbon *trans*-dihydrodiol specificity of four recombinant human dihydrodiol dehydrogenase isoforms. *Polycyc. aromat. Comp.*, **16**, 205–214

Burdick, A.D., Davis, J.W., Liu, K.J., Hudson, L.G., Shi, H., Monske, M.L. & Burchiel, S.W. (2003) Benzo(*a*)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. *Cancer Res*., **63**, 7825–7833

Busby, W.F., Jr, Smith, H., Crespi, C.L. & Penman, B.W. (1995) Mutagenicity of benzo[a]pyrene and dibenzopyrenes in the Salmonella typhimurium TM677 and the MCL-5 human cell forward mutation assays. *Mutat. Res*., **342**, 9–16

Buters, J.T.M., Sakai, S., Richter, T., Pineau, T., Alexander, D.L., Savas, U., Doehmer, J., Ward, J.M., Jefcoate, C.R. & Gonzalez, F.J. (1999) Cytochrome P450 CYP1B1 determines susceptibility to 7, 12-dimethylbenz[*a*]anthracene-induced lymphomas. *Proc. natl Acad. Sci. USA*, **96**, 1977–1982

Buters, J.T.M., Mahadevan, B., Quintanilla-Martinez, L., Gonzalez, F.J., Greim, H., Baird, W.M. & Luch, A. (2002) Cytochrome P450 1B1 determines susceptibility to dibenzo[*a*,*l*]pyrene-induced tumor formation. *Chem. Res. Toxicol*., **15**, 1127–1135

Cai, Y., Baer-Dubowska, W., Ashwood-Smith, M. & DiGiovanni, J. (1997) Inhibitory effects of naturally occurring coumarins on the metabolic activation of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene in cultured mouse keratinocytes. *Carcinogenesis*, **18**, 215–222

Camacho, I.A., Singh, N., Hegde, V.L., Nagarkatti, M. & Nagarkatti, P.S. (2005) Treatment of mice with 2,3,7,8-tetrachlorodibenzo-p-dioxin leads to aryl hydrocarbon receptor-dependent nuclear translocation of NF-kappaB and expression of Fas ligand in thymic stromal cells and consequent apoptosis in T cells. *J. Immunol*., **175**, 90–103

Carlson, D.B. & Perdew, G.H. (2002) A dynamic role for the Ah receptor in cell signaling? Insights from a diverse group of Ah receptor interacting proteins. *J. Biochem. mol. Toxicol*., **16**, 317–325

Carmichael, P.L., Platt, K.L., Ni She, M., Lecoq, S., Oesch, F., Phillips, D.H. & Grover, P.L. (1993) Evidence for the involvement of a bis-diol-epoxide in the metabolic activation of dibenz[*a*,*h*]anthracene to DNA-binding species in mouse skin. *Cancer Res*., **53**, 944–948

Carstensen, U., Alexandrie, A.K., Hogstedt, B., Rannug, A., Bratt, I. & Hagmar, L. (1993) B- and T-Lymphocyte micronuclei in chimney sweeps with respect to genetic polymorphism for CYP1A1 and GST1 (class Mu). *Mutat. Res*., **289**, 187–195

Carstensen, U., Hou, S.M., Alexandrie, A.K., Hogstedt, B., Tagesson, C., Warholm, M., Rannug, A., Lambert, B., Axmon, A. & Hagmar, L. (1999) Influence of genetic polymorphisms of biotransformation enzymes on gene mutations, strand breaks of deoxyribonucleic acid, and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine in potroom workers exposed to polyaromatic hydrocarbons. *Scand. J. Work Environ. Health*, **25**, 351–360

Casale, G.P., Cheng, Z., Liu, J., Cavalieri, E.L. & Singhal, M. (2000) Profiles of cytokine mRNAs in the skin and lymph nodes of SENCAR mice treated epicutaneously with dibenzo[a,l]pyrene or dimethylbenz[a]anthracene reveal a direct correlation between carcinogen-induced contact hypersensitivity and epidermal hyperplasia. *Mol. Carcinog*., **27**, 125–140

Casale, G.P., Singhal, M., Bhattacharya, S., RamaNathan, R., Roberts, K.P., Barbacci, D.C., Zhao, J., Jankowiak, R., Gross, M.L., Cavalieri, E.L., Small, G.J., Rennard, S.I., Mumford, J.L. & Shen, M. (2001) Detection and quantification of depurinated benzo[*a*]pyrene-adducted DNA bases in the urine of cigarette smokers and women exposed to household coal smoke. *Chem. Res. Toxicol.,* **14**, 192–201

Cascorbi, I., Brockmoller, J. & Roots, I. (1996) A C4887A polymorphism in exon 7 of human CYP1A1: Population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res.*, **56**, 4965–4969

Cavalieri, E. & Calvin, M. (1971) Molecular characteristics of some carcinogenic hydrocarbons. *Proc. natl Acad. Sci. USA*, **68**, 1251–1253

Cavalieri, E. & Rogan, E. (1985) Role of radical cations in aromatic hydrocarbon carcinogenesis. *Environ. Health Perspect*., **64**, 69–84

Cavalieri, E.L. & Rogan, E.G. (1992) The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic activation. *Pharmacol. Ther.*, **55**, 183–199

Cavalieri, E.L. & Rogan, E.G. (2002) Fluoro substitution of carcinogenic aromatic hydrocarbons: Models for understanding mechanisms of metabolic activation and of oxygen transfer catalyzed by cytochrome P450. In: Neilson, A.H., ed., *The Handbook of Environmental Chemistry*, Vol. 3N, *Organofluorine*, Heidelberg, Springer-Verlag, pp. 277–293

Cavalieri, E. & Roth, R. (1976) Reaction of methylbenzanthracenes and pyridine by one-electron oxidation: A model for metabolic activation and binding of carcinogenic aromatic hydrocarbons. *J. org. Chem.*, **41**, 2679–2684

Cavalieri, E., Roth, R., Grandjean, C., Althoff, J., Patil, K., Liakus, S. & Marsh, S. (1978) Carcinogenicity and metabolic profiles of 6-substituted benzo[*a*]pyrene derivatives on mouse skin. *Chem.-biol. Interact.*, **22**, 53–67

Cavalieri, E.L., Rogan, E.G., Roth, R.W., Saugier, R.K. & Hakam, A. (1983) The relationship between ionization potential and horseradish peroxidase/hydrogen peroxide-catalyzed binding of aromatic hydrocarbons to DNA. *Chem.-biol. Interact.*, **47**, 87–109

Cavalieri, E.L., Rogan, E.G., Cremonisi, P. & Devanesan, P.D. (1988) Radical cations as precursors in the metabolic formation of quinones from benzo[*a*]pyrene and 6-fluorobenzo[*a*]pyrene: Fluoro substitution as a probe for one-electron oxidation in aromatic substrates. *Biochem. Pharmacol.*, **37**, 2173–2182

Cavalieri, E.L., Rogan, E.G., Higginbotham, S., Cremonesi, P. & Salmasi, S. (1989) Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of dibenzo[*a*]pyrenes: The very potent environmental carcinogen dibenzo[*a,l*]pyrene. *J. Cancer Res. clin. Oncol.*, **115**, 67–72

Cavalieri, E.L., Rogan, E.G., Devanesan, P.D., Cremonesi, P., Cerny, R.L., Gross, M.L. & Bodell, W.J. (1990) Binding of benzo[a]pyrene to DNA by cytochrome P-450 catalyzed one-electron oxidation in rat liver microsomes and nuclei. *Biochemistry*, **29**, 4820–4827

Cavalieri, E.L., Higginbotham, S., RamaKrishna, N.V.S., Devanesan, P.D., Todorovic, R., Rogan, E.G. & Salmasi, S. (1991) Comparative dose–response tumorigenicity studies of dibenzo[*a,l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene, benzo[*a*]pyrene and two dibenzo[*a,l*]pyrene dihydrodiols in mouse skin and rat mammary gland. *Carcinogenesis*, **12**, 1939–1944

Cavalieri, E.L., Rogan, E.G., Li, K.-M., Todorovic, R., Ariese, F., Jankowiak, R., Grubor, N. & Small, G.J. (2005) Identification and quantification of the depurinating DNA adducts formed in mouse skin treated with dibenzo[*a,l*]pyrene (DB[*a,l*)P) or its metabolites and in rat mammary gland treated with DB[*a,l*]P. *Chem. Res. Toxicol.*, **18**, 976–983

Chaffin, C.L., Stouffer, R.L. & Duffy, D.M. (1999) Gonadotropin and steroid regulation of steroid receptor and aryl hydrocarbon receptor messenger ribonucleic acid in macaque granulosa cells during the periovulatory interval. *Endocrinology*, **140**, 4753–4760

Chakravarti, D., Pelling, J.C., Cavalieri, E.L. & Rogan, E.G. (1995) Relating aromatic hydrocarbon-induced DNA adducts and c-H-*ras* mutations in mouse skin papillomas: The role of apurinic sites. *Proc. natl Acad. Sci. USA*, **92**, 10422–10426

Chakravarti, D., Mailander, P., Franzen, J., Higginbotham, S., Cavalieri, E.L. & Rogan, E.G. (1998) Detection of dibenzo[*a,l*]pyrene-induced H-*ras* codon 61 mutant genes in preneoplastic SENCAR mouse skin using a new PCR-RFLP method. *Oncogene*, **16**, 3203–3210

Chakravarti, D., Mailander, P.C., Cavalieri, E.L. & Rogan, E.G. (2000) Evidence that error-prone DNA repair converts dibenzo[*a*,*l*]pyrene-induced depurinating lesions into mutations: Formation, clonal proliferation and regression of initiated cells carrying H-*ras* oncogene mutations in early preneoplasia. *Mutat. Res*., **456**, 17–32

Chang, L.H. (1943) The fecal excretion of polycyclic hydrocarbons following their administration to the rat. *J. biol. Chem.*, **151**, 93–99

Chang, R.L., Levin, W., Wood, A.W., Lehr, R.E., Kumar, S., Yagi, H., Jerina, D.M. & Conney, A.H. (1982) Tumorigenicity of bay-region diol-epoxides and other benzo-ring derivatives of dibenzo(*a*,*h*)pyrene and dibenzo(*a*,*i*)pyrene on mouse skin and in newborn mice. *Cancer Res*., **42**, 25–29

Chang, H., Wang, W.J., Chang, L.W. & Lin, P. (2005) A histochemical and pathological study on the interrelationship between TCDD-induced AhR expression, AhR activation, and hepatotoxicity in mice. *J. Toxicol .environ. Health,* **A68**, 1567–1579

Chen, R.-H., Maher, V.M., Brouwer, J., van de Putte, P. & McCormick, J.J. (1992) Preferential repair and strand-specific repair of benzo[*a*]pyrene diol epoxide adducts in the *HPRT* gene of diploid human fibroblasts. *Proc. natl Acad. Sci. USA*, **89**, 5413–5417

Chen, L., Devanesan, P.D., Higginbotham, S., Ariese, F., Jankowiak, R., Small, G.J., Rogan, E.G. & Cavalieri, E.L. (1996) Expanded analysis of benzo[*a*]pyrene–DNA adducts formed in vitro and in mouse skin: Their significance in tumor initiation. *Chem. Res. Toxicol*., **9**, 897–903

Chen, J.X., Zheng, Y., West, M. & Tang, M.-S. (1998) Carcinogens preferentially bind at methylated CpG in the p53 mutational hot spots. *Cancer Res*., **58**, 2070–2075

Chen, H., Lum, A., Seifried, A., Wilkens, L.R. & Le Marchand, L. (1999) Association of the NAD(P)H:quinone oxidoreductase $^{609}C \rightarrow T$ polymorphism with a decreased lung cancer risk. *Cancer Res*., **59**, 3045–3048

Chen, M.L., Lee, B.C., Lu, P.L., Mao, I.F. & Liu, T.Y. (2003) Polycyclic aromatic hydrocarbon–deoxyribonucleic acid (PAH–DNA) adduct levels and exposure to coke oven emissions among workers in Taiwan. *Arch. environ. Health*, **58**, 298–305

Chen, S., Nguyen, N., Tamura, K., Karin, M. & Tukey, R.H. (2003) The role of the Ah receptor and p38 in benzo[*a*]pyrene-7,8-dihydrodiol and benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-induced apoptosis. *J. biol. Chem*., **278**, 19526–19533

Chen, J.-H., Chou, F.-P., Lin, H.-H. & Wang, C.-J. (2005) Gaseous nitrogen oxide repressed benzo[*a*]pyrene-induced human lung fibroblast cell apoptosis via inhibiting JNK1 signals. *Arch. Toxicol*., **79**, 694–704

Cheung, Y.-L., Gray, T.J.B. & Ioannides, C. (1993) Mutagenicity of chrysene, its methyl and benzo derivatives, and their interactions with cytochromes P-450 and the Ah-receptor; relevance to their carcinogenic potency. *Toxicology*, **81**, 69–86

Chida, M., Yokoi, T., Fukui, T., Kinoshita, M., Yokota, J. & Kamataki, T. (1999) Detection of three genetic polymorphisms in the 5′-flanking region and intron 1 of human *CYP1A2* in the Japanese population. *Jpn. J. Cancer Res*., **90**, 899–902

Chou, H.-C., Ozawa, S., Fu, P.P., Lang, N.P. & Kadlubar, F.F. (1998) Metabolic activation of methyl-hydroxylated derivatives of 7,12-dimethylbenz[*a*]anthracene by human liver dehydroepiandrosterone-steroid sulfotransferase. *Carcinogenesis*, **19**, 1071–1076

Chramostova, K., Vondracek, J., Sindlerova, L., Vojtesek, B., Kozubik, A. & Machala, M. (2004) Polycyclic aromatic hydrocarbons modulate cell proliferation in rat hepatic epithelial stem-like WB-F344 cells. *Toxicol. appl. Pharmacol*., **196**, 136–148

Chun, Y.-J. & Kim, S. (2003) Discovery of cytochrome P450 1B1 inhibitors as new promising anti-cancer agents. *Med. Res. Rev.*, **23**, 657–668

Chun, Y.-J., Kim, M.Y. & Guengerich, F.P. (1999) Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem. biophys. Res. Commun.*, **262**, 20–24

Chun, Y.-J., Kim, S., Kim, D., Lee, S.-K. & Guengerich, F.P. (2001) A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. *Cancer Res.*, **61**, 8164–8170

Cizmas, L., Zhou, G.D., Safe, S.H., McDonald, T.J., Zhu, L. & Donnelly, K.C. (2004) Comparative in vitro and in vivo genotoxicities of 7H-benzo[c]fluorene, manufactured gas plant residue (MGP), and MGP fractions. *Environ. mol. Mutag.*, **43**, 159–168

Colapietro, A.-M., Goodell, A.L. & Smart, R.C. (1993) Characterization of benzo[*a*]pyrene-initiated mouse skin papillomas for Ha-*ras* mutations and protein kinase C levels. *Carcinogenesis*, **14**, 2289–2295

Comaish, J.S. (1987) The effect of tar and ultraviolet on the skin. *J. invest. Dermatol.*, **88**, 61s–64s

Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G.H.A. Clowes Memorial Lecture. *Cancer Res.*, **42**, 4875–4917

Cook, J.W., Hieger, I., Kennaway, E.L. & Mayneord, W.V. (1932) The production of cancer by pure hydrocarbons. Part I. *Proc. roy. Soc. London.*, **B111**, 455–484

Cook, J.W., Hewett, C.L. & Hieger, I. (1933) The isolation of a cancer-producing hydrocarbon from coal tar. *J. chem. Soc.*, 395–405

Cooper, C.S., Ribeiro, O., Hewer, A., Walsh, C., Pal, K., Grover, P.L. & Sims, P. (1980a) The involvement of a 'bay-region' and a non-'bay-region' diol-epoxide in the metabolic activation of benza[*a*]anthracene in mouse skin and hamster embryo cells. *Carcinogenesis*, **1**, 233–243

Cooper, C.S., Ribeiro, O., Farmer, P.B., Hewer, A., Walsh, C., Pal, K., Grover, P.L. & Sims, P. (1980b) The metabolic activation of benz[*a*]anthracene in hamster embryo cells: Evidence that diol-epoxides react with guanosine, deoxyguanosine and adenosine in nucleic acids. *Chem.-biol. Interact.*, **32**, 209–231

Cooper, C.S., MacNicoll, A.D., Ribeiro, O., Gervasi, P.G., Hewer, A., Walsh, C., Pal, K., Grover, P.L. & Sims, P. (1980c) The involvement of a non-'bay-region' diol-epoxide in the metabolic activation of benza[*a*]anthracene in hamster embryo cells. *Cancer Lett.*, **9**, 53–59

Cooper, C.S., Hewer, A., MacNicoll, A.D., Ribeiro, O., Grover, P.L. & Sims, P. (1980d) The metabolism of the 10,11-dihydrodiol of benz[*a*]anthracene to a vicinal diol-epoxide that is not involved in metabolic activation. *Carcinogenesis*, **1**, 937–943

Cooper, C.S., Grover, P.L. & Sims, P. (1983) The metabolism and activation of benzo[*a*]pyrene. *Progr. Drug Metab.*, **7**, 295–396

Crebelli, R., Carta, P., Andreoli, C., Aru, G., Dobrowolny, G., Rossi, S. & Zijno, A. (2002) Biomonitoring of primary aluminium industry workers: Detection of micronuclei and repairable DNA lesions by alkaline SCGE. *Mutat. Res.*, **516**, 63–70

Cremonesi, P., Cavalieri, E.L. & Rogan, E.G. (1989) One-electron oxidation of 6-substituted benzo[*a*]-pyrenes by manganic acetate. A model for metabolic activation. *J. org. Chem.*, **54**, 3561–3570

Cremonesi, P., Hietbrink, B., Rogan, E.G. & Cavalieri, E.L. (1992) One-electron oxidation of dibenzo[*a*]pyrenes by manganic acetate. *J. org. Chem.*, **57**, 3309–3312

Crofts, F., Cosma, G.N., Currie, D., Taioli, E., Toniolo, P. & Garte, S.J. (1993) A novel CYP1A1 gene polymorphism in African–Americans. *Carcinogenesis*, **14**, 1729–1731

Crofts, F., Taioli, E., Trachman, J., Cosma, G.N., Currie, D., Toniolo, P. & Garte, S.J. (1994) Functional significance of different human CYP1A1 genotypes. *Carcinogenesis*, **15**, 2961–2963

Culp, S.J. & Beland, F.A. (1994) Comparison of DNA adduct formation in mice fed coal tar or benzo[a]pyrene. *Carcinogenesis*, **15**, 247–252

Culp, S.J., Warbritton, A.R., Smith, B.A., Li, E.E. & Beland, F.A. (2000) DNA adduct measurements, cell proliferation and tumor mutation induction in relation to tumor formation in B6C3F1 mice fed coal tar or benzo[a]pyrene. *Carcinogenesis*, **21**, 1433–1440

Cunningham, M.J., Kurian, P. & Milo, G.E. (1989) Metabolism and binding of benzo[*a*]pyrene in randomly-proliferating, confluent and S-phase human skin fibroblasts. *Cell Biol. Toxicol*., **5**, 155–168

Dabestani, R. & Ivanov, I.N. (1999) A compilation of physical, spectroscopic and photophysical properties of polycyclic aromatic hydrocarbons. *Photochem. Photobiol*., **70**, 10–34

Dai, D., Tang, J., Rose, R., Hodgson, E., Bienstock, R.J., Mohrenweiser, H.W. & Goldstein, J.A. (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J. Pharmacol. exp. Ther*., **299**, 825–831

Dalhoff, K., Buus Jensen, K. & Enghusen Poulsen, H. (2005) Cancer and molecular biomarkers of phase 2. *Methods Enzymol.,* **400**, 618–627

Daly, A.K. (2003) Pharmacogenetics of the major polymorphic metabolizing enzymes. *Fundam. clin. Pharmacol*., **17**, 27–41

Davila, D.R., Davis, D.P., Campbell, K., Cambier, J.C., Zigmond, L.A. & Burchiel, S.W. (1995) Role of alterations in Ca^{2+}-associated signaling pathways in the immunotoxicity of polycyclic aromatic hydrocarbons. *J. Toxicol. environ. Health*, **45**, 101–126

Davila, D.R., Romero, D.L. & Burchiel, S.W. (1996) Human T cells are highly sensitive to suppression of mitogenesis by polycyclic aromatic hydrocarbons and this effect is differentially reversed by alpha-naphthoflavone. *Toxicol. appl. Pharmacol*., **139**, 333–341

Davila, D.R., Lane, J.L., Lauer, F.T. & Burchiel, S.W. (1999) Protein tyrosine kinase activation by polycyclic aromatic hydrocarbons in human HPB-ALL T cells. *J. Toxicol. environ. Health*, **A56**, 249–261

Davis, D.A. & Burchiel, S.W. (1992) Inhibition of calcium-dependent pathways of B-cell activation by DMBA. *Toxicol. appl. Pharmacol*., **116**, 202–208

Davis, D.P., Gomez, M.P., Barton, S.L., Montano, R.M. & Burchiel, S.W. (1991) Suppression of local gut-associated and splenic mitogen responsiveness of lymphoid cells following oral exposure of B6C3F1 mice to 7,12-dimethylbenz[a]anthracene. *Fundam. appl. Toxicol*., **17**, 429–431

Davis, J.W., Melendez, K., Salas, V.M., Lauer, F.T. & Burchiel, S.W. (2000) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) inhibits growth factor withdrawal-induced apoptosis in the human mammary epithelial cell line, MCF-10A. *Carcinogenesis*, **21**, 881–886

Davis, J.W., Jr, Burdick, A.D., Lauer, F.T. & Burchiel, S.W. (2003) The aryl hydrocarbon receptor antagonist, 3′-methoxy-4′-nitroflavone, attenuates 2,3,7,8-tetrachlorodibenzo-p-dioxin-dependent regulation of growth factor signaling and apoptosis in the MCF-10A cell line. *Toxicol. appl. Pharmacol*., **188**, 42–49

Dean, J.H., Ward, E.C., Murray, M.J., Lauer, L.D. & House, R.V. (1985) Mechanisms of dimethylbenzanthracene-induced immunotoxicity. *Clin. Physiol. Biochem*., **3**, 98–110

Dean, J.H., Ward, E.C., Murray, M.J., Lauer, L.D., House, R.V., Stillman, W., Hamilton, T.A. & Adams, D.O. (1986) Immunosuppression following 7,12-dimethylbenz[a]anthracene exposure in B6C3F1 mice—II. Altered cell-mediated immunity and tumor resistance. *Int. J. Immunopharmacol*., **8**, 189–198

De Jong, W.H., Kroese, E.D., Vos, J.G. & Van Loveren, H. (1999) Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. *Toxicol. Sci*., **50**, 214–220

Delescluse, C., Lemaire, G., de Sousa, G. & Rahmani, R. (2000) Is CYP1A1 induction always related to AHR signaling pathway? *Toxicology*, **153**, 73–82

van Delft, J.H., Steenwinkel, M.S., van Asten, J.G., de Vogel, N., Bruijntjes-Rozier, T.C., Schouten, T., Cramers, P., Maas, L., van Herwijnen, M.H., van Schooten, F. & Hopmans, P.M. (2001) Biological monitoring the exposure to polycyclic aromatic hydrocarbons of coke oven workers in relation to smoking and genetic polymorphisms for GSTM1 and GSTT1. *Ann. occup. Hyg.*, **45**, 395–408

DeMarini, D.M., Landi, S., Tian, D., Hanley, N.M., Li, X., Hu, F., Roop, B.C., Mass, M.J., Keohavong, P., Gao, W., Olivier, M., Hainaut, P. & Mumford, J.L. (2001) Lung tumor *KRAS* and *TP53* mutations in nonsmokers reflect exposure to PAH-rich coal combustion emissions. *Cancer Res*., **61**, 6679–6681

Denissenko, M.F., Pao, A., Tang, M.-S. & Pfeifer, G.P. (1996) Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hotspots in *P53*. *Science*, **274**, 430–432

Denissenko, M.F., Chen, J.X., Tang, M.-S. & Pfeifer, G.P. (1997) Cytosine methylation determines hot spots of DNA damage in the human *P53* gene. *Proc. natl Acad. Sci. USA*, **94**, 3893–3898

Denissenko, M.F., Pao, A., Pfeifer, G.P. & Tang, M.-S. (1998) Slow repair of bulky DNA adducts along the nontranscribed strand of the human *p53* gene may explain the strand bias of transverrsion mutations in cancers. *Oncogene*, **16**, 1241–1247

Dertinger, S.D., Nazarenko, D.A., Silverstone, A.E. & Gasiewicz, T.A. (2001) Aryl hydrocarbon receptor signaling plays a significant role in mediating benzo[a]pyrene- and cigarette smoke condensate-induced cytogenetic damage in vivo. *Carcinogenesis*, **22**, 171–177

Deutsch, J., Leutz, J.C., Yang, S.K., Gelboin, H.V., Chiang, Y.L., Vatsis, K.P. & Coon, M.J. (1978) Regio- and stereoselectivity of various forms of purified cytochrome P-450 in the metabolism of benzo[*a*]pyrene and (-)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene as shown by product formation and binding to DNA. *Proc. natl Acad. Sci. USA*, **75**, 3123–3127

Devanesan, P., Rogan, E. & Cavalieri, E. (1987) The relationship between ionization potential and prostaglandin H synthase-catalyzed binding of aromatic hydrocarbons to DNA. *Chem.-biol. Interact*., **61**, 89–95

Devanesan, P.D., Cremonesi, P., Nunnally, J.E., Rogan, E.G. & Cavalieri, E.L. (1990) Metabolism and mutagenicity of dibenzo[*a*,*e*]pyrene and the very potent environmental carcinogen dibenzo[*a*,*l*]pyrene. *Chem. Res. Toxicol*., **3**, 580–586

Devanesan, P.D., RamaKrishna, N.V.S., Padmavathi, N.S., Higginbotham, S., Rogan, E.G., Cavalieri, E.L., Marsch, G.A., Jankowiak, R. & Small, G.J. (1993) Identification and quantitation of 7,12-dimethylbenz[*a*]anthracene–DNA adducts formed in mouse skin. *Chem. Res. Toxicol*., **6**, 364–371

Devanesan, P., Ariese, F., Jankowiak, R., Small, G.J., Rogan, E.G. & Cavalieri, E.L. (1999) A novel method for the isolation and identification of stable DNA adducts formed by dibenzo[*a*,*l*]pyrene and dibenzo[*a*,*l*]pyrene 11,12-dihydrodiol 13,14-epoxides in vitro. *Chem. Res. Toxicol*., **12**, 796–801

Diamond, L., Cherian, K., Harvey, R.G. & DiGiovanni, J. (1984) Mutagenic activity of methyl- and fluoro-substituted derivatives of polycyclic aromatic hydrocarbons in a human hepatoma (HepG2) cell-mediated assay. *Mutat. Res*., **136**, 65–72

Dickmann, L.J., Rettie, A.E., Kneller, M.B., Kim, R.B., Wood, A.J.J., Stein, C.M., Wilkinson, G.R. & Schwarz, U.I. (2001) Identification and functional characterization of a new CYP2C9 variant (CYP2C9*5) expressed among African Americans. *Mol. Pharmacol*., **60**, 382–387

DiGiovanni, J., Miller, D.R., Singer, J.M., Viaje, A. & Slaga, T.J. (1982a) Benzo(a)pyrene metabolism in primary cultures of mouse epidermal cells and untransformed and transformed epidermal cell lines. *Cancer Res*., **42**, 2579-2586

DiGiovanni, J., Diamond, L., Singer, J.M., Daniel, F.B., Witiak, D.T. & Slaga, T.J. (1982b) Tumor-initiating activity of 4-fluoro-7,12-dimethylbenz[*a*]anthracene and 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene in female SENCAR mice. *Carcinogenesis*, **3**, 651–655

DiGiovanni, J., Beltran, L., Rupp, A., Harvey, R.G. & Gill, R.D. (1993) Further analysis of c-Ha-*ras* mutations in papillomas initiated by several polycyclic aromatic hydrocarbons and papillomas from uninitiated, promoter-treated skin in SENCAR mice. *Mol. Carcinog*., **8**, 272–279

Ding, X. & Kaminsky, L.S. (2003) Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu. Rev. Pharmacol. Toxicol*., **43**, 149–173

Dinkova-Kostova, A.T., Fahey, J.W. & Talalay, P. (2004) Chemical structures of inducers of nicotinamide quinone oxidoreductase 1 (NQO1). *Methods Enzymol.*, **382**, 423–448

Dipple, A., Moschel, R.C. & Bigger, C.A. (1984) Polynuclear aromatic hydrocarbons. In: Searle, C.E., ed., *Chemical Carcinogens*, 2nd Ed., rev. exp., Washington DC, American Chemical Society, pp. 41–163

Dogra, S., Doehmer, J., Glatt, H., Molders, H., Siegert, P., Friedberg, T., Seidel, A. & Oesch, F. (1990) Stable expression of rat cytochrome P-450IA1 cDNA in V79 Chinese hamster cells and their use in mutagenicity testing. *Mol. Pharmacol*., **37**, 608–613

Dong, S., Fu, P.P., Shirsat, R.N., Hwang, H.-M., Leszczynski, J. & Yu, H. (2002) UVA light-induced DNA cleavage by isomeric methylbenz[*a*]anthracenes. *Chem. Res. Toxicol*., **15**, 400–407

Dong, H., Bonala, R.R., Suzuki, N., Johnson, F., Grollman, A.P. & Shibutani, S. (2004) Mutagenic potential of benzo[*a*]pyrene-derived DNA adducts positioned in codon 273 of the human *P53* gene. *Biochemistry*, **43**, 15922–15928

Dreij, K., Sundberg, K., Johansson, A.-S., Nordling, E., Seidel, A., Persson, B., Mannervik, B. & Jernström, B. (2002) Catalytic activities of human alpha class glutathione transferases toward carcinogenic dibenzo[*a*,*l*]pyrene diol epoxides. *Chem. Res. Toxicol*., **15**, 825–831

Dreij, K., Seidel, A. & Jernstrom, B. (2005) Differential removal of DNA adducts derived from anti-diol epoxides of dibenzo[*a*,*l*]pyrene and benzo[*a*]pyrene in human cells. *Chem. Res. Toxicol*., **18**, 655–664

Drukteinis, J.S., Medrano, T., Ablordeppey, E.A., Kitzman, J.M. & Shiverick, K.T. (2005) Benzo[*a*]pyrene, but not 2,3,7,8-TCDD, induces G2/M cell cycle arrest, $p21^{CIP1}$ and p53

phosphorylation in human choriocarcinoma JEG-3 cells: A distinct signaling pathway. *Placenta*., **26** (Suppl. A), S87–S95

Dupont, B. (2002) Introduction: Current concepts in immunity to human cancer and therapeutic antitumor vaccines. *Immunol. Rev.*, **188**, 5–8

Durant, J.L., Busby, W.F., Jr, Lafleur, A.L., Penman, B.W. & Crespi, C.L. (1996) Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. *Mutat. Res*., **371**, 123–157

Durant, J.L., Lafleur, A.L., Busby, W.F., Jr, Donhoffner, L.L., Penman, B.W. & Crespi, C.L. (1999) Mutagenicity of C24H14 PAH in human cells expressing CYP1A1. *Mutat. Res*., **446**, 1–14

Ebert, B., Seidel, A. & Lampen, A. (2005) Identification of BCRP as transporter of benzo[*a*]pyrene conjugates metabolically formed in Caco-2 cells and its induction by Ah-receptor agonists. *Carcinogenesis*, **26**, 1754–1763

Eisenstadt, E., Shpizner, B. & Gold, A. (1981) Metabolism of cyclopenta(cd)pyrene at the K-region by microsomes and a reconstituted cytochrome P-450 system from rat liver. *Biochem. biophys. Res. Commun*., **100**, 965–971

Eisenstadt, E., Warren, A.J., Porter, J., Atkins, D. & Miller, J.H. (1982) Carcinogenic epoxides of benzo[*a*]pyrene and cyclopenta[*cd*]pyrene induce base substitutions via specific transversions. *Proc. natl Acad. Sci. USA*, **79**, 1945–1949

El-Bayoumy, K. & Sinha, R. (2004) Mechanisms of mammary cancer chemoprevention by organoselenium compounds. *Mutat. Res*., **551**, 181–197

El-Bayoumy, K., Chae, Y.-H., Upadhyaya, P., Meschter, C., Cohen, L.A. & Reddy, B.S. (1992) Inhibition of 7,12-dimethylbenz(a)anthracene-induced tumors and DNA adduct formation in the mammary glands of female Sprague-Dawley rats by the synthetic organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate. *Cancer Res*., **52**, 2402–2407

Elferink, C.J. (2003) Aryl hydrocarbon receptor-mediated cell cycle control. *Prog. Cell Cycle Res*., **5**, 261–267

Eling, T.E., Thompson, D.C., Foureman, G.L., Curtis, J.F. & Hughes, M.F. (1990) Prostaglandin H synthase and xenobiotic oxidation. *Annu. Rev. Pharmacol. Toxicol.,* **30**, 1–45

Engelke, C.E.H., Meinl, W., Boeing, H. & Glatt, H. (2000) Association between functional genetic polymorphisms of human sulfotransferases 1A1 and 1A2. *Pharmacogenetics*, **10**, 163–169

Ensell, M.-X., Whong, W.-Z., Heng, Z.-C., Nath, J. & Ong, T. (1998) In vitro and in vivo transformation in rat tracheal epithelial cells exposed to diesel emission particles and related compounds. *Mutat. Res*., **412**, 283–291

Ensell, M.-X., Hubbs, A., Zhou, G., Battelli, L., Nath, J. & Ong, T. (1999) Neoplastic potential of rat tracheal epithelial cell lines induced by 1-nitropyrene and dibenzo(*a*,*i*)pyrene. *Mutat. Res*., **444**, 193–199

Environmental Protection Agency (2005) *Supplemental Guidance for Assessing Susceptibility from Early-life Exposure to Carcinogens. Risk Assessment Forum* (EPA/630/R-03/003F), Washington, DC. Available from: http://www.epa.gov/iriswebp/iris/backgr-d.htm

Fang, J.L. & Lazarus, P. (2004) Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: Significantly decreased glucuronidating activity against benzo(a)pyrene-7,8-dihydrodiol(-) in liver microsomes from subjects with the UGT1A1*28 variant. *Cancer Epidemiol. Biomarkers Prev*., **13**, 102–109

Fang, A.H., Smith, W.A., Vouros, P. & Gupta, R.C. (2001) Identification and characterization of a novel benzo[*a*]pyrene-derived DNA adduct. *Biochem. biophys. Res. Commun*., **281**, 383–389

Fang, J.-L., Beland, F.A., Doerge, D.R., Wiener, D., Guillemette, C., Marques, M.M. & Lazarus, P. (2002) Characterization of benzo(a)pyrene-*trans*-7,8-dihydrodiol glucuronidation by human tissue microsomes and overexpressed UDP-glucuronosyltransferase enzymes. *Cancer Res*., **62**, 1978–1986

Feng, Z., Hu, W., Chen, J.X., Pao, A., Li, H., Rom, W., Hung, M.-C. & Tang, M.-S. (2002) Preferential DNA damage and poor repair determine ras gene mutational hotspot in human cancer. *J. natl Cancer Inst*., **94**, 1527–1536

Fernandez-Salguero, P., Pineau, T., Hilbert, D.M., McPhail, T., Lee, S.S., Kimura, S., Nebert, D.W., Rudikoff, S., Ward, J.M. & Gonzalez, F.J. (1995) Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. *Science*, **268**, 722–726

Field, J.M., Shen, Y.-M., Jin, Y., Troxel, A. & Penning, T.M. (2005) Mapping PAH *o*-quinone and *anti*-BPDE mutations on the tumor suppressor p53 using a yeast reporter system (Abstract). *Proc. Am. Assoc. Cancer Res*, **46**, 379

Finch, C.E. & Rose, M.R. (1995) Hormones and the physiological architecture of life history evolution. *Q. Rev. Biol.*, **70**, 1-52

Fine, J.S., Gasiewicz, T.A. & Silverstone, A.E. (1989) Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Mol. Pharmacol*., **35**, 18–25

Flesher, J.W. & Myers, S.R. (1985) Oxidative metabolism of 7-methylbenz[*a*]anthracene, 12-methylbenz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene by rat liver cytosol. *Cancer Lett.*, **26**, 83–88

Flesher, J.W. & Myers, S.R. (1990) Bioalkylation of benz[*a*]anthracene as a biochemical probe for carcinogenic activity. Lack of bioalkylation in a series of six noncarcinogenic polynuclear aromatic hydrocarbons. *Drug Metab. Dispos.*, **18**, 163–167

Flesher, J.W., Stansbury, K.H. & Sydnor, K.L. (1982) S-Adenosyl-L-methionine is a carbon donor in the conversion of benzo[*a*]pyrene to 6-hydroxymethylbenzo[*a*]pyrene by rat liver S-9. *Cancer Lett.*, **16**, 91–94

Flesher, J.W., Myers, S.R. & Blake, J.W. (1984) Biosynthesis of the potent carcinogen 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett.,* **24**, 335–343

Flesher, J.W., Myers, S.R., Bergo, C.H. & Blake, J.W. (1986) Bioalkylation of dibenz[*a,h*]anthracene in rat liver cytosol. *Chem.-biol. Interact*., **57**, 223–233

Flesher, J.W., Myers, S.R. & Stansbury, K.H. (1990) The site of substitution of the methyl group in the bioalkylation of benzo[*a*]pyrene. *Carcinogenesis*, **11**, 493–496

Flesher, J.W., Horn, J. & Lehner, A.F. (1997a) 6-Sulfooxymethylbenzo[*a*]pyrene is an ultimate electrophilic and carcinogenic form of the intermediary metabolite 6-hydroxymethylbenzo[*a*]pyrene. *Biochem. biophys. Res. Commun.*, **234**, 554–558

Flesher, J.W., Horn, J. & Lehner, A.F. (1997b) 7-Sulfooxymethyl-12-methylbenz[*a*]anthracene is an exceptionally reactive electrophilic mutagen and ultimate carcinogen. *Biochem. biophys. Res. Commun*., **231**, 144–148

Flesher, J., Horn, J. & Lehner, A.F. (2002) The meso-region theory of aromatic hydrocarbon carcinogenesis. *Polycyc. arom. Comp.*, **22**, 379–393

Flesher, J.W., Horn, J. & Lehner, A.F. (2004) Formation of benzylic alcohols and meso-aldehydes by one-electron oxidation of DMBA, a model for the first metabolic step in methylated carcinogenic hydrocarbon activation. *Polycyc. arom. Comp.*, **24**, 501–511

Flowers, L., Bleczinski, W.F., Burczynski, M.E., Harvey, R.G. & Penning, T.M. (1996) Disposition and biological activity of benzo[*a*]pyrene-7,8-dione. A genotoxic metabolite generated by dihydrodiol dehydrogenase. *Biochemistry*, **35**, 13664–13672

Flowers, L., Ohnishi, S.T. & Penning, T.M. (1997) DNA strand scission by polycyclic aromatic hydrocarbon *o*-quinones: Role of reactive oxygen species, Cu(II)/Cu(I) redox cycling, and *o*-semiquinone anion radicals. *Biochemistry*, **36**, 8640–8648

Flowers-Geary, L., Harvey, R.G. & Penning, T.M. (1992) Examination of polycyclic aromatic hydrocarbon *o*-quinones produced by dihydrodiol dehydrogenase as substrates for redox-cycling in rat liver. *Biochem. Life Sci. Adv.*, **11**, 49–58

Flowers-Geary, L., Harvey, R.G. & Penning, T.M. (1993) Cytotoxicity of polycyclic aromatic hydrocarbon *o*-quinones in rat and human hepatoma cells. *Chem. Res. Toxicol.*, **6**, 252–260

Flowers-Geary, L., Harvey, R.G. & Penning, T.M. (1995) Identification of benzo[*a*]pyrene-7,8-dione as an authentic metabolite of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene in isolated rat hepatocytes. *Carcinogenesis*, **16**, 2707–2715

Flowers-Geary, L., Bleczinski, W., Harvey, R.G. & Penning, T.M. (1996) Cytotoxicity and mutagenicity of polycyclic aromatic hydrocarbon *o*-quinones produced by dihydrodiol dehydrogenase. *Chem. biol. Interact.*, **99**, 55–72

Fritsche, E., Bruning, T., Jonkmanns, C., Ko, Y., Bolt, H.M. & Abel, J. (1999) Detection of cytochrome P450 *1B1 Bfr I* polymorphism: Genotype distribution in healthy German individuals and in patients with colorectal carcinoma. *Pharmacogenetics*, **9**, 405–408

Fuchs, J., Mlcoch, J., Platt, K.-L. & Oesch, F. (1993a) Characterization of highly polar bis-dihydrodiol epoxide–DNA adducts formed after metabolic activation of dibenz[*a*,*h*]anthracene. *Carcinogenesis*, **14**, 863–867

Fuchs, J., Mlcoch, J., Oesch, F. & Platt, K.L. (1993b) Characterization of highly polar DNA adducts derived from dibenz[*a*,*h*]anthracene (DBA), 3,4-dihydroxy-3,4-dihydro-DBA, and 3,4,10,11-tetrahydroxy-3,4,10,11-tetrahydro-DBA. *Toxicol. ind. Health*, **9**, 503–509

Fukumoto, S., Yamauchi, N., Moriguchi, H., Hippo, Y., Watanabe, A., Shibahara, J., Taniguchi, H., Ishikawa, S., Ito, H., Yamamoto, S., Iwanari, H., Hironaka, M., Ishikawa, Y., Niki, T., Sohara, Y., Kodama, T., Nishimura, M., Fukayama, M., Dosaka-Akita, H. & Aburatani, H. (2005) Overexpression of the aldo-keto reductase family protein AKR1B10 is highly correlated with smokers' non-small cell lung carcinomas. *Clin. Cancer Res.*, **11**, 1776–1785

Funatake, C.J., Dearstyne, E.A., Steppan, L.B., Shepherd, D.M., Spanjaard, E.S., Marshak-Rothstein, A. & Kerkvliet, N.I. (2004) Early consequences of 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on the activation and survival of antigen-specific T cells. *Toxicol. Sci.*, **82**, 129–142

Gabbani, G., Pavanello, S., Nardini, B., Tognato, O., Bordin, A., Fornasa, C.V., Bezze, G. & Clonfero, E. (1999) Influence of metabolic genotype GSTM1 on levels of urinary mutagens in patients treated topically with coal tar. *Mutat. Res.*, **440**, 27–33

Galati, R., Zijno, A., Crebelli, R., Falasca, G., Tomei, F., Iecher, F., Carta, P. & Verdina, A. (2001) Detection of antibodies to the benzo(a)pyrene diol epoxide–DNA adducts in sera from individuals exposed to low doses of polycyclic aromatic hydrocarbons. *J. exp. clin. Cancer Res.*, **20**, 359–364

Gallagher, J., Mumford, J., Li, X., Shank, T., Manchester, D. & Lewtas, J. (1993) DNA adduct profiles and levels in placenta, blood and lung in relation to cigarette smoking and smoky coal

emissions. In: Phillips, D.H., Castegnaro, M. & Bartsch, H., eds, *Postlabelling Methods for Detection of DNA Adducts* (IARC Scientific Publications No. 124), IARC, Lyon, pp. 283–292

Galvan, N., Jaskula-Sztul, R., MacWilliams, P.S., Czuprynski, C.J. & Jefcoate, C.R. (2003) Bone marrow cytotoxicity of benzo[a]pyrene is dependent on CYP1B1 but is diminished by Ah receptor-mediated induction of CYP1A1 in liver. *Toxicol. appl. Pharmacol*., **193**, 84–96

Galvan, N., Teske, D.E., Zhou, G., Moorthy, B., MacWilliams, P.S., Czuprynski, C.J. & Jefcoate, C.R. (2005) Induction of CYP1A1 and CYP1B1 in liver and lung by benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene do not affect distribution of polycyclic hydrocarbons to target tissue: Role of AhR and CYP1B1 in bone marrow cytotoxicity. *Toxicol. appl. Pharmacol*., **202**, 244–257

Gao, J., Lauer, F.T., Dunaway, S. & Burchiel, S.W. (2005a) Cytochrome P450 1B1 is required for 7,12-dimethylbenz(a)anthracene (DMBA) induced spleen cell immunotoxicity. *Toxicol. Sci*., **86**, 68–74

Gao, J., Voss, A.A., Pessah, I.N., Lauer, F.T., Penning, T.M. & Burchiel, S.W. (2005b) Ryanodine receptor-mediated rapid increase in intracellular calcium induced by 7,8-benzo(a)pyrene quinone in human and murine leukocytes. *Toxicol. Sci*., **87**, 419–426

Garcia-Martin, E., Martinez, C., Ladero, J.M., Gamito, F.J., Rodriguez-Lescure, A. & Agundez, J.A. (2002a) Influence of cytochrome P450 CYP2C9 genotypes in lung cancer risk. *Cancer Lett.*, **180**, 41–46

Garcia-Martin, E., Martinez, C., Pizarro, R.M., Garcia-Gamito, F.J., Gullsten, H., Raunio, H. & Agundez, J.A. (2002b) CYP3A4 variant alleles in white individuals with low CYP3A4 enzyme activity. *Clin. Pharmacol. Ther.*, **71**, 196–204

Garganta, F., Krause, G. & Scherer, G. (1999) Base-substitution profiles of externally activated polycyclic aromatic hydrocarbons and aromatic amines determined in a *lacZ* reversion assay. *Environ. mol. Mutag*., **33**, 75–85

Garner, R.C., Stanton, C.A., Martin, C.N., Harris, C.C. & Grafstrom, R.C. (1985) Rat and human explant metabolism, binding studies, and DNA adduct analysis of benzo(*a*)pyrene and its 6-nitro derivative. *Cancer Res*., **45**, 6225–6231

Garte, S., Gaspari, L., Alexandrie, A.K., Ambrosone, C., Autrup, H., Autrup, J.L., Baranova, H., Bathum, L., Benhamou, S., Boffetta, P., Bouchardy, C., Breskvar, K., Brockmoller, J., Cascorbi, I., Clapper, M.L., Coutelle, C., Daly, A., Dell'Omo, M., Dolzan, V., Dresler, C.M., Fryer, A., Haugen, A., Hein, D.W., Hildesheim, A., Hirvonen, A., Hsieh, L.L., Ingelman-Sundberg, M., Kalina, I., Kang, D., Kihara, M., Kiyohara, C., Kremers, P., Lazarus, P., Le Marchand, L., Lechner, M.C., van Lieshout, E.M., London, S., Manni, J.J., Maugard, C.M., Morita, S., Nazar-Stewart, V., Noda, K., Oda, Y., Parl, F.F., Pastorelli, R., Persson, I., Peters, W.H., Rannug, A., Rebbeck, T., Risch, A., Roelandt, L., Romkes, M., Ryberg, D., Salagovic, J., Schoket, B., Seidegard, J., Shields, P.G., Sim, E., Sinnet, D., Strange, R.C., Stucker, I., Sugimura, H., To-Figueras, J., Vineis, P., Yu, M.C. & Taioli, E. (2001) Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 1239–1248

Geacintov, N.E., Broyde, S., Buterin, T., Naegeli, H., Wu, M., Yan, S. & Patel, D.J. (2002) Thermodynamic and structural factors in the removal of bulky DNA adducts by the nucleotide excision repair machinery. *Biopolymers*, **65**, 202–210

Geddie, J.E., Amin, S., Huie, K. & Hecht, S.S. (1987) Formation and tumorigenicity of benzo[b]fluoranthene metabolites in mouse epidermis. *Carcinogenesis*, **8**, 1579–1584

Gehly, E.B., Landolph, J.R., Heidelberger, C., Nagasawa, H. & Little, J.B. (1982) Induction of cytotoxicity, mutation, cytogenetic changes, and neoplastic transformation by benzo(a)pyrene and derivatives in C3H/10T½ clone 8 mouse fibroblasts. *Cancer Res.*, **42**, 1866–1875

Gelboin, H.V. (1980) Benzo[alpha]pyrene metabolism, activation and carcinogenesis: Role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev.*, **60**, 1107–1166

Gelboin, H.V., Wiebel, F. & Diamond, L. (1970) Dimethylbenzanthracene tumorigenesis and aryl hydrocarbon hydroxylase in mouse skin: Inhibition by 7,8-benzoflavone. *Science*, **170**, 169–171

Gerde, P. & Scott, B.R. (2001) A model for absorption of low-volatile toxicants by the airway mucosa. *Inhal. Toxicol.*, **13**, 903–929

Gerde, P., Medinsky, M.A. & Bond, J.A. (1991a) The retention of polycyclic aromatic hydrocarbons in the bronchial airways and in the alveolar region—A theoretical comparison. *Toxicol. appl. Pharmacol.*, **107**, 239–252

Gerde, P., Medinsky, M.A. & Bond, J.A. (1991b) Particle-associated polycyclic aromatic hydrocarbons—a reappraisal of their possible role in pulmonary carcinogenesis. *Toxicol. appl. Pharmacol.*, **108**, 1–13

Gerde, P., Muggenburg, B.A. & Henderson, R.F. (1993a) Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. III. Mechanisms of the dosimetry. *Toxicol. appl. Pharmacol.*, **121**, 328–334

Gerde, P., Muggenburg, B.A., Hoover, M.D. & Henderson, R.F. (1993b) Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. I. The alveolar region. *Toxicol. appl. Pharmacol.*, **121**, 313–318

Gerde, P., Muggenburg, B.A., Sabourin, P.J., Harkema, J.R., Hotckiss, J.A., Hoover, M.D. & Henderson, R.F. (1993c) Disposition of polycyclic aromatic hydrocarbons in the respiratory tract of the beagle dog. II. The conducting airways. *Toxicol. appl. Pharmacol.*, **121**, 319–327

Gerde, P., Muggenburg, B.A., Thornton-Manning, J.R., Lewis, J.L., Pyon, K.H. & Dahl, A.R. (1997) Benzo[*a*]pyrene at an environmentally relevant dose is slowly absorbed by, and extensively metabolized in, tracheal epithelium. *Carcinogenesis*, **18**, 1825–1832

Gerde, P., Muggenburg, B.A., Scott, G.G., Lewis, J.L., Pyon, K.H. & Dahl, A.R. (1998) Local metabolism in lung airways increases the uncertainty of pyrene as a biomarker of polycyclic aromatic hydrocarbon exposure. *Carcinogenesis*, **19**, 493–500

Germolec, D.R., Henry, E.C., Maronpot, R., Foley, J.F., Adams, N.H., Gasiewicz, T.A. & Luster, M.I. (1996) Induction of CYP1A1 and ALDH-3 in lymphoid tissues from Fisher 344 rats exposed to 2,3,7,8-tetrachlorodibenzodioxin (TCDD). *Toxicol. appl. Pharmacol.*, **137**, 57–66

Giles, A.S., Seidel, A. & Phillips, D.H. (1996) Covalent DNA adducts formed in mouse epidermis by benzo[g]chrysene. *Carcinogenesis*, **17**, 1331–1336

Gill, R.D., Beltran, L., Nettikumara, A.N., Harvey, R.G., Kootstra, A. & DiGiovanni, J. (1992) Analysis of point mutations in murine c-Ha-*ras* of skin tumors initiated with dibenz[*a,j*]anthracene and derivatives. *Mol. Carcinog.*, **6**, 53–59

Gill, R.D., Rodriguez, H., Cortez, C., Harvey, R.G., Loechler, E.L. & DiGiovanni, J. (1993) Mutagenic specificity of the (+)*anti*-diol epoxide of dibenz[*a,j*]anthracene in the *supF* gene of an Escherichia coli plasmid. *Mol. Carcinog.*, **8**, 145–154

Gill, H.S., Kole, P.L., Wiley, J.C., Li, K.-M., Higginbotham, S., Rogan, E.G. & Cavalieri, E.L. (1994) Synthesis and tumor-initiating activity in mouse skin of dibenzo[*a,l*]pyrene *syn*- and *anti*-fjord-region diolepoxides. *Carcinogenesis*, **15**, 2455–2460

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S. & Goble, R. (2002) Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci*., **66**, 185–200

Glatt, H.R., Billings, R., Platt, K.L. & Oesch, F. (1981) Improvement of the correlation of bacterial mutagenicity with carcinogenicity of benzo(*a*)pyrene and four of its major metabolites by activation with intact liver cells instead of cell homogenate. *Cancer Res*., **41**, 270–277

Glatt, H., Piée, A., Pauly, K., Steinbrecher, T., Schrode, R., Oesch, F. & Seidel, A. (1991) Fjord- and bay-region diol-epoxides investigated for stability, SOS induction in *Escherichia coli*, and mutagenicity in *Salmonella typhimurium* and mammalian cells. *Cancer Res*., **51**, 1659–1667

Glatt, H., Engelke, C.E., Pabel, U., Teubner, W., Jones, A.L., Coughtrie, M.W., Andrae, U., Falany, C.N. & Meinl, W. (2000) Sulfotransferases: Genetics and role in toxicology. *Toxicol. Lett.*, **112–113**, 341–348

Godschalk, R.W., Ostertag, J.U., Moonen, E.J., Neumann, H.A., Kleinjans, J.C. & van Schooten, F.J. (1998) Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 767–773

Godschalk, R.W., Ostertag, J.U., Zandsteeg, A.M., Van Agen, B., Neuman, H.A., Van Straaten, H. & Van Schooten, F.J. (2001) Impact of GSTM1 on aromatic-DNA adducts and p53 accumulation in human skin and lymphocytes. *Pharmacogenetics*, **11**, 537–543

Gold, A. & Eisenstadt, E. (1980) Metabolic activation of cyclopenta(*cd*)pyrene to 3,4-epoxycyclopenta(*cd*)pyrene by rat liver microsomes. *Cancer Res*., **40**, 3940–3944

Gold, A., Nesnow, S., Moore, M., Garland, H., Curtis, G., Howard, B., Graham, D. & Eisenstadt, E. (1980) Mutagenesis and morphological transformation of mammalian cells by a non-bay-region polycyclic cyclopenta(*cd*)pyrene and its 3,4-oxide. *Cancer Res*., **40**, 4482–4484

Goldstein, L.S., Weyand, E.H., Safe, S., Steinberg, M., Culp, S.J., Gaylor, D.W., Beland, F.A. & Rodriguez, L.V. (1998) Tumors and DNA adducts in mice exposed to benzo[a]pyrene and coal tars: Implications for risk assessment. *Environ. Health Perspect.*, **106** (Suppl. 6), 1325–1330

Gong, Q.H., Cho, J.W., Huang, T., Potter, C., Gholami, N., Basu, N.K., Kubota, S., Carvalho, S., Pennington, M.W., Owens, I.S. & Popescu, N.C. (2001) Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. *Pharmacogenetics*, **11**, 357–368

Gonzalez, F.J. (1988) The molecular biology of cytochrome P450s. *Pharmacol. Rev*., **40**, 243–288

Gonzalez, F.J. (1990) Molecular genetics of the P-450 superfamily. *Pharmacol. Ther*., **45**, 1–38

Gonzalez, F.J., Crespi, C.L. & Gelboin, H.V. (1991) cDNA-expressed human cytochrome P450s: A new age of molecular toxicology and human risk assessment. *Mutat. Res*., **247**, 113–127

Granville, C.A., Hanley, N.M., Mumford, J.L. & DeMarini, D.M. (2003) Mutation spectra of smoky coal combustion emissions in *Salmonella* reflect the *TP53* and *KRAS* mutations in lung tumors from smoky coal-exposed individuals. *Mutat Res*., **525**, 77–83

van Grevenynghe, J., Rion, S., Le Ferrec, E., Le Vee, M., Amiot, L., Fauchet, R. & Fardel, O. (2003) Polycyclic aromatic hydrocarbons inhibit differentiation of human monocytes into macrophages. *J. Immunol*., **170**, 2374–2381

van Grevenynghe, J., Bernard, M., Langouet, S., Le Berre, C., Fest, T. & Fardel, O. (2005) Human CD34-positive hematopoietic stem cells constitute targets for carcinogenic polycyclic aromatic hydrocarbons. *J. Pharmacol. exp. Ther*., **314**, 693–702

Grzybowska, E., Butkiewicz, D., Motykiewicz, G. & Chorazy, M. (2000) The effect of the genetic polymorphisms of CYP1A1, CYP2D6, GSTM1 and GSTP1 on aromatic DNA adduct levels in the population of healthy women. *Mutat. Res.*, **469**, 271–277

Gsur, A., Zidek, T., Schnattinger, K., Feik, E., Haidinger, G., Hollaus, P., Mohn-Staudner, A., Armbruster, C., Madersbacher, S., Schatzl, G., Trieb, K., Vutuc, C. & Micksche, M. (2003) Association of microsomal epoxide hydrolase polymorphisms and lung cancer risk. *Br. J. Cancer*, **89**, 702–706

Guengerich, F.P. (1988) Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res.*, **48**, 2946–2954

Guengerich, F.P. (1999) Cytochrome P-450 3A4: Regulation and role in drug metabolism. *Annu. Rev. Pharmacol. Toxicol.*, **39**, 1–17

Guengerich, F.P. (2001) Uncommon P450-catalyzed reactions. *Curr. Drug Metab.*, **2**, 93–115

Guengerich, F.P. (2003) Cytochromes P450, drugs, and diseases. *Mol. Interv.*, **3**, 194–204

Guengerich, F.P. & Shimada, T. (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.*, **4**, 391–407

Guengerich, F.P. & Shimada, T. (1998) Activation of procarcinogens by human cytochrome P450 enzymes. *Mutat. Res.*, **400**, 201–213

Guengerich, F.P., Martin, M.V., Beaune, P.H., Kremers, P., Wolff, T. & Waxman, D.J. (1986) Characterization of rat and human liver microsomal cytochrome P-450 forms involved in nifedipine oxidation, a prototype for genetic polymorphism in oxidative drug metabolism. *J. biol. Chem.*, **261**, 5051–5060

Guillemette, C., Millikan, R.C., Newman, B. & Housman, D.E. (2000a) Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Res.*, **60**, 950–956

Guillemette, C., Ritter, J.K., Auyeung, D.J., Kessler, F.K. & Housman, D.E. (2000b) Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: Functional consequences of three novel missense mutations in the human *UGT1A7* gene. *Pharmacogenetics*, **10**, 629–644

Haimeur, A., Conseil, G., Deeley, R.G. & Cole, S.P.C. (2004) The MRP-related and BCRP/ABCG2 multidrug resistance proteins: Biology, substrate specificity and regulation. *Curr. Drug Metab.*, **5**, 21–53

Hainaut, P. & Pfeifer, G.P. (2001) Patterns of p53 G→T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. *Carcinogenesis*, **22**, 367–374

Haining, R.L., Hunter, A.P., Veronese, M.E., Trager, W.F. & Rettie, A.E. (1996) Allelic variants of human cytochrome P450 2C9: Baculovirus-mediated expression, purification, structural characterization, substrate stereoselectivity, and prochiral selectivity of the wild-type and I359L mutant forms. *Arch. Biochem. Biophys.*, **333**, 447–458

Hakkola, J., Tanaka, E. & Pelkonen, O. (1998a) Developmental expression of cytochrome P450 enzymes in human liver. *Pharmacol. Toxicol.*, **82**, 209–217

Hakkola, J., Pelkonen, O., Pasanen, M. & Raunio, H. (1998b) Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: Role in intrauterine toxicity. *Crit. Rev. Toxicol.*, **28**, 35–72

Hanelt, S., Helbig, R., Hartmann, A., Lang, M., Seidel, A. & Speit, G. (1997) A comparative investigation of DNA adducts, DNA strand breaks and gene mutations induced by benzo[a]pyrene and (+/-)-*anti*-benzo[*a*]pyrene-7,8-diol 9,10-oxide in cultured human cells. *Mutat. Res.*, **390**, 179–188

Hankinson, O. (1995) The aryl hydrocarbon receptor complex. *Annu. Rev. Pharmacol. Toxicol.*, **35**, 307–340

Hanson, A.A., Rogan, E.G. & Cavalieri, E.L. (1998) Synthesis of adducts formed by iodine oxidation of aromatic hydrocarbons in the presence of deoxyribonucleosides and nucleobases. *Chem. Res. Toxicol*., **11**, 1201–1208

Harper, P.A., Wong, J.Y., Lam, M.S. & Okey, A.B. (2002) Polymorphisms in the human AH receptor. *Chem.-biol. Interact*., **141**, 161–187

Harris, S.L. & Levine, A.J. (2005) The p53 pathway: Positive and negative feedback loops. *Oncogene*, **24**, 2899–2908

Harris, C.C., Vahakangas, K., Newman, M.J., Trivers, G.E., Shamsuddin, A., Sinopoli, N., Mann, D.L. & Wright, W.E. (1985) Detection of benzo[a]pyrene diol epoxide–DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. *Proc. natl Acad. Sci. USA*, **82**, 6672–6676

Harvey, R.G. (1991) *Polycyclic Aromatic Hydrocarbons Chemistry and Carcinogenicity*, Cambridge, University Press at Cambridge

Hashibe, M., Brennan, P., Strange, R.C., Bhisey, R., Cascorbi, I., Lazarus, P., Oude Ophuis, M.B., Benhamou, S., Foulkes, W.D., Katoh, T., Coutelle, C., Romkes, M., Gaspari, L., Taioli, E. & Boffetta, P. (2003) Meta- and pooled analyses of GSTM1, GSTT1, GSTP1, and CYP1A1 genotypes and risk of head and neck cancer. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 1509–1517

Hass, B.S., McKeown, C.K., Sardella, D.J., Boger, E., Ghoshal, P.K. & Huberman, E. (1982) Cell-mediated mutagenicity in Chinese hamster V79 cells of dibenzopyrenes and their bay-region fluorine-substituted derivatives. *Cancer Res*., **42**, 1646–1649

Hassett, C., Aicher, L., Sidhu, J.S. & Omiecinski, C.J. (1994) Human microsomal epoxide hydrolase: Genetic polymorphism and functional expression *in vitro* of amino acid variants. *Hum. mol. Genet*., **3**, 421–428

Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G.E., Newman, M.J. & Harris, C.C. (1986) Determination of polycyclic aromatic hydrocarbons in the urine, benzo(a)pyrene diol epoxide–DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. *Cancer Res*., **46**, 4178–4183

Hayashi, S.-I., Watanabe, J., Nakachi, K. & Kawajiri, K. (1991) Genetic linkage of lung cancer-associated *MspI* polymorphisms with amino acid replacement in the heme binding region of the human cytochrome P450IA1 gene. *J. Biochem*., **110**, 407–411

Hayashi, M., MacGregor, J.T., Gatehouse, D.G., Adler, I.D., Blakey, D.H., Dertinger, S.D., Krishna, G., Morita, T., Russo, A. & Sutou, S. (2000) In vivo rodent erythrocyte micronucleus assay. II. Some aspects of protocol design including repeated treatments, integration with toxicity testing, and automated scoring. *Environ. mol. Mutag*., **35**, 234–252

Hayashibara, T., Yamada, Y., Mori, N., Harasawa, H., Sugahara, K., Miyanishi, T., Kamihira, S. & Tomonaga, M. (2003) Possible involvement of aryl hydrocarbon receptor (AhR) in adult T-cell leukemia (ATL) leukemogenesis: Constitutive activation of AhR in ATL. *Biochem. biophys. Res. Commun*., **300**, 128–134

Hecht, S.S. (1998) Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chem. Res. Toxicol*., **11**, 559–603

Hecht, S.S. (2002a) Cigarette smoking and lung cancer: Chemical mechanisms and approaches to prevention. *Lancet Oncol.*, **3**, 461–469

Hecht, S.S. (2002b) Tobacco smoke carcinogens and breast cancer. *Environ. mol. Mutag.*, **39**, 119–126

Hecht, S.S., LaVoie, E., Mazzarese, R., Amin, S., Bedenko, V. & Hoffmann, D. (1978) 1,2-Dihydro-1,2-dihydroxy-5-methylchrysene, a major activated metabolite of the environmental carcinogen 5-methylchrysene. *Cancer Res.*, **38**, 2191–2194

Hecht, S.S., Rivenson, A. & Hoffmann, D. (1980) Tumor-initiating activity of dihydrodiols formed metabolically from 5-methylchrysene. *Cancer Res.*, **40**, 1396–1399

Hecht, S.S., LaVoie, E.J., Bendenko, V., Hoffmann, D., Sardella, D.J., Boger, E. & Lehr, R.E. (1981) On the metabolic activation of dibenzo[a,i]pyrene and dibenzo[a,h]pyrene. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons, Chemical Analyses and Biological Fate*, Columbus, OH, Battelle Press, pp. 43–54

Hecht, S.S., Radok, L., Amin, S., Huie, K., Melikian, A.A., Hoffmann, D., Pataki, J. & Harvey, R.G. (1985) Tumorigenicity of 5-methylchrysene dihydrodiols and dihydrodiol epoxides in newborn mice and on mouse skin. *Cancer Res.*, **45**, 1449–1452.

Hecht, S.S., Amin, S., Huie, K., Melikian, A.A. & Harvey, R.G. (1987) Enhancing effect of a bay region methyl group on tumorigenicity in newborn mice and mouse skin of enantiomeric bay region diol epoxides formed stereoselectively from methylchrysenes in mouse epidermis. *Cancer Res.*, **47**, 5310–5315

Hecht, S.S., El-Bayoumy, K., Rivenson, A. & Amin, S. (1994) Potent mammary carcinogenicity in female CD rats of a fjord region diol-epoxide of benzo[*c*]phenanthrene compared to a bay region diol-epoxide of benzo[*a*]pyrene. *Cancer Res.*, **54**, 21–24

Hecht, S.S., Rivenson, A., Amin, S., Krzeminski, J., El-Bayoumy, K., Reddy, B.S., Kurtzke, C. & La Voie, E.J. (1996) Mammary carcinogenicity of diol epoxide metabolites of benzo[j]fluoranthene in female CD rats. *Cancer Lett.*, **106**, 251–255

Hecht, S.S., Ronai, Z.A., Dolan, L., Desai, D. & Amin, S. (1998) Comparative mouse skin tumorigenicity and induction of Ha-*ras* mutations by bay region diol epoxides of 5-methylchrysene and 5,6-dimethylchrysene. *Carcinogenesis*, **19**, 157–160

Hegstad, S., Lundanes, E., Holme, J.A. & Alexander, J. (1999) Characterization of metabolites of benz(j)aceanthrylene in faeces, urine and bile from rat. *Xenobiotica*, **29**, 1257–1272

Heidel, S.M., Holston, K., Buters, J.T., Gonzalez, F.J., Jefcoate, C.R. & Czupyrynski, C.J. (1999) Bone marrow stromal cell cytochrome P4501B1 is required for pre-B cell apoptosis induced by 7,12-dimethylbenz[a]anthracene. *Mol. Pharmacol.*, **56**, 1317–1323

Heidel, S.M., MacWilliams, P.S., Baird, W.M., Dashwood, W.M., Buters, J.T., Gonzalez, F.J., Larsen, M.C., Czuprynski, C.J. & Jefcoate, C.R. (2000) Cytochrome P4501B1 mediates induction of bone marrow cytotoxicity and preleukemia cells in mice treated with 7,12-dimethylbenz[a]anthracene. *Cancer Res.*, **60**, 3454–3460

Heimler, I., Rawlins, R.G., Owen, H. & Hutz, R.J. (1998) Dioxin perturbs, in a dose- and time-dependent fashion, steroid secretion, and induces apoptosis of human luteinized granulosa cells. *Endocrinology*, **139**, 4373–4379

Heintz, N., Little, B., Bresnick, E. & Schaeffer, W.I. (1980) Malignant transformation of cultured rat hepatocytes by (+)- and (-)-trans-7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene. *Cancer Res.*, **40**, 1281–1285

Hemminki, K., Perera, F.P., Phillips, D.H., Randerath, K., Reddy, M.V. & Santella, R.M. (1988) Aromatic deoxyribonucleic acid adducts in white blood cells of foundry and coke oven workers. *Scand. J. Work Environ. Health*, **14** (Suppl. 1), 55–56

Henderson, P.T., Delaney, J.C., Muller, J.G., Neeley, W.L., Tannenbaum, S.R., Burrows, C.J. & Essigmann, J.M. (2003) The hydantoin lesions formed from oxidation of 7,8-dihydro-8-oxoguanine are potent sources of replication errors in vivo. *Biochemistry*, **42**, 9257–9262

Hengstler, J.G., Arand, M., Herrero, M.F. & Oesch, F. (1998) Polymorphisms of N-acetyltransferases, glutathione S-transferases, microsomal epoxide hydrolase and sulfortransferases: Influence on cancer susceptibility. *Recent Results Cancer Res.*, **154**, 47–85

Higginbotham, S., RamaKrishna, N.V.S., Johansson, S.L., Rogan, E.G. & Cavalieri, E.L. (1993) Tumor-initiating activity and carcinogenicity of dibenzo[*a,l*]pyrene *versus* 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene at low doses in mouse skin. *Carcinogenesis*, **14**, 875–878

Hirvonen, A., Husgafvel-Pursiainen, K., Karjalainen, A., Anttila, S. & Vainio, H. (1992) Point-mutational *MspI* and *Ile-Val* polymorphisms closely linked in the *CYP1A1* gene: Lack of association with susceptibility to lung cancer in a Finnish study population. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 485–489

Holladay, S.D. & Smialowicz, R.J. (2000) Development of the murine and human immune system: Differential effects of immunotoxicants depend on time of exposure. *Environ. Health Perspect.*, **108** (Suppl. 3), 463–473

Holladay, S.D. & Smith, B.J. (1995) Alterations in murine fetal thymus and liver hematopoietic cell populations following developmental exposure to 7,12-dimethylbenz[a]anthracene. *Environ. Res.*, **68**, 106–113

Holme, J.A., Bjorge, C., Soderlund, E.J., Brunborg, G., Becher, R., Lag, M., Schwarze, P.E., Ross, J., Nelson, G. & Nesnow, S. (1993) Genotoxic effects of cyclopenta-fused polycyclic aromatic hydrocarbons in isolated rat hepatocytes and rabbit lung cells. *Carcinogenesis*, **14**, 1125–1131

Holmen, A., Karlsson, A., Bratt, I., Raihle, G. & Hogstedt, B. (1994) Increased frequency of micronuclei in lymphocytes of Swedish chimney sweeps. *Int. Arch. occup. environ. Health*, **66**, 185–187

Holsapple, M.P., West, L.J. & Landreth, K.S. (2003) Species comparison of anatomical and functional immune system development. *Birth Defects Res. B Dev. Reprod. Toxicol.*, **68**, 321–334

Honkakoski, P. & Negishi, M. (2000) Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochem. J.*, **347**, 321–337

Hoogervorst, E.M., van Steeg, H. & de Vries, A. (2005) Nucleotide excision repair- and *p53*-deficient mouse models in cancer research. *Mutat. Res.*, **574**, 3–21

Hosagrahara, V.P., Rettie, A.E., Hassett, C. & Omiecinski, C.J. (2004) Functional analysis of human microsomal epoxide hydrolase genetic variants. *Chem.-biol. Interact.*, **150**, 149–159

Hou, L., Chatterjee, N., Huang, W.-Y., Baccarelli, A., Yadavalli, S., Yeager, M., Bresalier, R.S., Chanock, S.J., Caporaso, N.E., Ji, B.-T., Weissfeld, J.L. & Hayes, R.B. (2005) *CYP1A1* Val_{462} and *NQO1* Ser_{187} polymorphisms, cigarette use, and risk for colorectal adenoma. *Carcinogenesis*, **26**, 1122–1128

House, R.V., Lauer, L.D., Murray, M.J. & Dean, J.H. (1987) Suppression of T-helper cell function in mice following exposure to the carcinogen 7,12-dimethylbenz[a]anthracene and its restoration by interleukin-2. *Int. J. Immunopharmacol.*, **9**, 89–97

House, R.V., Pallardy, M.J. & Dean, J.H. (1989) Suppression of murine cytotoxic T-lymphocyte induction following exposure to 7,12-dimethylbenz[*a*]anthracene: Dysfunction of antigen recognition. *Int. J. Immunopharmacol.*, **11**, 207–215

Hsu, I.C., Stoner, G.D., Autrup, H., Trump, B.F., Selkirk, J.K. & Harris, C.C. (1978) Human bronchus-mediated mutagenesis of mammalian cells by carcinogenic polynuclear aromatic hydrocarbons. *Proc. natl Acad. Sci. USA*, **75**, 2003–2007

Hsu, C.-H., Skipper, P.L., Harris, T.M. & Tannenbaum, S.R. (1997) Characterization of DNA adducts formed by cyclopenta[*cd*]pyrene epoxide. *Chem. Res. Toxicol*., **10**, 248–253

Hsu, C.-H., Skipper, P.L. & Tannenbaum, S.R. (1999) DNA adduct formation by secondary metabolites of cyclopenta[*cd*]pyrene in vitro. *Cancer Lett*., **136**, 137–141

Hsu, N.-Y., Ho, H.-C., Chow, K.-C., Lin, T.-Y., Shih, C.-S., Wang, L.-S. & Tsai, C.-M. (2001) Overexpression of dihydrodiol dehydrogenase as a prognostic marker of non-small cell lung cancer. *Cancer Res*., **61**, 2727–2731

Hu, X. & Singh, S.V. (1997) Differential catalytic efficiency and enantioselectivity of murine glutathione *S*-transferase isoenzymes in the glutathione conjugation of carcinogenic *anti*-diol epoxides of chrysene and benzo(*g*)chrysene. *Arch. Biochem. Biophys*., **345**, 318–324

Hu, X., Pal, A., Krzeminski, J., Amin, S., Awasthi, Y.C., Zimniak, P. & Singh, S.V. (1998) Specificities of human glutathione *S*-transferase isozymes toward *anti*-diol epoxides of methylchrysenes. *Carcinogenesis*, **19**, 1685–1689

Hu, W., Feng, Z. & Tang, M.-S. (2003) Preferential carcinogen–DNA adduct formation at codons 12 and 14 in the human *K-ras* gene and their possible mechanisms. *Biochemistry*, **42**, 10012–10023

Hu, W., Feng, Z. & Tang, M.-S. (2004) Nickel (II) enhances benzo[a]pyrene diol epoxide-induced mutagenesis through inhibition of nucleotide excision repair in human cells: A possible mechanism for nickel (II)-induced carcinogenesis. *Carcinogenesis*, **25**, 455–462

Huang, Z., Fasco, M.J., Figge, H.L., Keyomarsi, K. & Kaminsky, L.S. (1996) Expression of cytochromes P450 in human breast tissue and tumors. *Drug Metab. Dispos*., **24**, 899–905

Huang, J.D., Guo, W.C., Lai, M.D., Guo, Y.L. & Lambert, G.H. (1999) Detection of a novel cytochrome P-450 1A2 polymorphism (F21L) in Chinese. *Drug Metab. Dispos.*, **27**, 98–101

Huang, W.-Y., Chatterjee, N., Chanock, S., Dean, M., Yeager, M., Schoen, R.E., Hou, L.-F., Berndt, S.I., Yadavalli, S., Johnson, C.C. & Hayes, R.B. (2005) Microsomal epoxide hydrolase polymorphisms and risk for advanced colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev*., **14**, 152–157

Huc, L., Sparfel, L., Rissel, M., Dimanche-Boitrel, M.-T., Guillouzo, A., Fardel, O. & Lagadic-Gossmann, D. (2004) Identification of Na^+/H^+ exchange as a new target for toxic polycyclic aromatic hydrocarbons. *FASEB J*., **18**, 344–346

Huckle, K.R., Smith, R.J., Watson, W.P. & Wright, A.S. (1986) Comparison of hydrocarbon–DNA adducts formed in mouse skin *in vivo* and in organ culture *in vitro* following treatment with benzo[a]pyrene. *Carcinogenesis*, **7**, 965–970

Hughes, N.C. & Phillips, D.H. (1990) Covalent binding of dibenzpyrenes and benzo[a]pyrene to DNA: Evidence for synergistic and inhibitory interactions when applied in combination to mouse skin. *Carcinogenesis*, **11**, 1611–1619

Hughes, N.C., Pfau, W., Hewer, A., Jacob, J., Grimmer, G. & Phillips, D.H. (1993) Covalent binding of polycyclic aromatic hydrocarbon components of coal tar to DNA in mouse skin. *Carcinogenesis*, **14**, 135–144

Hukkanen, J., Pelkonen, O., Hakkola, J. & Raunio, H. (2002) Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit. Rev. Toxicol.*, **32**, 391–411

Hung, R.J., Boffetta, P., Brennan, P., Malaveille, C., Hautefeuille, A., Donato, F., Gelatti, U., Spaliviero, M., Placidi, D., Carta, A., Scotto, D.C. & Porru, S. (2004) GST, NAT, SULT1A1, CYP1B1 genetic polymorphisms, interactions with environmental exposures and bladder cancer risk in a high-risk population. *Int. J. Cancer*, **110**, 598–604

Hussain, S.P., Amstad, P., Raja, K., Sawyer, M., Hofseth, L., Shields, P.G., Hewer, A., Phillips, D.H., Ryberg, D., Haugen, A. & Harris, C.C. (2001) Mutability of p53 hotspot codons to benzo(a)pyrene diol epoxide (BPDE) and the frequency of p53 mutations in nontumorous human lung. *Cancer Res*., **61**, 6350–6355

Hyndman, D., Bauman, D.R., Heredia, V.V. & Penning, T.M. (2003) The aldo-keto reductase superfamily homepage. *Chem.-biol. Interact*., **143–144**, 621–631

IARC (1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 32, *Polynuclear Aromatic Compounds. Part 1: Chemical, Environmental and Experimental Data*, Lyon

IARC (1984) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 34, *Polynuclear Aromatic Compounds. Part 3: Industrial Exposures in Aluminum Production, Coal Gasification, Coke Production, and Iron and Steel Founding*, Lyon

IARC (1985) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 35, *Polynuclear Aromatic Compounds. Part 4: Bitumens, Coal-Tars, and Derived Products, Shale Oils and Soots*, Lyon

IARC (1992) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 55, *Solar and Ultraviolet Radiation*, Lyon

IARC (2010) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 93, *Carbon Black, Titanium Dioxide and Non-Asbestiform Talc*, Lyon (in press)

Ichiba, M., Hagmar, L., Rannug, A., Hogstedt, B., Alexandrie, A.K., Carstensen, U. & Hemminki, K. (1994) Aromatic DNA adducts, micronuclei and genetic polymorphism for CYP1A1 and GST1 in chimney sweeps. *Carcinogenesis*, **15**, 1347–1352

Inoue, K., Asao, T. & Shimada, T. (2000) Ethnic-related differences in the frequency distribution of genetic polymorphisms in the CYP1A1 and CYP1B1 genes in Japanese and Caucasian populations. *Xenobiotica*, **30**, 285–295

Inyang, F., Ramesh, A., Kopsombut, P., Niaz, M.S., Hood, D.B., Nyanda, A.M. & Archibong, A.E. (2003) Disruption of testicular steroidogenesis and epididymal function by inhaled benzo(*a*)pyrene. *Reprod. Toxicol*., **17**, 527–537

Ip, C., El-Bayoumy, K., Upadhyaya, P., Ganther, H., Vadhanavikit, S. & Thompson, H. (1994) Comparative effect of inorganic and organic selenocyanate derivatives in mammary cancer chemoprevention. *Carcinogenesis*, **15**, 187–192

IPCS (1998) *Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons* (Environmental Health Criteria 202), Geneva, WHO, International Programme on Chemical Safety. Available at: http://www.inchem.org/documents/ehc/ehc202.htm

Ishibe, N., Hankinson, S.E., Colditz, G.A., Spiegelman, D., Willett, W.C., Speizer, F.E., Kelsey, K.T. & Hunter, D.J. (1998) Cigarette smoking, cytochrome P450 1A1 polymorphisms, and breast cancer risk in the Nurses' Health Study. *Cancer Res.*, **58**, 667–671

Ittah, Y., Thakker, D.R., Levin, W., Croisy-Delcey, M., Ryan, D.E., Thomas, P.E., Conney, A.H. & Jerina, D.M. (1983) Metabolism of benzo[*c*]phenanthrene by rat liver microsomes and by a

purified monooxygenase system reconstituted with different isozymes of cytochrome *P*-450. *Chem. biol. Interact.*, **45**, 15–28

Iyer, R.P., Lyga, J.W., Secrist, J.A.,III, Daub, G.H. & Slaga, T.J. (1980) Comparative tumor-initiating activity of methylated benzo(*a*)pyrene derivatives in mouse skin. *Cancer Res.*, **40**, 1073–1076

Jaiswal, A.K., Gonzalez, F.J. & Nebert, D.W. (1985) Human *P1-450* gene sequence and correlation of mRNA with genetic differences in benzo[a]pyrene metabolism. *Nucleic Acids Res.*, **13**, 4503–4520

Jaiswal, A.K., Nebert, D.W., McBride, O.W. & Gonzalez, F.J. (1987) Human P_3-450: cDNA and complete protein sequence, repetitive Alu sequences in the 3′ nontranslated region, and localization of gene to chromosome 15. *J. exp. Pathol.*, **3**, 1–17

Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W.W., Fong, H.H.S., Farnsworth, N.R., Kinghorn, A.D., Mehta, R.G., Moon, R.C. & Pezzuto, J.M. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, **275**, 218–220

Jankowiak, R. & Small, G.J. (1998) Analysis of PAH–DNA adducts – Fluorescence line-narrowing spectroscopy. In: Neilson, A.H. ed. *The Handbook of Environmental Chemistry*, Vol . 3J, *PAHs and Related Compounds*, Heidelberg, Springer-Verlag, pp. 120–145

Jankowiak, R., Ariese, F., Zamzow, D., Luch, A., Kroth, H., Seidel, A. & Small, G.J. (1997) Conformational studies of stereoisomeric tetrols derived from *syn*- and *anti*-dibenzo[*a*,*l*]pyrene diol epoxides. *Chem. Res. Toxicol.*, **10**, 677–686

Jankowiak, R., Ariese, F., Hewer, A., Luch, A., Zamzow, D., Hughes, N.C., Phillips, D., Seidel, A., Platt, K.-L., Oesch, F. & Small, G.J. (1998) Structure, conformations, and repair of DNA adducts from dibenzo[*a*,*l*]pyrene: ^{32}P-Postlabeling and fluorescence studies. *Chem. Res. Toxicol.*, **11**, 674–685

JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2005) Report of the 64th Meeting, Rome, FAO, pp. 32–38

Jeffrey, A.M., Weinstein, I.B., Jennette, K.W., Grzeskowiak, K., Nakanishi, K., Harvey, R.G., Autrup, H. & Harris, C. (1977) Structures of benzo(*a*)pyrene–nucleic acid adducts formed in human and bovine bronchial explants. *Nature*, **269**, 348–350

Jensen, B.A., Leeman, R.J., Schlezinger, J.J. & Sherr, D.H. (2003) Aryl hydrocarbon receptor (AhR) agonists suppress interleukin-6 expression by bone marrow stromal cells: An immunotoxicology study. *Environ. Health*, **2**, 16–28

Jerina, D.M. & Dansette, P.M. (1977) Hepatic microsomal epoxide hydrase: A sensitive radiometric assay for hydration of arene oxides of carcinogenic aromatic hydrocarbons. *Mol. Pharmacol.*, **13**, 342–351

Jerina, D.M., Lehr, R., Yagi, H., Hernandez, O., Dansette, P., Wislocki, P.G., Wood, A.W., Chang, R.L., Levin, W. & Conney, A.H. (1976) Mutagenicity of benzo(*a*)pyrene derivatives and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In: deSerres, F.J., Fouts, J.R., Bend, J.R. & Philpot, R.M., eds, *In Vitro Metabolic Activation and Mutagenesis Testing*, Amsterdam, Elsevier/North-Holland Biomedical, pp. 159–177

Jerina, D.M., Sayer, J.M., Agarwal, S.K., Yagi, H., Levin, W., Wood, A.W., Conney, A.H., Pruess-Schwartz, D., Baird, W.M., Pigott, M.A. & Dipple, A. (1986) Reactivity and tumorigenicity of

bay-region diol epoxides derived from polycyclic aromatic hydrocarbons. *Adv. exp. Med. Biol*., **197**, 11–30

Jernström, B., Funk, M., Frank, M., Mannervik, B. & Seidel, A. (1996) Glutathione S-transferase A1-1 catalyzed conjugation of bay and fjord region diol epoxides or polycyclic aromatic hydrocarbons with glutathione. *Carcinogenesis*, **17**, 1491–1498

Jiang, H., Shen, Y.-M., Quinn, A.M. & Penning, T.M. (2005a) Competing roles of cytochrome P450 1A1/1B1 and aldo-keto reductase 1A1 in the metabolic activation of (+/–)-7,8-dihydroxy-7,8-dihydro-benzo[*a*]pyrene in human bronchoalveolar cell extracts. *Chem. Res. Toxicol*., **18**, 365–374

Jiang, H., Vudathala, D., Blair, I.A. & Penning, T.M. (2005b) Activation of (±)-benzo[*a*]pyrene-7,8-diol to benzo[*a*]pyrene-7,8-dione by AKR1A1 in human bronchoalveolar H358 cells results in functional induction of P450 cytochrome 1A1/1B1. *Proc. Am. Assoc. Cancer Res*., **96**, 369

Jiménez, M., Aranda, F.J., Teruel, J.A. & Ortiz, A. (2002) The chemical toxic benzo[*a*]pyrene perturbs the physical organization of phosphatidylcholine membranes. *Environ Toxicol. Chem*., **21**, 787–793

Jinno, H., Saeki, M., Saito, Y., Tanaka-Kagawa, T., Hanioka, N., Sai, K., Kaniwa, N., Ando, M., Shirao, K., Minami, H., Ohtsu, A., Yoshida, T., Saijo, N., Ozawa, S. & Sawada, J.-I. (2003) Functional characterization of human UDP-glucuronosyltransferase 1A9 variant, D256N, found in Japanese cancer patients. *J. Pharmacol. exp. Ther*., **306**, 688–693

Johnsen, N.M., Schwarze, P.E., Nyholm, S.H., Lag, M., Becher, R., Brunborg, G. & Holme, J.A. (1997) Genotoxic effects of cyclopenta-fused polycyclic aromatic hydrocarbons in different types of isolated rat lung cells. *Carcinogenesis*, **18**, 193–199

Johnsen, N.M., Brunborg, G., Haug, K., Scholz, T. & Holme, J.A. (1998a) Metabolism and activation of cyclopenta polycyclic aromatic hydrocarbons in isolated human lymphocytes, HL-60 cells and exposed rats. *Chem.-biol. Interact*., **114**, 77–95

Johnsen, N.M., Nyholm, S.H., Haug, K., Scholz, T. & Holme, J.A. (1998b) Metabolism and activation of cyclopenta polycyclic aromatic hydrocarbons in liver tissue from rats and humans. *Chem.-biol. Interact*., **113**, 217–237

Joseph, P. & Jaiswal, A.K. (1994) NAD(P)H:quinone oxidoreductase1 (DT diaphorase) specifically prevents the formation of benzo[a]pyrene quinone–DNA adducts generated by cytochrome P4501A1 and P450 reductase. *Proc. natl Acad. Sci. USA*, **91**, 8413–8417

Jyonouchi, H., Sun, S., Iijima, K., Wang, M. & Hecht, S.S. (1999) Effects of anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene on human small airway epithelial cells and the protective effects of myo-inositol. *Carcinogenesis*, **20**, 139–145

Kagan, J., Tuveson, R.W. & Gong. H.-H. (1989) The light-dependent cytotoxicity of benzo[*a*]pyrene: Effect on human erythrocytes, *Escherichia coli* cells, and *Haemophilus influenzae* transforming DNA. *Mutat. Res.*, **216**, 231–242

Kalina, I., Brezani, P., Gajdosova, D., Binkova, B., Salagovic, J., Habalova, V., Mrackova, G., Dobias, L. & Šrám, R.J. (1998) Cytogenetic monitoring in coke oven workers. *Mutat. Res*., **417**, 9–17

Kamath, A.B., Xu, H., Nagarkatti, P.S. & Nagarkatti, M. (1997) Evidence for the induction of apoptosis in thymocytes by 2,3,7,8-tetrachlorodibenzo-p-dioxin in vivo. *Toxicol. appl. Pharmacol*., **142** , 367–377

Kapitulnik, J., Levin, W., Yagi, H., Jerina, D.M. & Conney, A.H. (1976) Lack of carcinogenicity of 4-, 5-, 6-, 7-, 8-, 9-, and 10-hydroxybenzo(*a*)pyrene on mouse skin. *Cancer Res.*, **36**, 3625–3628

Kapitulnik, J., Wislocki, P.G., Levin, W., Yagi, H., Jerina, D.M. & Conney, A.H. (1978a) Tumorigenicity studies with diol-epoxides of benzo(*a*)pyrene which indicate that (+/)-*trans*-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene is an ultimate carcinogen in newborn mice. *Cancer Res*., **38**, 354–358

Kapitulnik, J., Wislocki, P.G., Levin, W., Yagi, H., Thakker, D.R., Akagi, H., Koreeda, M., Jerina, D.M. & Conney, A.H. (1978b) Marked differences in the carcinogenic activity of optically pure (+)- and (-)-trans-7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene in newborn mice. *Cancer Res*., **38**, 2661–2665

Karakaya, A., Yucesoy, B., Turhan, A., Erdem, O., Burgaz, S. & Karakaya, A.E. (1999) Investigation of some immunological functions in a group of asphalt workers exposed to polycyclic aromatic hydrocarbons. *Toxicology*, **135**, 43–47

Karakaya, A., Ates, I. & Yucesoy, B. (2004) Effects of occupational polycyclic aromatic hydrocarbon exposure on T-lymphocyte functions and natural killer cell activity in asphalt and coke oven workers. *Hum. exp. Toxicol*., **23**, 317–322

Katz, A.K., Carrell, H.L. & Glusker, J.P. (1998) Dibenzo[*a*,*l*]pyrene (dibenzo[*def*,*p*]chrysene): Fjord-region distortions. *Carcinogenesis*, **19**, 1641–1648

Kawabata, T.T. & White, K.L., Jr (1987) Suppression of the in vitro humoral immune response of mouse splenocytes by benzo(a)pyrene metabolites and inhibition of benzo(a)pyrene-induced immunosuppression by alpha-naphthoflavone. *Cancer Res*., **47**, 2317–2322

Kawabata, T.T. & White, K.L., Jr (1989) Benzo(a)pyrene metabolism by murine spleen microsomes. *Cancer Res*., **49**, 5816–5822

Kawabata, T.T. & White, K.L., Jr (1990) Effects of naphthalene and naphthalene metabolites on the in vitro humoral immune response. *J. Toxicol. environ. Health*, **30**, 53–67

Kawajiri, K. & Fujii-Kuriyama, Y. (1991) P450 and human cancer. *Jpn. J. Cancer Res*., **82**, 1325–1335

Kawajiri, K., Eguchi, H., Nakachi, K., Sekiya, T. & Yamamoto, M. (1996) Association of CYP1A1 germ line polymorphisms with mutations of the p53 gene in lung cancer. *Cancer Res*., **56**, 72–76

Kel, A., Reymann, S., Matys, V., Nettesheim, P., Wingender, E. & Borlak, J. (2004) A novel computational approach for the prediction of networked transcription factors of aryl hydrocarbon-receptor-regulated genes. *Mol. Pharmacol*., **66**, 1557–1572

Kelley, M.J. & Littman, S.J. (2002) Etiology of the mutational spectrum of ras genes in human carcinoma. *J. natl Cancer Inst*., **94**, 1516–1517

Kelsey, K.T., Wiencke, J.K. & Spitz, M.R. (1994) A race-specific genetic polymorphism in the CYP1A1 gene is not associated with lung cancer in African Americans. *Carcinogenesis*, **15**, 1121–1124

Keohavong, P., Melacrinos, A. & Shukla, R. (1995) *In vitro* mutational spectrum of cyclopenta[*cd*]pyrene in the human HPRT gene. *Carcinogenesis*, **16**, 855–860

Kerkvliet, N.I., Shepherd, D.M. & Baecher-Steppan, L. (2002) T lymphocytes are direct, aryl hydrocarbon receptor (AhR)-dependent targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD): AhR expression in both CD4+ and CD8+ T cells is necessary for full suppression of a cytotoxic T lymphocyte response by TCDD. *Toxicol. appl. Pharmacol*., **185**, 146–152

Khan, Q.A., Vousden, K.H. & Dipple, A. (1997) Cellular response to DNA damage from a potent carcinogen involves stabilization of p53 without induction of $p21^{waf1/cip1}$. *Carcinogenesis*, **18**, 2313–2318

Khan, Q.A., Agarwal, R., Seidel, A., Frank, H., Vousden, K.H. & Dipple, A. (1998) DNA adduct levels associated with p53 induction and delay of MCF-7 cells in S phase after exposure to benzo[*g*]chrysene dihydrodiol epoxide enantiomers. *Mol. Carcinog*., **23**, 115–120

Khan, Q.A., Vousden, K.H. & Dipple, A. (1999) Lack of p53-mediated G1 arrest in response to an environmental carcinogen. *Oncology*, **57**, 258–264

Khan, G.A., Bhattacharya, G., Mailander, P.C., Meza, J.L., Hansen, L.A. & Chakravarti, D. (2005) Harvey-*ras* gene expression and epidermal cell proliferation in dibenzo[*a*,*l*]pyrene-treated early preneoplastic SENCAR mouse skin. *J. invest. Dermatol*., **125**, 567–574

Kidd, R.S., Curry, T.B., Gallagher, S., Edeki, T., Blaisdell, J. & Goldstein, J.A. (2001) Identification of a null allele of *CYP2C9* in an African–American exhibiting toxicity to phenytoin. *Pharmacogenetics*, **11**, 803–808

Kidd, L.C., Woodson, K., Taylor, P.R., Albanes, D., Virtamo, J. & Tangrea, J.A. (2003) Polymorphisms in glutathione-S-transferase genes (GST-M1, GST-T1 and GST-P1) and susceptibility to prostate cancer among male smokers of the ATBC cancer prevention study. *Eur. J. Cancer Prev.*, **12**, 317–320

King, L.C., George, M., Gallagher, J.E. & Lewtas, J. (1994) Separation of ^{32}P-postlabeled DNA adducts of polycyclic aromatic hydrocarbons and nitrated polycyclic aromatic hydrocarbons by HPLC. *Chem. Res. Toxicol*., **7**, 503–510

Kinoshita, N. & Gelboin, H.V. (1972) Aryl hydrocarbon hydroxylase and polycyclic hydrocarbon tumorigenesis: Effect of the enzyme inhibitor 7,8-benzoflavone on tumorigenesis and macromolecule binding. *Proc. natl Acad. Sci. USA*, **69**, 824–828

Kitada, M., Taneda, M., Itahashi, K. & Kamataki, T. (1991) Four forms of cytochrome P-450 in human fetal liver: Purification and their capacity to activate promutagens. *Jpn. J. Cancer Res*., **82**, 426–432

Kiyohara, C., Otsu, A., Shirakawa, T., Fukuda, S. & Hopkin, J.M. (2002a) Genetic polymorphisms and lung cancer susceptibility: A review. *Lung Cancer*, **37**, 241–256

Kiyohara, C., Shirakawa, T. & Hopkin, J.M. (2002b) Genetic polymorphisms and lung cancer susceptibility and the risk of lung cancer. *Environ. Health prevent. Med*., **7**, 47–59

Kleiner, H.E., Vulimiri, S.V., Reed, M.J., Uberecken, A. & DiGiovanni, J. (2002) Role of cytochrome P450 1a1 and 1b1 in the metabolic activation of 7,12-dimethylbenz[a]anthracene and the effects of naturally occurring furanocoumarins on skin tumor initiation. *Chem. Res. Toxicol*., **15**, 226–235

Kleiner, H.E., Reed, M.J. & DiGiovanni, J. (2003) Naturally occurring coumarins inhibit human cytochromes P450 and block benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene DNA adduct formation in MCF-7 cells. *Chem. Res. Toxicol*., **16**, 415–422

Kleiner, H.E., Vulimiri, S.V., Hatten, W.B., Reed, M.J., Nebert, D.W., Jefcoate, C.R. & DiGiovanni, J. (2004) Role of cytochrome P4501 family members in the metabolic activation of polycyclic aromatic hydrocarbons in mouse epidermis. *Chem. Res. Toxicol*., **17**, 1667–1674

Kligerman, A.D., Moore, M.M., Erexson, G.L., Brock, K.H., Doerr, C.L., Allen, J.W. & Nesnow, S. (1986) Genotoxicity studies of benz[*l*]aceanthrylene. *Cancer Lett*., **31**, 123–131

Knuckles, M.E., Inyang, F. & Ramesh, A. (2001) Acute and subchronic oral toxicities of benzo[a]pyrene in F-344 rats. *Toxicol. Sci*., **61**, 382–388

Ko, Y., Abel, J., Harth, V., Brode, P., Antony, C., Donat, S., Fischer, H.P., Ortiz-Pallardo, M.E., Thier, R., Sachinidis, A., Vetter, H., Bolt, H.M., Herbold, C. & Bruning, T. (2001) Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. *Cancer Res.*, **61**, 4398–4404

Ko, C.-B., Kim, S.-J., Park, C., Kim, B.-R., Shin, C.-H., Choi, S., Chung, S.-Y., Noh, J.-H., Jeun, J.-H., Kim, N.-S. & Park, R. (2004) Benzo(a)pyrene-induced apoptotic death of mouse hepatoma Hepa1c1c7 cells via activation of intrinsic caspase cascade and mitochondrial dysfunction. *Toxicology*, **199**, 35–46

Koehl, W., Amin, S., Staretz, M.E., Ueng, Y.-F., Yamazaki, H., Tateishi, T., Guengerich, F.P. & Hecht, S.S. (1996) Metabolism of 5-methylchrysene and 6-methylchrysene by human hepatic and pulmonary cytochrome P450 enzymes. *Cancer Res.*, **56**, 316–324

Koganti, A., Singh, R., Ma, B.L. & Weyand, E.H. (2001) Comparative analysis of PAH:DNA adducts formed in lung of mice exposed to neat coal tar and soils contaminated with coal tar. *Environ. Sci. Technol.*, **35**, 2704–2709

Kohan, M.J., Sangaiah, R., Ball, L.M. & Gold, A. (1985) Bacterial mutagenicity of aceanthrylene: A novel cyclopenta-fused polycyclic aromatic hydrocarbon of low molecular weight. *Mutat. Res.*, **155**, 95–98

Koizumi, A., Suzuki, T., Saitoh, N. & Kamiyama, S. (1994) A novel compound, 9-hydroxy-1-nitropyrene, is a major photodegraded compound of 1-nitropyrene in the environment. *Arch. environ. Health*, **49**, 87–92

Kong, A.N., Yu, R., Hebbar, V., Chen, C., Owuor, E., Hu, R., Ee, R. & Mandlekar, S. (2001) Signal transduction events elicited by cancer prevention compounds. *Mutat. Res.*, **480–481**, 231–241

Koreeda, M., Moore, P.D., Wislocki, P.G., Levin, W., Conney, A.H., Yagi, H. & Jerina, D.M. (1978) Binding of benzo[*a*]pyrene 7,8-diol-9,10-epoxides to DNA, RNA and protein of mouse skin occurs with high stereoselectivity. *Science*, **199**, 778–781

Kouri, R.E., Wood, A.W., Levin, W., Rude, T.H., Yagi, H., Mah, H.D., Jerina, D.M. & Conney, A.H. (1980) Carcinogenicity of benzo[*a*]pyrene and thirteen of its derivatives in C3H/fCum mice. *J. natl Cancer Inst.*, **64**, 617–623

Kozack, R.E. & Loechler, E.L. (1999) Molecular modeling of the major adduct of (+)-*anti*-B[*a*]PDE (N^2-dG) in the eight conformations and the five DNA sequences most relevant to base substitution mutagenesis. *Carcinogenesis*, **20**, 85–94

Krieger, J.A., Born, J.L. & Burchiel, S.W. (1994) Persistence of calcium elevation in the HPB-ALL human T cell line correlates with immunosuppressive properties of polycyclic aromatic hydrocarbons. *Toxicol. appl. Pharmacol.*, **127**, 268–274

Krieger, J.A., Davila, D.R., Lytton, J., Born, J.L. & Burchiel, S.W. (1995) Inhibition of sarcoplasmic/endoplasmic reticulum calcium ATPases (SERCA) by polycyclic aromatic hydrocarbons in HPB-ALL human T cells and other tissues. *Toxicol. appl. Pharmacol.*, **133**, 102–108

Kriek, E., Van Schooten, F.J., Hillebrand, M.J., Van Leeuwen, F.E., Den Engelse, L., De Looff, A.J. & Dijkmans, A.P. (1993) DNA adducts as a measure of lung cancer risk in humans exposed to polycyclic aromatic hydrocarbons. *Environ. Health Perspect.*, **99**, 71–75

Kristensen, P., Eilertsen, E., Einarsdóttir, E., Haugen, A., Skaug, V. & Øvrebo, S. (1995) Fertility in mice after prenatal exposure to benzo[*a*]pyrene and inorganic lead. *Environ. Health Perspect.*, **103**, 588–590

Kroese, E.D., Muller, J.J.A., Mohn, G.R., Dortant, P.M. & Wester, P.W. (2001) *Tumorigenic Effects in Wistar Rats Orally Administered Benzo[a]pyrene for Two Years: Implications for Human Cancer Risks Associated with Oral Exposure to Polycyclic Aromatic Hydrocarbons* (RIVM 658603), Bilthoven, National Institute of Public Health and the Environment

Krokje, A., Tiltnes, A., Mylius, E. & Gullvag, B. (1985) Testing for mutagens in filter samples from the work atmosphere of an aluminum plant. *Scand. J. Work Environ. Health*, **11**, 311–316

Krokje, A., Tiltnes, A., Mylius, E. & Gullvag, B. (1988) Testing for mutagens in an aluminium plant. The results of Salmonella typhimurium tests on urine from exposed workers. *Mutat. Res*., **204**, 163–172

Krolewski, B. & Little, J.B. (1985) Influence of isopropylvaleramide and allylisopropylacetamide on transformation of C3H/10T½ cells induced by benzo[*a*]pyrene derivatives. *Carcinogenesis*, **6**, 7–11

Krolewski, B., Nagasawa, H. & Little, J.B. (1986) Effect of aliphatic amides on oncogenic transformation, sister chromatid exchanges, and mutations induced by cyclopenta[cd]-pyrene and benzo[a]pyrene. *Carcinogenesis*, **7**, 1647–1650

Kuljukka-Rabb, T., Peltonen, K., Isotalo, S., Mikkonen, S., Rantanen, L. & Savela, K. (2001) Time- and dose-dependent DNA binding of PAHs derived from diesel particle extracts, benzo[a]pyrene and 5-methychrysene in a human mammary carcinoma cell line (MCF-7). *Mutagenesis*, **16**, 353–358

Kulkarni, M.S. & Anderson, M.W. (1984) Persistence of benzo(*a*)pyrene metabolite:DNA adducts in lung and liver of mice. *Cancer Res.*, **44**, 97–101

Kuroki, T., Malaveille, C., Drevon, C., Piccoli, C., Macleod, M. & Selkirk, J.K. (1979) Critical importance of microsome concentration in mutagenesis assay with V79 Chinese hamster cells. *Mutat. Res*., **63**, 259–272

Kuroki, T., Nemoto, N. & Kitano, Y. (1980) Metabolism of benzo[*a*]pyrene in human epidermal keratinocytes in culture. *Carcinogenesis*, **1**, 559–565

Kwon, H., Sahali, Y., Skipper, P.L. & Tannenbaum, S.R. (1992) Oxidation of cyclopenta[*cd*]pyrene by human and mouse liver microsomes and selected cytochrome P450 enzymes. *Chem. Res. Toxicol*., **5**, 760–764

Ladics, G.S., Kawabata, T.T. & White, K.L., Jr (1991) Suppression of the in vitro humoral immune response of mouse splenocytes by 7,12-dimethylbenz[a]anthracene metabolites and inhibition of immunosuppression by alpha-naphthoflavone. *Toxicol. appl. Pharmacol*., **110**, 31–44

Ladics, G.S., Kawabata, T.T., Munson, A.E. & White, K.L., Jr (1992a) Evaluation of murine splenic cell type metabolism of benzo[a]pyrene and functionality in vitro following repeated in vivo exposure to benzo[a]pyrene. *Toxicol. appl. Pharmacol*., **116**, 258–266

Ladics, G.S., Kawabata, T.T., Munson, A.E. & White, K.L., Jr (1992b) Metabolism of benzo[a]pyrene by murine splenic cell types. *Toxicol. appl. Pharmacol*., **116**, 248–257

Ladics, G.S., Kawabata, T.T., Munson, A.E. & White, K.L., Jr (1992c) Generation of 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene by murine splenic macrophages. *Toxicol. appl. Pharmacol*., **115**, 72–79

Lafleur, A.L., Longwell, J.P., Marr, J.A., Monchamp, P.A., Plummer, E.F., Thilly, W.G., Mulder, P.P.Y., Boere, B.B., Cornelisse, J. & Lugtenburg, J. (1993) Bacterial and human cell mutagenicity study of some C18H10 cyclopenta-fused polycyclic aromatic hydrocarbons associated with fossil fuels combustion. *Environ. Health Perspect*., **101**, 146–153

Laib, R.J., Bolt, H.M., Cartier, R. & Bartsch, H. (1989) Increased alkylation of liver DNA and cell turnover in young versus old rats exposed to vinyl chloride correlates with cancer susceptibility. *Toxicol. Lett*., **45**, 231–239

Lampen, A., Ebert, B., Stumkat, L., Jacob, J. & Seidel, A. (2004) Induction of gene expression of xenobiotic metabolism enzymes and ABC-transport proteins by PAH and a reconstituted PAH mixture in human Caco-2 cells. *Biochim. biophys. Acta*, **1681**, 38–46

Lan, Q., Chen, W., Chen, H. & He, X.-Z. (1993) Risk factors for lung cancer in non-smokers in Xuanwei County of China. *Biomed. environ. Sci*., **6**, 112–118

Lan, Q., He, X., Costa, D.J., Tian, L., Rothman, N., Hu, G. & Mumford, J.L. (2000) Indoor coal combustion emissions, GSTM1 and GSTT1 genotypes, and lung cancer risk: A case–control study in Xuan Wei, China. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 605–608

Lan, Q., Feng, Z., Tian, D., He, X., Rothman, N., Tian, L., Lu, X., Terry, M.B. & Mumford, J.L. (2001) p53 Gene expression in relation to indoor exposure to unvented coal smoke in Xuan Wei, China. *J. occup. environ. Med*., **43**, 226–230

Lan, Q., Mumford, J.L., Shen, M., Demarini, D.M., Bonner, M.R., He, X., Yeager, M., Welch, R., Chanock, S., Tian, L., Chapman, R.S., Zheng, T., Keohavong, P., Caporaso, N. & Rothman, N. (2004) Oxidative damage-related genes *AKR1C3* and *OGG1* modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions. *Carcinogenesis*, **25**, 2177–2181

Landrigan, P.J., Lioy, P.J., Thurston, G., Berkowitz, G., Chen, L.C., Chillrud, S.N., Gavett, S.H., Georgopoulos, P.G., Geyh, A.S., Levin, S., Perera, F., Rappaport, S.M., Small, C. & the NIEHS World Trade Center Working Group (2004) Health and environmental consequences of the World Trade Center disaster. *Environ. Health Perspect*., **112**, 731–739

Lane, D.A. & Gundel, L. (1996) Gas and particle sampling of airborne polycyclic aromatic compounds. *Polycyc. arom. Comp.*, **9**, 67–73

Lang, N.P., Butler, M.A., Massengill, J., Lawson, M., Stotts, R.C., Hauer-Jensen, M. & Kadlubar, F.F. (1994) Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 675–682

Laupeze, B., Amiot, L., Sparfel, L., Le Ferrec, E., Fauchet, R. & Fardel, O. (2002) Polycyclic aromatic hydrocarbons affect functional differentiation and maturation of human monocyte-derived dendritic cells. *J. Immunol*., **168**, 2652–2658

LaVoie, E.J., Hecht, S.S., Amin, S., Bedenko, V. & Hoffmann, D. (1980) Identification of mutagenic dihydrodiols as metabolites of benzo(j)fluoranthene and benzo(k)fluoranthene. *Cancer Res*., **40**, 4528–4532

LaVoie, E.J., Amin, S., Hecht, S.S., Furuya, K. & Hoffmann, D. (1982) Tumour initiating activity of dihydrodiols of benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene. *Carcinogenesis*, **3**, 49–52

LaVoie, E.J., He, Z.-M., Meegalla, R.L. & Weyand, E.H. (1993) Exceptional tumor-initiating activity of 4-fluorobenzo[*j*]fluoranthene on mouse skin: Comparison with benzo[*j*]fluoranthene, 10-fluorobenzo[*j*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a,l*]pyrene and 7,12-dimethyl-benz[*a*]anthracene. *Cancer Lett*., **70**, 7–14

LaVoie, E.J., He, Z.-M., Wu, Y., Meschter, C.L. & Weyand, E.H. (1994) Tumorigenic activity of the 4,5- and 9,10-dihydrodiols of benzo[j]fluoranthene and their *syn*- and *anti*-diol epoxides in newborn mice. *Cancer Res*., **54**, 962–968

Le Calvez, F., Mukeria, A., Hunt, J.D., Kelm, O., Hung, R.J., Taniere, P., Brennan, P., Boffetta, P., Zaridze, D.G. & Hainaut, P. (2005) *TP53* and *KRAS* mutation load and types in lung cancers in relation to tobacco smoke: Distinct patterns in never, former, and current smokers. *Cancer Res*., **65**, 5076–5083

Le Marchand, L., Sivaraman, L., Pierce, L., Seifried, A., Lum, A., Wilkens, L.R. & Lau, A.F. (1998) Associations of CYP1A1, GSTM1, and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. *Cancer Res.*, **58**, 4858–4863

Lecoq, S., Perin, F., Plessis, M.J., Strapelias, H. & Duquesne, M. (1989) Comparison of the *in vitro* metabolisms and mutagenicities of dibenzo[*a,c*]anthracene, dibenzo[*a,h*]anthracene and dibenzo[*a,j*]anthracene: Influence of norharman. *Carcinogenesis*, **10**, 461–469

Lecoq, S., Chalvet, O., Strapelias, H., Grover, P.L., Phillips, D.H. & Duquesne, M. (1991a) Microsomal metabolism of dibenz[*a,c*]anthracene, dibenz[*a,h*]anthracene and dibenz[*a,j*]anthracene to bis-dihydrodiols and polyhydroxylated products. *Chem.-biol. Interact*., **80**, 261–279

Lecoq, S., Ni She, M., Grover, P.L., Platt, K.L., Oesch, F. & Phillips, D.H. (1991b) The *in vitro* metabolic activation of dibenz[*a,h*]anthracene, catalyzed by rat liver microsomes and examined by ^{32}P-postlabelling. *Cancer Lett*., **57**, 261–269

Lecoq, S., Ni She, M., Hewer, A., Grover, P.L., Platt, K.L., Oesch, F. & Phillips, D.H. (1991c) The metabolic activation of dibenz[*a,h*]anthracene in mouse skin examined by ^{32}P-postlabelling: Minor contribution of the 3,4-diol 1,2-oxides to DNA binding. *Carcinogenesis*, **12**, 1079–1083

Lecoq, S., Pfau, W., Grover, P.L. & Phillips, D.H. (1992) HPLC separation of ^{32}P-postlabelled DNA adducts formed from dibenz[*a,h*]anthracene in skin. *Chem.-biol. Interact*., **85**, 173–185

Lee, W.J., Brennan, P., Boffetta, P., London, S.J., Benhamou, S., Rannug, A., To-Figueras, J., Ingelman-Sundberg, M., Shields, P., Gaspari, L. & Taioli, E. (2002) Microsomal epoxide hydrolase polymorphisms and lung cancer risk: A quantitative review. *Biomarkers*, **7**, 230–241

Lee, K.H., Ichiba, M., Zhang, J., Tomokuni, K., Hong, Y.C., Ha, M., Kwon, H.J., Koh, S.B., Choi, H.R., Park, C.G., Cho, S.H., Hirvonen, A., Strickland, P.T., Vermeulen, R., Hayes, R.B. & Kang, D. (2003) Multiple biomarkers study in painters in a shipyard in Korea. *Mutat. Res.*, **540**, 89–98

Lee-Ruff, E., Kazarians-Modhaddam, H. & Katz, M. (1988) Controlled oxidation studies of benzo[a]pyrene. In: Cooke, M. & Dennis, A.J., eds, *Polynuclear Aromatic Hydrocarbons: A Decade of Progress*, Columbus, OH, Batelle Press, pp. 519–534

Lehman, T.A. & Harris, C.C. (1994) Mutational spectra of protooncogenes and tumour suppressor genes: Clues in predicting cancer etiology. In: Hemminki, K., Dipple, A., Shuker, D.E.G., Kadlubar, F.F., Segerbäck, D. & Bartsch, H., eds, *DNA Adducts: Identification and Biological Significance* (IARC Scientific Publications No. 125), Lyon, IARC, pp. 399–412

Lehner, A.F., Horn, J. & Flesher, J.W. (2004) Formation of radical cations in a model for the metabolism of aromatic hydrocarbons. *Biochem. biophys. Res. Commun*., **322**, 1018–1023

Lei, W., Yu, R., Mandlekar, S. & Kong, A.N. (1998) Induction of apoptosis and activation of interleukin 1beta-converting enzyme/Ced-3 protease (caspase-3) and c-Jun NH2-terminal kinase 1 by benzo(a)pyrene. *Cancer Res*., **58**, 2102–2106

Leng, S., Cheng, J., Pan, Z., Huang, C., Niu, Y., Dai, Y., Li, B., He, F. & Zheng, Y. (2004a) Associations between XRCC1 and ERCC2 polymorphisms and DNA damage in peripheral blood lymphocytes among coke oven workers. *Biomarkers*, **9**, 395–406

Leng, S., Dai, Y., Niu, Y., Pan, Z., Li, X., Cheng, J., He, F. & Zheng, Y. (2004b) Effects of genetic polymorphisms of metabolic enzymes on cytokinesis-block micronucleus in peripheral blood lymphocytes among coke-oven workers. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1631–1639

Lesko, S.A., Jr, Smith, A., Ts'o, P.O.P. & Umans, R.S. (1968) Interaction of nucleic acids. IV. The physical binding of 3,4-benzpyrene to nucleosides, nucleotides, nucleic acids and nucleoprotein. *Biochemistry*, **7**, 434–447

Leslie, E.M., Deeley, R.G. & Cole, S.P. (2001) Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. *Toxicology*, **167**, 3–23

Levin, W., Wood, A.W., Yagi, H., Dansette, P.M., Jerina, D.M. & Conney, A.H. (1976) Carcinogenicity of benzo[*a*]pyrene 4,5-, 7,8-, and 9,10-oxides on mouse skin. *Proc. natl Acad. Sci. USA*, **73**, 243–247

Levin, W., Wood, A.W., Chang, R.L., Slaga, T.J., Yagi, H., Jerina, D.M. & Conney, A.H. (1977a) Marked differences in the tumor-initiating activity of optically pure (+)- and (–)-trans-7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene on mouse skin. *Cancer Res.*, **37**, 2721–2725

Levin, W., Wood, A.W., Wislocki, P.G., Kapitulnik, J., Yagi, H., Jerina, D.M. & Conney, A.H. (1977b) Carcinogenicity of benzo-ring derivatives of benzo(*a*)pyrene on mouse skin. *Cancer Res.*, **37**, 3356–3361

Levin, W., Thakker, D.R., Wood, A.W., Chang, R.L., Lehr, R.E., Jerina, D.M. & Conney, A.H. (1978) Evidence that benzo(*a*)anthracene 3,4-diol-1,2-epoxide is an ultimate carcinogen on mouse skin. *Cancer Res.*, **38**, 1705–1710

Levin, W., Buening, M.K., Wood, A.W., Chang, R.L., Kedzierski, B., Thakker, D.R., Boyd, D.R., Gadaginamath, G.S., Armstrong, R.N., Yagi, H., Karle, J.M., Slaga, T.J., Jerina, D.M. & Conney, A.H. (1980) An enantiomeric interaction in the metabolism and tumorigenicity of (+)- and (-)-benzo[*a*]pyrene 7,8-oxide. *J. biol. Chem.*, **255**, 9067–9074

Levin, W., Chang, R.L., Wood, A.W., Yagi, H., Thakker, D.R., Jerina, D.M. & Conney, A.H. (1984) High stereoselectivity among the optical isomers of the diastereomeric bay-region diol-epoxides of benz(a)anthracene in the expression of tumorigenic activity in murine tumor models. *Cancer Res.*, **44**, 929–933

Levin, W., Chang, R.L., Wood, A.W., Thakker, D.R., Yagi, H., Jerina, D.M. & Conney, A.H. (1986) Tumorigenicity of optical isomers of the diastereomeric bay-region 3,4-diol-1,2-epoxides of benzo(c)phenanthrene in murine tumor models. *Cancer Res.*, **46**, 2257–2261

Lewis, S.J., Cherry, N.M., Niven, R.M., Barber, P.V. & Povey, A.C. (2001) Polymorphisms in the NAD(P)H:quinone oxidoreductase gene and small cell lung cancer risk in a UK population. *Lung Cancer*, **34**, 177–183

Lewis-Bevan, L., Little, S.B. & Rabinowitz, J.R. (1995) Quantum mechanical studies of the structure and reactivities of the diol epoxides of benzo[*c*]phenanthrene. *Chem. Res. Toxicol.*, **8**, 499–505

Li, K.-M., Todorovic, R., Rogan, E.G., Cavalieri, E.L., Ariese, F., Suh, M., Jankowiak, R. & Small, G.J. (1995) Identification and quantitation of dibenzo[*a,l*]pyrene–DNA adducts formed by rat liver microsomes *in vitro*: Preponderance of depurinating adducts. *Biochemistry*, **34**, 8043–8049

Li, J., Chen, H., Tang, M.-S., Shi, X., Amin, S., Desai, D., Costa, M. & Huang, C. (2004a) PI-3K and Akt are mediators of AP-1 induction by 5-MCDE in mouse epidermal Cl41 cells. *J. Cell Biol.*, **165**, 77–86

Li, J., Chen, H., Ke, Q., Feng, Z., Tang, M.-S., Liu, B., Amin, S., Costa, M. & Huang, C. (2004b) Differential effects of polycyclic aromatic hydrocarbons on transactivation of AP-1 and NF-kappaB in mouse epidermal Cl41 cells. *Mol. Carcinog*., **40**, 104–115

Li, D., Jiao, L., Li, Y., Doll, M.A., Hein, D.W., Bondy, M.L., Evans, D.B., Wolff, R.A., Lenzi, R., Pisters, P.W., Abbruzzese, J.L. & Hassan, M.M. (2006) Polymorphisms of cytochrome P4501A2 and N-acetyltransferase genes, smoking, and risk of pancreatic cancer. *Carcinogenesis*, **27**, 103–111

Liang, G., Miao, X., Zhou, Y., Tan, W. & Lin, D. (2004) A functional polymorphism in the SULT1A1 gene (G638A) is associated with risk of lung cancer in relation to tobacco smoking. *Carcinogenesis*, **25**, 773–778

Liehr, J.G., Ricci, M.J., Jefcoate, C.R., Hannigan, E.V., Hokanson, J.A. & Zhu, B.T. (1995) 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: Implications for the mechanism of uterine tumorigenesis. *Proc. natl Acad. Sci. USA*, **92**, 9220–9224

Lilienblum, W., Platt, K.L., Schirmer, G., Oesch, F. & Bock, K.W. (1987) Regioselectivity of rat liver microsomal UDP-glucuronosyltransferase activities toward phenols of benzo(*a*)pyrene and dibenz(*a*,*h*)anthracene. *Mol. Pharmacol*., **32**, 173–177

Liu, Z., Muehlbauer, K.-R., Schmeiser, H.H., Hergenhahn, M., Belharazem, D. & Hollstein, M.C. (2005) p53 Mutations in benzo(a)pyrene-exposed human p53 knock-in murine fibroblasts correlate with p53 mutations in human lung tumors. *Cancer Res*., **65**, 2583–2587

Lloyd, D.R. & Hanawalt, P.C. (2002) p53 Controls global nucleotide excision repair of low levels of structurally diverse benzo(*g*)chrysene–DNA adducts in human fibroblasts. *Cancer Res*., **62**, 5288–5294

Lodovici, M., Akpan, V., Giovannini, L., Migliani, F. & Dolara, P. (1998) Benzo[*a*]pyrene diol-epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. *Chem.-biol. Interact*., **116**, 199–212

Lodovici, M., Luceri, C., Guglielmi, F., Bacci, C., Akpan, V., Fonnesu, M.L., Boddi, V. & Dolara, P. (2004) Benzo(a)pyrene diolepoxide (BPDE)–DNA adduct levels in leukocytes of smokers in relation to polymorphism of CYP1A1, GSTM1, GSTP1, GSTT1, and mEH. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1342–1348

Lohmann, R. & Lammel, G. (2004) Adsorptive and absorptive contributions to the gas-particle partitioning of polycyclic aromatic hydrocarbons: State of knowledge and recommended parametrization for modeling. *Environ. Sci. Technol*., **38**, 3793–3803

London, S.J., Daly, A.K., Fairbrother, K.S., Holmes, C., Carpenter, C.L., Navidi, W.C. & Idle, J.R. (1995) Lung cancer risk in African-Americans in relation to a race-specific CYP1A1 polymorphism. *Cancer Res*., **55**, 6035–6037

London, S.J., Yuan, J.M., Coetzee, G.A., Gao, Y.T., Ross, R.K. & Yu, M.C. (2000) CYP1A1 I462V genetic polymorphism and lung cancer risk in a cohort of men in Shanghai, China. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 987–991

Long, D.J., Waikel, R.L., Wang, X.-J., Perlaky, L., Roop, D.R. & Jaiswal, A.K. (2000) NAD(P)H:quinone oxidoreductase 1 deficiency increases susceptibility to benzo(*a*)pyrene-induced mouse skin carcinogenesis. *Cancer Res*., **60**, 5913–5915

Long, D.J., Waikel, R.L., Wang, X.-J., Roop, D.R. & Jaiswal, A.K. (2001) NAD(P)H:quinone oxidoreductase 1 deficiency and increased susceptibility to 7,12-dimethylbenz[*a*]-anthracene-induced carcinogenesis in mouse skin. *J. natl Cancer Inst*., **93**, 1166–1170

Lu, A.Y. & West, S.B. (1979) Multiplicity of mammalian microsomal cytochromes P-450. *Pharmacol. Rev.*, **31**, 277–295

Lu, R., Nash, H.M. & Verdine, G.L. (1997) A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer. *Curr. Biol.*, **7**, 397–407

Luch, A., Glatt, H., Platt, K.L., Oesch, F. & Seidel, A. (1994) Synthesis and mutagenicity of the diastereomeric fjord-region 11,12-dihydrodiol 13,14-epoxides of dibenzo[*a,l*]pyrene. *Carcinogenesis*, **15**, 2507–2516

Luch, A., Seidel, A., Glatt, H. & Platt, K.L. (1997) Metabolic activation of the (+)-*S,S*- and (–)-*R,R*-enantiomers of *trans*-11,12-dihydroxy-11,12-dihydrodibenzo[*a,l*]pyrene: Stereoselectivity, DNA adduct formation, and mutagenicity in Chinese hamster V79 cells. *Chem. Res. Toxicol.*, **10**, 1161–1170

Luch, A., Platt, K.-L. & Seidel, A. (1998a) Synthesis of fjord region tetraols and their use in hepatic biotransformation studies of dihydrodiols of benzo[*c*]chrysene, benzo[*g*]chrysene and dibenzo[*a,l*]pyrene. *Carcinogenesis*, **19**, 639–648

Luch, A., Coffing, S.L., Tang, Y.M., Schneider, A., Soballa, V., Greim, H., Jefcoate, C.R., Seidel, A., Greenlee, W.F., Baird, W.M. & Doehmer, J. (1998b) Stable expression of human cytochrome P450 1B1 in V79 Chinese hamster cells and metabolically catalyzed DNA adduct formation of dibenzo[*a,l*]pyrene. *Chem. Res. Toxicol.*, **11**, 686–695

Luch, A., Schober, W., Soballa, V.J., Raab, G., Greim, H., Jacob, J., Doehmer, J. & Seidel, A. (1999a) Metabolic activation of dibenzo[*a,l*]pyrene by human cytochrome P450 1A1 and P450 1B1 expressed in V79 Chinese hamster cells. *Chem. Res. Toxicol.*, **12**, 353–364

Luch, A., Kudla, K., Seidel, A., Doehmer, J., Greim, H. & Baird, W.M. (1999b) The level of DNA modification by (+)-*syn*-(11*S*,12*R*,13*S*,14*R*)- and (–)-anti-(11*R*,12*S*,13*S*,14*R*)-dihydrodiol epoxides of dibenzo[*a,l*]pyrene determined the effect on the proteins p53 and $p21^{WAF1}$ in the human mammary carcinoma cell line MCF-7. *Carcinogenesis*, **20**, 859–865

Luch, A., Friesel, H., Seidel, A. & Platt, K.L. (1999c) Tumor-initiating activity of the (+)-(*S,S*)- and (–)-(*R,R*)-enantiomers of trans-11,12-dihydroxy-11,12-dihydrodibenzo[*a,l*]pyrene in mouse skin. *Cancer Lett.*, **136**, 119–128

Luo, W., Muller, J.G., Rachlin, E.M. & Burrows, C.J. (2001) Characterization of hydantoin products from one-electron oxidation of 8-oxo-7,8-dihydroguanosine in a nucleoside model. *Chem. Res. Toxicol.*, **14**, 927–938

Luster, M.I., Hong, L.H., Boorman, G.A., Clark, G., Hayes, H.T., Greenlee, W.F., Dold, K. & Tucker, A.N. (1985) Acute myelotoxic responses in mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Toxicol. appl. Pharmacol.*, **81**, 156–165

Luster, M.I., Germolec, D.R., Clark, G., Wiegand, G. & Rosenthal, G.J. (1988) Selective effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin and corticosteroid on in vitro lymphocyte maturation. *J. Immunol.*, **140**, 928–935

Luster, M.I., Portier, C., Pait, D.G., White, K.L., Jr, Gennings, C., Munson, A.E. & Rosenthal, G.J. (1992) Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam. appl. Toxicol.*, **18**, 200–210

Lyte, M. & Bick, P.H. (1985) Differential immunotoxic effects of the environmental chemical benzo[a]pyrene in young and aged mice. *Mech. Ageing Dev.*, **30**, 333–341

MacKenzie, K.M. & Angevine, D.M. (1981) Infertility in mice exposed in utero to benzo(*a*)pyrene. *Biol. Reprod.*, **24**, 183–191

Mackenzie, P.I., Bock, K.W., Burchell, B., Guillemette, C., Ikushiro, S.-I., Iyanagi, T., Miners, J.O., Owens, I.S. & Nebert, D.W. (2005) Nomenclature update for the mammalian UDP glycosyltransferase (*UGT*) gene superfamily. *Pharmacogenet. Genom.*, **15**, 677–685

MacNicoll, A.D., Grover, P.L. & Sims, P. (1980) The metabolism of a series of polycyclic hydrocarbons by mouse skin maintained in short-term organ culture. *Chem.-biol. Interact.*, **29**, 169–188

Mahadevan, B., Luch, A., Seidel, A., Pelling, J.C. & Baird, W.M. (2001) Effects of the (–)-anti-11R,12S-dihydrodiol 13S,14R-epoxide of dibenzo. *Carcinogenesis*, **22**, 161–169

Mahadevan, B., Luch, A., Bravo, C.F., Atkin, J., Steppan, L.B., Pereira, C., Kerkvliet, N.I. & Baird, W.M. (2005a) Dibenzo[*a*,*l*]pyrene induced DNA adduct formation in lung tissue in vivo. *Cancer Lett.*, **227**, 25–32

Mahadevan, B., Keshava, C., Musafia-Jeknic, T., Pecaj, A., Weston, A. & Baird, W.M. (2005b) Altered gene expression patterns in MCF-7 cells induced by the urban dust particulate complex mixture standard reference material 1649a. *Cancer Res.*, **65**, 1251–1258

Mallakin, A., Dixon, D.G. & Greenberg, B.M. (2000) Pathway of anthracene modification under simulated solar radiation. *Chemosphere*, **40**, 1435–1441

Mann, K.K., Matulka, R.A., Hahn, M.E., Trombino, A.F., Lawrence, B.P., Kerkvliet, N.I. & Sherr, D.H. (1999) The role of polycyclic aromatic hydrocarbon metabolism in dimethylbenz[a]anthracene-induced pre-B lymphocyte apoptosis. *Toxicol. appl. Pharmacol.*, **161**, 10–22

Marczynski, B., Rihs, H. P., Rossbach, B., Holzer, J., Angerer, J., Scherenberg, M., Hoffmann, G., Bruning, T. & Wilhelm, M. (2002) Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: Comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. *Carcinogenesis*, **23**, 273–281

Marlowe, J.L., Knudsen, E.S., Schwemberger, S. & Puga, A. (2004) The aryl hydrocarbon receptor displaces p300 from E2F-dependent promoters and represses S phase-specific gene expression. *J. biol. Chem.*, **279**, 29013–29022

Marnett, L.J. (1981) Polycyclic aromatic hydrocarbon oxidation during prostaglandin biosynthesis. *Life Sci.*, **29**, 531–546

Marnett, L.J. (1987) Peroxyl free radicals: Potential mediators of tumor initiation and promotion. *Carcinogenesis*, **8**, 1365–1373

Marsch, G.A., Jankowiak, R., Small, G.J., Hughes, N.C. & Phillips, D.H. (1992) Evidence of involvement of multiple sites of metabolism in the in vivo covalent binding of dibenzo[*a*,*h*]pyrene to DNA. *Chem. Res. Toxicol.*, **5**, 765–772

Marshall, M.V., He, Z.-M., Weyand, E.H., Rice, J.E. & LaVoie, E.J. (1993) Mutagenic activity of the 4,5- and 9,10-dihydrodiols of benzo[j]fluoranthene and their syn- and anti-dihydrodiol epoxides in Salmonella typhimurium. *Environ. mol. Mutag.*, **22**, 34–45

Mass, M.J. & Kaufman, D.G. (1978) [^{3}H]Benzo(a)pyrene metabolism in tracheal epithelial microsomes and tracheal organ cultures. *Cancer Res.*, **38**, 3861–3866

Mass, M.J., Jeffers, A.J., Ross, J.A., Nelson, G., Galati, A.J., Stoner, G.D. & Nesnow, S. (1993) Ki-*ras* Oncogene mutations in tumors and DNA adducts formed by benz[*j*]aceanthrylene and benzo[*a*]pyrene in the lungs of strain A/J mice. *Mol. Carcinog.*, **8**, 186–192

Mass, M.J., Abu-Shakra, A., Roop, B.C., Nelson, G., Galati, A.J., Stoner, G.D., Nesnow, S. & Ross, J.A. (1996) Benzo[*b*]fluoranthene: Tumorigenicity in strain A/J mouse lungs, DNA adducts and mutations in the Ki-*ras* oncogene. *Carcinogenesis*, **17**, 1701–1704

Masson, L.F., Sharp, L., Cotton, S.C. & Little, J. (2005) Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: A HuGE review. *Am. J. Epidemiol*., **161**, 901–915

Matikainen, T., Perez, G.I., Jurisicova, A., Pru, J.K., Schlezinger, J.J., Ryu, H.Y., Laine, J., Sakai, T., Korsmeyer, S.J., Casper, R.F., Sherr, D.H. & Tilly, J.L. (2001) Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. *Nat. Genet*., **28**, 355–360

McCann, J., Choi, E., Yamasaki, E., & Ames, B.N. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test of 300 chemicals. *Proc. natl Acad. Sci. USA*, **72**, 5135–5139

McCoull, K.D., Rindgen, D., Blair, I.A. & Penning, T.M. (1999) Synthesis and characterization of polycyclic aromatic hydrocarbon *o*-quinone depurinating N7-guanine adducts. *Chem. Res. Toxicol*., **12** , 237–246

McGarry, M.A., Charles, G.D., Medrano, T., Bubb, M.R., Grant, M.B., Campbell-Thompson, M. & Shiverick, K.T. (2002) Benzo(*a*)pyrene, but not 2,3,7,8-tetrachlorodibenzo-p-dioxin, alters cell adhesion proteins in human uterine RL95-2 cells. *Biochem. biophys. Res. Commun*., **294**, 101–107

Meek, D.W. (2004) The p53 response to DNA damage. *DNA Repair*, **3**, 1049–1056

Melendez-Colon, V.J., Smith, C.A., Seidel, A., Luch, A., Platt, K.L. & Baird, W.M. (1997) Formation of stable adducts and absence of depurinating DNA adducts in cells and DNA treated with the potent carcinogen dibenzo[a,l]pyrene or its diol epoxides. *Proc. natl Acad. Sci. USA*, **94**, 13542–13547

Melendez-Colon, V.J., Luch, A., Seidel, A. & Baird, W.M. (1999a) Comparison of cytochrome P450- and peroxidase-dependent metabolic activation of the potent carcinogen dibenzo[*a*,*l*]pyrene in human cell lines: Formation of stable DNA adducts and absence of a detectable increase in apurinic sites. *Cancer Res*., **59**, 1412–1416

Melendez-Colon, V.J., Luch, A., Seidel, A. & Baird, W.M. (1999b) Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. *Carcinogenesis*, **20**, 1885–1891

Melendez-Colon, V.J., Luch, A., Seidel, A. & Baird, W.M. (2000) Formation of stable DNA adducts and apurinic sites upon metabolic activation of bay and fjord region polycyclic aromatic hydrocarbons in human cell cultures. *Chem. Res. Toxicol*., **13**, 10–17

Melikian, A.A., LaVoie, E.J., Hecht, S.S. & Hoffmann, D. (1982) Influence of bay-region methyl group on formation of 5-methylchrysene dihydrodiol epoxide:DNA adducts in mouse skin. *Cancer Res*., **42**, 1239–1242

Melikian, A.A., LaVoie, E.J., Hecht, S.S. & Hoffmann, D. (1983) 5-Methylchrysene metabolism in mouse epidermis *in vivo*, diol epoxide–DNA adduct persistence, and diol epoxide reactivity with DNA as potential factors influencing the predominance of 5-methylchrysene-1,2-diol-3,4-epoxide–DNA adducts in mouse epidermis. *Carcinogenesis*, **4**, 843–849

Melikian, A.A., Amin, S., Hecht, S.S., Hoffmann, D., Pataki, J. & Harvey, R.G. (1984) Identification of the major adducts formed by reaction of 5-methylchrysene anti-dihydrodiol-epoxides with DNA *in vitro*. *Cancer Res*., **44**, 2524–2529

Melikian, A.A., Bagheri, K. & Hecht, S.S. (1987) Contrasting disposition and metabolism of topically applied benzo(*a*)pyrene, *trans*-7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene, and

7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene in mouse epidermis in vivo. *Cancer Res*., **47**, 5354–5360

Melikian, A.A., Amin, S., Huie, K., Hecht, S.S. & Harvey, R.G. (1988) Reactivity with DNA bases and mutagenicity toward *Salmonella typhimurium* of methylchrysene diol epoxide enantiomers. *Cancer Res*., **48**, 1781–1787

Melikian, A.A., Bagheri, K., Hecht, S.S. & Hoffmann, D. (1989) Metabolism of benzo[*a*]pyrene and 7beta, 8alpha-dihydroxy-9alpha, 10alpha-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene in lung and liver of newborn mice. *Chem.-biol. Interact*., **69**, 245–257

Melikian, A.A., Prahalad, K.A., Amin, S. & Hecht, S.S. (1991) Comparative DNA binding of polynuclear aromatic hydrocarbons and their dihydrodiol and bay region diolepoxide metabolites in newborn mouse lung and liver. *Carcinogenesis*, **12**, 1665–1670

Meranze, D.R., Gruenstein, M. & Shimkin, M.B. (1969) Effect of age and sex on the development of neoplasms in wistar rats receiving a single intragastric instillation of 7,12-dimethylbenz(a)anthracene. *Int. J. Cancer*, **4**, 480–486

Miller, M.L., Andringa, A., Cody, T., Dixon, K. & Albert, R.E. (1996) Cell proliferation and nuclear abnormalities are increased and apoptosis is decreased in the epidermis of the p53 null mouse after topical application of benzo[a]pyrene. *Cell Prolif*., **29**, 561–576

Mimura, J. & Fujii-Kuriyama, Y. (2003) Functional role of AhR in the expression of toxic effects by TCDD. *Biochim. biophys. Acta*, **1619**, 263–268

Mimura, J., Yamashita, K., Nakamura, K., Morita, M., Takagi, T.N., Nakao, K., Ema, M., Sogawa, K., Yasuda, M., Katsuki, M. & Fujii-Kuriyama, Y. (1997) Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. *Genes Cells*, **2**, 645–654

Mimura, J., Ema, M., Sogawa, K. & Fujii-Kuriyama, Y. (1999) Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. *Genes Dev*., **13**, 20–25

Mitchell, C.E. (1982) Distribution and retention of benzo(a)pyrene in rats after inhalation. *Toxicol. Lett.*, **11**, 35–42

Mitchell, C.E. (1983) The metabolic fate of benzo[a]pyrene in rats after inhalation. *Toxicology*, **28**, 65–73

Mitchell, C.E. & Tu, K.W. (1979) Distribution, retention, and elimination of pyrene in rats after inhalation. *J. Toxicol. environ. Health*, **5**, 1171–1179

Mlcoch, J., Fuchs, J., Oesch, F. & Platt, K.L. (1993) Characterization of DNA adducts at the bay region of dibenz[*a*,*h*]anthracene formed *in vitro*. *Carcinogenesis*, **14**, 469–473

Modica, R., Fiume, M., Guaitani, A. & Bartosek, I. (1983) Comparative kinetics of benz(*a*)anthracene, chrysene and triphenylene in rats after oral administration. I. Study with single compounds. *Toxicol. Lett.*, **18**, 103–109

Mohapatra, N., MacNair, P., Bryant, B.J., Ellis, S., Rudo, K., Sangaiah, R., Gold, A. & Nesnow, S. (1987) Morphological transforming activity and metabolism of cyclopenta-fused isomers of benz[*a*]anthracene in mammalian cells. *Mutat. Res*., **188**, 323–334

Moore, B.P. & Cohen, G.M. (1978) Metabolism of benzo(*a*)pyrene and its major metabolites to ethyl acetate-soluble and water-soluble metabolites by cultured rodent trachea. *Cancer Res*., **38**, 3066–3075

Moore, C.J., Pruess-Schwartz, D., Mauthe, R.J., Gould, M.N. & Baird, W.M. (1987) Interspecies differences in the major DNA adducts formed from benzo(*a*)pyrene but not 7,12-

dimethylbenz(*a*)anthracene in rat and human mammary cell cultures. *Cancer Res*., **47**, 4402–4406

Moos, A.B. & Kerkvliet, N.I. (1995) Inhibition of tumor necrosis factor activity fails to restore 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced suppression of the antibody response to sheep red blood cells. *Toxicol. Lett*., **81**, 175–181

Moreno, V., Glatt, H., Guino, E., Fisher, E., Meinl, W., Navarro, M., Badosa, J.M. & Boeing, H. (2005) Polymorphisms in sulfotransferases SULT1A1 and SULT1A2 are not related to colorectal cancer. *Int. J. Cancer*, **113**, 683–686

Morinello, E.J., Ham, A.J., Ranasinghe, A., Nakamura, J., Upton, P.B. & Swenberg, J.A. (2002a) Molecular dosimetry and repair of N2,3-ethenoguanine in rats exposed to vinyl chloride. *Cancer Res*., **62**, 5189–5195

Morinello, E.J., Koc, H., Ranasinghe, A. & Swenberg, J.A. (2002b) Differential induction of N2,3-ethenoguanine in rat brain and liver after exposure to vinyl chloride. *Cancer Res*., **62**, 5183–5188

Mounho, B.J. & Burchiel, S.W. (1998) Alterations in human B cell calcium homeostasis by polycyclic aromatic hydrocarbons: Possible associations with cytochrome P450 metabolism and increased protein tyrosine phosphorylation. *Toxicol. appl. Pharmacol*., **149**, 80–89

Mounho, B.J., Davila, D.R. & Burchiel, S.W. (1997) Characterization of intracellular calcium responses produced by polycyclic aromatic hydrocarbons in surface marker-defined human peripheral blood mononuclear cells. *Toxicol. appl. Pharmacol*., **145**, 323–330

Mukhtar, H. & Bresnick, E. (1976) Glutathione-S-epoxide transferase activity during development and the effect of partial hepatectomy. *Cancer Res*., **36**, 937–940

Mukhtar, H., Elmamlouk, T.H., Philpot, R.M. & Bend, J.R. (1979) Rat hepatic nuclear cytochrome P-450 and epoxide hydrase in membranes prepared by two methods: Similarities with the microsomal enzymes. *Mol. Pharmacol.*, **15**, 192–196

Mukhtar, H., DelTito, B.J., Jr, Matgouranis, P.M., Das, M., Asokan, P. & Bickers, D.R (1986) Additive effects of ultraviolet B and crude coal tar on cutaneous carcinogen metabolism: Possible relevance to the tumorigenicity of the Goeckerman regimen. *J. invest. Dermatol*., **87**, 348–353

Mulder, P.P.J., Chen, L., Sekhar, B.C., George, M., Gross, M.L., Rogan, E.G. & Cavalieri, E.L. (1996) Synthesis and structure determination of the adducts formed by electrochemical oxidation of 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene in the presence of deoxyribonucleosides or adenine. *Chem. Res. Toxicol*., **9**, 1264–1277

Mulder, P.P.J., Devanesan, P., van Alem, K., Lodder, G., Rogan, E.G. & Cavalieri, E.L. (2003) Fluorobenzo[a]pyrenes as probes of the mechanism of cytochrome P450-catalyzed oxygen transfer in aromatic oxygenations. *Free Rad. Biol. Med.*, **34**, 734–745

Mumford, J.L., Lee, X., Lewtas, J., Young, T.L. & Santella, R.M. (1993) DNA adducts as biomarkers for assessing exposure to polycyclic aromatic hydrocarbons in tissues from Xuan Wei women with high exposure to coal combustion emissions and high lung cancer mortality. *Environ. Health Perspect*., **99**, 83–87

Murante, F.G. & Gasiewicz, T.A. (2000) Hemopoietic progenitor cells are sensitive targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6J mice. *Toxicol. Sci*., **54**, 374–383

Murata, M., Watanabe, M., Yamanaka, M., Kubota, Y., Ito, H., Nagao, M., Katoh, T., Kamataki, T., Kawamura, J., Yatani, R. & Shiraishi, T. (2001) Genetic polymorphisms in cytochrome

P450 (CYP) 1A1, CYP1A2, CYP2E1, glutathione S-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. *Cancer Lett.*, **165**, 171–177

Murayama, N., Soyama, A., Saito, Y., Nakajima, Y., Komamura, K., Ueno, K., Kamakura, S., Kitakaze, M., Kimura, H., Goto, Y., Saitoh, O., Katoh, M., Ohnuma, T., Kawai, M., Sugai, K., Ohtsuki, T., Suzuki, C., Minami, N., Ozawa, S. & Sawada, J. (2004) Six novel nonsynonymous *CYP1A2* gene polymorphisms: Catalytic activities of the naturally occurring variant enzymes. *J. Pharmacol. exp. Ther*., **308**, 300–306

Murray, G.I., Melvin, W.T., Greenlee, W.F. & Burke, M.D. (2001) Regulation, function, and tissue-specific expression of cytochrome P450 CYP1B1. *Annu. Rev. Pharmacol. Toxicol.*, **41**, 297–316

Murty, V.S. & Penning, T.M. (1992a) Polycyclic aromatic hydrocarbon (PAH) *ortho*-quinone conjugate chemistry: Kinetics of thiol addition to PAH *ortho*-quinones and structures of thioether adducts of naphthalene-1,2-dione. *Chem.-biol. Interact*., **84**, 169–188

Murty, V.S. & Penning, T.M. (1992b) Characterization of mercapturic acid and glutathionyl conjugates of benzo[*a*]pyrene-7,8-dione by two-dimensional NMR. *Bioconjug. Chem*., **3**, 218–224

Mushtaq, M., Weems, H.B. & Yang, S.K. (1989) Stereoselective formations of enantiomeric K-region epoxide and *trans*-dihydrodiols in dibenz[*a,h*]anthracene metabolism. *Chem. Res. Toxicol*., **2**, 84–93

Myers, S.R. & Flesher, J.W. (1989) Biooxidation of 7,12-dimethylbenz[*a*]anthracene in rat subcutaneous tissue. *Biochem. Pharmacol.*, **38**, 3199–3206

Myers, S.R. & Flesher, J.W. (1991) Metabolism of chrysene, 5-methylchrysene, 6-methylchrysene and 5,6-dimethylchrysene in rat liver cytosol, *in vitro*, and in rat subcutaneous tissue, *in vivo*. *Chem.-biol. Interact.*, **77**, 203–221

Myers, M.J., Schook, L.B. & Bick, P.H. (1987) Mechanisms of benzo(a)pyrene-induced modulation of antigen presentation. *J. Pharmacol. exp. Ther*., **242**, 399–404

Myers, M.J., Blanton, R.H. & Bick, P.H. (1988) Inhibition of IL-2 responsiveness following exposure to benzo(a)pyrene is due to alterations in accessory cell function. *Int. J. Immunopharmacol*., **10**, 177–186

Nagai, A., Kano, Y., Funaska, R. & Nakamuro, K. (2002) Mutagenic characteristics and contribution of polycyclic aromatic hydrocarbons to mutagenicity of concentrates from municipal river water by blue chitin column. *J. Health Sci*., **48**, 232–241

Nagata, K. & Yamazoe, Y. (2002) Genetic polymorphism of human cytochrome P450 involved in drug metabolism. *Drug Metab. Pharmacokinet*., **17**, 167–189

Nair, R.V., Gill, R.D., Cortez, C., Harvey, R.G. & DiGiovanni, J. (1989) Characterization of DNA adducts derived from (±)-*trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydrodibenz-[*a,j*]anthracene and (±)-7-methyl-*trans*-3,4-dihydroxy- *anti*-1,2-epoxy-1,2,3,4-tetrahydrodibenz[*a,j*]anthracene. *Chem. Res. Toxicol*., **2**, 341–348

Nair, R.V., Gill, R.D., Nettikumara, A.N., Baer-Dubowska, W., Cortez, C., Harvey, R.G. & DiGiovanni, J. (1991) Characterization of covalently modified deoxyribonucleosides formed from dibenz[*a,j*]anthracene in primary cultures of mouse keratinocytes. *Chem. Res. Toxicol*., **4**, 115–122

Nair, R.V., Nettikumara, A.N., Cortez, C., Harvey, R.G. & DiGiovanni, J. (1992) Comparative metabolism of dibenz[*a,j*]anthracene and 7-methyldibenz[*a,j*]anthracene in primary cultures of mouse keratinocytes. *Chem. Res. Toxicol*., **5**, 532–540

Nakachi, K., Imai, K., Hayashi, S.-I. & Kawajiri, K. (1993) Polymorphisms of the *CYP1A1* and glutathione *S*-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Res.*, **53**, 2994–2999

Nakajima, M., Yokoi, T., Mizutani, M., Kinoshita, M., Funayama, M. & Kamataki, T. (1999) Genetic polymorphism in the 5′-flanking region of human *CYP1A2* gene: Effect on the CYP1A2 inducibility in humans. *J. Biochem.*, **125**, 803–808

National Toxicology Program (1993) *Eighth Report on Carcinogens*, Research Triangle Park, NC, US Department of Health and Human Services, Integrated Laboratory Systems, pp. 178–181

Near, R.I., Matulka, R.A., Mann, K.K., Gogate, S.U., Trombino, A.F. & Sherr, D.H. (1999) Regulation of preB cell apoptosis by aryl hydrocarbon receptor/transcription factor-expressing stromal/adherent cells. *Proc. Soc. exp. Biol. Med.*, **221**, 242–252

Nebert, D.W. (1978) Genetic control of carcinogen metabolism leading to individual differences in cancer risk. *Biochimie*, **60**, 1019–1029

Nebert, D.W. (1980) The Ah locus. A gene with possible importance in cancer predictability. *Arch. Toxicol.*, **Suppl. 3**, 195–207

Nebert, D.W. (2005a) Role of host susceptibility to toxicity and cancer caused by pesticides: Cytochromes P450. *J. Biochem. mol. Toxicol.*, **19**, 184–186

Nebert, D.W. (2005b) Inter-individual susceptibility to environmental toxicants—A current assessment. *Toxicol. appl. Pharmacol.*, **207** (Suppl. 2), 34–42

Nebert, D.W. & Jensen, N.M. (1979) The Ah locus: Genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. *CRC crit. Rev. Biochem.*, **6**, 401–437

Nebert, D.W., Robinson, J.R., Niwa, A., Kumaki, K. & Poland, A.P. (1975) Genetic expression of aryl hydrocarbon hydroxylase activity in the mouse. *J. Cell Physiol.*, **85**, 393–414

Nebert, D.W., Puga, A. & Vasiliou, V. (1993) Role of the Ah receptor and the dioxin-inducible [Ah] gene battery in toxicity, cancer, and signal transduction. *Ann. N.Y. Acad. Sci.*, **685**, 624–640

Nebert, D.W., Ingelman-Sundberg, M. & Daly, A.K. (1999) Genetic epidemiology of environmental toxicity and cancer susceptibility: Human allelic polymorphisms in drug-metabolizing enzyme genes, their functional importance, and nomenclature issues. *Drug Metab. Rev.*, **31**, 467–487

Nebert, D.W., Roe, A.L., Dieter, M.Z., Solis, W.A., Yang, Y. & Dalton, T.P. (2000) Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. *Biochem. Pharmacol.*, **59**, 65–85

Nebert, D.W., Dalton, T.P., Okey, A.B. & Gonzalez, F.J. (2004) Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J. biol. Chem.*, **279**, 23847–23850

Nemoto, N., Hirakawa, T. & Takayama, S. (1979) Metabolism of benzo[*a*]pyrene in hamster embryo cells. Effect of the concentration of benzo[*a*]pyrene on its metabolism. *Chem.-biol. Interact.*, **25**, 177–188

Nerurkar, P.V., Okinaka, L., Aoki, C., Seifried, A., Lum-Jones, A., Wilkens, L.R. & Le Marchand, L. (2000) CYP1A1, GSTM1, and GSTP1 genetic polymorphisms and urinary 1-hydroxypyrene excretion in non-occupationally exposed individuals. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 1119–1122

Nesnow, S., Huisingh, J., Bergman, H., Easterling, R., Inmon, J., King, L., Morris, M.S., Williams, K., Hyatt, B., Montgomery, L. & Cudak, C. (1980) Carcinogen metabolism in rat liver cell culture. *Ann. N.Y. Acad. Sci*., **349**, 247–263

Nesnow, S., Leavitt, S., Garland, H., Vaughan, T.O., Hyatt, B., Montgomery, L. & Cudak, C. (1981) Identification of cocarcinogens and their potential mechanisms of action using C3H10T½CL8 mouse embryo fibroblasts. *Cancer Res*., **41**, 3071–3076

Nesnow, S., Leavitt, S., Easterling, R., Watts, R., Toney, S.H., Claxton, L., Sangaiah, R., Toney, G.E., Wiley, J., Fraher, P. & Gold, A. (1984) Mutagenicity of cyclopenta-fused isomers of benz(*a*)anthracene in bacterial and rodent cells and identification of the major rat liver microsomal metabolites. *Cancer Res*., **44**, 4993–5003

Nesnow, S., Easterling, R.E., Ellis, S., Watts, R. & Ross, J. (1988) Metabolism of benz[*j*]aceanthrylene (cholanthrylene) and benz[*l*]aceanthrylene by induced rat liver S9. *Cancer Lett*., **39**, 19–27

Nesnow, S., Milo, G., Kurian, P., Sangaiah, R. & Gold, A., (1990) Induction of anchorage-independent growth in human diploid fibroblasts by the cyclopenta-polycyclic aromatic hydrocarbon, benz[*l*]aceanthrylene. *Mutat. Res*., **244**, 221–225

Nesnow, S., Lasley, J., Curti, S., Ross, J., Nelson, G., Sangaiah, R. & Gold, A. (1991) Morphological transformation and DNA adduct formation by benz[*j*]aceanthrylene and its metabolites in C3H10T1/2CL8 cells: Evidence for both cyclopenta-ring and bay-region metabolic activation pathways. *Cancer Res*., **51**, 6163–6169

Nesnow, S., Ross, J.A., Nelson, G., Wilson, K., Roop, B.C., Jeffers, A.J., Galati, A.J., Stoner, G.D., Sangaiah, R., Gold, A. & Mass, M.J. (1994a) Cyclopenta[cd]pyrene-induced tumorigenicity, Ki-ras codon 12 mutations and DNA adducts in strain A/J mouse lung. *Carcinogenesis*, **15**, 601–606

Nesnow, S., Ross, J., Beck, S., Lasley, J., Nelson, G., Lambert, G., Platt, K.L. & Agarwal, S.C. (1994b) Morphological transformation and DNA adduct formation by dibenz[*a,h*]anthracene and its metabolites in C3H10T1/2CL8 cells. *Carcinogenesis*, **15**, 2225–2231

Nesnow, S., Ross, J.A., Stoner, G.D. & Mass, M.J. (1995) Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. *Toxicology*, **105**, 403–413

Nesnow, S., Davis, C., Nelson, G., Ross, J.A., Allison, J., Adams, L. & King, L.C. (1997) Comparison of the morphological transforming activities of dibenzo[*a,l*]pyrene and benzo[*a*]pyrene in C3H10T1/2CL8 cells and characterization of the dibenzo[*a,l*]pyrene–DNA adducts. *Carcinogenesis*, **18**, 1973–1978

Nesnow, S., Ross, J.A., Mass, M.J. & Stoner, G.D. (1998a) Mechanistic relationships between DNA adducts, oncogene mutations, and lung tumorigenesis in strain A mice. *Exp. Lung Res.*, **24**, 395–405

Nesnow, S., Mass, M.J., Ross, J.A., Galati, A.J., Lambert, G.R., Gennings, C., Carter, W.H., Jr & Stoner, G.D. (1998b) Lung tumorigenic interactions in strain A/J mice of five environmental polycyclic aromatic hydrocarbons. *Environ. Health Perspect*., **106** (Suppl. 6), 1337–1346

Newcomb, K.O., Sangaiah, R., Gold, A. & Ball, L.M. (1993) Activation and metabolism of benz[*j*]aceanthrylene-9,10-dihydrodiol, the precursor to bay-region metabolism of the genotoxic cyclopenta-PAH benz[*j*]aceanthrylene. *Mutat. Res*., **287**, 181–190

Nguyen, T., Sherratt, P.J., Nioi, P., Yang, C.S. & Pickett, C.B. (2005) Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. *J. biol. Chem.*, **280**, 32485–32492

Nikonova, T.V. (1977) [Transplacental effect of benz(a)pyrene and pyrene.] *Biull. eksp. Biol. Med.*, **84**, 88–91 (in Russian)

Nohara, K., Pan, X., Tsukumo, S., Hida, A., Ito, T., Nagai, H., Inouye, K., Motohashi, H., Yamamoto, M., Fujii-Kuriyama, Y. & Tohyama, C. (2005) Constitutively active aryl hydrocarbon receptor expressed specifically in T-lineage cells causes thymus involution and suppresses the immunization-induced increase in splenocytes. *J. Immunol.*, **174**, 2770–2777

Nordqvist, M., Thakker, D.R., Levin, W., Yagi, H., Ryan, D.E., Thomas, P.E., Conney, A.H. & Jerina, D.M. (1979) The highly tumorigenic 3,4-dihydrodiol is a principal metabolite formed from dibenzo[a,h]anthracene by liver enzymes. *Mol. Pharmacol.*, **16**, 643–655

Nyholm, S.H., Alexander, J., Lundanes, E., Frandsen, H., Andersson, R., Grivas, S., Nesnow, S. & Holme, J.A. (1996) Biotransformation of the cyclopenta-fused polycyclic aromatic hydrocarbon benz[*j*]aceanthrylene in isolated rat liver cell: Identification of nine new metabolites. *Carcinogenesis*, **17**, 1111–1120

Nylund, L., Heikkila, P., Hameila, M., Pyy, L., Linnainmaa, K. & Sorsa, M. (1992) Genotoxic effects and chemical compositions of four creosotes. *Mutat. Res.*, **265**, 223–236

Oesch-Bartlomowicz, B., Huelster, A., Wiss, O., Antoniou-Lipfert, P., Dietrich, C., Arand, M., Weiss, C., Bockamp, E. & Oesch, F. (2005) Aryl hydrocarbon receptor activation by cAMP vs. dioxin: Divergent signaling pathways. *Proc. natl Acad. Sci. USA*, **102**, 9218–9223

Ogba, N., Wang, C. & Raychoudhury, S. (2005) Differential effects of fluoranthene and benzo[*a*]pyrene in MCF-7 cells. *J. environ. Sci. Health*, **A40,** 927–936

Ogura, K., Sohtome, T., Sugiyama, A., Okuda, H., Hiratsuka, A. & Watabe, T. (1990) Rat liver cytosolic hydroxysteroid sulfotransferase (sulfotransferase a) catalyzing the formation of reactive sulfate esters from carcinogenic polycyclic hydroxymethylarenes. *Mol. Pharmacol.*, **37**, 848–854

Oguri, T., Singh, S.V., Nemoto, K. & Lazo, J.S. (2003) The carcinogen (7*R*,8*S*)-dihydroxy-(9*S*,10*R*)-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene induces Cdc25B expression in human bronchial and lung cancer cells. *Cancer Res.*, **63**, 771–775

Oh, E., Lee, E., Im, H., Kang, H.S., Jung, W.W., Won, N.H., Kim, E.M. & Sul, D. (2005) Evaluation of immuno- and reproductive toxicities and association between immunotoxicological and genotoxicological parameters in waste incineration workers. *Toxicology*, **210**, 65–80

Okey, A.B., Riddick, D.S. & Harper, P.A. (1994) The Ah receptor: Mediator of the toxicity of 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. *Toxicol. Lett.*, **70**, 1–22

Okuda, H., Hiratsuka, A., Nojima, H. & Watabe, T. (1986) A hydroxymethyl sulphate ester as an active metabolite of the carcinogen, 5-hydroxymethylchrysene. *Biochem. Pharmacol.*, **35**, 535–538

Okuda, H., Nojima, H., Miwa, K., Watanabe, N. & Watabe, T. (1989) Selective covalent binding of the active sulfate ester of the carcinogen 5-(hydroxymethyl)chrysene to the adenine residue of calf thymus DNA. *Chem. Res. Toxicol.*, **2**, 15–22

Olshan, A.F., Weissler, M.C., Watson, M.A. & Bell, D.A. (2000) GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 185–191

Omiecinski, C.J., Hassett, C. & Hosagrahara, V. (2000) Epoxide hydrolase—Polymorphism and role in toxicology. *Toxicol. Lett*., **112–113**, 365–370

O'Neill, I.K., Goldberg, M.T., el Ghissassi, F. & Rojas-Moreno, M. (1991) Dietary fibre, fat and beef modulation of colonic nuclear aberrations and microcapsule-trapped gastrointestinal metabolites of benzo[*a*]pyrene-treated C57/B6 mice consuming human diets. *Carcinogenesis*, **12** , 175–180

Otto, S., Marcus, C., Pidgeon, C. & Jefcoate, C. (1991) A novel adrenocorticotropin-inducible cytochrome P450 from rat adrenal microsomes catalyzes polycyclic aromatic hydrocarbon metabolism. *Endocrinology*, **129**, 970–982

Ovrebo, S., Haugen, A., Hemminki, K., Szyfter, K., Drablos, P.A. & Skogland, M. (1995) Studies of biomarkers in aluminum workers occupationally exposed to polycyclic aromatic hydrocarbons. *Cancer Detect. Prev*., **19**, 258–267

Page, J.E., Pataki, J., Harvey, R.G. & Dipple, A. (1996) Mutational specificity of the *syn* 1,2-dihydrodiol 3,4-epoxide of 5-methylchrysene. *Cancer Lett*., **110**, 249–252

Page, T.J., O'Brien, S., Jefcoate, C.R. & Czuprynski, C.J. (2002) 7,12-Dimethylbenz[a]anthracene induces apoptosis in murine pre-B cells through a caspase-8-dependent pathway. *Mol. Pharmacol*., **62**, 313–319

Page, T.J., O'Brien, S., Holston, K., MacWilliams, P.S., Jefcoate, C.R. & Czuprynski, C.J. (2003) 7,12-Dimethylbenz[a]anthracene-induced bone marrow toxicity is p53-dependent. *Toxicol. Sci*., **74**, 85–92

Pal, K., Grover, P.L. & Sims, P. (1980) The induction of sister-chromatid exchanges in Chinese hamster ovary cells by some epoxides and phenolic derivatives of benzo[a]pyrene. *Mutat. Res*., **78**, 193–199

Palackal, N.T., Burczynski, M.E., Harvey, R.G. & Penning, T.M. (2001a) The ubiquitous aldehyde reductase (AKR1A1) oxidizes proximate carcinogen *trans*-dihydrodiols to *o*-quinones: Potential role in polycyclic aromatic hydrocarbon activation. *Biochemistry*, **40**, 10901–10910

Palackal, N.T., Burczynski, M.E., Harvey, R.G. & Penning, T.M. (2001b) Metabolic activation of polycyclic aromatic hydrocarbon *trans*-dihydrodiols by ubiquitously expressed aldehyde reductase (AKR1A1). *Chem.-biol. Interact*., **130–132**, 815–824

Palackal, N.T., Lee, S.H., Harvey, R.G., Blair, I.A. & Penning, T.M. (2002a) Activation of polycyclic aromatic hydrocarbon *trans*-dihydrodiol proximate carcinogens by human aldo-keto reductase (AKR1C) enzymes and their functional overexpression in human lung carcinoma (A549) cells. *J. biol. Chem*., **277**, 24799–24808

Palackal, N.T., Lee, S.H., Havey, R.G., Blair, I.A. & Penning, T.M. (2002b) Human AKR1C members oxidize the potent proximate carcinogen 7,12-dimethylbenz[*a*]anthracene-3,4-diol in vitro in the human lung A549 carcinoma cell line. *Polycyc. arom. Comp.*, **22**, 801–810

Paleologo, M., van Schooten, F.J., Pavanello, S., Kriek, E., Zordan, M., Clonfero, E., Bezze, C. & Levis, A.G. (1992) Detection of benzo[a]pyrene-diol-epoxide–DNA adducts in white blood cells of psoriatic patients treated with coal tar. *Mutat. Res.*, **281**, 11–16

Pallardy, M.J., House, R.V. & Dean, J.H. (1989) Molecular mechanism of 7,12-dimethylbenz[a]anthracene-induced immunosuppression: Evidence for action via the interleukin-2 pathway. *Mol. Pharmacol*., **36**, 128–133

Park, J.-H., Gopishetty, S., Szewczuk, L.M., Troxel, A.B., Harvey, R.G. & Penning, T.M. (2005) Formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) by PAH *o*-quinones:

Involvement of reactive oxygen species and copper(II)/copper(I) redox cycling. *Chem. Res. Toxicol.*, **18** , 1026–1037

Park, J.Y., Chen, L., Elahi, A., Lazarus, P. & Tockman, M.S. (2005) Genetic analysis of microsomal epoxide hydrolase gene and its association with lung cancer risk. *Eur. J. Cancer Prev.*, **14**, 223–230

Parker, P.J. & Murray-Rust, J. (2004) PKC at a glance. *J. Cell Sci.*, **117**, 131–132

Pataki, J. & Balick, R. (1972) Relative carcinogenicity of some diethyl-benz(*a*)anthracenes. *J. med. Chem.*, **15**, 905–909

Paules, R.S., Poirier, M.C., Mass, M.J., Yuspa, S.H. & Kaufman, D.G. (1985) Quantitation by electron microscopy of the binding of highly specific antibodies to benzo[a]pyrene–DNA adducts. *Carcinogenesis*, **6**, 193–198

Pavanello, S. & Levis, A.G. (1994) Human peripheral blood lymphocytes as a cell model to evaluate the genotoxic effect of coal tar treatment. *Environ. Health Perspect.*, **102** (Suppl. 9), 95–99

Pavanello, S., Favretto, D., Brugnone, F., Mastrangelo, G., Dal Pra, G. & Clonfero, E. (1999a) HPLC/fluorescence determination of anti-BPDE–DNA adducts in mononuclear white blood cells from PAH-exposed humans. *Carcinogenesis*, **20**, 431–435

Pavanello, S., Gabbani, G., Mastrangelo, G., Brugnone, F., Maccacaro, G. & Clonfero, E. (1999b) Influence of GSTM1 genotypes on anti-BPDE–DNA adduct levels in mononuclear white blood cells of humans exposed to PAH. *Int. Arch. occup. environ. Health*, **72**, 238–246

Pavanello, S., Siwinska, E., Mielzynska, D. & Clonfero, E. (2004) GSTM1 null genotype as a risk factor for anti-BPDE–DNA adduct formation in mononuclear white blood cells of coke-oven workers. *Mutat. Res.*, **558**, 53–62

Pavanello, S., Pulliero, A., Siwinska, E., Mielzynska, D. & Clonfero, E. (2005) Reduced nucleotide excision repair and GSTM1-null genotypes influence anti-B[a]PDE–DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. *Carcinogenesis*, **26**, 169–175

Pelkonen, O. & Nebert, D.W. (1982) Metabolism of polycyclic aromatic hydrocarbons: Etiologic role in carcinogenesis. *Pharmacol. Rev.*, **34**, 189–222

Penning, T.M. (2004) Aldo-keto reductases and formation of polycyclic aromatic hydrocarbon o-quinones. *Methods Enzymol.*, **378**, 31–67

Penning, T.M. & Talalay, P. (1983) Inhibition of a major NAD(P)-linked oxidoreductase from rat liver cytosol by steroidal and nonsteroidal anti-inflammatory agents and by prostaglandins. *Proc. natl Acad. Sci. USA*, **80**, 4504–4508

Penning, T.M., Ohnishi, S.T., Ohnishi, T. & Harvey, R.G. (1996) Generation of reactive oxygen species during the enzymatic oxidation of polycyclic aromatic hydrocarbon *trans*-dihydrodiols catalyzed by dihydrodiol dehydrogenase. *Chem. Res. Toxicol.*, **9**, 84–92

Penning, T.M., Burczynski, M.E., Hung, C.-F., McCoull, K.D., Palackal, N.T. & Tsuruda, L.S. (1999) Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: Generation of reactive and redox active *o*-quinones. *Chem. Res. Toxicol.*, **12**, 1–18

Penning, T.M., Shen, Y.-M., Mick, R., Shults, J. & Field, J.M. (2004) Polycyclic aromatic hydrocarbon *o*-quinones mutate p53 in human lung adenocarcinoma (A549) cells. *Polycyc. arom. Comp.*, **24**, 583–596

Perera, F.P., Hemminki, K., Young, T.L., Brenner, D., Kelly, G. & Santella, R.M. (1988) Detection of polycyclic aromatic hydrocarbon–DNA adducts in white blood cells of foundry workers. *Cancer Res*., **48**, 2288–2291

Perera, F.P., Whyatt, R.M., Jedrychowski, W., Rauh, V., Manchester, D., Santella, R.M. & Ottman, R. (1998) Recent developments in molecular epidemiology: A study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. *Am. J. Epidemiol*., **147**, 309–314

Perera, F.P., Jedrychowski, W., Rauh, V. & Whyatt, R.M. (1999) Molecular epidemiologic research on the effects of environmental pollutants on the fetus. *Environ. Health Perspect*., **107** (Suppl 3), 451–460

Perera, F.P., Rauh, V., Whyatt, R.M., Tsai, W.-Y., Bernert, J.T., Tu, Y.-H., Andrews, H., Ramirez, J., Qu, L. & Tang, D. (2004) Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population. *Environ. Health Perspect*., **112**, 626–630

Perera, F.P., Tang, D., Rauh, V., Lester, K., Tsai, W.Y., Tu, Y.H., Weiss, L., Hoepner, L., King, J., Del Priore, G. & Lederman, S.A. (2005) Relationships among polycyclic aromatic hydrocarbon–DNA adducts, proximity to the World Trade Center, and effects on fetal growth. *Environ. Health Perspect*., **113**, 1062–1067

Persson, I., Johansson, I. & Ingelman-Sundberg, M. (1997) *In vitro* kinetics of two human *CYP1A1* variant enzymes suggested to be associated with interindividual differences in cancer susceptibility. *Biochem. biophys. Res. Commun*., **231**, 227–230

Peters, J.M., Narotsky, M.G., Elizondo, G., Fernandez-Salguero, P.M., Gonzalez, F.J. & Abbott, B.D. (1999) Amelioration of TCDD-induced teratogenesis in aryl hydrocarbon receptor (AhR)-null mice. *Toxicol. Sci*., **47**, 86–92

Pfau, W., Hughes, N.C., Grover, P.L. & Phillips, D.H. (1992) HPLC separation of ^{32}P-postlabelled benzo[b]fluoranthene–DNA adducts. *Cancer Lett*., **65**, 159–167

Pfeifer, G.P. & Denissenko, M.F. (1998) Formation and repair of DNA lesions in the p53 gene: Relation to cancer mutations? *Environ. mol. Mutag*., **31**, 197–205

Pfeifer, G.P. & Hainaut, P. (2003) On the origin of G→T transversions in lung cancer. *Mutat. Res*., **526**, 39–43

Phillips, D.H., Hewer, A., Seidel, A., Steinbrecher, T., Schrode, R., Oesch, F. & Glatt, H. (1991) Relationship between mutagenicity and DNA adduct formation in mammalian cells for fjord- and bay-region diol-epoxides of polycyclic aromatic hydrocarbons. *Chem.-biol. Interact*., **80**, 177–186

Phillips, D.H., Farmer, P.B., Beland, F.A., Nath, R.G., Poirier, M.C., Reddy, M.V. & Turteltaub, K.W. (2000) Methods of DNA adduct determination and their application to testing compounds for genotoxicity. *Environ. mol. Mutagen.*, **35**, 222–233

Pietra, G., Rappaport, H. & Shubik, P. (1961) The effects of carcinogenic chemicals in newborn mice. *Cancer*, **14**, 308–317

Pittelkow, M.R., Perry, H.O., Muller, S.A., Maughan, W.Z. & O'Brien, P.C. (1981) Skin cancer in patients with psoriasis treated with coal tar. A 25-year follow-up study. *Arch. Dermatol*., **8**, 465–468

Platt, K.L. & Reischmann, I. (1987) Regio- and stereoselective metabolism of dibenz[*a,h*]anthracene: Identification of 12 new microsomal metabolites. *Mol. Pharmacol*., **32**, 710–722

Platt, K.L. & Schollmeier, M. (1994) Bisdihydrodiols, rather than dihydrodiol oxides, are the principal microsomal metabolites of tumorigenic *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,h*]anthracene. *Chem. Res. Toxicol*., **7**, 89–97

Platt, K.L., Bücker, M., Golan, M. & Oesch, F. (1982) The mutagenicity of dibenz[*a,h*]anthracene activated by phenobarbital-inducible mouse-liver mono-oxygenase is potentiated by the presence of hydrophilic residues at the K-region of the molecule. *Mutat. Res*., **96**, 1–13

Platt, K.L., Schollmeier, M., Frank, H. & Oesch, F. (1990) Stereoselective metabolism of dibenz(*a,h*)anthracene to *trans*-dihydrodiols and their activation to bacterial mutagens. *Environ. Health Perspect*., **88**, 37–41

Platt, K.L., Dienes, H.P., Tommasone, M. & Luch, A. (2004) Tumor formation in the neonatal mouse bioassay indicates that the potent carcinogen dibenzo[*def,p*]chrysene (dibenzo[*a,l*]pyrene) is activated in vivo via its *trans*-11,12-dihydrodiol. *Chem.-biol. Interact*., **148**, 27–36

Pliskova, M., Vondracek, J., Vojtesek, B., Kozubik, A. & Machala, M. (2005) Deregulation of cell proliferation by polycyclic aromatic hydrocarbons in human breast carcinoma MCF-7 cells reflects both genotoxic and nongenotoxic events. *Toxicol. Sci*., **83**, 246–256

Plummer, S.J., Conti, D.V., Paris, P.L., Curran, A.P., Casey, G. & Witte, J.S. (2003) CYP3A4 and CYP3A5 genotypes, haplotypes, and risk of prostate cancer. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 928–932

Pocar, P., Fischer, B., Klonisch, T. & Hombach-Klonisch, S. (2005) Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. *Reproduction*, **129**, 379–389

Pollack, M., Oe, T., Lee, S.H., Silva Elipe, M.V., Arison, B.H. & Blair, I.A. (2003) Characterization of 2'-deoxycytidine adducts derived from 4-oxo-2-nonenal, a novel lipid peroxidation product. *Chem. Res. Toxicol*., **16**, 893–900

Popp, W., Vahrenholz, C., Schell, C., Grimmer, G., Dettbarn, G., Kraus, R., Brauksiepe, A., Schmeling, B., Gutzeit, T., von Bulow, J. & Norpoth, K. (1997) DNA single strand breakage, DNA adducts, and sister chromatid exchange in lymphocytes and phenanthrene and pyrene metabolites in urine of coke oven workers. *Occup. environ. Med.*, **54**, 176–183

Pottenger, L.H. & Jefcoate, C.R. (1990) Characterization of a novel cytochrome P450 from the transformable cell line, C3H/10T1/2. *Carcinogenesis*, **11**, 321–327

Pottenger, L.H., Christou, M. & Jefcoate, C.R. (1991) Purification and immunological characterization of a novel cytochrome P450 from C3H/10T1/2 cells. *Arch. Biochem. Biophys*., **286**, 488–497

Potter, G.A., Patterson, L.H., Wanogho, E., Perry, P.J., Butler, P.C., Ijaz, T., Ruparelia, K.C., Lamb, J.H., Farmer, P.B., Stanley, L.A. & Burke, M.D. (2002) The cancer preventive agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. *Br. J. Cancer*, **86**, 774–778

Prahalad, A.K., Ross, J.A., Nelson, G.B., Roop, B.C., King, L.C., Nesnow, S. & Mass, M.J. (1997) Dibenzo[*a,l*]pyrene-induced DNA adduction, tumorigenicity, and Ki-*ras* oncogene mutations in strain A/J mouse lung. *Carcinogenesis*, **18**, 1955–1963

Primiano, T., Sutter, T.R. & Kensler, T.W. (1997) Antioxidant-inducible genes. *Adv. Pharmacol*., **38**, 293–328

Prodi, G., Grilli, S., Mazzullo, M., Colacci, A. & Arfellini, G. (1984) Comparison between photo-induction and microsomal activation of polycyclic hydrocarbons with different oncogenic potency. *Toxicol. Pathol.*, **12**, 185–188

Prokopczyk, B., Rosa, J.G., Desai, D., Amin, S., Sohn, O.S., Fiala, E.S. & El-Bayoumy, K. (2000) Chemoprevention of lung tumorigenesis induced by a mixture of benzo(a)pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by the organoselenium compound 1,4-phenylenebis(methylene)selenocyanate. *Cancer Lett.*, **161**, 35–46

Pruess-Schwartz, D., Nimesheim, A. & Marnett, L.J. (1989) Peroxyl radical- and cytochrome P-450-dependent metabolic activation of (+)-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene in mouse skin *in vitro* and *in vivo*. *Cancer Res.*, **49**, 1732–1737

Puga, A., Xia, Y. & Elferink, C. (2002) Role of the aryl hydrocarbon receptor in cell cycle regulation. *Chem.-biol. Interact.*, **20**, 117–130

Qin, C., Wilson, C., Blancher, C., Taylor, M., Safe, S. & Harris, A.L. (2001) Association of ARNT splice variants with estrogen receptor-negative breast cancer, poor induction of vascular endothelial growth factor under hypoxia, and poor prognosis. *Clin. Cancer Res.*, **7**, 818–823

Quiñones, L., Berthou, F., Varela, N., Simon, B., Gil, L. & Lucas, D. (1999) Ethnic susceptibility to lung cancer: Differences in CYP2E1, CYP1A1 and GSTM1 genetic polymorphisms between French Caucasian and Chilean populations. *Cancer Lett.*, **141**, 167–171

Raftogianis, R.B., Wood, T.C., Otterness, D.M., Van Loon, J.A. & Weinshilboum, R.M. (1997) Phenol sulfotransferase pharmacogenetics in humans: Association of common *SULT1A1* alleles with TS PST phenotype. *Biochem. biophys. Res. Commun.*, **239**, 298–304

Raftogianis, R.B., Wood, T.C. & Weinshilboum, R.M. (1999) Human phenol sulfotransferases SULT1A2 and SULT1A1: Genetic polymorphisms, allozyme properties, and human liver genotype–phenotype correlations. *Biochem. Pharmacol.*, **58**, 605–616

Rahman, A., Barrowman, J.A. & Rahimtula, A. (1986) The influence of bile on the bioavailability of polynuclear aromatic hydrocarbons from the rat intestine. *Can. J. Physiol. Pharmacol.*, **64**, 1214–1218

Ralston, S.L., Seidel, A., Luch, A., Platt, K.L. & Baird, W.M. (1995) Stereoselective activation of dibenzo[*a,l*]pyrene to (–)-*anti*(11*R*,12*S*,13*S*,14*R*)- and (+)-*syn*(11*S*,12*R*,13*S*,14*R*)-11,12-diol-13,14-epoxides which bind extensively to deoxyadenosine residues of DNA in the human mammary carcinoma cell line MCF-7. *Carcinogenesis*, **16**, 2899–2907

Ralston, S.L., Coffing, S.L., Seidel, A., Luch, A., Platt, K.L. & Baird, W.M. (1997) Stereoselective activation of dibenzo[*a,l*]pyrene and its *trans*-11,12-dihydrodiol to fjord region 11,12-diol 13,14-epoxides in a human mammary carcinoma MCF-7 cell-mediated V79 cell mutation assay. *Chem. Res. Toxicol.*, **10**, 687–693

RamaKrishna, N.V.S., Devanesan, P.D., Rogan, E.G., Cavalieri, E.L., Jeong, H., Jankowiak, R. & Small, G.J. (1992) Mechanism of metabolic activation of the potent carcinogen 7,12-dimethylbenz[*a*]anthracene. *Chem. Res. Toxicol.*, **5**, 220–226

RamaKrishna, N.V.S., Padmavathi, N.S., Cavalieri, E.L., Rogan, E.G., Cerny, R.L. & Gross, M.L. (1993a) Synthesis and structure determination of the adducts formed by electrochemical oxidation of the potent carcinogen dibenzo[*a,l*]pyrene in the presence of nucleosides. *Chem. Res. Toxicol.*, **6**, 554–560

RamaKrishna, N.V.S., Li, K.-M., Rogan, E.G., Cavalieri, E.L., George, M., Cerny, R.L. & Gross, M.L. (1993b) Adducts of 6-methylbenzo[*a*]pyrene and 6-fluorobenzo[*a*]pyrene formed by

electrochemical oxidation in the presence of deoxyribonucleosides. *Chem. Res. Toxicol*., **6**, 837–845

Ramesh, A., Greenwood, M., Inyang, F. & Hood, D.B. (2001) Toxicokinetics of inhaled benzo[*a*]pyrene: Plasma and lung bioavailability. *Inhal. Toxicol.*, **13**, 533–555

Rämet, M., Castren, K., Järvinen, K., Pekkala, K., Turpeenniemi-Hujanen, T., Soini, Y., Pääkkö, P. & Vahakangas, K. (1995) p53 Protein expression is correlated with benzo[a]pyrene–DNA adducts in carcinoma cell lines. *Carcinogenesis*, **16**, 2117–2124

Randerath, E., Zhou, G.D., Donnelly, K.C., Safe, S.H. & Randerath, K. (1996) DNA damage induced in mouse tissues by organic wood preserving waste extracts as assayed by ^{32}P-postlabeling. *Arch. Toxicol*., **70**, 683–695

Randerath, K., Zhou, G.D., Randerath, E., Safe, S.H. & Donnelly, K.C. (1997) Comparative ^{32}P-postlabeling analysis of exogenous and endogenous DNA adducts in mouse skin exposed to a wood-preserving waste extract, a complex mixture of polycyclic and polychlorinated chemicals. *Environ. mol. Mutag*., **29**, 372–378

Rasmussen, H., Isales, C.M., Calle, R., Throckmorton, D., Anderson, M., Gasalla-Herraiz, J. & McCarthy, R. (1995) Diacylglycerol production, Ca^{2+} influx, and protein kinase C activation in sustained cellular responses. *Endocr. Rev.,* **16**, 649–681

Raveh, D., Slaga, T.J. & Huberman, E. (1982) Cell-mediated mutagenesis and tumor-initiating activity of the ubiquitous polycyclic hydrocarbon, cyclopenta[c,d]pyrene. *Carcinogenesis*, **3**, 763–766

Ravi Kumar, M.N., Vadhanam, M.V., Horn, J., Flesher, J.W. & Gupta, R.C. (2005) Formation of benzylic–DNA adducts resulting from 7,12-dimethylbenz[*a*]anthracene in vivo. *Chem. Res. Toxicol*., **18**, 686–691

Raychoudhury, S.S. & Kubinski, D. (2003) Polycyclic aromatic hydrocarbon-induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. *Environ. Health Perspect*., **111**, 33–38

Reardon, D.B., Prakash, A.S., Hilton, B.D., Roman, J.M., Pataki, J., Harvey, R.G. & Dipple, A. (1987) Characterization of 5-methylchrysene-1,2-dihydrodiol-3,4-epoxide–DNA adducts. *Carcinogenesis*, **8**, 1317–1322

Recio, L. & Hsie, A.W. (1987) Modulation of the cytotoxicity and mutagenicity of benzo[a]pyrene and benzo[a]pyrene 7,8-diol by glutathione and glutathione S-transferases in mammalian cells (CHO/HGPRT assay). *Mutat. Res*., **178**, 257–269

Reed, G.A. & Ryan, M.J. (1990) Peroxyl radical-dependent epoxidation of cyclopenteno[*c,d*]pyrene. *Carcinogenesis*, **11**, 1825–1829

Reed, G.A., Grafstrom, R.C., Krauss, R.S., Autrup, H. & Eling, T.E. (1984) Prostaglandin H synthase-dependent co-oxygenation of (+/-)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in hamster trachea and human bronchus explants. *Carcinogenesis*, **5**, 955–960

Reed, G.A., Layton, M.E. & Ryan, M.J. (1988) Metabolic activation of cyclopenteno[*c,d*]pyrene by peroxyl radicals. *Carcinogenesis*, **9**, 2291–2295

Reed, M., Monske, M., Lauer, F., Meserole, S., Born, J. & Burchiel, S. (2003) Benzo[a]pyrene diones are produced by photochemical and enzymatic oxidation and induce concentration-dependent decreases in the proliferative state of human pulmonary epithelial cells. *J. Toxicol. environ. Health*, **A66**, 1189–1205

Rees, E.D., Mandelstam, P., Lowry, J.Q. & Lipscomb, H. (1971) A study of the mechanism of intestinal absorption of benzo(*a*)pyrene. *Biochim. biophys. Acta*, **225**, 96–107

Rettie, A.E., Wienkers, L.C., Gonzalez, F.J., Trager, W.F. & Korzekwa, K.R. (1994) Impaired (*S*)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. *Pharmacogenetics*, **4**, 39–42

Rhee, S.G., Kang, S.W., Jeong, W., Chang, T.S., Yang, K.S. & Woo, H.A. (2005) Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. *Curr. Opin. Cell Biol*., **17**, 183–189

Rice, J.E., Coleman, D.T., Hosted, T.J., Jr, LaVoie, E.J., McCaustland, D.J. & Wiley, J.C., Jr (1985) Identification of mutagenic metabolites of indeno[1,2,3-*cd*]pyrene formed *in vitro* with rat liver enzymes. *Cancer Res*., **45**, 5421–5425

Rice, J.E., Hosted, T.J., Jr, DeFloria, M.C., LaVoie, E.J., Fischer, D.L. & Wiley, J.C., Jr (1986) Tumor-initiating activity of major *in vivo* metabolites of indeno[1,2,3-*cd*]pyrene on mouse skin. *Carcinogenesis*, **7**, 1761–1764

Rice, J.E., Weyand, E.H., Geddie, N.G., DeFloria, M.C. & LaVoie, E.J. (1987) Identification of tumorigenic metabolites of benzo[*j*]fluoranthene formed *in vivo* in mouse skin. *Cancer Res*., **47**, 6166–6170

Rice, J.E., Jordan, K., Little, P. & Hussain, N. (1988) Comparative tumor-initiating activity of methylene-bridged and bay-region methylated derivatives of benz[*a*]anthracene and chrysene. *Carcinogenesis*, **9**, 2275–2278

Rice, J.E., Weyand, E.H., Burrill, C. & LaVoie, E.J. (1990) Fluorine probes for investigating the mechanism of activation of indeno[1,2,3-*cd*]pyrene to a tumorigenic agent. *Carcinogenesis*, **11**, 1971–1974

Rideout, W.M., III, Coetzee, G.A., Olumi, A.F. & Jones, P.A. (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. *Science*, **249**, 1288–1290

Rier, S.E., Coe, C.L., Lemieux, A.M., Martin, D.C., Morris, R., Lucier, G.W. & Clark, G.C. (2001) Increased tumor necrosis factor-alpha production by peripheral blood leukocytes from TCDD-exposed rhesus monkeys. *Toxicol. Sci*., **60**, 327–337

Rodenhuis, S. (1992) ras and Human tumors. *Semin. Cancer Biol*., **3**, 241–247

Rodin, S.N. & Rodin, A.S. (2005) Origins and selection of p53 mutations in lung carcinogenesis. *Semin. Cancer Biol*., **15**, 103–112

Rodriguez, H. & Loechler, E.L. (1993) Mutational specificity of the (+)-*anti*-diol epoxide of benzo[*a*]pyrene in a *supF* gene of an Escherichia coli plasmid: DNA sequence context influences hotspots, mutagenic specificity and the extent of SOS enhancement of mutagenesis. *Carcinogenesis*, **14**, 373–383

Rodriguez, H. & Loechler, E.L. (1995) Are base substitution and frameshift mutagenesis pathways interrelated? An analysis based upon studies of the frequencies and specificities of mutations induced by the (+)-*anti* diol epoxide of benzo[*a*]pyrene. *Mutat. Res*., **326**, 29–37

Rodriguez, L.V., Dunsford, H.A., Steinberg, M., Chaloupka, K.K., Zhu, L., Safe, S., Womack, J.E. & Goldstein, L.S. (1997) Carcinogenicity of benzo[a]pyrene and manufactured gas plant residues in infant mice. *Carcinogenesis*, **18**, 127–135

Rogan, E.G., Cavalieri, E.L., Walker, B.A., Balasubramanian, R., Wislocki, P.G., Roth, R.W. & Saugier, R.K. (1986) Mutagenicity of benzylic acetates, sulfates and bromides of polycyclic aromatic hydrocarbons. *Chem.-biol. Interact.*, **58**, 253–275

Rogan, E., Cavalieri, E., Tibbels, S., Cremonesi, P., Warner, C., Nagel, D., Tomer, K., Cerny, R. & Gross, M. (1988) Synthesis and identification of benzo[*a*]pyrene–guanine nucleoside adducts

formed by electrochemical oxidation and horseradish peroxidase-catalyzed reaction of benzo[*a*]pyrene with DNA. *J. Am. chem. Soc*., **110**, 4023–4029

Rogan, E.G., Devanesan, P.D., RamaKrishna, N.V.S., Higginbotham, S., Padmavathi, N.S., Chapman, K., Cavalieri, E.L., Jeong, H., Jankowiak, R. & Small, G.J. (1993) Identification and quantitation of benzo[*a*]pyrene–DNA adducts formed in mouse skin. *Chem. Res. Toxicol*., **6**, 356–363

Rojas, M. & Alexandrov, K. (1986) *In vivo* formation and persistence of DNA adducts in mouse and rat skin exposed to (+/-)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene and (+/-)-7beta,8alpha-dihydroxy-9alpha,10alpha-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Carcinogenesis*, **7**, 1553–1560

Rojas, M., Alexandrov, K., Auburtin, G., Wastiaux-Denamur, A., Mayer, L., Mahieu, B., Sebastien, P. & Bartsch, H. (1995) Anti-benzo[*a*]pyrene diolepoxide–DNA adduct levels in peripheral mononuclear cells from coke oven workers and the enhancing effect of smoking. *Carcinogenesis*, **16**, 1373–1376

Rojas, M., Alexandrov, K., Cascorbi, I., Brockmoller, J., Likhachev, A., Pozharisski, K., Bouvier, G., Auburtin, G., Mayer, L., Kopp-Schneider, A., Roots, I. & Bartsch, H. (1998) High benzo[a]pyrene diol-epoxide DNA adduct levels in lung and blood cells from individuals with combined CYP1A1 MspI/Msp-GSTM1*0/*0 genotypes. *Pharmacogenetics*, **8**, 109–118

Rojas, M., Cascorbi, I., Alexandrov, K., Kriek, E., Auburtin, G., Mayer, L., Kopp-Schneider, A., Roots, I. & Bartsch, H. (2000) Modulation of benzo[a]pyrene diolepoxide–DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogenesis*, **21**, 35–41

Rojas, M., Marie, B., Vignaud, J.M., Martinet, N., Siat, J., Grosdidier, G., Cascorbi, I. & Alexandrov, K. (2004) High DNA damage by benzo[*a*]pyrene 7,8-diol-9,10-epoxide in bronchial epithelial cells from patients with lung cancer: Comparison with lung parenchyma. *Cancer Lett.*, **207**, 157–163

Romero, D.L., Mounho, B.J., Lauer, F.T., Born, J.L. & Burchiel, S.W. (1997) Depletion of glutathione by benzo(a)pyrene metabolites, ionomycin, thapsigargin, and phorbol myristate in human peripheral blood mononuclear cells. *Toxicol. appl. Pharmacol*., **144**, 62–69

van Rooij, J.G.M., Bodelier-Bade, M.M. & Jongeneelen, F.J. (1993a) Estimation of individual dermal and respiratory uptake of polycyclic aromatic hydrocarbons in 12 coke oven workers. *Br. J. ind. Med.*, **50**, 623–632

van Rooij, J.G.M., van Lieshout, E.M.A., Bodelier-Bade, M.M. & Jongeneelen, F.J. (1993b) Effect of the reduction of skin contamination on the internal dose of creosote workers exposed to polycyclic aromatic hydrocarbons. *Scand. J. Work Environ. Health*, **19**, 200–207

Ross, D. (2000) The role of metabolism and specific metabolites in benzene-induced toxicity: evidence and issues. *J. Toxicol. environ. Health*, **A61**, 357–372

Ross, J.A. & Nesnow, S. (1999) Polycyclic aromatic hydrocarbons: Correlations between DNA adducts and *ras* oncogene mutations. *Mutat. Res*., **424**, 155–166

Ross, D. & Siegel, D. (2004) NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase), functions and pharmacogenetics. *Methods Enzymol*., **382**, 115–144

Ross, J., Nelson, G., Kligerman, A., Erexson, G., Bryant, M., Earley, K., Gupta, R. & Nesnow, S. (1990) Formation and persistence of novel benzo(*a*)pyrene adducts in rat lung, liver, and peripheral blood lymphocyte DNA. *Cancer Res*., **50**, 5088–5094

Ross, J.A., Nelson, G.B., Holden, K.L., Kligerman, A.D., Erexson, G.L., Bryant, M.F., Earley, K., Beach, A.C., Gupta, R.C. & Nesnow, S. (1992) DNA adducts and induction of sister chromatid exchanges in the rat following benzo[b]fluoranthene administration. *Carcinogenesis*, **13**, 1731–1734

Ross, J.A., Nelson, G.B., Wilson, K.H., Rabinowitz, J.R., Galati, A., Stoner, G.D., Nesnow, S. & Mass, M.J. (1995) Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Res*., **55**, 1039–1044

Ross, D., Kepa, J.K., Winski, S.L., Beall, H.D., Anwar, A. & Siegel, D. (2000) NAD(P)H:quinone oxidoreductase 1 (NQO1): Chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chem.-biol. Interact*., **129**, 77–97

Rossner, P., Jr, Binkova, B. & Šrám, R.J. (2003) The influence of occupational exposure to PAHs on the blood plasma levels of p53 and p21WAF1 proteins. *Mutat. Res.*, **535**, 87–94

Rothman, N., Poirier, M.C., Haas, R.A., Correa-Villasenor, A., Ford, P., Hansen, J.A., O'Toole, T. & Strickland, P.T. (1993) Association of PAH–DNA adducts in peripheral white blood cells with dietary exposure to polyaromatic hydrocarbons. *Environ. Health Perspect*., **99**, 265–267

Ruggenenti, P., Perico, N., Mosconi, L., Gaspari, F., Benigni, A., Amuchastegui, F., Bruzzi, I. & Remuzzi, J. (1993) Calcium channel blockers protect transplant patients from cyclosporine-induced daily renal hypoperfusion. *Kidney int.*, **43**, 706–711

Ruggeri, B., DiRado, M., Zhang, S.Y., Bauer, B., Goodrow, T. & Klein-Szanto, A.J.P. (1993) Benzo[*a*]pyrene-induced murine skin tumors exhibit frequent and characteristic G to T mutations in the p53 gene. *Proc. natl Acad. Sci. USA*, **90**, 1013–1017

Russo, J., Wilgus, G. & Russo, I.H. (1979) Susceptibility of the mammary gland to carcinogenesis: I. Differentiation of the mammary gland as determinant of tumor incidence and type of lesion. *Am. J. Pathol*., **96**, 721–736

Ryan, D.E. & Levin, W. (1990) Purification and characterization of hepatic microsomal cytochrome P-450. *Pharmacol. Ther*., **45**, 153–239

Rylander-Rudqvist, T., Wedren, S., Granath, F., Humphreys, K., Ahlberg, S., Weiderpass, E., Oscarson, M., Ingelman-Sundberg, M. & Persson, I. (2003) Cytochrome P450 1B1 gene polymorphisms and postmenopausal breast cancer risk. *Carcinogenesis*, **24**, 1533–1539

Safe, S. (2001) Molecular biology of the Ah receptor and its role in carcinogenesis. *Toxicol. Lett*., **120**, 1–7

Safe, S., Wormke, M. & Samudio, I. (2000) Mechanisms of inhibitory aryl hydrocarbon receptor–estrogen receptor crosstalk in human breast cancer cells. *J. mamm. Gland Biol. Neoplasia*, **5**, 295–306

Sagher, D. & Strauss, B. (1983) Insertion of nucleotides opposite apurinic/apyrimidinic sites in deoxyribonucleic acid during in vitro synthesis: Uniqueness of adenine nucleotides. *Biochemistry*, **22**, 4518–4526

Sahali, Y., Kwon, H., Skipper, P.L. & Tannenbaum, S.R. (1992) Microsomal metabolism of cyclopenta[*cd*]pyrene. Characterization of new metabolites and their mechanism of formation. *Chem. Res. Toxicol*., **5**, 157–162

Sakaeda, T., Nakamura, T. & Okumura, K. (2004) Pharmacogenetics of drug transporters and its impact on the pharmacotherapy. *Curr. Top. Med. Chem.*, **4**, 1383–1396

Saldivar, S.J., Wang, Y., Zhao, H., Shao, L., Lin, J., Spitz, M.R. & Wu, X. (2005) An association between a NQO1 genetic polymorphism and risk of lung cancer. *Mutat. Res*., **582**, 71–78

Santamaria, L., Giordano, G.G., Alfisi, M. & Cascione, F. (1966) Effects of light on 3,4-benzpyrene carcinogenesis. *Nature*, **210**, 824–825

Santella, R., Kinoshita, T. & Jeffrey, A.M. (1982) Mutagenicity of some methylated benzo[*a*]pyrene derivatives. *Mutat. Res.*, **104**, 209–213

Santella, R.M., Perera, F.P., Young, T.L., Zhang, Y.J., Chiamprasert, S., Tang, D., Wang, L.W., Beachman, A., Lin, J.H. & DeLeo, V.A. (1995) Polycyclic aromatic hydrocarbon–DNA and protein adducts in coal tar treated patients and controls and their relationship to glutathione S-transferase genotype. *Mutat. Res.*, **334**, 117–124

Sarmanova, J., Susova, S., Gut, I., Mrhalova, M., Kodet, R., Adamek, J., Roth, Z. & Soucek, P. (2004) Breast cancer: Role of polymorphisms in biotransformation enzymes. *Eur. J. hum. Genet.*, **12**, 848–854

Sarto, F., Zordan, M., Tomanin, R., Mazzotti, D., Canova, A., Cardin, E.L., Bezze, G. & Levis, A.G. (1989) Chromosomal alterations in peripheral blood lymphocytes, urinary mutagenicity and excretion of polycyclic aromatic hydrocarbons in six psoriatic patients undergoing coal tar therapy. *Carcinogenesis*, **10**, 329–334

Savas, U., Bhattacharyya, K.K., Christou, M., Alexander, D.L. & Jefcoate, C.R. (1994) Mouse cytochrome P-450EF, representative of a new *1B* subfamily of cytochrome P-450s. Cloning, sequence determination, and tissue expression. *J. biol. Chem*., **269**, 14905–14911

Savas, U., Carstens, C.P. & Jefcoate, C.R. (1997) Biological oxidations and P450 reactions. Recombinant mouse CYP1B1 expressed in *Escherichia coli* exhibits selective binding by polycyclic hydrocarbons and metabolism which parallels C3H10T1/2 cell microsomes, but differs from human recombinant CYP1B1. *Arch. Biochem. Biophys*., **347**, 181–192

Sawyer, T.W., Baer-Dubowska, W., Chang, K., Crysup, S.B., Harvey, R.G. & DiGiovanni, J. (1988) Tumor-initiating activity of the bay-region dihydrodiols and diol-epoxides of dibenz[*a,j*]anthracene and cholanthrene on mouse skin. *Carcinogenesis*, **9**, 2203–2207

Scherer, G., Frank, S., Riedel, K., Meger-Kossien, I. & Renner, T. (2000) Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. *Cancer Epidemiol. Biomarkers Prev*., **9**, 373–380

Schieven, G.L., Mittler, R.S., Nadler, S.G., Kirihara, J.M., Bolen, J.B., Kanner, S.B. & Ledbetter, J.A. (1994) ZAP-70 tyrosine kinase, CD45, and T cell receptor involvement in UV- and H_2O_2-induced T cell signal transduction. *J. biol. Chem*., **269**, 20718–20726

Schinkel, A.H. & Jonker, J.W. (2003) Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: An overview. *Adv. Drug Deliv. Rev.*, **55**, 3–29

Schmidt, J.V. & Bradfield, C.A. (1996) Ah receptor signaling pathways. *Annu. Rev. Cell Dev. Biol*., **12**, 55–89

Schmidt, J.V., Su, G.H., Reddy, J.K., Simon, M.C. & Bradfield, C.A. (1996) Characterization of a murine Ahr null allele: Involvement of the Ah receptor in hepatic growth and development. *Proc. natl Acad. Sci. USA*, **93**, 6731–6736

Schoket, B. (1999) DNA damage in humans exposed to environmental and dietary polycyclic aromatic hydrocarbons. *Mutat. Res*., **424**, 143–153

Schoket, B., Hewer, A., Grover, P.L. & Phillips, D.H. (1988a) Covalent binding of components of coal-tar, creosote and bitumen to the DNA of the skin and lungs of mice following topical application. *Carcinogenesis*, **9**, 1253–1258

Schoket, B., Hewer, A., Grover, P.L. & Phillips, D.H. (1988b) Formation of DNA adducts in human skin maintained in short-term organ culture and treated with coal-tar, creosote or bitumen. *Int. J. Cancer*, **42**, 622–626

Schoket, B., Horkay, I., Kósa, A., Páldeák, L., Hewer, A., Grover, P.L. & Phillips, D.H. (1990) Formation of DNA adducts in the skin of psoriasis patients, in human skin in organ culture, and in mouse skin and lung following topical application of coal-tar and juniper tar. *J. invest. Dermatol.*, **94**, 241–246

Schoket, B., Doty, W.A., Vincze, I., Strickland, P.T., Ferri, G.M., Assennato, G. & Poirier, M.C. (1993a) Increased sensitivity for determination of polycyclic aromatic hydrocarbon–DNA adducts in human DNA samples by dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA). *Cancer Epidemiol. Biomarkers Prev*., **2**, 349–353

Schoket, B., Phillips, D.H., Poirier, M.C. & Vincze, I. (1993b) DNA adducts in peripheral blood lymphocytes from aluminum production plant workers determined by ^{32}P-postlabeling and enzyme-linked immunosorbent assay. *Environ. Health Perspect*., **99**, 307–309

Schoket, B., Poirier, M.C. & Vincze, I. (1995) Biomonitoring of genotoxic exposure in aluminium plant workers by determination of DNA adducts in human peripheral blood lymphocytes. *Sci. total Environ*., **163**, 153–163

Schoket, B., Poirier, M.C., Mayer, G., Torok, G., Kolozsi-Ringelhann, A., Bognar, G., Bigbee, W.L. & Vincze, I. (1999) Biomonitoring of human genotoxicity induced by complex occupational exposures. *Mutat. Res*., **445**, 193–203

Schoket, B., Papp, G., Levay, K., Mrackova, G., Kadlubar, F.F. & Vincze, I. (2001) Impact of metabolic genotypes on levels of biomarkers of genotoxic exposure. *Mutat. Res*., **482**, 57–69

van Schooten, F.J., Jongeneelen, F.J., Hillebrand, M.J., van Leeuwen, F.E., de Looff, A.J., Dijkmans, A.P., van Rooij, J.G., den Engelse, L. & Kriek, E. (1995) Polycyclic aromatic hydrocarbon–DNA adducts in white blood cell DNA and 1-hydroxypyrene in the urine from aluminum workers: Relation with job category and synergistic effect of smoking. *Cancer Epidemiol. Biomarkers Prev*., **4**, 69–77

Schwartz, R.H. (2003) T Cell anergy. *Annu. Rev. Immunol*., **21**, 305–334

Schwarzmann, N., Kunerth, S., Weber, K., Mayr, G.W. & Guse, A.H. (2002) Knock-down of the type 3 ryanodine receptor impairs sustained Ca^{2+} signaling via the T cell receptor/CD3 complex. *J. biol. Chem*., **277**, 50636–50642

Schwerdtle, T., Walter, I. & Hartwig, A. (2003) Arsenite and its biomethylated metabolites interfere with the formation and repair of stable BPDE-induced DNA adducts in human cells and impair XPAzf and Fpg. *DNA Repair*, **2**, 1449–1463

Sebti, S.M. & Baird, W.M. (1984) Rat liver homogenate (S9)-mediated binding of benzo[*a*]pyrene to DNA in V79 cells, V79 cell nuclei and aqueous solution. *Mutat. Res*., **125**, 307–314

Sebti, S.M., Moynihan, C.G., Anderson, J.N. & Baird, W.M. (1985) Benzo[a]pyrene–DNA adduct formation in target and activator cells in a Wistar rat embryo cell-mediated V79 cell mutation assay. *Carcinogenesis*, **6**, 983–988

Sei, Y., Gallagher, K.L. & Basile, A.S. (1999) Skeletal muscle type ryanodine receptor is involved in calcium signaling in human B lymphocytes. *J. biol. Chem*., **274**, 5995–6002

Seidel, A., Luch, A., Platt, K.L., Oesch, F. & Glatt, H. (1994) Activated fjord-region metabolites of dibenzo[*a,l*]pyrene: Synthesis and mutagenic activities of the diastereoisomeric *syn*- and *anti*-11,12-dihydrodiol-13,14-epoxides. *Polycyc. arom. Comp.*, **6**, 191–198

Selkirk, J.K., Huberman, E. & Heidelberger, C. (1971) An epoxide is an intermediate in the microsomal metabolism of the chemical carcinogen, dibenz(a,h)anthracene. *Biochem. biophys. Res. Commun*., **43**, 1010–1016

Senft, A.P., Dalton, T.P., Nebert, D.W., Genter, M.B., Puga, A., Hutchinson, R.J., Kerzee, J.K., Uno, S. & Shertzer, H.G. (2002) Mitochondrial reactive oxygen production is dependent on the aromatic hydrocarbon receptor. *Free Radic. Biol. Med*., **33**, 1268–1278

Shaw, G.R. & Connell, D.W. (1994) Prediction and monitoring of the carcinogenicity of polycyclic aromatic compounds (PACs). *Rev. environ. Contam. Toxicol.*, **135**, 1–62

Shen, A.L., Fahl, W.E. & Jefcoate, C.R. (1980) Metabolism of benzo(*a*)pyrene by isolated hepatocytes and factors affecting covalent binding of benzo(*a*)pyrene metabolites to DNA in hepatocyte and microsomal systems. *Arch. Biochem. Biophys*., **204**, 511–523

Shen, Y.-M., Troxel, A.B., Vedantam, S., Penning, T.M. & Field, J. (2006) Comparison of *p53* mutations induced by PAH *o*-quinones with those caused by *anti*-benzo[*a*]pyrene diol epoxide *in vitro*: Role of reactive oxygen and biological selection. *Chem. Res. Toxicol.*, **19**, 1441–1450

Shimada, T. (2000) Human cytochrome P450 1B1 and chemical carcinogenesis. *Rev. Toxicol*., **3**, 139–166

Shimada, T. & Fujii-Kuriyama, Y. (2004) Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer Sci*., **95**, 1–6

Shimada, T. & Guengerich, F.P. (1989) Evidence for cytochrome P-450$_{NF}$, the nifedipine oxidase, being the principal enzyme involved in the bioactivation of aflatoxins in human liver. *Proc. natl Acad. Sci. USA*, **86**, 462–465

Shimada, T. & Guengerich, F.P. (1991) Activation of amino-alpha-carboline, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and a copper phthalocyanine cellulose extract of cigarette smoke condensate by cytochrome P-450 enzymes in rat and human liver microsomes. *Cancer Res*., **51**, 5284–5291

Shimada, T. & Okuda, Y. (1988) Metabolic activation of environmental carcinogens and mutagens by human liver microsomes. Role of cytochrome P-450 homologous to a 3-methylcholanthrene-inducible isozyme in rat liver. *Biochem. Pharmacol*., **37**, 459–465

Shimada, T., Iwasaki, M., Martin, M.V. & Guengerich, F.P. (1989a) Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by umu gene response in Salmonella typhimurium TA1535/pSK1002. *Cancer Res*., **49**, 3218–3228

Shimada, T., Martin, M.V., Pruess-Schwartz, D., Marnett, L.J. & Guengerich, F.P. (1989b) Roles of individual human cytochrome P-450 enzymes in the bioactivation of benzo(a)pyrene, 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Res*., **49**, 6304–6312

Shimada, T., Yun, C.H., Yamazaki, H., Gautier, J.C., Beaune, P.H. & Guengerich, F.P. (1992) Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens. *Mol. Pharmacol*., **41**, 856–864

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y. & Guengerich, F.P. (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: Studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. exp. Ther*., **270**, 414–423

Shimada, T., Hayes, C.L., Yamazaki, H., Amin, S., Hecht, S.S., Guengerich, F.P. & Sutter, T.R. (1996) Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res*., **56** , 2979–2984

Shimada, T., Gillam, E.M., Sutter, T.R., Strickland, P.T., Guengerich, F.P. & Yamazaki, H. (1997a) Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. *Drug Metab. Dispos*., **25**, 617–622

Shimada, T., El-Bayoumy, K., Upadhyaya, P., Sutter, T.R., Guengerich, F.P. & Yamazaki, H. (1997b) Inhibition of human cytochrome P450-catalyzed oxidations of xenobiotics and procarcinogens by synthetic organoselenium compounds. *Cancer Res*., **57**, 4757–4764

Shimada, T., Wunsch, R.M., Hanna, I.H., Sutter, T.R., Guengerich, F.P. & Gillam, E.M. (1998a) Recombinant human cytochrome P450 1B1 expression in *Escherichia coli*. *Arch. Biochem. Biophys*., **357**, 111–120

Shimada, T., Yamazaki, H., Foroozesh, M., Hopkins, N.E., Alworth, W.L. & Guengerich, F.P. (1998b) Selectivity of polycyclic inhibitors for human cytochrome P450s 1A1, 1A2, and 1B1. *Chem. Res. Toxicol*., **11**, 1048–1056

Shimada, T., Watanabe, J., Kawajiri, K., Sutter, T.R., Guengerich, F.P., Gillam, E.M.J. & Inoue, K. (1999a) Catalytic properties of polymorphic human cytochrome P450 1B1 variants. *Carcinogenesis*, **20**, 1607–1613

Shimada, T., Gillam, E.M.J., Oda, Y., Tsumura, F., Sutter, T.R., Guengerich, F.P. & Inoue, K. (1999b) Metabolism of benzo[*a*]pyrene to *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene by recombinant human cytochrome P450 1B1 and purified liver epoxide hydrolase. *Chem. Res. Toxicol*., **12**, 623–629

Shimada, T., Oda, Y., Gillam, E.M.J., Guengerich, F.P. & Inoue, K. (2001a) Metabolic activation of polycyclic aromatic hydrocarbons and other procarcinogens by cytochromes P450 1A1 and P450 1B1 allelic variants and other human cytochromes P450 in *Salmonella typhimurium* NM2009. *Drug Metab. Dispos*., **29**, 1176–1182

Shimada, T., Watanabe, J., Inoue, K., Guengerich, F.P. & Gillam, E.M. (2001b) Specificity of 17beta-oestradiol and benzo[a]pyrene oxidation by polymorphic human cytochrome P4501B1 variants substituted at residues 48, 119 and 432. *Xenobiotica*, **31**, 163–176

Shimada, T., Inoue, K., Suzuki, Y., Kawai, T., Azuma, E., Nakajima, T., Shindo, M., Kurose, K., Sugie, A., Yamagishi, Y., Fujii-Kuriyama, Y. & Hashimoto, M. (2002) Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice. *Carcinogenesis*, **23**, 1199–1207

Shimada, T., Sugie, A., Shindo, M., Nakajima, T., Azuma, E., Hashimoto, M. & Inoue, K. (2003a) Tissue-specific induction of cytochromes P450 1A1 and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered C57BL/6J mice of *arylhydrocarbon receptor* gene. *Toxicol. appl. Pharmacol*., **187**, 1–10

Shimada, T., Sugie, A., Yamada, T., Kawazoe, H., Hashimoto, M., Azuma, E., Nakajima, T., Inoue, K. & Oda, Y. (2003b) Dose–response studies on the induction of liver cytochromes P4501A1 and 1B1 by polycyclic aromatic hydrocarbons in arylhydrocarbon-responsive C57BL/6J mice. *Xenobiotica*, **33**, 957–971

Shimizu, Y., Nakatsuru, Y., Ichinose, M., Takahashi, Y., Kume, H., Mimura, J., Fujii-Kuriyama, Y. & Ishikawa, T. (2000) Benzo[a]pyrene carcinogenicity is lost in mice lacking the aryl hydrocarbon receptor. *Proc. natl Acad. Sci. USA*, **97**, 779–782

Shinohara, K. & Cerutti, P.A. (1977) Formation of benzo(*a*)pyrene–DNA adducts in peripheral human lung tissue. *Cancer Lett*., **3**, 303–309

Shintani, M., Ieiri, I., Inoue, K., Mamiya, K., Ninomiya, H., Tashiro, N., Higuchi, S. & Otsubo, K. (2001) Genetic polymorphisms and functional characterization of the 5'-flanking region of the human *CYP2C9* gene: In vitro and in vivo studies. *Clin. Pharmacol. Ther.*, **70**, 175–182

Shiue, G.-H., El-Bayoumy, K. & Hecht, S.S. (1987) Comparative metabolism and DNA binding of 6-nitro-5-methylchrysene and 5-methylchrysene. *Carcinogenesis*, **8**, 1327–1331

Shou, M., Harvey, R.G. & Penning, T.M. (1993) Reactivity of benzo[a]pyrene-7,8-dione with DNA. Evidence for the formation of deoxyguanosine adducts. *Carcinogenesis*, **14**, 475–482

Shou, M., Krausz, K.W., Gonzalez, F.J. & Gelboin, H.V. (1996a) Metabolic activation of the potent carcinogen dibenzo[*a,h*]anthracene by cDNA-expressed human cytochromes P450. *Arch. Biochem. Biophys.*, **328**, 201–207

Shou, M., Krausz, K.W., Gonzalez, F.J. & Gelboin, H.V. (1996b) Metabolic activation of the potent carcinogen dibenzo[*a,l*]pyrene by human recombinant cytochromes P450, lung and liver microsomes. *Carcinogenesis*, **17**, 2429–2433

Siegel, D., Gibson, N.W., Preusch, P.C. & Ross, D. (1990) Metabolism of mitomycin C by DT-diaphorase: Role in mitomycin C-induced DNA damage and cytotoxicity in human colon carcinoma cells. *Cancer Res.*, **50**, 7483–7489

Siegfried, J.M., Rudo, K., Bryant, B.J., Ellis, S., Mass, M.J. & Nesnow, S. (1986) Metabolism of benzo(a)pyrene in monolayer cultures of human bronchial epithelial cells from a series of donors. *Cancer Res.*, **46**, 4368–4371

Sigman, M.E., Schuler, P.F., Ghosh, M.M. & Dabestani, R.T. (1998) Mechanism of pyrene photochemical oxidation in aqueous and surfactant solutions. *Environ. Sci. Technol.*, **32**, 3980–3985

Silberstein, G.B. (2001) Tumour–stromal interactions. Role of the stroma in mammary development. *Breast Cancer Res.*, **3**, 218–223

Simioli, P., Lupi, S., Gregorio, P., Siwinska, E., Mielzynska, D., Clonfero, E. & Pavanello, S. (2004) Non-smoking coke oven workers show an occupational PAH exposure-related increase in urinary mutagens. *Mutat. Res.*, **562**, 103–110

Sims, P. (1970) Studies on the metabolism of 7-methylbenz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene and its hydroxymethyl derivatives in rat liver and adrenal homogenates. *Biochem. Pharmacol.*, **19**, 2261–2275

Sims, P., Grover, P.L., Swaisland, A., Pal, K. & Hewer, A. (1974) Metabolic activation of benzo[*a*]pyrene proceeds by a diol epoxide. *Nature*, **252**, 326–328

Sinha, B.K. & Chignell, C.F. (1983) Binding of anthracene to cellular macromolecules in the presence of light. *Photochem. Photobiol.*, **37**, 33–37

Slaga, T.J., Viaje, A., Berry, D.L., Bracken, W., Buty, S.G. & Scribner, J.D. (1976) Skin tumor initiating ability of benzo(a)pyrene 4,5- 7,8- and 7,8-diol-9,10-epoxides and 7,8-diol. *Cancer Lett.*, **2**, 115–121

Slaga, T.J., Thompson, S., Berry, D.L., DiGiovanni, J., Juchau, M.R. & Viaje, A. (1977a) The effects of benzoflavones on polycyclic hydrocarbon metabolism and skin tumor initiation. *Chem.-biol. Interact.*, **17**, 297–312

Slaga, T.J., Bracken, W.M., Viaje, A., Levin, W., Yagi, H., Jerina, D.M. & Conney, A.H. (1977b) Comparison of the tumor-initiating activities of benzo[*a*]pyrene arene oxides and diol-epoxides. *Cancer Res.*, **37**, 4130–4133

Slaga, T.J., Huberman, E., Selkirk, J.K., Harvey, R.G. & Bracken, W.M. (1978a) Carcinogenicity and mutagenicity of benz(*a*)anthracene diols and diol-epoxides. *Cancer Res*., **38**, 1699–1704

Slaga, T.J., Bracken, W.M., Dresner, S., Levin, W., Yagi, H., Jerina, D.M. & Conney, A.H. (1978b) Skin tumor-initiating activities of the twelve isomeric phenols of benzo(a)pyrene. *Cancer Res*., **38**, 678–681

Slaga, T.J., Bracken, W.M., Viaje, A., Berry, D.L., Fischer, S.M. & Miller, D.R. (1978c) Lack of involvement of 6-hydroxymethylation in benzo[*a*]pyrene skin tumor initiation in mice. *J. natl Cancer Inst.*, **61**, 451–455

Slaga, T.J., Bracken, W.J., Gleason, G., Levin, W., Yagi, H., Jerina, D.M. & Conney, A.H. (1979) Marked differences in the skin tumor-initiating activities of the optical enantiomers of the diastereomeric benzo(a)pyrene 7,8-diol-9,10-epoxides. *Cancer Res*., **39**, 67–71

Slaga, T.J., Gleason, G.L., Mills, G., Ewald, L., Fu, P.P., Lee, H.M. & Harvey, R.G. (1980) Comparison of the skin tumor-initiating activities of dihydrodiols and diol-epoxides of various polycyclic aromatic hydrocarbons. *Cancer Res*., **40**, 1981–1984

Slikker, W., III, Mei, N. & Chen, T. (2004) N-Ethyl-N-nitrosourea (ENU) increased brain mutations in prenatal and neonatal mice but not in the adults. *Toxicol. Sci*., **81**, 112–120

Smith, L.E., Denissenko, M.F., Bennett, W.P., Li, H., Amin, S., Tang, M. & Pfeifer, G.P. (2000) Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. *J. natl Cancer Inst*., **92**, 803–811

Smith, W.A., Arif, J.M. & Gupta, R.C. (2001) 1,2-Dithiole-3-thione and its structural analogue oltipraz are potent inhibitors of dibenz[*a,l*]pyrene-DNA adduction in female Sprague-dawley rats. *Int. J. Cancer*, **91**, 132–136

Smithgall, T.E., Harvey, R.G. & Penning, T.M. (1986) Regio- and stereospecificity of homogeneous 3α-hydroxysteroid-dihydrodiol dehydrogenase for *trans*-dihydrodiol metabolites of polycyclic aromatic hydrocarbons. *J. biol. Chem*., **261**, 6184–6191

Smithgall, T.E., Harvey, R.G. & Penning, T.M. (1988a) Spectroscopic identification of *ortho*-quinones as the products of polycyclic aromatic *trans*-dihydrodiol oxidation catalyzed by dihydrodiol dehydrogenase. A potential route of proximate carcinogen metabolism. *J. biol. Chem*., **263**, 1814–1820

Smithgall, T.E., Harvey, R.G. & Penning, T.M. (1988b) Oxidation of the trans-3,4-dihydrodiol metabolites of the potent carcinogen 7,12-dimethylbenz(*a*)anthracene and other benz(*a*)anthracene derivatives by 3α-hydroxysteroid-dihydrodiol dehydrogenase: Effects of methyl substitution on velocity and stereochemical course of *trans*-dihydrodiol oxidation. *Cancer Res*., **48**, 1227–1232

Sogawa, K., Numayama-Tsuruta, K., Takahashi, T., Matsushita, N., Miura, C., Nikawa, J., Gotoh, O., Kikuchi, Y. & Fujii-Kuriyama, Y. (2004) A novel induction mechanism of the rat *CYP1A2* gene mediated by Ah receptor-Arnt heterodimer. *Biochem. biophys. Res. Commun*., **318**, 746–755

Solhaug, A., Refsnes, M., Lag, M., Schwarze, P.E., Husoy, T. & Holme, J.A. (2004a) Polycyclic aromatic hydrocarbons induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells. *Carcinogenesis*, **25**, 809–819

Solhaug, A., Refsnes, M. & Holme, J.A. (2004b) Role of cell signalling involved in induction of apoptosis by benzo[a]pyrene and cyclopenta[c,d]pyrene in Hepa1c1c7 cells. *J. cell. Biochem*., **93**, 1143–1154

Solhaug, A., Øvrebø, S., Mollerup, S., Lag, M., Schwarze, P.E., Nesnow, S. & Holme, J.A. (2005) Role of cell signaling in B[*a*]P-induced apoptosis: Characterization of unspecific effects of cell signaling inhibitors and apoptotic effects of B[*a*]P metabolites. *Chem. biol. Interact.*, **151**, 101–119

Sørensen, M., Autrup, H., Tjønneland, A., Overvad, K. & Raaschou-Nielsen, O. (2005) Genetic polymorphisms in *CYP1B1*, *GSTA1*, *NQO1* and *NAT2* and the risk of lung cancer. *Cancer Lett.*, **221**, 185–190

Spencer, D.L., Masten, S.A., Lanier, K.M., Yang, X., Grassman, J.A., Miller, C.R., Sutter, T.R., Lucier, G.W. & Walker, N.J. (1999) Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 139–146

Spink, D.C., Spink, B.C., Cao, J.Q., Gierthy, J.F., Hayes, C.L., Li, Y. & Sutter, T.R. (1997) Induction of cytochrome P450 1B1 and catechol estrogen metabolism in ACHN human renal adenocarcinoma cells. *J. steroid Biochem. mol. Biol.*, **62**, 223–232

Spink, D.C., Spink, B.C., Cao, J.Q., DePasquale, J.A., Pentecost, B.T., Fasco, M.J., Li, Y. & Sutter, T.R. (1998) Differential expression of CYP1A1 and CYP1B1 in human breast epithelial cells and breast tumor cells. *Carcinogenesis*, **19**, 291–298

Spurdle, A.B., Webb, P.M., Purdie, D.M., Chen, X., Green, A. & Chenevix-Trench, G. (2001) Polymorphisms at the glutathione S-transferase GSTM1, GSTT1 and GSTP1 loci: Risk of ovarian cancer by histological subtype. *Carcinogenesis*, **22**, 67–72

Šrám, R.J., Binková, B., Rössner, P., Rubeš, J., Topinka, J. & Dejmek, J. (1999) Adverse reproductive outcomes from exposure to environmental mutagens. *Mutat. Res.*, **428**, 203–215

Šrám, R.J., Binková, B., Dejmek, J. & Bobak, M. (2005) Ambient air pollution and pregnancy outcomes: A review of the literature. *Environ. Health Perspect.*, **113**, 375–382

Sridhar, G.R., Murty, V.S., Lee, S.H., Blair, I.A. & Penning, T.M. (2001) Amino acid adducts of PAH *o*-quinones: Model studies with naphthalene-1,2-dione. *Tetrahedron*, **57**, 407–412

Srivastava, S.K., Watkins, S.C., Schuetz, E. & Singh, S.V. (2002) Role of glutathione conjugate efflux in cellular protection against benzo[*a*]pyrene-7,8-diol-9,10-epoxide-induced DNA damage. *Mol. Carcinog.*, **33**, 156–162

Stack, D.E., Cremonesi, P., Hanson, A., Rogan, E.G. & Cavalieri, E.L. (1995) Radical cations of benzo[*a*]pyrene and 6-substituted derivatives: Reaction with nucleophiles and DNA. *Xenobiotica*, **25**, 755–760

Stansbury, K.H., Flesher, J.W. & Gupta, R.C. (1994) Mechanism of aralkyl–DNA adduct formation from benzo[*a*]pyrene in vivo. *Chem. Res. Toxicol.*, **7**, 254–259

Steckelbroeck, S., Jin, Y., Gopishetty, S., Oyesanmi, B. & Penning, T.M. (2004) Human cytosolic 3α-hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3β-hydroxysteroid dehydrogenase activity: Implications for steroid hormone metabolism and action. *J. biol. Chem.*, **279**, 10784–10795

Stern, R.S., Zierler, S. & Parrish, J.A. (1980) Skin carcinoma in patients with psoriasis treated with topical tar and artificial ultraviolet radiation. *Lancet*, **i**, 732–735

Stoilov, I., Akarsu, A.N. & Sarfarazi, M. (1997) Identification of three different truncating mutations in cytochrome P4501B1 (*CYP1B1*) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. *Hum. mol. Genet.*, **6**, 641–647

Stoilov, I., Akarsu, A.N., Alozie, I., Child, A., Barsoum-Homsy, M., Turacli, M.E., Or, M., Lewis, R.A., Ozdemir, N., Brice, G., Aktan, S.G., Chevrette, L., Coca-Prados, M. & Sarfarazi, M. (1998) Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *Am. J. hum. Genet*., **62**, 573–584

Strange, R.C., Spiteri, M.A., Ramachandran, S. & Fryer, A.A. (2001) Glutathione-*S*-transferase family of enzymes. *Mutat. Res*., **482**, 21–26

Sulentic, C.E., Holsapple, M.P. & Kaminski, N.E. (2000) Putative link between transcriptional regulation of IgM expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin and the aryl hydrocarbon receptor/dioxin-responsive enhancer signaling pathway. *J. Pharmacol. exp. Ther*., **295**, 705–716

Sullivan-Klose, T.H., Ghanayem, B.I., Bell, D.A., Zhang, Z.-Y., Kaminsky, L.S., Shenfield, G.M., Miners, J.O., Birkett, D.J. & Goldstein, J.A. (1996) The role of the *CYP2C9*-Leu^{359} allelic variant in the tolbutamide polymorphism. *Pharmacogenetics*, **6**, 341–349

Sun, J.D., Wolff, R.K. & Kanapilly, G.M. (1982) Deposition, retention, and biological fate of inhaled benzo(a)pyrene adsorbed onto ultrafine particles and as a pure aerosol. *Toxicol. appl. Pharmacol*., **65**, 231–244

Sun, J.D., Wolff, R.K., Kanapilly, G.M. & McClellan, R.O. (1984) Lung retention and metabolic fate of inhaled benzo(*a*)pyrene associated with diesel exhaust particles. *Toxicol. appl. Pharmacol.*, **73**, 48–59

Sundberg, K., Johannsoon, A.-S., Stenberg, G., Widersten, M., Siedel, A., Mannervik, B. & Jernström, B. (1998) Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol-epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis*, **19**, 433–436

Sundberg, K., Dreij, K., Seidel, A. & Jernstrom, B. (2002) Glutathione conjugation and DNA adduct formation of dibenzo[*a,l*]pyrene and benzo[*a*]pyrene diol epoxides in V79 cells stably expressing different human glutathione transferases. *Chem. Res. Toxicol*., **15**, 170-179

Surh, Y.-J. & Tannenbaum, S.R. (1995) Sulfotransferase-mediated activation of 7,8,9,10-tetrahydro-7-ol, 7,8-dihydrodiol, and 7,8,9,10-tetraol derivatives of benzo[*a*]pyrene. *Chem. Res. Toxicol*., **8**, 693–698

Surh, Y.-J., Lai, C.-C., Miller, J.A. & Miller, E.C. (1987) Hepatic DNA and RNA adduct formation from the carcinogen 7-hydroxymethyl-12-methylbenz[a]anthracene and its electrophilic sulfuric acid ester metabolite in preweanling rats and mice. *Biochem. biophys. Res. Commun*., **144** , 576–582

Surh, Y.-J., Liem, A., Miller, E.C. & Miller, J.A. (1990) The strong hepatocarcinogenicity of the electrophilic and mutagenic metabolite 6-sulfooxymethylbenzo[*a*]pyrene and its formation of benzylic DNA adducts in the livers of infant male B6C3F$_1$ mice. *Biochem. biophys. Res. Commun.*, **172**, 85–91

Surh, Y.-J., Liem, A., Miller, E.C. & Miller, J.A. (1991) 7-Sulfooxymethyl-12-methylbenz[*a*]anthracene is an electrophilic mutagen, but does not appear to play a role in carcinogenesis by 7,12-dimethylbenz[*a*]anthracene or 7-hydroxymethyl-12-methylbenz[*a*]anthracene. *Carcinogenesis*, **12**, 339–347

Surh, Y.-J., Kwon, H. & Tannenbaum, S.R. (1993) Sulfotransferase-mediated activation of 4-hydroxy- and 3,4-dihydroxy-3,4-dihydrocyclopenta[*c,d*]pyrene, major metabolites of cyclopenta[*c,d*]pyrene. *Cancer Res*., **53**, 1017–1022

Sutter, T.R., Guzman, K., Dold, K.M. & Greenlee, W.F. (1991) Targets for dioxin: Genes for plasminogen activator inhibitor-2 and interleukin-1 beta. *Science*, **254**, 415–418

Sutter, T.R., Tang, Y.M., Hayes, C.L., Wo, Y.-Y., Jabs, E.W., Li, X., Yin, H., Cody, C.W. & Greenlee, W.F. (1994) Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J. biol. Chem.*, **269**, 13092–13099

Swartz, R.C., Ferraro, S.P., Lamberson, J.O., Cole, F.A., Ozretich, R.J., Boese, B.L., Schults, D.W., Behrenfeld, M. & Ankley, G.T. (1997) Photoactivation and toxicity of mixtures of polycyclic aromatic hydrocarbon compounds in marine sediment. *Environ. Toxicol. Chem.*, **16**, 2151–2157

Szczeklik, A., Szczeklik, J., Galuszka, Z., Musial, J., Kolarzyk, E. & Targosz, D. (1994) Humoral immunosuppression in men exposed to polycyclic aromatic hydrocarbons and related carcinogens in polluted environments. *Environ. Health Perspect.*, **102**, 302–304

Szeliga, J., Lee, H., Harvey, R.G., Page, J.E., Ross, H.L., Routledge, M.N., Hilton, B.D. & Dipple, A. (1994) Reaction with DNA and mutagenic specificity of syn-benzo[*g*]chrysene 11,12-dihydrodiol 13,14-epoxide. *Chem. Res. Toxicol.*, **7**, 420–427

Szeliga, J., Page, J.E., Hilton, B.D., Kiselyov, A.S., Harvey, R.G., Dunayevskiy, Y.M., Vouros, P. & Dipple, A. (1995) Characterization of DNA adducts formed by anti-benzo[*g*]chrysene 11,12-dihydrodiol 13,14-epoxide. *Chem. Res. Toxicol.*, **8**, 1014–1019

Szeliga, J., Amin, S., Zhang, F.-J. & Harvey, R.G. (1999) Reactions of dihydrodiol epoxides of 5-methylchrysene and 5,6-dimethylchrysene with DNA and deoxyribonucleotides. *Chem. Res. Toxicol.*, **12**, 347–352

Taioli, E., Crofts, F., Trachman, J., Demopoulos, R., Toniolo, P. & Garte, S.J. (1995a) A specific African–American CYP1A1 polymorphism is associated with adenocarcinoma of the lung. *Cancer Res.*, **55**, 472–473

Taioli, E., Trachman, J., Chen, X., Toniolo, P. & Garte, S.J. (1995b) A CYP1A1 restriction fragment length polymorphism is associated with breast cancer in African–American women. *Cancer Res.*, **55** , 3757–3758

Takahashi, H., Ieiri, I., Wilkinson, G.R., Mayo, G., Kashima, T., Kimura, S., Otsubo, K. & Echizen, H. (2004) 5'-Flanking region polymorphisms of *CYP2C9* and their relationship to S-warfarin metabolism in white and Japanese patients. *Blood*, **103**, 3055–3057

Tang, Y.M., Wo, Y.-Y.P., Stewart, J., Hawkins, A.L., Griffin, C.A., Sutter, T.R. & Greenlee, W.F. (1996) Isolation and characterization of the human cytochrome P450 *CYP1B1* gene. *J. Biol. Chem.*, **271**, 28324–28330

Tang, M.-S., Zheng, J.B., Denissenko, M.F., Pfeifer, G.P. & Zheng, Y. (1999) Use of UvrABC nuclease to quantify benzo[*a*]pyrene diol epoxide–DNA adduct formation at methylated versus unmethylated CpG sites in the p53 gene. *Carcinogenesis*, **20**, 1085–1089

Tannheimer, S.L., Barton, S.L., Ethier, S.P. & Burchiel, S.W. (1997) Carcinogenic polycyclic aromatic hydrocarbons increase intracellular Ca^{2+} and cell proliferation in primary human mammary epithelial cells. *Carcinogenesis*, **18**, 1177–1182

Tannheimer, S.L., Ethier, S.P., Caldwell, K.K. & Burchiel, S.W. (1998) Benzo[*a*]pyrene- and TCDD-induced alterations in tyrosine phosphorylation and insulin-like growth factor signaling pathways in the MCF-10A human mammary epithelial cell line. *Carcinogenesis*, **19**, 1291–1297

Tannheimer, S.L., Lauer, F.T., Lane, J. & Burchiel, S.W. (1999) Factors influencing elevation of intracellular Ca^{2+} in the MCF-10A human mammary epithelial cell line by carcinogenic polycyclic aromatic hydrocarbons. *Mol. Carcinog.*, **25**, 48–54

Tapiainen, T., Järvinen, K., Pääkkö, P., Bjelogrlic, N. & Vähäkangas, K. (1996) TPA decreases the p53 response to benzo[*a*]pyrene–DNA adducts in vivo in mouse skin. *Carcinogenesis*, **17**, 1377–1380

Tefre, T., Ryberg, D., Haugen, A., Nebert, D.W., Skaug, V., Brogger, A. & Borresen, A.-L. (1991) Human CYP1A1 (cytochrome P_1450) gene: Lack of association between the *Msp* I restriction fragment length polymorphism and incidence of lung cancer in a Norwegian population. *Pharmacogenetics*, **1**, 20–25

Temchura, V.V., Frericks, M., Nacken, W. & Esser, C. (2005) Role of the aryl hydrocarbon receptor in thymocyte emigration in vivo. *Eur. J. Immunol.*, **35**, 2738–2747

Thakker, D.R., Yagi, H., Lu, A.Y.H., Levin, W. & Conney, A.H. (1976) Metabolism of benzo[*a*]pyrene: Conversion of (+/-)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to highly mutagenic 7,8-diol-9,10-epoxides. *Proc. natl Acad. Sci. USA*, **73**, 3381–3385

Thakker, D.R., Yagi, H., Akagi, H., Koreeda, M., Lu, A.Y.H., Levin, W., Wood, A.W., Conney, A.H. & Jerina, D.M. (1977a) Metabolism of benzo[a]pyrene. VI. Stereoselective metabolism of benzo[a]pyrene and benzo[a]pyrene 7,8-dihydrodiol to diol epoxides. *Chem.-biol. Interact.*, **16**, 281–300

Thakker, D.R., Yagi, H., Levin, W., Lu, A.Y.H., Conney, A.H.Y. & Jerina, D.M. (1977b) Stereospecificity of microsomal and purified epoxide hydrase from rat liver. Hydration of arene oxides of polycyclic hydrocarbons. *J. biol. Chem.*, **252**, 6328–6334

Thakker, D.R., Levin, W., Yagi, H., Ryan, D., Thomas, P.E., Karle, J.M., Lehr, R.E., Jerina, D.M. & Conney, A.H. (1979a) Metabolism of benzo[a]anthracene to its tumorigenic 3,4-dihydrodiol. *Mol. Pharmacol.*, **15**, 138–153

Thakker, D.R., Levin, W., Yagi, H., Turujman, S., Kapadia, D., Conney, A.H. & Jerina, D.M. (1979b) Absolute stereochemistry of the trans-dihydrodiols formed from benzo[*a*]anthracene by liver microsomes. *Chem.-biol. Interact.*, **27**, 145–161

Thakker, D.R., Levin, W., Yagi, H., Tada, M., Ryan, D.E., Thomas, P.E., Conney, A.H. & Jerina, D.M. (1982) Stereoselective metabolism of the (+)- and (–)-enantiomers of *trans*-3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene by rat liver microsomes and by a purified and reconstituted cytochrome P-450 system. *J. biol. Chem.*, **257**, 5103–5110

Thastrup, O., Cullen, P.J., Drobak, B.K., Hanley, M.R. & Dawson, A.P. (1990) Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum $Ca2^{+}$-ATPase. *Proc. natl Acad. Sci. USA*, **87**, 2466–2470

Thein, N., Moller, P., Amtoft, H., Vogel, U., Korsholm, B., Autrup, H.P. & Wallin, H. (2000) A strong genotoxic effect in mouse skin of a single painting of coal tar in hairless mice and in MutaMouse. *Mutat. Res.*, **468**, 117–124

Thomas, R.S., Penn, S.G., Holden, K., Bradfield, C.A. & Rank, D.R. (2002) Sequence variation and phylogenetic history of the mouse Ahr gene. *Pharmacogenetics*, **12**, 151–163

Thurmond, L.M., Lauer, L.D., House, R.V., Cook, J.C. & Dean, J.H. (1987) Immunosuppression following exposure to 7,12-dimethylbenz[a]anthracene (DMBA) in Ah-responsive and Ah-nonresponsive mice. *Toxicol. appl. Pharmacol.*, **91**, 450–460

Thurmond, L.M., House, R.V., Lauer, L.D. & Dean, J.H. (1988) Suppression of splenic lymphocyte function by 7,12-dimethylbenz[a]anthracene (DMBA) in vitro. *Toxicol. appl. Pharmacol*., **93**, 369–377

Thurmond, T.S., Staples, J.E., Silverstone, A.E. & Gasiewicz, T.A. (2000) The aryl hydrocarbon receptor has a role in the in vivo maturation of murine bone marrow B lymphocytes and their response to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. appl. Pharmacol*., **165**, 227–236

Tian, Y., Ke, S., Denison, M.S., Rabson, A.B. & Gallo, M.A. (1999) Ah receptor and NF-kappaB interactions, a potential mechanism for dioxin toxicity. *J. biol. Chem*., **274**, 510–515

Tian, Y., Rabson, A.B. & Gallo, M.A. (2002) Ah receptor and NF-kappaB interactions: Mechanisms and physiological implications. *Chem.-biol. Interact*., **141**, 97–115

Tiemersma, E.W., Bunschoten, A., Kok, F.J., Glatt, H., de Boer, S.Y. & Kampman, E. (2004) Effect of SULT1A1 and NAT2 genetic polymorphism on the association between cigarette smoking and colorectal adenomas. *Int. J. Cancer*, **108**, 97–103

Todorovic, R., Devanesan, P., Rogan, E. & Cavalieri, E. (2005) Identification and quantification of stable DNA adducts formed from dibenzo[*a,l*]pyrene or its metabolites *in vitro*, and in mouse skin and rat mammary gland. *Chem. Res. Toxicol*., **18**, 984–990

Toide, K., Yamazaki, H., Nagashima, R., Itoh, K., Iwano, S., Takahashi, Y., Watanabe, S. & Kamataki, T. (2003) Aryl hydrocarbon hydroxylase represents CYP1B1, and not CYP1A1, in human freshly isolated white cells: Trimodal distribution of Japanese population according to induction of CYP1B1 mRNA by environmental dioxins. *Cancer Epidemiol. Biomarkers Prev*., **12**, 219–222

Tolbert, L.M., Khanna, R.K., Popp, A.E., Gelbaum, L. & Bottomley, L.A. (1990) Sterioelectronic effects in the deprotonation of arylalkylradical cations: *Meso*-ethylanthracenes. *J. Am. chem. Soc*., **112**, 2373–2378

Tonks, N.K. (2005) Redox redux: Revisiting PTPs and the control of cell signaling. *Cell*, **121**, 667–670

Tornaletti, S. & Pfeifer, G.P. (1995) Complete and tissue-independent methylation of CpG sites in the p53 gene: Implications for mutations in human cancers. *Oncogene*, **10**, 1493–1499

Tsai, K.S., Yang, R.S. & Liu, S.H. (2004) Benzo[*a*]pyrene regulates osteoblast proliferation through an estrogen receptor-related cyclooxygenase-2 pathway. *Chem. Res. Toxicol*., **17**, 679–684

Tsuruda, L., Hou, Y. & Penning, T.M. (2001) Stable expression of rat dihydrodiol dehydrogenase (AKR1C9) in human breast MCF-7 cells results in the formation of PAH-*o*-quinones and enzyme mediated cell death. *Chem. Res. Toxicol*., **14**, 856–862

Tukey, R.H. & Strassburg, C.P. (2000) Human UDP-glucuronosyltransferases: Metabolism, expression, and disease. *Annu. Rev. Pharmacol. Toxicol*., **40**, 581–616

Tuominen, R., Baranczewski, P., Warholm, M., Hagmar, L., Moller, L. & Rannug, A. (2002) Susceptibility factors and DNA adducts in peripheral blood mononuclear cells of aluminium smelter workers exposed to polycyclic aromatic hydrocarbons. *Arch. Toxicol*., **76**, 178–186

Tuveson, R.W., Wang, G.R., Wang, T.P. & Kagan, J. (1990) Light-dependent cytotoxic reactions of anthracene. *Photochem. Photobiol*., **52**, 993–1002

Uno, S., Dalton, T.P., Derkenne, S., Curran, C.P., Miller, M.L., Shertzer, H.G. & Nebert, D.W. (2004) Oral exposure to benzo[a]pyrene in the mouse: Detoxication by inducible cytochrome P450 is more important than metabolic activation. *Mol. Pharmacol*., **65**, 1225–1237

Upham, B.L., Masten, S.J., Lockwood, B.R. & Trosko, J.E. (1994) Nongenotoxic effects of polycyclic aromatic hydrocarbons and their ozonation by-products on the intercellular communication of rat liver epithelial cells. *Fundam. appl. Toxicol*., **23**, 470–475

Vähäkangas, K., Haugen, A. & Harris, C.C. (1985) An applied synchronous fluorescence spectrophotometric assay to study benzo[*a*]pyrene-diolepoxide–DNA adducts. *Carcinogenesis*, **6**, 1109–1115

Vähäkangas, K.H., Bennett, W.P., Castren, K., Welsh, J.A., Khan, M.A., Blömeke, B., Alavanja, M.C. & Harris, C.C. (2001) *p53* and K-*ras* Mutations in lung cancers from former and never-smoking women. *Cancer Res*., **61**, 4350–4356

Vanden Heuvel, J.P., Clark, G.C., Kohn, M.C., Tritscher, A.M., Greenlee, W.F., Lucier, G.W. & Bell, D.A. (1994) Dioxin-responsive genes: Examination of dose–response relationships using quantitative reverse transcriptase–polymerase chain reaction. *Cancer Res*., **54**, 62–68

Vaziri, C., Schneider, A., Sherr, D.H. & Faller, D.V. (1996) Expression of the aryl hydrocarbon receptor is regulated by serum and mitogenic growth factors in murine 3T3 fibroblasts. *J. biol. Chem*., **271**, 25921–25927

Vecchi, A., Sironi, M., Canegrati, M.A., Recchia, M. & Garattini, S. (1983) Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. *Toxicol. appl. Pharmacol*., **68**, 434–441

Vesselinovitch, S.D., Kyriazis, A.P., Mihailovich, N. & Rao, K.V. (1975) Conditions modifying development of tumors in mice at various sites by benzo(a)pyrene. *Cancer Res*., **35**, 2948–2953

Viaje, A., Lu, J.-Y., Hopkins, N.E., Nettikumara, A.N., DiGiovanni, J., Alworth, W.L. & Slaga, T.J. (1990) Inhibition of the binding of 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene to DNA in mouse skin epidermis by 1-ethynylpyrene. *Carcinogenesis*, **11**, 1139–1143

Vialaton, D., Richard, C., Baglio, D. & Paya-Perez, A.-B. (1999) Mechanism of the photochemical transformation of naphthalene in water. *J. Photochem. Photobiol.*, **A123**, 15–19

Vigny, P., Ginot, Y.M., Kindts, M., Cooper, C.S., Grover, P.L. & Sims, P. (1980) Fluorescence spectral evidence that benzo[a]pyrene is activated by metabolism in mouse skin to a diol-epoxide and a phenol-epoxide. *Carcinogenesis*, **1**, 945–950

Vogel, A., Ockenga, J., Ehmer, U., Barut, A., Kramer, F.-J., Tukey, R.H., Manns, M.P. & Strassburg, C.P. (2002) Polymorphisms of the carcinogen detoxifying UDP-glucuronosyltransferase UGT1A7 in proximal digestive tract cancer. *Z. Gastroenterol*., **40**, 497–502

Vogelstein, B. & Kinzler, K.W. (1992) p53 Function and dysfunction. *Cell*, **70**, 523–526

Vogl, F.D., Taioli, E., Maugard, C., Zheng, W., Pinto, L.F., Ambrosone, C., Parl, F.F., Nedelcheva-Kristensen, V., Rebbeck, T.R., Brennan, P. & Boffetta, P. (2004) Glutathione S-transferases M1, T1, and P1 and breast cancer: A pooled analysis. *Cancer Epidemiol. Biomarkers Prev.*, **13**, 1473–1479

Vulimiri, S.V., Baer-Dubowska, W., Harvey, R.G., Zhang, J.-T. & DiGiovanni, J. (1999) Analysis of highly polar DNA adducts formed in SENCAR mouse epidermis following topical application of dibenz[*a,j*]anthracene. *Chem. Res. Toxicol*., **12**, 60–67

Walker, N.J., Gastel, J.A., Costa, L.T., Clark, G.C., Lucier, G.W. & Sutter, T.R. (1995) Rat CYP1B1: An adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. *Carcinogenesis*, **16**, 1319–1327

Walters, M.A. (1966) The induction of lung tumours by the injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) into newborn suckling and young adult mice. A dose response study. *Br. J. Cancer*, **20**, 148–160

Wang, Y., Spitz, M.R., Tsou, A.M., Zhang, K., Makan, N. & Wu, X. (2002) Sulfotransferase (SULT) 1A1 polymorphism as a predisposition factor for lung cancer: A case–control analysis. *Lung Cancer*, **35**, 137–142

Wang, J., Deng, Y., Cheng, J., Ding, J. & Tokudome, S. (2003) GST genetic polymorphisms and lung adenocarcinoma susceptibility in a Chinese population. *Cancer Lett.*, **201**, 185–193

Wang, Y., Gao, D., Atencio, D.P., Perez, E., Saladi, R., Moore, J., Guevara, D., Rosenstein, B.S., Lebwohl, M. & Wei, H. (2005) Combined subcarcinogenic benzo[*a*]pyrene and UVA synergistically caused high tumor incidence and mutations in H-*ras* gene, but not *p53*, in SKH-1 hairless mouse skin. *Int. J. Cancer*, **116**, 193–199

Ward, E.C., Murray, M.J., Lauer, L.D., House, R.V., Irons, R. & Dean, J.H. (1984) Immunosuppression following 7,12-dimethylbenz[a]anthracene exposure in B6C3F1 mice. I. Effects on humoral immunity and host resistance. *Toxicol. appl. Pharmacol*., **75**, 299–308

Ward, E.C., Murray, M.J., Lauer, L.D., House, R.V. & Dean, J.H. (1986) Persistent suppression of humoral and cell-mediated immunity in mice following exposure to the polycyclic aromatic hydrocarbon 7,12-dimethylbenz[a]anthracene. *Int. J. Immunopharmacol*., **8**, 13–22

Watabe, T., Ishizuka, T., Isobe, M. & Ozawa, N. (1982) A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylbenz[alpha]anthracene. *Science*, **215**, 403–405

Watabe, T., Fujieda, T., Hiratsuka, A., Ishizuka, T., Hakamata, Y. & Ogura, K. (1985) The carcinogen, 7-hydroxymethyl-12-methylbenz[*a*]anthracene, is activated and covalently binds to DNA via a sulphate ester. *Biochem. Pharmacol.*, **34**, 3002–3005

Watabe, T., Hakamata, Y., Hiratsuka, A. & Ogura, K. (1986) A 7-hydroxymethyl sulphate ester as an active metabolite of the carcinogen, 7-hydroxymethylbenz[*a*]anthracene. *Carcinogenesis*, **7**, 207–214

Watanabe, M., Maher, V.M. & McCormick, J.J. (1985) Excision repair of UV- or benzo[a]pyrene diol epoxide-induced lesions in xeroderma pigmentosum variant cells is 'error free'. *Mutat. Res*., **146**, 285–294

Watanabe, J., Shimada, T., Gillam, E.M., Ikuta, T., Suemasu, K., Higashi, Y., Gotoh, O. & Kawajiri, K. (2000) Association of *CYP1B1* genetic polymorphism with incidence to breast and lung cancer. *Pharmacogenetics*, **10**, 25–33

Wattenberg, L.W., Jerina, D.M., Lam, L.K.T. & Yagi, H. (1979) Neoplastic effects of oral administration of (+/-)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene and their inhibition by butylated hydroxyanisole. *J. natl Cancer Inst*., **62**, 1103–1106

Wei, S.-J., Chang, R.L., Bhachech, N., Cui, X.X., Merkler, K.A., Wong, C.-Q., Hennig, E., Yagi, H., Jerina, D.M. & Conney, A.H. (1993) Dose-dependent differences in the profile of mutations induced by (+)-7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene in the coding region of the hypoxanthine (guanine) phosphoribosyltransferase gene in Chinese hamster V-79 cells. *Cancer Res*., **53**, 3294–3301

Wei, Q., Gu, J., Cheng, L., Bondy, M.L., Jiang, H., Hong, W.K. & Spitz, M.R. (1996) Benzo(*a*)pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. *Cancer Res*., **56**, 3975–3979

Weinstein, I.B. (1991) Nonmutagenic mechanisms in carcinogenesis: Role of protein kinase C in signal transduction and growth control. *Environ. Health Perspect*., **93**, 175–179

Wells, P.G., Mackenzie, P.I., Chowdhury, J.R., Guillemette, C., Gregory, P.A., Ishii, Y., Hansen, A.J., Kessler, F.K., Kim, P.M., Chowdhury, N.R. & Ritter, J.K. (2004) Glucuronidation and the UDP-glucuronosyltransferases in health and disease. *Drug Metab. Dispos.*, **32**, 281–290

Wen, S., Zhao, J., Sheng, G., Fu, J. & Peng, P. (2002) Photocatalytic reactions of phenanthrene at TiO_2/water interfaces. *Chemosphere*, **46**, 871–877

Weyand, E.H., Rice, J.E. & LaVoie, E.J. (1987) ^{32}P-Postlabeling analysis of DNA adducts from non-alternant PAH using thin-layer and high performance liquid chromatography. *Cancer Lett.*, **37**, 257–266

Weyand, E.H., Geddie, N., Rice, J.E., Czech, A., Amin, S. & LaVoie, E.J. (1988) Metabolism and mutagenic activity of benzo[*k*]fluoranthene and 3-, 8- and 9-fluorobenzo[*k*]fluoranthene. *Carcinogenesis*, **9**, 1277–1281

Weyand, E.H., Wu, Y., Patel, S., Taylor, B.B. & Mauro, D.M. (1991) Urinary excretion and DNA binding of coal tar components in B6C3F1 mice following ingestion. *Chem. Res. Toxicol.*, **4**, 466–473

Weyand, E.H., Bryla, P., Wu, Y., He, Z.-M. & LaVoie, E.J. (1993a) Detection of the major DNA adducts of benzo[*j*]fluoranthene in mouse skin: Nonclassical dihydrodiol epoxides. *Chem. Res. Toxicol.*, **6**, 117–124

Weyand, E.H., Cai, Z.-W., Wu, Y., Rice, J.E., He, Z.-M. & LaVoie, E.J. (1993b) Detection of the major DNA adducts of benzo[*b*]fluoranthene in mouse skin: Role of phenolic dihydrodiols. *Chem. Res. Toxicol.*, **6**, 568–577

Wheeler, L.A., Saperstein, M.D. & Lowe, N.J. (1981) Mutagenicity of urine from psoriatic patients undergoing treatment with coal tar and ultraviolet light. *J. invest. Dermatol.*, **77**, 181–185

White, P.A. (2002) The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. *Mutat. Res.*, **515**, 85–98

White, K.L., Jr & Holsapple, M.P. (1984) Direct suppression of in vitro antibody production by mouse spleen cells by the carcinogen benzo(a)pyrene but not by the noncarcinogenic congener benzo(e)pyrene. *Cancer Res.*, **44**, 3388–3393

White, K.L., Jr, Lysy, H.H. & Holsapple, M.P. (1985) Immunosuppression by polycyclic aromatic hydrocarbons: A structure–activity relationship in B6C3F1 and DBA/2 mice. *Immunopharmacology*, **9**, 155–164

Whitlock, J.P., Jr (1999) Induction of cytochrome P4501A1. *Annu. Rev. Pharmacol. Toxicol.*, **39**, 103–125

Whitlock, J.P., Jr, Cooper, H.L. & Gelboin, V.H. (1972) Aryl hydrocarbon (benzopyrene) hydroxylase is stimulated in human lymphocytes by mitogens and benz(a)anthracene. *Science*, **177**, 618–619

Whong, W.-Z., Stewart, J.D., Cutler, D. & Ong, T. (1994) Induction of in vivo DNA adducts by 4 industrial by-products in the rat-lung-cell system. *Mutat. Res.*, **312**, 165–172

Wilkinson, G.R. (1996) Cytochrome P4503A (CYP3A) metabolism: Prediction of *in vivo* activity in humans. *J. Pharmacokinet. Biopharm.*, **24**, 475–490

Williams, J.A. (2001) Single nucleotide polymorphisms, metabolic activation and environmental carcinogenesis: Why molecular epidemiologists should think about enzyme expression. *Carcinogenesis*, **22**, 209–214

Williams, J.A. & Phillips, D.H. (2000) Mammary expression of xenobiotic metabolizing enzymes and their potential role in breast cancer. *Cancer Res.*, **60**, 4667–4677

Winker, N., Tuschl, H., Kovac, R. & Weber, E. (1997) Immunological investigations in a group of workers exposed to various levels of polycyclic aromatic hydrocarbons. *J. appl. Toxicol.*, **17**, 23–29

Wislocki, P.G., Kapitulnik, J., Levin, W., Lehr, R., Schaefer-Ridder, M., Karle, J.M., Jerina, D.M. & Conney, A.H. (1978a) Exceptional carcinogenic activity of benz[a]anthracene 3,4-dihydrodiol in the newborn mouse and the bay region theory. *Cancer Res*., **38**, 693–696

Wislocki, P.G., Kapitulnik, J., Levin, W. & Conney, A.H. (1978b) Tumorigenicity of benzo[*a*]pyrene 4,5-, 7,8-, 9,10- and 11,12-oxides in newborn mice. *Cancer Lett*., **5**, 191–197

Wislocki, P.G., Buening, M.K., Levin, W., Lehr, R.E., Thakker, D.R., Jerina, D.M. & Conney, A.H. (1979) Tumorigenicity of the diastereomeric benz[a]anthracene 3,4-diol-1,2-epoxides and the (+)- and (–)-enantiomers of benz[a]anthracene 3,4-dihydrodiol in newborn mice. *J. natl Cancer Inst*., **63**, 201–204

Wislocki, P.G., Bagan, E.S., Lu, A.Y., Dooley, K.L., Fu, P.P., Han-Hsu, H., Beland, F.A. & Kadlubar, F.F. (1986) Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. *Carcinogenesis*, **7**, 1317–1322

Withey, J.R., Shedden, J., Law, F.C.P. & Abedini, S. (1993a) Distribution of benzo[a]pyrene in pregnant rats following inhalation exposure and a comparison with similar data obtained with pyrene. *J. appl. Toxicol*., **13**, 193–202

Withey, J.R., Law, F.C.P. & Endrenyi, L. (1993b) Percutaneous uptake, distribution, and excretion of pyrene in rats. *J. Toxicol. environ. Health*, **40**, 601–612

Withey, J.R., Burnett, R., Law, F.C.P., Abedini, S. & Endrenyi, L. (1994) Pharmacokinetics of inhaled pyrene in rats. *J. Toxicol. environ. Health*, **43**, 103–116

Wojdani, A. & Alfred, L.J. (1984) Alterations in cell-mediated immune functions induced in mouse splenic lymphocytes by polycyclic aromatic hydrocarbons. *Cancer Res*., **44**, 942–945

Wojdani, A., Attarzadeh, M., Wolde-Tsadik, G. & Alfred, L.J. (1984) Immunocytotoxicity effects of polycyclic aromatic hydrocarbons on mouse lymphocytes. *Toxicology*, **31**, 181–189

Wolff, M.S., Teitelbaum, S.L., Lioy, P.J., Santella, R.M., Wang, R.Y., Jones, R.L., Caldwell, K.L., Sjödin, A., Turner, W.E., Li, W., Georgopoulos, P. & Berkowitz, G.S. (2005) Exposures among pregnant women near the World Trade Center site on 11 September 2001. *Environ. Health Perspect*., **113**, 739–748

Wood, A.W., Goode, R.L., Chang, R.L., Levin, W., Conney, A.H., Yagi, H., Dansette, P.M. & Jerina, D.M. (1975) Mutagenic and cytotoxic activity of benzol[*a*]pyrene 4,5-, 7,8-, and 9,10-oxides and the six corresponding phenols. *Proc. natl. Acad. Sci. USA*, **72**, 3176–3180

Wood, A.W., Wislocki, P.G., Chang, R.L., Levin, W., Lu, A.Y.H., Yagi, H., Hernandez, O., Jerina, D.M. & Conney, A.H. (1976) Mutagenicity and cytotoxicity of benzo(*a*)pyrene benzo-ring epoxides. *Cancer Res.*, **36**, 3358–3366

Wood, A.W., Levin, W., Chang, R.L., Lehr, R.E., Schaefer-Ridder, M., Karle, J.M., Jerina, D.M. & Conney, A.H. (1977a) Tumorigenicity of five dihydrodiols of benz(a)anthracene on mouse skin: Exceptional activity of benz(a)anthracene 3,4-dihydrodiol. *Proc. natl Acad. Sci. USA*, **74**, 3176–3179

Wood, A.W., Chang, R.L., Levin, W., Lehr, R.E., Schaefer-Ridder, M., Karle, J.M., Jerina, D.M. & Conney, A.H. (1977b) Mutagenicity and cytotoxicity of benz[*a*]anthracene diol epoxides and tetrahydro-epoxides: Exceptional activity of the bay region 1,2-epoxides. *Proc. natl Acad. Sci. USA*, **74**, 2746–2750

Wood, A.W., Levin, W., Lu, A.Y.H., Ryan, D., West, S.B., Yagi, H., Mah, H.D., Jerina, D.M. & Conney, A.H. (1977c) Structural requirements for the metabolic activation of benzo[*a*]pyrene to mutagenic products: Effects of modifications in the 4,5-, 7,8- and 9,10-positions. *Mol. Pharmacol*., **13**, 1116–1125

Wood, A.W., Levin, W., Thomas, P.E., Ryan, D., Karle, J.M., Yagi, H., Jerina, D.M. & Conney, A.H. (1978) Metabolic activation of dibenzo(*a,h*)anthracene and its dihydrodiols to bacterial mutagens. *Cancer Res*., **38**, 1967–1973

Wood, J.L., Barker, C.L. & Grubbs, C.J. (1979) Photooxidation products of 7,12-dimethylbenz[*a*]anthracene. *Chem.-biol. Interact*., **26**, 339–347

Wood, A.W., Chang, R.L., Levin, W., Ryan, D.E., Thomas, P.E., Croisy-Delcey, M., Ittah, Y., Yagi, H., Jerina, D.M. & Conney, A.H. (1980) Mutagenicity of the dihydrodiols and bay-region diol-epoxides of benzo(*c*)phenanthrene in bacterial and mammalian cells. *Cancer Res.*, **40**, 2876–2883

Wood, A.W., Chang, R.L., Levin, W., Ryan, D.E., Thomas, P.E., Lehr, R.E., Kumar, S., Sardella, D.J., Boger, E., Yagi, H., Sayer, J.M., Jerina, D.M. & Conney, A.H. (1981) Mutagenicity of the bay-region diol-epoxides and other benzo-ring derivatives of dibenzo(*a,h*)pyrene and dibenzo(*a,i*)pyrene. *Cancer Res*., **41**, 2589–2597

Wood, A.W., Chang, R.L., Levin, W., Yagi, H., Thakker, D.R., van Bladeren, P.J., Jerina, D.M. & Conney, A.H. (1983) Mutagenicity of the enantiomers of the diastereomeric bay-region benz(a)anthracene 3,4-diol-1,2-epoxides in bacterial and mammalian cells. *Cancer Res*., **43**, 5821–5825

Wormke, M., Stoner, M., Saville, B. & Safe, S. (2000) Crosstalk between estrogen receptor alpha and the aryl hydrocarbon receptor in breast cancer cells involves unidirectional activation of proteasomes. *FEBS Lett*., **478**, 109–112

Wormley, D.D., Ramesh, A. & Hood, D.B. (2004) Environmental contaminant-mixture effects on CNS development, plasticity, and behavior. *Toxicol. appl. Pharmacol*., **197**, 49–65

Wu, M.T., Simpson, C.D., Christiani, D.C. & Hecht, S.S. (2002) Relationship of exposure to coke-oven emissions and urinary metabolites of benzo(a)pyrene and pyrene in coke-oven workers. *Cancer Epidemiol. Biomarkers Prev*., **11**, 311–314

Wu, M.T., Pan, C.H., Huang, Y.L., Tsai, P.J., Chen, C.J. & Wu, T.N. (2003) Urinary excretion of 8-hydroxy-2-deoxyguanosine and 1-hydroxypyrene in coke-oven workers. *Environ. mol. Mutag.*, **42**, 98–105

Xu, L.L., Wain, J.C., Miller, D.P., Thurston, S.W., Su, L., Lynch, T.J. & Christiani, D.C. (2001) The NAD(P)H:quinone oxidoreductase 1 gene polymorphism and lung cancer: Differential susceptibility based on smoking behavior. *Cancer Epidemiol. Biomarkers Prev*., **10**, 303–309

Xu, C., Li, C.Y. & Kong, A.N. (2005) Induction of phase I, II and III drug metabolism/transport by xenobiotics. *Arch. pharm. Res*., **28**, 249–268

Xue, W. & Warshawsky, D. (2005) Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review. *Toxicol. appl. Pharmacol*., **206**, 73–93

Yadav, J.S. & Seth, N. (1998) Effect of polycyclic aromatic hydrocarbons on somatic chromosomes of coal tar workers. *Cytobios*, **93**, 165–173

Yamaguchi, K., Matulka, R.A., Shneider, A.M., Toselli, P., Trombino, A.F., Yang, S., Hafer, L.J., Mann, K.K., Tao, X.J., Tilly, J.L., Near, R.I. & Sherr, D.H. (1997a) Induction of PreB cell apoptosis by 7,12-dimethylbenz[a]anthracene in long-term primary murine bone marrow cultures. *Toxicol. appl. Pharmacol*., **147**, 190–203

Yamaguchi, K., Near, R.I., Matulka, R.A., Shneider, A., Toselli, P., Trombino, A.F. & Sherr, D.H. (1997b) Activation of the aryl hydrocarbon receptor/transcription factor and bone marrow stromal cell-dependent preB cell apoptosis. *J. Immunol*., **158**, 2165–2173

Yamazaki, H. & Shimada, T. (1997) Human liver cytochrome P450 enzymes involved in the 7-hydroxylation of R- and S-warfarin enantiomers. *Biochem. Pharmacol*., **54**, 1195–1203

Yamazaki, H., Gillam, E.M.J., Dong, M.-S., Johnson, W.W., Guengerich, F.P. & Shimada, T. (1997) Reconstitution of recombinant cytochrome P450 2C10(2C9) and comparison with cytochrome P450 3A4 and other forms: Effects of cytochrome P450-P450 and cytochrome P450-b5 interactions. *Arch. Biochem. Biophys*., **342**, 329–337

Yamazaki, H., Inoue, K., Chiba, K., Ozawa, N., Kawai, T., Suzuki, Y., Goldstein, J.A., Guengerich, F.P. & Shimada, T. (1998a) Comparative studies on the catalytic roles of cytochrome P450 2C9 and its Cys- and Leu-variants in the oxidation of warfarin, flurbiprofen, and diclofenac by human liver microsomes. *Biochem. Pharmacol*., **56**, 243–251

Yamazaki, H., Inoue, K. & Shimada, T. (1998b) Roles of two allelic variants (Arg144Cys and Ile359Leu) of cytochrome P4502C9 in the oxidation of tolbutamide and warfarin by human liver microsomes. *Xenobiotica*, **28**, 103–115

Yan, J., Wang, L., Fu, P.P. & Yu, H. (2004) Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. *Mutat. Res*., **557**, 99–108

Yang, S.K. (1988) Stereoselectivity of cytochrome P-450 isozymes and epoxide hydrolase in the metabolism of polycyclic aromatic hydrocarbons. *Biochem. Pharmacol*., **37**, 61–70

Yang, D., Louden, C., Reinhold, D.S., Kohler, S.K., Maher, V.M. & McCormick, J.J. (1992) Malignant transformation of human fibroblast cell strain MSU-1.1 by (±)-7beta,8alpha-dihydroxy-9alpha,10 alpha-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Proc. natl. Acad. Sci. USA*, **89** , 2237–2241

Yin, L., Pu, Y., Liu, T.Y., Tung, Y.H., Chen, K.W. & Lin, P. (2001) Genetic polymorphisms of NAD(P)H quinone oxidoreductase, CYP1A1 and microsomal epoxide hydrolase and lung cancer risk in Nanjing, China. *Lung Cancer*, **33**, 133–141

Yoon, J.-H., Smith, L.E., Feng, Z., Tang, M.-S., Lee, C.-S. & Pfeifer, G.P. (2001) Methylated CpG dinucleotides are the preferential targets for G-to-T transversion mutations induced by benzo[*a*]pyrene diol epoxide in mammalian cells: Similarities with the p53 mutation spectrum in smoking-associated lung cancers. *Cancer Res.*, **61**, 7110–7117

Yoon, J.-H., Besaratinia, A., Feng, Z., Tang, M.-S., Amin, S., Luch, A. & Pfeifer, G.P. (2004) DNA damage, repair, and mutation induction by (+)-*syn* and (–)-*anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-epoxides in mouse cells. *Cancer Res*., **64**, 7321–7328

Yoshii, S., Tanaka, M., Otsuki, Y., Fujiyama, T., Kataoka, H., Arai, H., Hanai, H. & Sugimura, H. (2001) Involvement of alpha-PAK-interacting exchange factor in the PAK1-c-Jun NH_2-terminal kinase 1 activation and apoptosis induced by benzo[*a*]pyrene. *Mol. cell. Biol*., **21**, 6796–6807

You, L., Wang, D., Galati, A.J., Ross, J.A., Mass, M.J., Nelson, G.B., Wilson, K.H., Amin, S., Stoner, J.C., Nesnow, S. & Stoner, G.D. (1994) Tumor multiplicity, DNA adducts and K-*ras* mutation pattern of 5-methylchrysene in strain A/J mouse lung. *Carcinogenesis*, **15**, 2613–2618

Yu, H. (2002) Environmental carcinogenic polycyclic aromatic hydrocarbons: Photochemistry and phototoxicity. *J. environ. Sci. Health C environ. Carcinog. Ecotoxic. Rev*., **20**, 149–183

Yu, D., Berlin, J.A., Penning, T.M. & Field, J. (2002a) Reactive oxygen species generated by PAH *o*-quinones cause change-in-function mutations in *p53*. *Chem. Res. Toxicol*., **15**, 832–842

Yu, D., Penning, T.M. & Field, J.M. (2002b) Benzo[*a*]pyrene-7,8-dione is more mutagenic than *anti*-BPDE on *p53* and is dependent upon the generation of ROS. *Polycyc. arom. Comp.*, **22**, 881–891

Yuan, W., Kiselyov, A.S., Harvey, R.G. & Carothers, A.M. (1995) Mutagenic specificity of *syn*-benzo[g]chrysene 11,12-dihydrodiol 13,14-epoxide in the dihydrofolate reductase gene of Chinese hamster ovary cells. *Carcinogenesis*, **16**, 2869–2873

Yun, C.-H., Shimada, T. & Guengerich, F.P. (1992) Roles of human liver cytochrome P4502C and 3A enzymes in the 3-hydroxylation of benzo(a)pyrene. *Cancer Res*., **52**, 1868–1874

Zaher, H., Fernandez-Salguero, P.M., Letterio, J., Sheikh, M.S., Fornace, A.J., Jr, Roberts, A.B. & Gonzalez, F.J. (1998) The involvement of aryl hydrocarbon receptor in the activation of transforming growth factor-beta and apoptosis. *Mol. Pharmacol*., **54**, 313–321

Zanesi, N., Mognato, M., Pizzato, M., Viezzer, C., Ferri, G. & Celotti, L. (1998) Determination of HPRT mutant frequency and molecular analysis of T-lymphocyte mutants derived from coke-oven workers. *Mutat. Res.*, **412**, 177–186

Zeng, K., Hwang, H.M., Fu, P.P. & Yu, H. (2002) Identification of 1-hydroxypyrene photoproducts and study of the effect by humic substances on its photolysis. *Polycyc. arom. Comp*., **22**, 459–467

Zeng, K., Hwang, H.M., Dong, S., Shi, X., Wilson, K., Green, J., Jiao, Y. & Yu, H. (2004) Photochemical transformation and phototoxicity of 1-aminopyrene. *Environ. Toxicol. Chem.*, **23**, 1400–1407

Zhang, L., Connor, E.E., Chegini, N. & Shiverick, K.T. (1995) Modulation by benzo[*a*]pyrene of epidermal growth factor receptors, cell proliferation, and secretion of human chorionic gonadotropin in human placental cell lines. *Biochem. Pharmacol.*, **50**, 1171–1180

Zhang, Z.Y., Fasco, M.J., Huang, L., Guengerich, F.P. & Kaminsky, L.S. (1996) Characterization of purified human recombinant cytochrome P4501A1-Ile^{462} and -Val^{462}: Assessment of a role for the rare allele in carcinogenesis. *Cancer Res*., **56**, 3926–3933

Zhao, M., Lytton, J., Burchiel, S.W. (1996) Inhibition of sarco-endoplasmic reticulum calcium ATPases (SERCA) by polycyclic aromatic hydrocarbons: Lack of evidence for direct effects on cloned rat enzymes. *Int. J. Immunopharmacol.*, **18**, 589–598

Zhao, H., Spitz, M.R., Gwyn, K.M. & Wu, X. (2002) Microsomal epoxide hydrolase polymorphisms and lung cancer risk in non-Hispanic whites. *Mol. Carcinog*., **33**, 99–104

Zheng, W., Xie, D.W., Jin, F., Cheng, J.R., Dai, Q., Wen, W.Q., Shu, X.O. & Gao, Y.T. (2000) Genetic polymorphism of cytochrome P450-1B1 and risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 147–150

Zhong, B.-Z., Gu, Z.-W., Stewart, J. & Ong, T. (1995) Micronucleus formation induced by three polycyclic aromatic hydrocarbons in rat bone marrow and spleen erythrocytes following intratracheal instillation. *Mutat. Res*., **326**, 147–153

Zhu, H., Li, Y. & M.A. (1995) Characterization of benzo[a]pyrene quinone-induced toxicity to primary cultured bone marrow stromal cells from DBA/2 mice: Potential role of mitochondrial dysfunction. *Toxicol. appl. Pharmacol*., **130**, 108–120

Zhu, H., Smith, C., Ansah, C. & Gooderham, N.J. (2005) Responses of genes involved in cell cycle control to diverse DNA damaging chemicals in human lung adenocarcinoma A549 cells. *Cancer Cell int*., **5**, 28–40

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Polycyclic aromatic hydrocarbons (PAHs) are products of the incomplete combustion or pyrolysis of organic material. They are ubiquitous in the environment, which leads to measurable background levels of exposure in the general population. Tobacco smoke is a significant source of exposure to PAHs; in the nonsmoking, non-occupationally exposed population, diet is frequently the major source of exposure to PAHs.

High levels of occupational exposure can occur during the conversion of coal to coke and coal tar, and during the processing and use of coal-tar derived products. Industries and occupations for which exposures are reviewed in this monograph include coal gasification, coke production, coal-tar distillation, paving and roofing that involve coal tar, the use of creosote as a wood preservative, aluminium production (including anode manufacturing), carbon electrode manufacture, calcium carbide production, occupation as a chimney sweep and other exposures to soot, and thermoelectric power plants.

Occupational exposures to benzo[*a*]pyrene in these industries can be as high as 100 μg/m^3 compared with typical ambient air concentrations of a few nanograms per cubic metre. Similarly, levels of 1-hydroxypyrene, a urinary metabolite, can reach 100 μmol/mol creatinine in highly exposed workers but are generally less than 0.1 μmol/mol creatinine in people who are not exposed occupationally. The highest reported levels of exposure to benzo[*a*]pyrene by inhalation have been measured in the aluminium production industry when the Söderberg process is used. Levels of exposure to PAHs and profiles of exposure to multiple PAHs are, to a large extent, dependent on the industry, the job tasks performed within an industry, and the time period and country of exposure. Available information on dermal exposure suggests that it is a major route in many of the industries considered. Few measurements have been made of exposure to PAHs with a molecular weight greater than 300.

5.2 Human carcinogenicity data

Coal gasification

Epidemiological studies that are of sufficient size to be informative have consistently shown an excess of lung cancer associated with gas production. A large epidemiological cohort study of over 11 000 British gas production workers showed an excess incidence of lung cancer and urinary bladder cancer. A study of nearly 5000 German gas production workers showed an excess incidence of lung, stomach and colorectal cancer among gas furnace workers; the risk for lung cancer was related to duration of employment. A large

study of over 3000 Chinese gas plant workers that was only reported briefly showed an excess incidence of lung cancer among workers in the gas department. A case–control study nested within a cohort of gas and electricity production workers in France supported an association between coal gasification and the excess incidence of lung cancer. The Working Group noted that the findings are probably not explained by tobacco smoking habits, although no study fully adjusted for this.

Coke production

Most but not all of the epidemiological studies provided evidence of an excess risk for lung cancer among coke production workers. A large cohort study of coke plant workers in the USA and Canada showed an excess in mortality from lung cancer. The risk was highest in work areas close to the ovens and was especially high among workers in top-side jobs; an exposure–response trend was found. A very large study of coke plant workers in China that was only reported briefly also showed an increased risk for lung cancer. Cohort studies from France, Italy, Japan and the Netherlands supported an increased risk for lung cancer, although the studies were not fully conclusive when considered individually. Two mortality studies of coke plant workers in the United Kingdom showed no excess of lung cancer, although a record linkage study from the United Kingdom indicated an elevated risk. A case–control study among Chinese women who were exposed to coke oven emissions showed a positive exposure–response relationship after adjustment for tobacco smoking and supported an association between the excess incidence of lung cancer and coke production.

Coal-tar distillation

Two large surveillance programmes provide evidence of an increased risk for skin cancer among coal-tar distillers. Notifications of skin cancer in England during 1911–38 were analysed in relation to occupation, and more than 700 skin cancers that were attributed to exposure to coal tar among coal-tar distillers had been notified; crude mortality rates for scrotal cancer were very high among coal-tar distillers. Occupational health surveillance in a German coal-tar distillation plant identified more than 600 individuals with skin lesions during 1946–96, a third of whom also had malignant skin tumours; 20 cases of scrotal cancer were observed. More recent cohort mortality studies showed no indication of an increased risk for skin cancer, but this design is not sufficiently sensitive to identify potential risks for skin cancer. The findings for other cancer sites were inconsistent; a modest, non-significant increase in mortality was reported for lung cancer in one British and one Dutch study, and a significant excess in the incidence of buccal cavity and pharyngeal cancers was reported in a French study.

Paving and roofing with coal-tar pitch

Studies of pavers and roofers who presumably have been exposed to coal-tar pitch (and often also to bitumen) have suggested increased cancer risks in these occupations; studies of members of a roofer's union in the USA, analyses of registry-based data on pavers in the United Kingdom and roofers in the USA, and follow-up studies of cancer incidence among pavers in Finland and the Netherlands all showed an excess risk for lung cancer. Mortality from urinary bladder, laryngeal and skin (non-melanoma) cancer was increased in one of these cohorts, but this finding was not widely supported by other studies. Three case–control studies (conducted in the USA) reported a tobacco smoking-adjusted increase in the risk for lung cancer among roofers; none of these increases was statistically significant, but a meta-analysis of the case–control studies reported a statistically significant meta-relative risk.

Creosote

A number of cases of skin cancer, including scrotal cancer, have been reported among workers who had been occupationally exposed to creosote. A cohort study of wood impregnators in Norway and Sweden and a cohort study of timber workers in Finland, who had been exposed to creosote, reported a statistically significant excess incidence of non-melanoma skin cancer. A study of power linesmen in Sweden did not report any statistically significant increase in the incidence of cancer, although the risk for non-melanoma cancer was slightly increased. A nested case–control study of lung cancer among a cohort of gas and electricity workers in France reported an increased risk for exposure to creosote, with evidence of an exposure–response relationship. A cohort study of workers in the USA who had used creosote for the treatment of wood indicated the possibility of an increase in mortality from lung cancer. A study that applied a job–exposure matrix to job titles in the Swedish census and linked this to cancer incidence found an increase in the incidence of cancers of the renal pelvis and urinary bladder that was related to exposure to creosote.

Aluminium production

The first reports of risk for cancer associated with work in the aluminium production industry were made in the 1970s in the former USSR. A series of reports from Québec, Canada, showed statistically significant excess risks and positive exposure–response relationships for lung and urinary bladder cancer after adjustment for tobacco smoking. A study of an aluminium production plant in British Columbia, Canada, found statistically significant exposure-related trends in risk for both lung and urinary bladder cancer. A Norwegian cohort study showed an increase in the risk for cancer of the urinary bladder but not of the lung. In a study of multiple plants in the USA, the risk for lung cancer was close to that expected, but a statistically significant excess risk for bladder cancer was found. A study in the French aluminium industry reported excesses in the risk for cancer

of the lung and urinary bladder. In a recent meta-analysis of studies that used benzo[*a*]-pyrene as an index of exposure to PAHs, results from eight cohort studies of lung cancer and six studies of urinary bladder cancer in aluminium workers were pooled. Pooled risk estimates indicated a positive exposure–response relationship between cumulative exposure to benzo[*a*]pyrene and both urinary bladder and lung cancer.

In two studies, statistically significant increases in the incidence of lymphatic and haematopoietic cancers were reported for aluminium workers. Increased risks for pancreatic cancer were also reported in two studies.

Carbon electrode manufacture

A study of carbon electrode workers in China showed an excess risk for lung cancer and a positive exposure–response relationship between increasing exposure to carbon compounds and lung cancer risk. When the study was limited to nonsmokers, the increased risk was still observed. However, the study included both carbon electrode workers and pot-room workers in an aluminium smelter, and it is questionable how much of the excess risk may be attributed to exposures in carbon electrode manufacture. A small study of carbon electrode manufacturing workers in Japan showed an excess incidence of lung cancer. A large study of workers at a carbon product department of a plant in the USA showed no excess incidence of respiratory cancer and no exposure–response trend in internal analyses. A cohort study of two plants in France and two cohort studies from Italy provided no evidence for an increased risk for lung cancer associated with carbon electrode manufacture. A small study from Sweden based on only two cases was uninformative due to small numbers.

Calcium carbide production

One study of calcium carbide production workers showed an increased risk for cancers of the prostate and of the colon but not of the lung. The study provided little information on risk for cancer in relation to exposure to PAHs.

Chimney sweeps and other exposures to soot

A large cohort study of chimney sweeps in Sweden showed an excess incidence of cancers of the lung, oesophagus, urinary bladder and haematolymphatic system; the excess remained after adjustment for tobacco smoking and a trend in exposure–response was observed. A smaller cohort study of chimney sweeps in Germany showed excess mortality from lung cancer, and a Danish cohort study showed an excess of cancer deaths among chimney sweeps. Record linkage studies from Finland and Norway showed increased risks for lung cancer, although the results from the Finnish study were not statistically significant. A record linkage study from the United Kingdom showed no increased mortality from lung cancer. A large number of case series reported an increased risk for skin cancer, especially scrotal cancer, among chimney sweeps.

Case–control studies by cancer site

Lung

Smoking-adjusted increased risks for lung cancer were reported for several industries and for general occupational exposures to PAHs in Germany, Norway and Sweden. The two larger studies (Germany and Sweden) also found positive exposure–response relationships. A study of lung cancer in Canada found an excess risk for exposure to PAHs from any occupational source among light smokers and nonsmokers only; a Dutch study reported inconsistent results.

Other sites

Six studies of renal-cell carcinoma and exposure to PAHs were reviewed. One showed an increased risk among coke-oven workers and another study found a statistically non-significant positive association with exposure to coke. Exposure to coal tar and to coal-tar pitch was assessed in three studies of renal-cell carcinoma; a statistically significant or nearly significant increase in odds ratios was found in all three studies. Three studies investigated occupational exposures to PAHs and the incidence of renal-cell carcinoma; one was uninformative due to the small number of exposed cases and the other two found no excess risk for renal-cell carcinoma.

Among eight case–control studies of urinary bladder cancer and occupational exposure to PAHs, exposure to mixtures of coal-tar pitch, coal tar and asphalt was assessed in three, two of which found a statistically significant excess risk. Assessment of exposure based on industries or job titles gave inconsistent results for bladder cancer risks. Four studies assessed general occupational exposure to PAHs and one found a borderline significantly increased risk for cancer of the urinary bladder.

Three studies of laryngeal cancer consistently showed statistically significant associations with exposure to PAHs, as assessed from lifetime occupational history. Case–control studies of skin cancer (two studies), pancreatic cancer (three studies and one meta-analysis of four case–control studies), stomach cancer (two studies), oesophageal cancer (two studies) and prostate cancer (two studies) assessed jobs that entailed high known occupational exposure to PAHs; the results were inconsistent.

Dietary intake

Few epidemiological studies have directly investigated the association between dietary intake of PAHs and risk for cancer. The studies conducted to date have all used a questionnaire with a meat-cooking module in conjunction with a database of levels of benzo[*a*]pyrene that was used as a marker for total intake of PAHs. Three case–control studies of colorectal adenoma, a precursor of cancer, showed small to moderate increases in risk with higher estimated intake of benzo[*a*]pyrene. In contrast, there was no association with intake of benzo[*a*]pyrene in a case–control study of colon cancer. One case–control study of pancreatic cancer found a moderate increase in risk associated with

benzo[*a*]pyrene intake; no association was found in one study of prostatic cancer and non-Hodgkin lymphoma. The available information is at present too limited to draw definitive conclusions.

5.3 Animal carcinogenicity data

Studies on the carcinogenicity in experimental animals of anthanthrene, anthracene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*c*]fluorene, benzo[*ghi*]perylene, benzo[*c*]-phenanthrene, benzo[*a*]pyrene, benzo[*e*]pyrene, chrysene, coronene, cyclopenta[*cd*]pyrene, dibenz[*a,c*]anthracene, dibenz[*a,h*]anthracene, dibenz[*a,j*]anthracene, dibenzo[*a,e*]fluoranthene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, 1-methylchrysene, 2-methylchrysene, 3-methylchrysene, 4-methylchrysene, 5-methylchrysene, 6-methylchrysene, 2-methylfluoranthene, 3-methylfluoranthene, perylene, phenanthrene, pyrene and triphenylene were considered by previous working groups. For these compounds, only studies that were evaluated since that time are summarized below.

Acenaphthene was tested for carcinogenicity by dermal administration in two experiments in mice, both of which were considered to be inadequate for an evaluation.

2,3-Acepyrene (cyclopentano[*cd*]pyrene) was tested for carcinogenicity in one initiation–promotion study and one study of repeated dermal application in mice, both of which gave negative results.

Anthanthrene was tested for carcinogenicity on mouse skin in one initiation–promotion study that gave negative results. Anthanthrene also gave negative results when tested for carcinogenicity by intramamillary administration to rats.

Anthracene was tested for carcinogenicity on mouse skin in two experiments, one of which was an initiation–promotion study. Both gave negative results. It also gave negative results when administered by subcutaneous injection to mice in one study.

11*H*-Benz[*bc*]aceanthrylene gave positive results when tested for carcinogenicity in one initiation–promotion study on mouse skin, but gave negative results after subcutaneous injection to mice.

Benz[*j*]aceanthrylene was tested for carcinogenicity in one dermal initiation–promotion study on mouse skin and after intraperitoneal administration to mice. In both instances, highly significant increases in tumour incidence and/or in the number of tumours per animal were observed.

Benz[*l*]aceanthrylene was tested for carcinogenicity in one dermal initiation–promotion experiment in mice, in which it was positive as a tumour initiator.

Benz[*a*]anthracene was tested for carcinogenicity in a number of bioassays. It gave negative results in one study of repeated application on mouse skin and positive results in four initiation–promotion studies on mouse skin. In three assays in newborn mice, results were positive in two studies and questionable in one study, possibly due to the low dose

tested. One study of intratracheal instillation and one of buccal pouch application in hamsters and one study of intramammary administration to rats gave negative results.

Benzo[*b*]chrysene was tested for carcinogenicity on mouse skin in one initiation–promotion study that gave positive results.

No data were available to the Working Group on the carcinogenicity of **benzo[*g*]chrysene** in experimental animals.

Benzo[*a*]fluoranthene was tested for carcinogenicity in one initiation–promotion experiment in mice that gave positive results.

Benzo[*b*]fluoranthene was tested for carcinogenicity by dermal application in mice in multiple studies, by intraperitoneal injection into mice in one study and by intrapulmonary implantation into rats in one study. In all of these studies, benzo[*b*]-fluoranthene exhibited significant carcinogenic activity.

No new studies were available to the Working Group to evaluate the carcinogenicity of **benzo[*ghi*]fluoranthene** in experimental animals.

Benzo[*j*]fluoranthene was tested for carcinogenicity by dermal application in mice in four studies, by intraperitoneal injection into newborn mice in two studies and by intrapulmonary implantation into rats in one study. With the exception of one study in newborn female mice, benzo[*j*]fluoranthene exhibited significant carcinogenic activity in all of the assays.

Benzo[*k*]fluoranthene was tested for carcinogenicity by dermal application in mice in one study, by intraperitoneal injection into newborn mice in one study and by intrapulmonary implantation into rats in one study. Benzo[*k*]fluoranthene exhibited significant carcinogenic activity in the dermal and intrapulmonary assays.

No new studies were available to the Working Group to evaluate the carcinogenicity of **benzo[*a*]fluorene** in experimental animals.

No new studies were available to the Working Group to evaluate the carcinogenicity of **benzo[*b*]fluorene** in experimental animals.

Benzo[*c*]fluorene was tested for carcinogenicity by oral and intraperitoneal administration to mice; both studies gave positive results.

No new studies were available to the Working Group to evaluate the carcinogenicity of **benzo[*ghi*]perylene** in experimental animals.

Benzo[*c*]phenanthrene was tested for carcinogenicity by intraperitoneal injection into infant mice, which resulted in a substantial induction of lung tumours.

In several studies in which **benzo[*a*]pyrene** was applied to the skin of different strains of mice, benign and malignant skin tumours were observed. No skin tumours developed in mice that lacked the aryl hydrocarbon receptor ($AhR^{-/-}$ mice). In a large number of initiation–promotion studies, benzo[*a*]pyrene was active as an initiator when applied to the skin of mice.

In a series of studies in newborn and adult mice, intraperitoneal injection of benzo[*a*]pyrene increased the incidence of liver and lung tumours and, occasionally, that of forestomach and lymphoreticular tumours.

Subcutaneous injection of benzo[*a*]pyrene induced malignant tumours (mainly fibrosarcomas) at the injection site in mice, rats and hamsters. $AhR^{-/-}$ mice did not develop tumours.

Intratracheal administration of benzo[*a*]pyrene alone or mixed with particulates and suspended in saline with or without suspendents resulted in benign and malignant respiratory tumours in numerous studies in hamsters and in a few studies in rats and mice. Larger benzo[*a*]pyrene particles were generally more effective than smaller ones. Mice that lack the nucleotide excision repair gene *XPA* ($XPA^{-/-}$ mice) showed a stronger lung tumour response after intratracheal instillation of benzo[*a*]pyrene than their similarly treated $XPA^{+/+}$ and $XPA^{+/-}$ counterparts.

Following administration of benzo[*a*]pyrene by gavage or in the diet to mice and rats of different strains, increased tumour responses were found in several organs, including the lung, forestomach, liver, lymphoreticular tissue, oesophagus and tongue. Oral administration of benzo[*a*]pyrene to $XPA^{-/-}$ mice resulted in a significantly higher increase in lymphomas than that observed in similarly treated $XPA^{+/-}$ and $XPA^{+/+}$ mice. Benzo[*a*]pyrene administered by gavage to $XPA^{-/-}/p53^{+/-}$ double transgenic mice induced tumours (mainly splenic lymphomas and forestomach tumours) much earlier and at a higher incidence than in similarly treated single transgenic and wild-type counterparts. These cancer-prone $XPA^{-/-}$ or $XPA^{-/-}/p53^{+/-}$ mice also developed a high incidence of (mainly) forestomach tumours when fed benzo[*a*]pyrene in the diet.

Dose-related increases in the incidence of malignant lung tumours were found after injection of benzo[*a*]pyrene in beeswax/tricaprylin or beeswax/trioctanoin into the lung tissue of rats.

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

Repeated application of benzo[*a*]pyrene to the buccal pouch mucosa of male hamsters resulted in a high incidence of forestomach papillomas.

In rat subcutaneous tracheal grafts exposed to benzo[*a*]pyrene in beeswax, a high incidence of squamous-cell carcinomas was found. In one of 13 benzo[*a*]pyrene-exposed human bronchial grafts transplanted subcutaneously into nude mice, a squamous-cell carcinoma developed, while four other grafts developed preneoplastic epithelial changes.

In three studies in rats, benign and malignant mammary gland tumours developed after intramamillary administration of benzo[*a*]pyrene.

In three studies in mice, intracolonic instillation of benzo[*a*]pyrene induced a variety of benign and malignant tumours in various organs but no colonic tumours.

No new studies were available to the Working Group to evaluate the carcinogenicity of **benzo[*e*]pyrene** in experimental animals.

The carcinogenicity of **chrysene** was assessed in three studies in newborn mice, in three initiation–promotion studies in mice and after pulmonary implantation into rats.

Chrysene gave a positive response in two of three assays in newborn mice, in one of three initiation–promotion studies and in the pulmonary implantation study.

No new studies were available to the Working Group to evaluate the carcinogenicity of **coronene** in experimental animals.

4*H*-Cyclopenta[*def*]chrysene was tested for carcinogenicity in two initiation–promotion experiments in mice, in which a positive response was observed.

Cyclopenta[*cd*]pyrene was tested for carcinogenicity by repeated dermal exposure of mice and by intraperitoneal administration to newborn and adult mice. In all of these studies, cyclopenta[*cd*]pyrene gave a positive response.

5,6-Cyclopenteno-1,2-benzanthracene was tested for carcinogenicity in mouse skin in two similarly designed studies that were conducted in the same laboratory; both studies gave positive results.

Dibenz[*a,c*]anthracene was tested for carcinogenicity in an initiation–promotion study in mouse skin, but the results were questionable, presumably due to the low initiating doses of dibenz[*a,c*]anthracene that were used. Dibenz[*a,c*]anthracene gave negative results when administered subcutaneously to mice, and tumour incidence was not significantly increased over that observed in controls when the compound was administered intraperitoneally to newborn mice.

Dibenz[*a,h*]anthracene was tested for carcinogenicity by dermal application in mice, subcutaneous injection into mice and rats, intraperitoneal injection into mice, intrapulmonary injection into mice and rats and intramammary and intratracheal injection into rats. In the majority of these studies, dibenz[*a,h*]anthracene exhibited significant carcinogenic activity.

Dibenz[*a,j*]anthracene was tested for carcinogenicity in three studies on mouse skin, each of which showed a positive response.

No new studies were available to the Working Group to evaluate the carcinogenicity of **dibenzo[*a,e*]fluoranthene** in experimental animals.

13*H*-Dibenzo[*a,g*]fluorene was tested for carcinogenicity in two bioassays in which mice received repeated dermal applications. Both assays yielded positive results, although control groups were not included for comparison.

Dibenzo[*h,rst*]pentaphene was tested for carcinogenicity in one study in mice by subcutaneous injection and gave positive results.

Dibenzo[*a,e*]pyrene was assessed in two initiation–promotion studies on mouse skin. One of the studies gave positive results, while the other gave negative results. Dibenzo[*a,e*]pyrene gave negative results when tested for carcinogenicity by intramamillary administration to rats.

Dibenzo[*a,h*]pyrene was tested for carcinogenicity in one study on mouse skin, in three tumour initiation–promotion studies on mouse skin, by intraperitoneal injection into mice and by intramammary injection into rats. It exhibited significant carcinogenic activity in all of these studies.

Dibenzo[*a,i*]pyrene was tested for carcinogenicity in three initiation–promotion studies in mouse skin, one study of subcutaneous injection into mice, one study of

intraperitoneal administration to newborn mice, two studies of intratracheal administration to hamsters and one study of intramamillary administration to rats. All studies gave positive results.

Dibenzo[*a*,*l*]pyrene was tested for carcinogenicity in studies of single and repeated dermal application to mice, as well as several initiation–promotion studies on mouse skin; all studies gave positive results. Dibenzo[*a*,*l*]pyrene also induced oral cavity tumours when applied dermally to the tongue of hamsters, and lung tumours in mice following intraperitoneal injection. In addition to lung tumours, dibenzo[*a*,*l*]pyrene induced hepatic tumours and a variety of tumours at other sites when administered intraperitoneally to newborn mice. Multiple tumour sites were also observed following intragastric application of dibenzo[*a*,*l*]pyrene in mice. Intramamillary administration to rats also yielded positive results. Furthermore, two studies in fish demonstrated that non-mammalian species are also susceptible to dibenzo[*a*,*l*]pyrene-induced tumorigenicity.

Dibenzo[*e*,*l*]pyrene was tested for carcinogenicity in one study and in one initiation–promotion study on mouse skin, both of which gave negative results.

1,2-Dihydroaceanthrylene was tested in an early design-limited study by subcutaneous injection into mice and yielded negative results. More recently, it was tested in two intraperitoneal bioassays in newborn mice. In one assay, an increase in lung tumours was observed at the highest dose administered in males and females combined.

1,4-Dimethylphenanthrene was tested in two initiation–promotion studies in mice and gave positive results.

Fluoranthene was tested for carcinogenicity in four studies in newborn mice and in one study in mice by dermal application. All the studies in newborn mice gave positive results, whereas the study in mouse skin gave negative results.

No new studies were available to the Working Group to evaluate the carcinogenicity of **fluorene** in experimental animals.

Indeno[1,2,3-*cd*]pyrene was tested for carcinogenicity in two initiation–promotion studies in mice, both of which gave positive results, and by pulmonary injection into rats, which also yielded positive results. Indeno[1,2,3-*cd*]pyrene did not induce tumours in newborn mice following intraperitoneal injection. Indeno[1,2,3-*cd*]pyrene was administered subcutaneously to mice in an experiment that did not include control animals; sarcomas were observed.

No new studies were available to the Working Group to evaluate the carcinogenicity of **1-methylchrysene** in experimental animals.

No new studies were available to the Working Group to evaluate the carcinogenicity of **2-methylchrysene** in experimental animals.

No new studies were available to the Working Group to evaluate the carcinogenicity of **3-methylchrysene** in experimental animals.

No new studies were available to the Working Group to evaluate the carcinogenicity of **4-methylchrysene** in experimental animals.

5-Methylchrysene was tested for carcinogenicity in numerous initiation–promotion studies in mice and by intraperitoneal administration to newborn mice in one study and to adult mice in another study. All of these studies gave positive results.

No new studies were available to the Working Group to evaluate the carcinogenicity of **6-methylchrysene** in experimental animals.

2-Methylfluoranthene was tested for carcinogenicity in one study by intraperitoneal administration to newborn mice which gave positive results.

3-Methylfluoranthene was tested for carcinogenicity in one study by intraperitoneal injection to newborn male mice which gave positive results.

1-Methylphenanthrene was tested for carcinogenicity in an initiation–promotion study in mice and was negative.

Naphtho[1,2-*b*]fluoranthene was tested for carcinogenicity in one initiation–promotion experiment in mice that gave positive results.

Naphtho[2,1-*a*]fluoranthene was tested for carcinogenicity in one initiation–promotion experiment in mice that gave positive results.

Naphtho[2,3-*e*]pyrene was tested for carcinogenicity in one initiation–promotion experiment in mice and in one study by repeated dermal application in mice. The initiation–promotion study gave positive results; repeated dermal application gave negative results.

Perylene was tested for carcinogenicity by dermal application in mice (repeated dose and initiation–promotion protocols) and by intraperitoneal injection into mice. All studies gave negative results.

Phenanthrene was tested for carcinogenicity in one repeated-dose study on mouse skin and in one study of intrapulmonary injection into rats. Both studies gave negative results.

Picene was tested for carcinogenicity in an early design-limited study by dermal application in mice which gave negative results. More recently, picene was tested in three studies in mice by dermal application, two of which were initiation–promotion experiments. All three studies gave positive results. Picene has also been tested by subcutaneous injection into newborn and adult mice, and young rats. It gave positive results in mice and negative results in rats.

Pyrene was tested for carcinogenicity on mouse skin, in multiple studies in newborn mice and in A/J mice. All studies gave negative results.

No new studies were available to the Working Group to evaluate the carcinogenicity of **triphenylene** in experimental animals.

A number of studies assessed the carcinogenicity of **defined mixtures of PAHs** in different species using various treatment regimens. Examples include the co-administration of binary mixtures of strong (e.g. benzo[*a*]pyrene) and weak (e.g. benzo[*e*]pyrene) carcinogens onto mouse skin, using repeated dosing or initiation–promotion protocols, and the subcutaneous injection of binary mixtures of strong (e.g. benzo[*a*]pyrene) and weak (e.g. benzo[*e*]pyrene) or two strong (e.g. benzo[*a*]pyrene and dibenz[*a,h*]anthracene) carcinogens into mice. In some experiments, tumorigenic responses were consistent

with an additive effect. In other instances, the tumorigenic responses were less than or greater than the anticipated additive response. The reasons for these variations in tumorigenicity were not apparent. Additional experiments included the administration of multiple PAHs, including those with both strong and weak carcinogenic potential. As with the binary mixtures, the responses were varied; nevertheless, at low doses of the mixtures, greater than additive responses were observed while, at high doses, these were less than additive, perhaps due to metabolic saturation.

The carcinogenicity of a variety of **coal-tar preparations** has been assessed in various species. The carcinogenicity of solvent-refined coal distillates and their subfractions was assessed in two initiation–promotion studies in mice, both of which gave positive results. Coaltar-based paints were evaluated in one initiation–promotion study and one study by oral administration to mice; both gave positive results. Crude coal-tar preparations were assessed in two studies in mouse skin (one of which used repeated doses and the other used an initiation–promotion protocol). The study with repeated doses was inadequate for an evaluation of carcinogenicity, but the initiation–promotion study gave positive results. Coal-tar pitch was assessed by repeated application onto mouse skin in four studies, all of which gave positive results. Manufactured gas plant residues were evaluated in two studies of oral administration to mice and one study in newborn mice. All gave positive results.

5.4 Mechanism of action and other relevant data

Exposure to PAHs, most of which are associated with a solid or liquid matrix in aerosols of polluted air or food, occurs through the airways, skin and digestive tract. The bioavailabilities of individual PAHs may differ and contribute to differences in activity, particularly for those that contain five or more aromatic rings. Bioavailable fractions of PAHs are absorbed into the circulation through all three routes. Metabolic activation of lipophilic PAHs occurs primarily in the liver, but also in many other tissues, including the epithelial barriers. Although distribution through the circulatory system is widespread, slow absorption through most epithelia results in higher levels of enzymes that activate PAH substrates at the site of entry. This uneven distribution of dose is a factor that may contribute to the high propensity of PAHs to act as carcinogens at the sites where they enter the body.

PAHs are metabolized by phase I enzymes and peroxidases, which produce DNA-reactive metabolites, and phase II enzymes, which form polar conjugates. Phase I enzymes, such as cytochrome P450s, catalyse the mono-oxygenation of PAHs to form phenols and epoxides. Specific cytochrome P450 isozymes and epoxide hydrolase can form reactive diol epoxides that comprise one class of ultimate carcinogenic metabolites of many PAHs. Both cytochrome P450s and peroxidases can form radical cations by one-electron oxidation that comprise another class of ultimate carcinogenic metabolites. Further oxidation of PAH phenols leads to the formation of PAH quinones. The major cytochrome P450s that are involved in the formation of diol epoxides are 1A1, 1A2 and

1B1, while 2C9 and 3A4 play a minor role in the activation of PAHs. PAHs induce increased expression of activating cytochrome P450s via enhanced aryl hydrocarbon receptor-mediated transcription. Polymorphisms in human cytochrome P450s have been identified, some of which may be associated with increased susceptibility. Additional enzymes that may play a role in the further activation of some PAH diols include members of the aldo-keto reductase family, among which polymorphisms that influence susceptibility have been identified. Nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1 catalyses the reduction of PAH quinones to hydroquinones which may be re-oxidized and generate reactive oxygen species. Polymorphisms in this gene have also been described.

The major phase II enzymes include the glutathione *S*-transferases, uridine 5′-diphosphate glucuronosyltransferases and sulfotransferases . The major glutathione *S*-transferases involved in the conjugation of PAH metabolites are M1, P1 and T1. Multiple polymorphisms of these as well as polymorphisms in both uridine 5′-diphosphate glucuronosyl- and sulfotransferases have been identified, some of which can modulate susceptibility to cancer.

The current understanding of the carcinogenesis of PAHs in experimental animals is almost solely based on two complementary mechanisms: those of the diol epoxide and the radical cation. Each provides a different explanation for the data observed in experimental animals.

The diol epoxide mechanism features a sequence of metabolic transformations of PAHs, each of which leads to potentially reactive genotoxic forms. In general, PAHs are converted to oxides and dihydrodiols, which are in turn oxidized to diol epoxides. Both oxides and diol epoxides are ultimate DNA-reactive metabolites. PAH oxides can form stable DNA adducts and diol epoxides can form stable and depurinating adducts with DNA through electrophilic carbonium ions. The inherent reactivities of oxides and diol epoxides are dependent on topology (e.g. bay regions, fjord regions, cyclopenta rings), and the reactivity of diol epoxides is further dependent on factors such as stereochemistry and degree of planarity. Both stable and depurinating adducts are formed primarily with guanines and adenines, and induce mutations (e.g. in *ras* proto-oncogenes) that are strongly associated with the tumorigenic process. Some mutagenic PAH diols, oxides and diol epoxides are tumorigenic in experimental animals.

One-electron oxidation creates radical cations at a specific position on some PAHs. The ease of formation and relative stabilities of radical cations are related to the ionization potential of the PAH. Additional important factors in the radical cation mechanism are localization of charge in the PAH radical cation and optimal geometric configuration, particularly the presence of an angular ring. The radical cation mechanism results in the formation of depurinating DNA adducts with guanines and adenines, which generate apurinic sites that can induce mutations in *ras* proto-oncogenes, which are strongly associated with tumorigenesis.

There is strong evidence that the diol epoxide mechanism operates in the mouse lung tumorigenesis of many PAHs evaluated in this monograph. For some PAHs, there is

strong evidence that both radical cation and diol epoxide mechanisms induce mouse skin carcinogenesis. Many of the pathways that lead to PAH carcinogenesis involve genotoxicity, and the genotoxic effects of PAHs and their metabolites were included in the overall evaluation of each PAH discussed.

The genotoxic effects of exposure to complex mixtures that contain PAHs have been studied in some populations exposed in industrial settings and in patients who undergo coal-tar therapy. Measured end-points include mutagenicity in urine and the presence of aromatic DNA adducts in the peripheral lymphocytes of exposed workers. In some studies, specific benzo[*a*]pyrene–DNA adducts have been measured. Cytogenetic effects such as micronucleus formation have also been reported.

Other mechanisms of carcinogenesis have been proposed for PAHs, but these are less well developed. The *ortho*-quinone/reactive oxygen species mechanism features enzymatic oxidation of non-K-region PAH diols to *ortho*-quinones by aldo-keto reductases, and has been studied only in in-vitro systems. These PAH *ortho*-quinones are highly reactive towards DNA; they yield DNA adducts and damage DNA. PAH *ortho*-quinones induce mutations in the *p53* tumour-suppressor gene *in vitro*; they can also undergo repetitive redox cycling and generate reactive oxygen species, which have been associated with oxidative DNA-base damage as well as the induction of pro-oxidant signals that may have consequences on growth. Reactive oxygen species can also be produced by other mechanisms such as the formation of PAH quinones through peroxidase reactions. Thus, this pathway has the potential to contribute to the complete carcinogenicity of a parent PAH.

The mechanism of meso-region biomethylation and benzylic oxidation features biomethylation of parent PAHs to methyl PAHs. Methyl PAHs are further metabolized by cytochrome P450s to hydroxymethyl PAHs that are converted into reactive sulfate ester forms that are capable of forming DNA adducts. Studies on this mechanism have been limited to subcutaneous tissues in rats that are susceptible to PAH tumorigenesis.

Several of the biological effects of PAHs, such as enzyme induction of xenobiotic metabolizing enzymes, immunosuppression, teratogenicity and carcinogenicity, are thought to be mediated by activation of the aryl hydrocarbon receptor. This receptor is widely distributed and has been detected in most cells and tissues. There is also evidence that the aryl hydrocarbon receptor acts through a variety of pathways and, more recently, that cross-talk with other nuclear receptors enables cell type-specific and tissue-specific control of gene expression. Translocation of the activated aryl hydrocarbon receptor to the nucleus may require threshold concentrations of the ligand. Various oxidative and electrophilic PAH metabolites are also known to induce enzyme systems via anti-oxidant receptor elements. The biological effects of aryl hydrocarbon receptor and anti-oxidant receptor element signalling involve a variety of cellular responses, including regulation of phase I and II metabolism, lipid peroxidation, production of arachidonic acid-reactive metabolites, decreased levels of serum thyroxine and vitamin A and persistent activation of the thyroid hormone receptor. Aryl hydrocarbon receptor signalling may result in adaptive and toxic responses or perturbations of endogenous pathways. Furthermore,

metabolic activation of PAHs produces cellular stress. This in turn activates mitogen-mediated protein kinase pathways, notably of Nrf2. The Nrf2 protein dimerizes with Maf-oncoproteins to enable binding to an anti-oxidant/electrophilic response element, which has been identified in many phase I/II and other cellular defence enzymes and controls their expression. Therefore, cellular stress may be regulated independently of aryl hydrocarbon receptor-mediated xenobiotic metabolizing enzymes.

PAHs exert many important effects on the immune system of many species. The dose and route of exposure determine the nature of the effect of specific and adaptive immune responses. Extremely limited or no data are available on many of the pure PAHs (with the exception of benzo[*a*]pyrene) and complex mixtures considered in this monograph. Studies with those PAHs that have been investigated indicate that the aryl hydrocarbon receptor plays a critical role in the activation of immunotoxic PAHs via diol epoxide mechanisms that lead to DNA interactions, cause genotoxicity and suppress immunity by p53-dependent pathways. Benzo[*a*]pyrene diol epoxide may also affect protein targets and modulate lymphocyte signalling pathways via non-genotoxic (epigenetic) mechanisms. Certain PAH metabolites, such as benzo[*a*]pyrene quinones, may be formed via cytochrome P450-dependent and -independent (peroxidase) pathways, and redox-cycling PAH quinones may exert oxidative stress in lymphoid cells. Human exposures usually involve complex mixtures of PAHs, and it is difficult to attribute the relative contributions of individual PAHs to the overall immunotoxic effects. There is some evidence that human environmental exposures to PAHs may produce immunotoxicity, but further studies are needed.

Since PAHs are ubiquitous in the environment, and concomitant exposure of humans to PAHs and ultraviolet light is inevitable, the photomutagenicity and photocarcinogenicity of these compounds are of considerable importance to human health. PAH-contaminated skin may be exposed to sunlight, which is of greatest concern in outdoor workers who handle products that contain PAHs. Such workers include roofers, tanners and road construction workers. Some commercial medicines also contain PAHs. For example, coal tar, a complex mixture of PAHs, is widely used in creams, ointments, lotions and shampoos for the treatment of psoriasis (the Goeckerman therapy). A topical application of coal tar on the skin followed by irradiation with ultraviolet light has been found to increase the risk for developing skin cancer.

PAHs must be metabolically activated in order to induce tumours. However, individuals differ in their ability to metabolize PAHs: people who are deficient in particular enzymes that activate PAHs to reactive metabolites may be at a lower risk for chemical carcinogenesis, whereas deficiencies in enzymes that detoxify reactive metabolites may increase this risk. Some of the epidemiological studies that have been conducted to date have shown positive relationships between genetic polymorphisms of drug-metabolizing enzymes and susceptibility to cancer, while others have been inconclusive. Many factors, including race, age, sex, tobacco smoking, alcohol intake and genetic factors, could induce or inhibit drug-metabolizing activities which indicates that a

complex interaction exists. Multi-gene and exposure interactions may also play a complex role in the interpretation of any increases in risk.

Newborn animals treated with benzo[*a*]pyrene or dimethylbenz[*a*]anthracene have been shown to develop higher incidences of liver and lung tumours than animals treated later in life. The age-dependent susceptibility to these two compounds may be dependent on their ability to act as mutagens. Analyses of data on a variety of chemicals for which tumour incidence following perinatal exposure was compared with that following adult exposure show that the increased susceptibility may be linked to a mutagenic mechanism of action. In addition, studies on the carcinogenicity of dimethylbenz[*a*]anthracene indicate that a potential period of increased susceptibility to mammary tumorigenesis exists during the pubertal period of development.

The available data on the effects of PAHs other than cancer are sparse, with the exception of reproductive or developmental and immunological studies. Chronic bioassays are available for benzo[*a*]pyrene, but they do not include histological analyses. Short-term studies, however, indicate that limited hepato- and nephrotoxic effects occur at high doses. Human exposure to polluted air containing mixtures of PAHs and animal studies with individual PAHs indicate that these compounds may affect the developing fetus and impair male and female reproductive performance.

Mechanistic considerations pertinent to the evaluations

In making its evaluations of the compounds below, the Working Group considered the following mechanistic data.

Benz[*j*]aceanthrylene is mutagenic in bacteria and in mammalian cells. It causes morphological cell transformation in mouse embryonic fibroblasts. K-*Ras* codon 12 mutations are found in benz[*j*]aceanthrylene-induced lung adenomas in mice. Benz[*j*]-aceanthrylene-1,2-diol, which is formed by cyclopenta-ring oxidation of the parent compound, is found in rodent liver and lung tissue. This metabolite is also formed *in vitro* in the presence of human microsomes. Benz[*j*]aceanthrylene-1,2-oxide is mutagenic in bacteria and causes malignant cell transformation in mouse embryonic fibroblasts. Benz[*j*]aceanthrylene-9,10-diol and benz[*j*]aceanthrylene-9,10-diol-7,8-oxide, which are formed via the diol epoxide pathway, are mutagenic in bacteria, cause malignant cell transformation in mouse embryonic fibroblasts and form DNA adducts in these cells. Benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts have been found in benz[*j*]aceanthrylene-induced lung tumours in mice.

There is strong evidence that benz[*j*]aceanthrylene is activated via cyclopenta-ring oxidation and the formation of its diol epoxide.

The Working Group did not review all the mechanistic data on **benzo[*c*]phenanthrene**, but concluded that the formation of its diol epoxide as a mechanism for tumorigenesis is consistent with its activity as a mouse skin tumour initiator in a limited number of studies.

The complete sequence of steps in the metabolic activation pathway of **benzo[*a*]pyrene** to mutagenic and carcinogenic diol epoxides has been demonstrated in experimental animals, in human tissues and in humans. Following exposure, humans metabolically activate benzo[*a*]pyrene to benzo[*a*]pyrene diol epoxides that form DNA adducts: the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct has been measured in populations (e.g. coke-oven workers, chimney sweeps) exposed to PAH mixtures that contain benzo[*a*]pyrene. The reactive *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells. Mutations (G→T transversions) in the K-*ras* proto-oncogene in lung tumours from benzo[*a*]pyrene-treated mice are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts. Similar mutations in the K-*RAS* proto-oncogene and mutations in *TP53* were found in lung tumours from nonsmokers exposed to PAH-rich products of coal combustion that are known to contain benzo[*a*]pyrene (as well as many other PAHs). In an in-vitro study, the codons in the tumour-suppressor gene *TP53* that are most frequently mutated in human lung cancer were shown to be targets for DNA adduct formation and mutations induced by benzo[*a*]pyrene.

Cyclopenta[*cd*]pyrene induces mutation in bacteria, in mammalian cells and in various human cell lines that express cytochrome P450 1A1. It causes sister chromatid exchange and morphological cell transformation in mouse embryonic fibroblasts. Two major classes of K-*ras* oncogene mutations are found in lung adenomas from cyclopenta[*cd*]pyrene-treated mice. Cyclopenta[*cd*]pyrene-3,4-oxide is mutagenic in bacteria and in mammalian cells, and induces morphological cell transformation in mouse embryo fibroblasts. DNA adducts with deoxyguanosine and deoxyadenosine are found in the lungs of mice treated with cyclopenta[*cd*]pyrene. Cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydro-cyclopenta[*cd*]pyrene are mutagenic in bacteria in the presence of 3′-phosphadenosine-phosphosulfate. 4-Sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene is a direct mutagen in bacteria. Human liver microsomes and recombinant human cytochrome P450 1A1, 1A2 and 3A4 metabolize cyclopenta[*cd*]pyrene at the cyclopenta ring.

There is strong evidence that activation via cyclopenta-ring oxidation is the mechanism by which cyclopenta[*cd*]pyrene is tumorigenic in mouse lung.

Dibenz[*a,h*]anthracene induces DNA damage and mutation in bacteria, DNA damage in cultured rodent and human cells, mutation and chromosomal damage in rodent cells in culture and morphological cell transformation in mouse embryonic fibroblasts. It induces sister chromatid exchange and micronucleus formation in lung cells and micronucleus formation in bone marrow and spleen erythrocytes of rats treated by intratracheal instillation. Dibenz[*a,h*]anthracene-3,4-diols (racemic mixture and the individual stereoisomers) are mutagenic in bacteria. Racemic dibenz[*a,h*]anthracene-3,4-diol induces tumours in mouse skin and lung. Dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide induces morphological cell transformation in mouse embryonic fibroblasts. Racemic dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide is tumorigenic in mouse skin. Dibenz[*a,h*]-anthracene is metabolized to dibenz[*a,h*]anthracene-3,4-diol by recombinant human liver cytochrome P450s and by human liver microsomes. Human skin treated with dibenz[*a,h*]-

anthracene in short-term culture shows an adduct profile that is qualitatively similar to that seen in mouse skin treated *in vivo*. There is moderate evidence that activation via the formation of the diol epoxide is the mechanism by which dibenz[*a,h*]anthracene is tumorigenic in mouse skin.

The complete sequence of metabolic activation of **dibenzo[*a,l*]pyrene** has been demonstrated in experimental animals and in human cells in culture. Dibenzo[*a,l*]pyrene is mutagenic in bacteria, in mammalian cells and in human cell lines. It causes morphological cell transformation in mouse embryonic fibroblasts. Dibenzo[*a,l*]pyrene induces Ki-*ras* mutations at codon 12 and 61 in mouse lung adenomas, and Ha-*ras* mutations at codon 61 in mouse skin papillomas. Dibenzo[*a,l*]pyrene-11,12-diol forms DNA adducts in Chinese hamster V79 cells that express recombinant human cytochrome P450 1A1 and is mutagenic in Chinese hamster V79 cells in the presence of metabolic activation systems. Recombinant human cytochrome P450 1B1, expressed in Chinese hamster V79 cells, metabolizes dibenzo[*a,l*]pyrene-11,12-diol to reactive metabolites that form dibenzo[*a,l*]pyrene–11,12-diol-13,14-oxide adducts. Dibenzo[*a,l*]pyrene-11,12-diols (racemic mixture) induce tumours in mouse skin and lung. Dibenzo[*a,l*]pyrene-11,12-diol-13,14-epoxides (racemic mixture) are mutagenic in bacteria and in mammalian cells and induce tumours in the skin and lung of mice and in breast tissue of rats.

There is strong evidence that the diol epoxide metabolic pathway is a mechanism in the induction of lung tumours in dibenzo[*a,l*]pyrene-treated mice and that the radical cation and diol epoxide metabolic pathways are mechanisms in dibenzo[*a,l*]pyrene-induced mouse skin tumorigenesis. The enzymes that metabolize dibenzo[*a,l*]pyrene to its reactive diol epoxides (cytochrome P450 1A1 and 1B1) are present in human tissues and in human mammary cells in culture. Mutations (G→T transversions) in the K-*ras* proto-oncogene in lung tumours from dibenzo[*a,l*]pyrene-treated mice are associated with *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide–DNA adducts. Similar mutations in the K-*RAS* proto-oncogene were found in lung tumours from nonsmokers exposed to PAH-rich products of coal combustion that are known to contain dibenzo[*a,l*]pyrene (as well as many other PAHs).

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during coal gasification. There is *sufficient evidence* in experimental animals for the carcinogenicity of manufactured gas plant residues.

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during coke production. There is *sufficient evidence* in experimental animals for the carcinogenicity of coke-oven coal-tar samples. There is *inadequate evidence* in experimental animals for the carcinogenicity of crude coal-tar preparations.

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during coal-tar distillation. There is *sufficient evidence* in experimental animals for the carcinogenicity of solvent-refined coal distillates.

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposure as a chimney sweep. There is *sufficient evidence* in experimental animals for the carcinogenicity of soot extracts. There is *inadequate evidence* in experimental animals for the carcinogenicity of soots.

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during paving and roofing with coal-tar pitch. There is *sufficient evidence* in experimental animals for the carcinogenicity of coal-tar pitch.

There is *sufficient evidence* in humans for the carcinogenicity of occupational exposures during aluminium production.

There is *limited evidence* in humans for the carcinogenicity of occupational exposures during carbon electrode manufacture.

There is *inadequate evidence* in humans for the carcinogenicity of occupational exposures during calcium carbide production.

There is *limited evidence* in humans for the carcinogenicity of creosotes. There is *sufficient evidence* in experimental animals for the carcinogenicity of creosotes.

There is *sufficient evidence* in experimental animals for the carcinogenicity of benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a*,*h*]anthracene, dibenzo[*a*,*h*]-pyrene, dibenzo[*a*,*i*]pyrene, dibenzo[*a*,*l*]pyrene, indeno[1,2,3-*cd*]pyrene and 5-methyl-chrysene.

There is *limited evidence* in experimental animals for the carcinogenicity of anthanthrene, benz[*j*]aceanthrylene, benzo[*c*]fluorene, benzo[*c*]phenanthrene, dibenz[*a*,*c*]-anthracene, dibenz[*a*,*j*]anthracene, dibenzo[*a*,*e*]fluoranthene, 13*H*-dibenzo[*a*,*g*]fluorene, dibenzo[*a*,*e*]pyrene, fluoranthene, 2-methylchrysene, 3-methylchrysene, 4-methylchrysene, 6-methylchrysene, 2-methylfluoranthene and picene.

There is *inadequate evidence* in experimental animals for the carcinogenicity of acenaphthene, acepyrene (3,4-dihydrocyclopenta[*cd*]pyrene), anthracene, 11*H*-benz[*bc*]aceanthrylene, benz[*l*]aceanthrylene, benzo[*b*]chrysene, benzo[*g*]chrysene, benzo[*a*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, 4*H*-cyclopenta[*def*]chrysene, 5,6-cyclopenteno-1,2-benzanthracene, dibenzo[*h*,*rst*]pentaphene, dibenzo[*e*,*l*]pyrene, 1,2-dihydroaceanthrylene, 1,4-dimethylphenanthrene, fluorene, 1-methylchrysene, 3-methylfluoranthene, 1-methylphenanthrene, naphtho[1,2-*b*]fluoranthene, naphtho[2,1-*a*]fluoranthene, naphtho[2,3-*e*]pyrene, perylene, phenanthrene, pyrene and triphenylene.

Overall evaluation

Occupational exposures during coal gasification are *carcinogenic to humans (Group 1)*.

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

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

Occupational exposure as a chimney sweep is *carcinogenic to humans (Group 1)*.

Occupational exposures during paving and roofing with coal-tar pitch are *carcinogenic to humans (Group 1)*.

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

Occupational exposures during carbon electrode manufacture are probably *carcinogenic to humans (Group 2A)*.

Occupational exposures during calcium carbide production are *not classifiable as to their carcinogenicity to humans (Group 3)*.

Creosotes are *probably carcinogenic to humans (Group 2A)*.

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

Cyclopenta[*cd*]pyrene, dibenz[*a*,*h*]anthracene and dibenzo[*a*,*l*]pyrene are *probably carcinogenic to humans (Group 2A)*.

Benz[*j*]aceanthrylene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*c*]phenanthrene, chrysene, dibenzo[*a*,*h*]pyrene, dibenzo[*a*,*i*]-pyrene, indeno[1,2,3-*cd*]pyrene and 5-methylchrysene are *possibly carcinogenic to humans (Group 2B)*.

Acenaphthene, acepyrene (3,4-dihydrocyclopenta[*cd*]pyrene), anthanthrene, anthracene, 11*H*-benz[*bc*]aceanthrylene, benz[*l*]aceanthrylene, benzo[*b*]chrysene, benzo[*g*]-chrysene, benzo[*a*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*c*]fluorene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, 4*H*-cyclopenta-[*def*]chrysene, 5,6-cyclopenteno-1,2-benzanthracene, dibenz[*a*,*c*]anthracene, dibenz[*a*,*j*]-anthracene, dibenzo[*a*,*e*]fluoranthene, 13*H*-dibenzo[*a*,*g*]fluorene, dibenzo[*h*,*rst*]pentaphene, dibenzo[*a*,*e*]pyrene, dibenzo[*e*,*l*]pyrene, 1,2-dihydroaceanthrylene, 1,4-dimethyl-phenanthrene, fluoranthene, fluorene, 1-methylchrysene, 2-methylchrysene, 3-methyl-chrysene, 4-methylchrysene, 6-methylchrysene, 2-methylfluoranthene, 3-methyl-fluoranthene, 1-methylphenanthrene, naphtho[1,2-*b*]fluoranthene, naphtho[2,1-*a*]-fluoranthene, naphtho[2,3-*e*]pyrene, perylene, phenanthrene, picene, pyrene and triphenylene are *not classifiable as to their carcinogenicity to humans (Group 3)*.

In making the overall evaluations of benz[*j*]aceanthrylene, benzo[*c*]phenanthrene, benzo[*a*]pyrene, cyclopenta[*cd*]pyrene, dibenzo[*a*,*h*]anthracene and dibenzo[*a*,*l*]pyrene, the Working Group took into consideration the mechanistic data detailed in Section 5.4.

APPENDIX

CHEMICAL AND PHYSICAL DATA FOR SOME NON-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS

The molecular formulae and relative molecular masses of these compounds are given in Table 1. Their structural formulae are given in Figure 1 and their selected physical and chemical properties are summarized in Table 2.

1. Acenaphthene

1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 83-32-9
Chem. Abstr. Name: Acenaphthene
IUPAC Systematic Name: Acenaphthene
Synonyms: 1,8-dihydroacenaphthene; 1,2-dihydroacenaphthylene; 1,8-ethylene-naphthalene; peri-ethylenenaphthalene; naphthyleneethylene

1.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006) unless otherwise specified

(a) *Description*: Orthorhombic bipyramidal needles from alcohol
(b) *Boiling-point*: 279 °C
(c) *Melting-point*: 95 °C
(d) *Density*: 1.189
(e) *Spectroscopy data*: ultraviolet (UV)/visible (VIS), infrared, fluorescence, mass and nuclear magnetic resonance (NMR) spectral data have been reported (Karcher *et al.*, 1988; NIST, 2005).
(f) *Water solubility*: 3.9 mg/L at 25 °C (Miller *et al.*, 1985)
(g) *Vapour pressure*: 0.29 Pa at 25 °C (Sonnefeld *et al.*, 1983)
(h) *Log K_{ow} (octanol-water)*: 3.92 (Sangster Research Laboratories, 2005)

(i) *Henry's law constant*: 18.5 Pa m^3/mol at 25 °C (Bamford *et al.*, 1999)
(j) *Atmospheric OH rate constant*: $8.0 \pm 0.4 \times 10^{-11}$ cm^3/mol/s (Reisen & Arey, 2002)

2. Acepyrene

2.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 25732-74-5
Chem. Abstr. Name: Cyclopenta[cd]pyrene, 3,4-dihydro-
IUPAC Systematic Name: 3,4-Dihydrocyclopenta[*cd*]pyrene
Synonyms: 2,3-Acepyrene*; 3,4-dimethylenepyrene
*Alternative numbering convention

2.2 *Chemical and physical properties of the pure substance*

(a) *Description*: White crystals (Tintel *et al.*, 1983)
(b) *Melting-point*: 133–134 °C (Tintel *et al.*, 1983; Otero-Lobato, 2005)
(c) *Spectroscopy data*: NMR (Tintel *et al.*, 1983; Otero-Lobato *et al.*, 2005), UV, infrared and mass spectral data have been reported (Tintel *et al.*, 1983; NIST, 1998)

3. Anthanthrene

3.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 191-26-4
Chem. Abstr. Name: Dibenzo[def,mno]chrysene
IUPAC Systematic Name: Dibenzo[*def,mno*]chrysene
Synonym: Dibenzo[*cd,jk*]pyrene

3.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Golden yellow plates (recrystallized from xylene) (Clar, 1964)
(b) *Melting-point*: 264 °C (Karcher *et al.*, 1985)
(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1985; NIST, 1998).
(d) *Log K_{ow} (octanol–water)*: 7.04 (Howard & Meylan, 1997; Sangster Research Laboratories, 2005)

4. Anthracene

4.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 120-12-7
Chem. Abstr. Name: Anthracene
IUPAC Systematic Name: Anthracene*
Synonyms: Anthracin; paranaphthalene
*Numbering exception

4.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified

(a) *Description*: Monoclinic plates from alcohol. When pure, colourless with violet fluorescence; when impure (due to tetracene, naphthacene), yellow with green fluorescence
(b) *Boiling-point*: 342 °C
(c) *Melting-point*: 218 °C; 216.4 °C (Karcher *et al.*, 1985; Lide, 2005)
(d) *Density*: 1.25 at 27 °C relative to water at 4 °C; 1.283 at 25 °C relative to water at 4 °C (Lide, 2005)
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1985; NIST, 1998).
(f) *Water solubility*: 0.0436 at 25 °C (May *et al.*, 1983)
(g) *Vapour pressure*: 8.0×10^{-4} Pa at 25 °C (Sonnefeld *et al.*, 1983)
(h) *Log K_{ow} (octanol–water)*: 4.45 (Howard & Meylan, 1997); 4.54 (Miller *et al.*, 1985)
(i) *Henry's law constant*: 5.64 Pa m^3/mol at 25 °C (Bamford *et al.*, 1999)

5. 11*H*-Benz[*bc*]aceanthrylene

5.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 202-94-8
Chem. Abstr. Name: 11H-Benz[bc]aceanthrylene
IUPAC Systematic Name: 11H-Benz[*bc*]aceanthrylene
Synonyms: Benz[a]anthracene, 1,12-methylene-; 1′,9-methylene-1,2-benzanthracene

5.2 *Chemical and physical properties of the pure substance*

(a) *Melting-point*: 123 °C (Ray & Harvey, 1983); 120.5-121 °C (Harvey *et al.*, 1991)
(b) *Spectroscopy data*: NMR (Ray & Harvey, 1983) and UV/VIS (Harvey *et al.*, 1991) spectra have been reported.

6. Benz[*j*]aceanthrylene

6.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 202-33-5
Chem. Abstr. Name: Benz[j]aceanthrylene
IUPAC Systematic Name: Benz[*j*]aceanthrylene
Synonyms: Benz[7,8]aceanthrylene; cholanthrylene; naphth[2,1-*d*]acenaphthylene

6.2 *Chemical and physical properties of the pure substance*

From Sangaiah *et al.* (1983)
(a) Description: Orange plates from hexane
(b) Melting-point: 170–171 °C
(c) Spectroscopy data: UV/VIS, mass and NMR spectral data have been reported.

7. Benz[*l*]aceanthrylene

7.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 211-91-6
Chem. Abstr. Name: Benz[l]aceanthrylene
IUPAC Systematic Name: Benz[*l*]aceanthrylene
Synonym: Naphth[1,2-*d*]acenaphthylene

7.2 *Chemical and physical properties of the pure substance*

From Sangaiah *et al.* (1983)
(a) Melting-point: 157–158 °C
(b) Spectroscopy data: UV/VIS, mass and NMR spectral data have been reported.

8. Benz[*a*]anthracene

8.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 56-55-3
Chem. Abstr. Name: Benz[a]anthracene
IUPAC Systematic Name: Benz[*a*]anthracene
Synonyms: 1,2-Benz[*a*]anthracene; benzanthracene; 1,2-benzanthracene; benzanthrene; 1,2-benzanthrene; benzo[*a*]anthracene; benzoanthracene; 1,2-benzoanthracene; benzo[*b*]phenanthrene; 2,3-benzophenanthrene; tetraphene

8.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified

(a) Description: Plates from glacial acetic acid or alcohol with greenish-yellow fluorescence

(b) Melting-point: 160.7 °C (Karcher *et al.*, 1985)

(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

(d) Water solubility: 0.0090 mg/L at 25 °C (May *et al*., 1983)

(e) Vapour pressure: 2.8×10^{-5} Pa at 25 °C (Sonnefeld *et al*., 1983)

(f) Log K_{ow} (octanol–water): 5.91 (Miller *et al*., 1985; Sangster Research Laboratories, 2005)

(g) Henry's law constant: 1.22 Pa m^3/mol at 25 °C (Bamford *et al*., 1999)

9. Benzo[*b*]chrysene

9.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 214-17-5
Chem. Abstr. Name: Benzo[b]chrysene
IUPAC Systematic Name: Benzo[*b*]chrysene
Synonyms: 2,3-Benzochrysene; benzo[*c*]tetraphene; 3,4-benzotetraphene; 1,2:6,7-dibenzophenanthrene; 2,3:7,8-dibenzophenanthrene*; naphth[2,1-*a*]anthracene
*Alternative numbering convention

9.2 *Chemical and physical properties of the pure substance*

(a) Description: Pale green-yellow leaves from xylene (Lide, 1992)

(b) Melting-point: 294 °C (Lide, 1992); 299.7 °C (Karcher *et al.*, 1985)

(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

(d) Log K_{ow} (octanol–water): 7.11 (Sangster Research Laboratories, 2005)

10. Benzo[*g*]chrysene

10.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 196-78-1
Chem. Abstr. Name: Benzo[g]chrysene
IUPAC Systematic Name: Benzo[*g*]chrysene
Synonyms: Benzo[*a*]triphenylene; 1,2:3,4-dibenzophenanthrene; 1,2,3,4-dibenzphenanthrene; 1,2:3,4:7,8-tribenznaphthalene

10.2 *Chemical and physical properties of the pure substance*

(a) *Melting-point*: 116 °C (Sukumaran & Harvey, 1981); 114 °C (Agarwal *et al.*, 1985); 112–114 °C (Utermoehlen *et al*., 1987)

(b) *Spectroscopy data*: Mass and NMR spectral data have been reported (Sukumaran & Harvey, 1981; Agarwal *et al.*, 1985).

11. Benzo[*a*]fluoranthene

11.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 203-33-8
Chem. Abstr. Name: Benz[a]aceanthrylene
IUPAC Systematic Name: Benz[*a*]aceanthrylene
Synonyms: 1,2-Benzfluoranthene; benzo[*a*]aceanthrylene; 1,2-benzofluoranthene; dibenzo[*c,lm*]fluorene

11.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Golden needles from petroleum ether (Cho & Harvey, 1987a)

(b) *Melting-point*: 146.3 °C (Karcher *et al.*, 1985); 143-145 °C (Cho & Harvey, 1987a)

(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; Cho & Harvey, 1987b)

12. Benzo[*b*]fluoranthene

12.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 205-99-2
Chem. Abstr. Name: Benz[e]acephenanthrylene
IUPAC Systematic Name: Benz[*e*]acephenanthrylene
Synonyms: 3,4-Benz[*e*]acephenanthrylene*; 2,3-benzfluoranthene; 3,4-benzfluoranthene*; benzo[*e*]fluoranthene; 3,4-benzofluoranthene*
*Alternative numbering convention

12.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Needles from benzene (Lide, 2005)

(b) *Melting-point*: 168 °C (Lide, 2005); 168.3 °C (Karcher *et al.*, 1985)

(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

(d) *Water solubility*: 0.0015 mg/L (Howard & Meylan, 1997)

(e) *Log K_{ow} (octanol–water)*: 5.78 (Sangster Research Laboratories, 2005)

(f) Henry's law constant: 0.051 Pa m^3/mol at 20 °C (ten Hulscher *et al.*, 1992)

13. Benzo[*ghi*]fluoranthene

13.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 203-12-3
Chem. Abstr. Name: Benzo[ghi]fluoranthene
IUPAC Systematic Name: Benzo[*ghi*]fluoranthene
Synonyms: 2,13-Benzofluoranthene*; 7,10-benzofluoranthene*; benzo[*mno*]fluoranthene
*Alternative numbering convention

13.2 *Chemical and physical properties of the pure substance*

(a) Description: Yellow needles with greenish-yellow fluorescence (recrystallized from petroleum ether); blue fluorescence in solution (Buckingham, 1996)
(b) Melting-point: 128.4 °C (Karcher *et al.*, 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1985; NIST, 1998).
(d) Log K_{ow} (octanol–water): 6.63 (Sangster Research Laboratories, 2005)

14. Benzo[*j*]fluoranthene

14.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 205-82-3
Chem. Abstr. Name: Benzo[j]fluoranthene
IUPAC Systematic Name: Benzo[*j*]fluoranthene
Synonyms: 7,8-Benzfluoranthene; 10,11-benzofluoranthene*; benzo-12,13-fluoranthene*; dibenzo[*a,jk*]fluorene
*Alternative numbering convention

14.2 *Chemical and physical properties of the pure substance*

(a) Description: Yellow plates from alcohol; needles from acetic acid (Lide, 2005)
(b) Melting-point: 165.4 (Karcher *et al.*, 1985); 166 °C (Lide, 2005)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1985; NIST, 1998).
(d) Water solubility: 0.0025 mg/L (Howard & Meylan, 1997)

15. Benzo[*k*]fluoranthene

15.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 207-08-9
Chem. Abstr. Name: Benzo[k]fluoranthene
IUPAC Systematic Name: Benzo[*k*]fluoranthene
Synonyms: 8,9-Benzfluoranthene; 8,9-benzofluoranthene; 11,12-benzofluoranthene*;
2,3,1′,8′-binaphthylene; dibenzo[*b*,*jk*]fluorene
*Alternative numbering convention

15.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Pale yellow needles from benzene (Lide, 2005)
(b) *Boiling-point*: 480 °C (Lide, 2005)
(c) *Melting-point*: 215.7 °C (Karcher *et al.*, 1985); 217 °C (Lide, 2005)
(d) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(e) *Water solubility*: 0.0008 mg/L at 25 °C (Pearlman *et al.*, 1984)
(f) *Log K_{ow} (octanol–water)*: 6.11 (Sangster Research Laboratories, 2005)
(g) *Henry's law constant*: 0.044 Pa m^3/mol at 20 °C (ten Hulscher *et al*., 1992)

16. Benzo[*a*]fluorene

16.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 238-84-6
Chem. Abstr. Name: 11H-Benzo[a]fluorene
IUPAC Systematic Name: 11*H*-Benzo[*a*]fluorene
Synonyms: 1,2-Benzofluorene; chrysofluorene

16.2 *Chemical and physical properties of the pure substance*

From Lide (2005), unless otherwise specified
(a) *Description*: Plates from acetone or acetic acid
(b) *Boiling-point*: 413 °C
(c) *Melting-point*: 189–190 °C
(d) *Spectroscopy data*: UV/VIS, infrared and mass spectral data have been reported (NIST, 1998, 2005).
(e) *Water solubility*: 0.045 mg/L at 25 °C (Miller *et al*., 1985)
(f) *Log K_{ow} (octanol–water)*: 5.40 (Sangster Research Laboratories, 2005)
(g) *Henry's law constant*: 2.70 Pa m^3/mol at 25 °C (Bamford *et al*., 1999)

17. Benzo[*b*]fluorene

17.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 243-17-4
Chem. Abstr. Name: 11H-Benzo[b]fluorene
IUPAC Systematic Name: 11*H*-Benzo[*b*]fluorene
Synonym: 2,3-Benzofluorene

17.2 *Chemical and physical properties of the pure substance*

(a) Description: Crystals from petroleum ether or acetic acid (Buckingham, 1996)
(b) Boiling-point: 401–402 °C (Buckingham, 1996)
(c) Melting-point: 208–209 °C (Buckingham, 1996); 213.5 °C (Karcher *et al.*, 1988); 212 °C (Lide, 2005)
(d) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1988; NIST, 1998).
(e) Water solubility: 0.002 mg/L at 25 °C (Pearlman *et al.*, 1984; Miller *et al.*, 1985)
(f) Log K_{ow} (octanol–water): 5.75 (Sangster Research Laboratories, 2005)

18. Benzo[*c*]fluorene

18.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 205-12-9
Chem. Abstr. Name: 7H-Benzo[c]fluorene
IUPAC Systematic Name: 7*H*-Benzo[*c*]fluorene
Synonym: 3,4-Benzofluorene

18.2 *Chemical and physical properties of the pure substance*

(a) Description: Plates recrystallized from ethanol (Buckingham, 1996)
(b) Melting-point: 130–131 °C (Buckingham, 1996); 126.5 °C (Karcher *et al.*, 1988)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1988; NIST, 1998).

19. Benzo[*ghi*]perylene

19.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 191-24-2
Chem. Abstr. Name: Benzo[ghi]perylene

IUPAC Systematic Name: Benzo[*ghi*]perylene
Synonyms: 1,12-Benzoperylene; 1,12-benzperylene

19.2 *Chemical and physical properties of the pure substance*

(a) Description: Large, pale yellow-green plates from xylene (Clar, 1964)
(b) Melting-point: 278.3 °C (Karcher *et al*., 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) Water solubility: 0.00026 mg/L at 25 °C (Miller *et al*., 1985)
(e) Log K_{ow} (octanol–water): 6.90 (Sangster Research Laboratories, 2005)
(f) Henry's law constant: 0.027 Pa m^3/mol at 20 °C (ten Hulscher *et al.*, 1992)

20. Benzo[*c*]phenanthrene

20.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 195-19-7
Chem. Abstr. Name: Benzo[c]phenanthrene
IUPAC Systematic Name: Benzo[*c*]phenanthrene
Synonyms: 3,4-Benzophenanthrene; tetrahelicene

20.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) Description: Needles from alcohol
(b) Melting-point: 68 °C; 66.1 °C (Karcher *et al.*, 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998)

21. Benzo[*a*]pyrene

21.1 *Nomenclature*

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

21.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified

(a) Description: Yellowish plates, needles from benzene/methanol; crystals may be monoclinic or orthorhombic
(b) Boiling-point: 310–312 °C at 10 mm Hg
(c) Melting-point: 179–179.3 °C; 178.1 °C (Karcher *et al.*, 1985)
(d) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(e) Water solubility: 0.00162 mg/L at 25 °C (May *et al.*, 1983); 0.0038 mg/L at 25 °C (Miller *et al.*, 1985)
(f) Log K_{ow} (octanol–water): 6.35 (Sangster Research Laboratories, 2005)
(g) Henry's law constant: 0.034 Pa m^3/mol at 20 °C (ten Hulscher *et al*., 1992)

22. Benzo[*e*]pyrene

22.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 192-97-2
Chem. Abstr. Name: Benzo[e]pyrene
IUPAC Systematic Name: Benzo[*e*]pyrene
Synonyms: 1,2-Benzopyrene*; 4,5-benzopyrene; 1,2-benzpyrene*; 4,5-benzpyrene
*Alternative numbering convention

22.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) Description: Prisms or plates from benzene
(b) Melting-point: 178–179 °C; 178.7 (Karcher *et al.*, 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) Water solubility: 0.0063 mg/L at 25 °C (Pearlman *et al.*, 1984)
(e) Log K_{ow} (octanol–water): 6.44 (Sangster Research Laboratories, 2005)

23. Chrysene

23.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 218-01-9
Chem. Abstr. Name: Chrysene
IUPAC Systematic Name: Chrysene
Synonyms: Benzo[*a*]phenanthrene; 1,2-benzophenanthrene; 1,2-benzphenanthrene

23.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified

(a) *Description*: Orthorhombic bipyramidal plates from benzene
(b) *Boiling-point*: 448 °C
(c) *Melting-point*: 254°C; 253.8 °C (Karcher *et al*., 1985)
(d) *Density*: 1.274 at 20 °C relative to water at 4 °C
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(f) *Water solubility*: 0.00179 mg/L at 25 °C (May *et al*., 1983); 0.0020 mg/L at 25 °C (Miller *et al*., 1985)
(g) *Log K_{ow} (octanol–water)*: 5.79 (Miller *et al.*, 1985); 5.86 (Sangster Research Laboratories, 2005)
(h) *Henry's law constant*: 0.53 Pa m^3/mol at 25 °C (Bamford *et al*., 1999)

24. Coronene

24.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 191-07-1
Chem. Abstr. Name: Coronene
IUPAC Systematic Name: Coronene
Synonyms: Dibenzo[*ghi,pqr*]perylene; hexabenzobenzene

24.2 *Chemical and physical properties of the pure substance*

From Lide (2005), unless otherwise specified
(a) *Description*: Yellow needles from benzene
(b) *Boiling-point*: 525 °C
(c) *Melting-point*: 437.4 °C; 439 °C (Karcher *et al*., 1988)
(d) *Density*: 1.371 at 25 °C
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).
(f) *Water solubility*: 0.00014 mg/L at 25 °C (Miller *et al.*, 1985)
(g) *Log K_{ow} (octanol–water)*: 6.50 (Sangster Research Laboratories, 2005)

25. 4*H*-Cyclopenta[*def*]chrysene

25.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 202-98-2
Chem. Abstr. Name: 4H-Cyclopenta[def]chrysene
IUPAC Systematic Name: 4*H*-Cyclopenta[*def*]chrysene
Synonyms: 4,5-Methanochrysene; 4,5-methylenechrysene

25.2 *Chemical and physical properties of the pure substance*

(a) Melting-point: 171–173 °C (Nagel *et al.*, 1977); 172.5–173.5 (Harvey *et al.*, 1991)

(b) Spectroscopy data: UV/VIS, infrared and NMR spectral data have been reported (Harvey *et al.*, 1991).

26. Cyclopenta[*cd*]pyrene

26.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 27208-37-3
Chem. Abstr. Name: Cyclopenta[cd]pyrene
IUPAC Systematic Name: Cyclopenta[*cd*]pyrene
Synonyms: Acepyrene; acepyrylene; cyclopenta[*c*,*d*]pyrene

26.2 *Chemical and physical properties of the pure substance*

(a) Melting-point: 170 °C (Karcher *et al.*, 1985)

(b) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al.*, 1985; NIST, 1998).

27. 5,6-Cyclopenteno-1,2-benzanthracene

27.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 7099-43-6
Chem. Abstr. Name: 1H-Benzo[a]cyclopent[h]anthracene, 2,3-dihydro-
IUPAC Systematic Name: 2,3-Dihydro-1*H*-benzo[*a*]cyclopent[*h*]anthracene
Synonym: 5,6-Cyclopenteno-1,2-benzanthracene

27.2 *Chemical and physical properties of the pure substance*

(a) Melting-point: 197–199 °C (Cooke, 1932)

(b) Spectroscopy data: UV/VIS spectral data have been reported (Mayneord & Roe, 1935).

28. Dibenz[*a,c*]anthracene

28.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 215-58-7
Chem. Abstr. Name: Benzo[b]triphenylene
IUPAC Systematic Name: Benzo[*b*]triphenylene

Synonyms: 2,3-Benzotriphenylene; 1,2:3,4-dibenzanthracene; dibenzo[*a*,*c*]anthracene; 1,2:3,4-dibenzoanthracene

28.2 *Chemical and physical properties of the pure substance*

(a) Description: Needles from acetic acid or alcohol (Lide, 2005)
(b) Melting-point: 205 °C (Lide, 2005); 205.6 °C (Karcher *et al*., 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) Water solubility: 0.0016 mg/L at 25 °C (Howard & Meyland, 1997)
(e) Log K_{ow} (octanol–water): 6.17 (Sangster Research Laboratories, 2005)

29. Dibenz[*a,h*]anthracene

29.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 53-70-3
Chem. Abstr. Name: Dibenz[a,h]anthracene
IUPAC Systematic Name: Dibenz[*a,h*]anthracene
Synonyms: 1,2:5,6-Benzanthracene; 1,2:5,6-dibenz[*a*]anthracene; 1,2:5,6-dibenzanthracene; dibenzo[*a*,*h*]anthracene; 1,2:5,6-dibenzoanthracene

29.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) Description: Plates or leaflets from acetic acid; crystals may be monoclinic or orthorhombic
(b) Melting-point: 266 °C; 266.6 °C (Karcher *et al.*, 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) Water solubility: 0.00050 mg/L at 25 °C (Miller *et al*., 1985)
(e) Log K_{ow} (octanol–water): 6.75 (Sangster Research Laboratories, 2005)

30. Dibenz[*a,j*]anthracene

30.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 224-41-9
Chem. Abstr. Name: Dibenz[a,j]anthracene
IUPAC Systematic Name: Dibenz[*a,j*]anthracene
Synonyms: 1,2:7,8-Dibenzanthracene; 3,4:5,6-dibenzanthracene; dibenzo-1,2,7,8-anthracene

30.2 *Chemical and physical properties of the pure substance*

(a) Description: Orange leaves or needles from benzene (Lide, 2005)
(b) Melting-point: 197.3 °C (Karcher *et al.*, 1985); 197.5 °C (Lide, 2005)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) Water solubility: 0.012 mg/L at 25 °C (Pearlman *et al*., 1984)
(e) Log K_{ow} (octanol–water): 7.11 (Sangster Research Laboratories, 2005)

31. Dibenzo[*a,e*]fluoranthene

31.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 5385-75-1
Chem. Abstr. Name: Dibenz[a,e]aceanthrylene
IUPAC Systematic Name: Dibenz[*a,e*]aceanthrylene
Synonym: 2,3,5,6-Dibenzofluoranthene

31.2 *Chemical and physical properties of the pure substance*

(a) Description: Yellow needles (recrystallized from benzene) (Buckingham, 1996)
(b) Melting-point: 232 °C (Buckingham, 1996); 232 °C (Karcher *et al.*, 1988)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).

32. 13*H*-Dibenzo[*a,g*]fluorene

32.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 207-83-0
Chem. Abstr. Name: 13H-Dibenzo[a,g]fluorene
IUPAC Systematic Name: 13H-Dibenzo[*a,g*]fluorene
Synonyms: Dibenzo[*a,g*]fluorene; 1,2,5,6-dibenzofluorene

32.2 *Chemical and physical properties of the pure substance*

(a) Melting-point: 176–178 °C (Hopkinson *et al.*, 1986); 175–175.5 °C (Harvey *et al.*, 1991)
(b) Spectroscopy data: UV/VIS and NMR spectral data have been reported (Hopkinson *et al.*, 1986; Harvey *et al.*, 1991).

33. Dibenzo[*h,rst*]pentaphene

33.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 192-47-2
Chem. Abstr. Name: Dibenzo[h,rst]pentaphene
IUPAC Systematic Name: Dibenzo[*h,rst*]pentaphene
Synonyms: Tribenzo[*a,e,i*]pyrene; 1,2:4,5:7,8-tribenzpyrene

33.2 *Chemical and physical properties of the pure substance*

(a) Melting-point: 315 °C (Blümer et al., 1976)
(b) Spectroscopy data: Infrared and mass spectral data have been reported (NIST, 1998, 2005).

34. Dibenzo[*a,e*]pyrene

34.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 192-65-4
Chem. Abstr. Name: Naphtho[1,2,3,4-def]chrysene
IUPAC Systematic Name: Naphtho[1,2,3,4-*def*]chrysene
Synonym: 1,2:4,5-Dibenzopyrene

34.2 *Chemical and physical properties of the pure substance*

(a) Description: Pale yellow needles from xylene (Lide, 2005)
(b) Melting-point: 244.4 °C (Karcher *et al*., 1985); 233.5 °C (Lide, 2005)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

35. Dibenzo[*a,h*]pyrene

35.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 189-64-0
Chem. Abstr. Name: Dibenzo[b,def]chrysene
IUPAC Systematic Name: Dibenzo[*b,def*]chrysene
Synonym: 3,4:8,9-Dibenzopyrene*
*Alternative numbering convention

35.2 *Chemical and physical properties of the pure substance*

(a) Description: Golden-yellow plates, recrystallized from xylene or trichlorobenzene (Lide, 2005)
(b) Melting-point: 317 °C (Karcher *et al.*, 1988); 315 °C (Lide, 2005)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).

36. Dibenzo[*a,i*]pyrene

36.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 189-55-9
Chem. Abstr. Name: Benzo[rst]pentaphene
IUPAC Systematic Name: Benzo[*rst*]pentaphene
Synonyms: Benzo[*rst*]pentacene; dibenzo[*b,h*]pyrene; 1,2:7,8-dibenzpyrene

36.2 *Chemical and physical properties of the pure substance*

(a) Description: Greenish yellow needles, prisms or lamellae (IARC, 1983)
(b) Melting-point: 282 °C (Karcher *et al.*, 1988); 281.5 °C (Lide, 2005)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).

37. Dibenzo[*a,l*]pyrene

37.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 191-30-0
Chem. Abstr. Name: Dibenzo[def,p]chrysene
IUPAC Systematic Name: Dibenzo[*def,p*]chrysene
Synonym: 1,2,9,10-Dibenzopyrene

37.2 *Chemical and physical properties of the pure substance*

(a) Description: Yellow plates, from ethanol–benzene (Lide, 2005)
(b) Melting-point: 162.4 °C (Karcher *et al*., 1985)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; 1988; NIST, 1998).
(d) Log K_{ow} (octanol–water): 7.71 (Sangster Research Laboratories, 2005)

38. Dibenzo[*e,l*]pyrene

38.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 192-51-8
Chem. Abstr. Name: Dibenzo[fg,op]naphthacene
IUPAC Systematic Name: Dibenzo[*fg,op*]naphthacene
Synonyms: 4,5,9,10-Dibenzopyrene; dibenzotetracene

38.2 *Chemical and physical properties of the pure substance*

(a) *Spectroscopy data*: UV/VIS (Yu & Campiglia, 2004), infrared (Weisman *et al.*, 2005), phosphorescence (Schmidt *et al.*, 1987) and mass (NIST, 1998) spectral data have been reported.

39. 1,2-Dihydroaceanthrylene

39.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 641-48-5
Chem. Abstr. Name: Aceanthrylene, 1,2-dihydro-
IUPAC Systematic Name: 1,2-Dihydroaceanthrylene
Synonym: Aceanthrene

39.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Yellow, flaky crystals (Becker *et al.*, 1985); colourless solid (Otero-Lobato *et al.*, 2005)
(b) *Melting-point*: 118–119 °C (Becker *et al.*, 1985); 112–113 °C (Olde Boerrigter *et al.*, 1989; Otero-Lobato *et al.*, 2005)
(c) *Spectroscopy data*: Proton and carbon-13 NMR and mass spectral data have been reported (Becker *et al.*, 1985; Olde Boerrigter *et al.*, 1989; Otero-Lobato *et al.*, 2005).

40. 1,4-Dimethylphenanthrene

40.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 22349-59-3
Chem. Abstr. Name: Phenanthrene, 1,4-dimethyl-
IUPAC Systematic Name: 1,4-Dimethylphenanthrene

40.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Long, white needles recrystallized from methanol (Papa *et al*., 1938); colourless needles from petroleum ether (Jung & Koreeda, 1989)
(b) *Melting-point*: 49.5–50.5 °C (Papa *et al.*, 1938); 50–51 °C (Jung & Koreeda, 1989)
(c) *Spectroscopy data*: Infrared, and proton and carbon-13 NMR spectral data have been reported (Jung & Koreeda, 1989).

41. Fluoranthene

41.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 206-44-0
Chem. Abstr. Name: Fluoranthene
IUPAC Systematic Name: Fluoranthene
Synonyms: 1,2-Benzacephthalene; 1,2-benzoacenaphthylene; benzo[*jk*]fluorene; 1,2-idryl; 1,2-(1,8-naphthalenediyl)benzene

41.2 *Chemical and physical properties of the pure substance*

From Lide (2005), unless otherwise specified
(a) *Description*: Pale yellow needles or plates from alcohol
(b) *Boiling-point*: 384 °C
(c) *Melting-point*: 110.19 °C; 108.8 °C (Karcher *et al*., 1985)
(d) *Density*: 1.252 at 0 °C relative to water at 4 °C
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(f) *Water solubility*: 0.205 mg/L at 25 °C (May *et al*., 1983); 0.26 mg/L at 25 °C (Miller *et al*., 1985)
(g) *Vapour pressure*: 0.00123 Pa at 25 °C (Sonnefeld *et al.*, 1983)
(h) *Log K_{ow} (octanol–water)*: 5.20 (Sangster Research Laboratories, 2005)
(i) *Henry's law constant*: 1.96 Pa m^3/mol at 25 °C (Bamford *et al*., 1999)
(j) *Atmospheric OH rate constant*: ~1.8×10^{-11} cm^3/mol/s at 100 °C (Calvert *et al.*, 2002)

42. Fluorene

42.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 86-73-7
Chem. Abstr. Name: 9H-Fluorene
IUPAC Systematic Name: 9*H*-Fluorene

Synonyms: *ortho*-Biphenylenemethane; diphenylenemethane; methane, diphenylene-; 2,2′-methylenebiphenyl

42.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified

(a) *Description*: White leaflets or flakes from alcohol
(b) *Boiling-point*: 295 °C
(c) *Melting-point*: 116–117 °C; 115–116 °C (Karcher *et al*., 1988)
(d) *Density*: 1.20
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).
(f) *Water solubility*: 1.68 mg/L at 25 °C (May *et al*., 1983)
(g) *Vapour pressure*: 0.08 Pa at 25 °C (Sonnefeld *et al*., 1983)
(h) *Log K_{ow} (octanol–water)*: 4.18 (Miller *et al*., 1985); 4.18 (Sangster Research Laboratories, 2005)
(i) *Henry's law constant*: 9.81 Pa m^3/mol at 25 °C (Bamford *et al.*, 1999)
(j) *Atmospheric OH rate constant*: 1.4×10^{-11} cm^3/ mol/s at 25 °C (Calvert *et al*., 2002)

43. Indeno[1,2,3-*cd*]pyrene

43.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 193-39-5
Chem. Abstr. Name: Indeno[1,2,3-cd]pyrene
IUPAC Systematic Name: Indeno[1,2,3-*cd*]pyrene
Synonyms: 1,10-(*ortho*-Phenylene)pyrene; 1,10-(1,2-phenylene)pyrene

43.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Yellow plates or needles (recrystallized from light petroleum solution) showing a greenish yellow fluorescence; yellow cristals from cyclohexane (IARC, 1983; Lide, 2005)
(b) *Melting-point*: 163.6 °C (Karcher *et al.*, 1985)
(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) *Water solubility*: 0.00019 mg/L at 25 °C (Pearlman *et al*., 1984)
(e) *Henry's law constant*: 0.029 Pa m^3/mol at 20 °C (ten Hulscher *et al*., 1992)

44. 1-Methylchrysene

44.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 3351-28-8
Chem. Abstr. Name: Chrysene, 1-methyl-
IUPAC Systematic Name: 1-Methylchrysene

44.2 *Chemical and physical properties of the pure substance*

(a) Description: Leaflets recrystallized from benzene, hexane or toluene (Lide, 1992)
(b) Melting-point: 254.4 °C (Karcher *et al.*, 1985); 256–257 °C (Lide, 1992)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

45. 2-Methylchrysene

45.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 3351-32-4
Chem. Abstr. Name: Chrysene, 2-methyl-
IUPAC Systematic Name: 2-Methylchrysene

45.2 *Chemical and physical properties of the pure substance*

(a) Description: Leaflets recrystallized from benzene–alcohol (Lide, 1992)
(b) Melting-point: 230.2 °C (Karcher *et al.*, 1985); 229–230 °C (Lide, 1992)
(c) Spectroscopy data: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

46. 3-Methylchrysene

46.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 3351-31-3
Chem. Abstr. Name: Chrysene, 3-methyl-
IUPAC Systematic Name: 3-Methylchrysene

46.2 *Chemical and physical properties of the pure substance*

(a) Description: Leaflets recrystallized from benzene–petroleum ether (Lide, 2005)
(b) Melting-point: 171.9 °C (Karcher *et al.*, 1985); 173.3 °C (Lide, 2005)

(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).

47. 4-Methylchrysene

47.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 3351-30-2
Chem. Abstr. Name: Chrysene, 4-methyl-
IUPAC Systematic Name: 4-Methylchrysene

47.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Highly fluorescent plates recrystallized from benzene–ethanol (Buckingham, 1996)
(b) *Melting-point*: 150.6 °C (Karcher *et al.*, 1985); 151–152 °C (Buckingham, 1996)
(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported spectra (Karcher *et al*., 1985; NIST, 1998).

48. 5-Methylchrysene

48.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 3697-24-3
Chem. Abstr. Name: Chrysene, 5-methyl-
IUPAC Systematic Name: 5-Methylchrysene

48.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Needles recrystallized from benzene–ethanol with a brilliant bluish violet fluorescence in UV light (Buckingham, 1996)
(b) *Melting-point*: 117.1 °C (Karcher *et al.*, 1985); 118–119 °C (Buckingham, 1996)
(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) *Water solubility*: 0.062 mg/L at 27 °C (Howard & Meylan, 1997)

49. 6-Methylchrysene

49.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 1705-85-7
Chem. Abstr. Name: Chrysene, 6-methyl-

IUPAC Systematic Name: 6-Methylchrysene

49.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Fluorescent needles recrystallized from ethyl acetate–ethanol (Buckingham, 1996)
(b) *Melting-point*: 160–161 °C (Karcher *et al.*, 1985); 161–162 °C (Buckingham, 1996)
(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(d) *Water solubility*: 0.065 mg/L at 27 °C (Howard & Meylan, 1997)

50. 2-Methylfluoranthene

50.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 33543-31-6
Chem. Abstr. Name: Fluoranthene, 2-methyl-
IUPAC Systematic Name: 2-Methylfluoranthene
Note: Previously 3-methylfluoranthene

50.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Ill-defined needles after crystallization from methanol (Tucker, 1952)
(b) *Melting-point*: 79–81 °C (Tucker, 1952)
(c) *Spectroscopy data*: Mass spectral data have been reported (NIST, 1998).

51. 3-Methylfluoranthene

51.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 1706-01-0
Chem. Abstr. Name: Fluoranthene, 3-methyl-
IUPAC Systematic Name: 3-Methylfluoranthene
Note: Previously 4-methylfluoranthene

51.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Pale green sword blades recrystallized from ethanol (Stubbs & Tucker, 1950)
(b) *Melting-point*: 65–66 °C (Stubbs & Tucker, 1950); 66–68 °C (Karcher *et al.*, 1991)

(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1991).

52. 1-Methylphenanthrene

52.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 832-69-9
Chem. Abstr. Name: Phenanthrene, 1-methyl-
IUPAC Systematic Name: 1-Methylphenanthrene

52.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Leaves or plates recrystallized from diluted alcohol (Lide, 2005)
(b) *Melting-point*: 123 °C (Lide, 2005; Karcher *et al.*, 1991)
(c) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1991; NIST, 1998).
(d) *Water solubility*: 0.269 mg/L at 25 °C (May *et al.*, 1983)
(e) *Log K_{ow} (octanol–water)*: 5.08 (Sangster Research Laboratories, 2005)
(f) *Henry's law constant*: 5.00 Pa m^3/mol at 25 °C (Bamford *et al*., 1999)
(g) *Atmospheric OH rate constant*: 2.88×10^{-11} cm^3/mol/s at 25 °C (Lee *et al.*, 2003)

53. Naphtho[1,2-*b*]fluoranthene

53.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 111189-32-3
Chem. Abstr. Name: Indeno[1,2,3-hi]chrysene
IUPAC Systematic Name: Indeno[1,2,3-*hi*]chrysene

53.2 *Chemical and physical properties of the pure substance*

(a) *Description*: Long greenish yellow needles from ethyl acetate/hexane (Cho & Harvey, 1987a)
(b) *Melting-point*: 188–189 °C (Cho & Harvey, 1987a)
(c) *Spectroscopy data*: UV/VIS, and proton and carbon-13 NMR spectral data have been reported (Cho & Harvey, 1987a,b).

54. Naphtho[2,1-*a*]fluoranthene

54.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 203-20-3

Chem. Abstr. Name: Dibenz[a,j]aceanthrylene
IUPAC Systematic Name: Dibenz[*a,j*]aceanthrylene
Synonym: 15,16-Benzodehydrocholanthrene

54.2 *Chemical and physical properties of the pure substance*

(a) Description: Bright yellow, cottony needles recrystallized from benzene (Fieser & Seligman, 1935)
(b) Melting-point: 181–181.3 °C (Fieser & Seligman, 1935); 180 °C (Ray & Harvey, 1982; Cho & Harvey, 1987a)
(c) Spectroscopy data: NMR spectral data have been reported (Ray & Harvey, 1982; Cho & Harvey, 1987a).

55. Naphtho[2,3-*e*]pyrene

55.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 193-09-9
Chem. Abstr. Name: Dibenzo[de,qr]naphthacene
IUPAC Systematic Name: Dibenzo[*de,qr*]naphthacene
Synonyms: Dibenzo[*de,qr*]tetracene; naphtho-(2′,3′:4,5)-pyrene

55.2 *Chemical and physical properties of the pure substance*

(a) Spectroscopy data: UV/VIS and fluorescence spectral data have been reported (Schmidt *et al.*, 1987; NIST, 2005).

56. Perylene

56.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 198-55-0
Chem. Abstr. Name: Perylene
IUPAC Systematic Name: Perylene
Synonyms: Dibenz[*de,kl*]anthracene; *peri*-dinaphthalene; α-perylene

56.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) Description: Yellow to colourless crystals from toluene
(b) Boiling-point: Sublimes at 350–400 °C
(c) Melting-point: 273–274 °C; 277.5 °C (Karcher *et al.*, 1988)
(d) Density: 1.35

(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).
(f) *Water solubility*: 0.00040 mg/L at 25 °C (Miller *et al.*, 1985)
(g) *Vapour pressure*: 5.85×10^{-7} Pa at 25 °C (Howard & Meylan, 1997)
(h) *Log K_{ow} (octanol–water)*: 6.25 (Sangster Research Laboratories, 2005)

57. Phenanthrene

57.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 85-01-8
Chem. Abstr. Name: Phenanthrene
IUPAC Systematic Name: Phenanthrene*
Synonym: Phenanthrin
*Numbering exception

57.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) *Description*: Monoclinic plates from alcohol
(b) *Boiling-point*: 340 °C
(c) *Melting-point*: 100 °C; 100.5 °C (Karcher *et al.*, 1985); 99.24 °C (Lide, 2005)
(d) *Density*: 1.179 at 25 °C; 0.9800 relative to water at 4 °C (Lide, 2005)
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(f) *Water solubility*: 0.977 mg/L at 25 °C (May *et al.*, 1983); 1.18 mg/L at 25 °C (Miller *et al.*, 1985)
(g) *Vapour pressure*: 0.016 Pa at 25 °C (Sonnefeld *et al.*, 1983)
(h) *Log K_{ow} (octanol–water)*: 4.57 (Miller *et al.*, 1985); 4.52 (Sangster Research Laboratories, 2005)
(i) *Henry's law constant*: 4.29 Pa m^3/mol at 25 °C (Bamford *et al.*, 1999)
(j) *Atmospheric OH rate constant*: 1.8×10^{-11} cm^3/mol/s at 25 °C (Calvert *et al.*, 2002)

58. Picene

58.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 213-46-7
Chem. Abstr. Name: Picene
IUPAC Systematic Name: Picene
Synonyms: Benzo[*a*]chrysene; dibenzo[*a*,*i*]phenanthrene; 1,2;7,8-dibenzophenanthrene

58.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) *Description*: Fluorescent plates from ethyl acetate
(b) *Boiling-point*: 518–520 °C
(c) *Melting-point*: 366–367 °C; 364 °C (Karcher *et al*., 1988)
(d) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).
(e) *Water solubility*: 0.00431 mg/L at 20 °C (Howard & Meylan, 1997)
(f) *Log K_{ow} (octanol–water)*: 7.11 (Sangster Research Laboratories, 2005)

59. Pyrene

59.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 129-00-0
Chem. Abstr. Name: Pyrene
IUPAC Systematic Name: Pyrene
Synonyms: Benzo[*def*]phenanthrene; β-pyrene

59.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified
(a) *Description*: Monoclinic prismatic tablets from alcohol
(b) *Boiling-point*: 404 °C
(c) *Melting-point*: 156 °C; 150.4 °C (Karcher *et al*., 1985); 150.6 °C (Lide, 2005)
(d) *Density*: 1.271 at 23 °C; 1.271 at 23 °C relative to water at 4 °C (Lide, 2005)
(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1985; NIST, 1998).
(f) *Water solubility*: 0.130 mg/L at 25 °C (May *et al*., 1983); 0.135 mg/L (Miller *et al*., 1985)
(g) *Vapour pressure*: 0.00060 Pa at 25 °C (Sonnefeld *et al*., 1983)
(h) *Log K_{ow} (octanol–water)*: 5.18 (Miller *et al*., 1985); 5.00 (Sangster Research Laboratories, 2005)
(i) *Henry's law constant*: 1.71 Pa m^3/mol at 25 °C (Bamford *et al*., 1999)

60. Triphenylene

60.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 217-59-4
Chem. Abstr. Name: Triphenylene
IUPAC Systematic Name: Triphenylene

Synonyms: Benzo[*l*]phenanthrene; 9,10-benzophenanthrene; 9,10-benzphenanthrene; 1,2,3,4-dibenznaphthalene; isochrysene

60.2 *Chemical and physical properties of the pure substance*

From O'Neil (2006), unless otherwise specified

(a) *Description*: Long needles recrystallized from alcohol or chloroform

(b) *Boiling-point*: 425 °C

(c) *Melting-point*: 199 °C; 199 ^{o}C (Karcher *et al.*, 1988)

(d) *Density*: 1.302

(e) *Spectroscopy data*: UV/VIS, infrared, fluorescence, mass and NMR spectral data have been reported (Karcher *et al*., 1988; NIST, 1998).

(f) *Water solubility*: 0.00632 mg/L at 25 °C (May *et al.*, 1983)

(g) *Log K_{ow} (octanol–water)*: 5.49 (Sangster Research Laboratories, 2005)

Table 1. Identification of the polycyclic aromatic hydrocarbons covered in this monograph

Common name (name used in this volume)	IUPAC name	CAS Registry No.	Molecular formula	Relative molecular mass
Acenaphthene	Acenaphthene	83-32-9	$C_{12}H_{10}$	154.2
Acepyrene	3,4-Dihydrocyclopenta[*cd*]pyrene	25732-74-5	$C_{18}H_{12}$	228.3
Anthanthrene	Dibenzo[*def,mno*]chrysene	191-26-4	$C_{22}H_{12}$	276.3
Anthracene	Anthracene	120-12-7	$C_{14}H_{10}$	178.2
11*H*-Benz[*b,c*]aceanthrylene	11*H*-Benz[*b,c*]aceanthrylene	202-94-8	$C_{19}H_{12}$	240.3
Benz[*j*]aceanthrylene	Benz[*j*]aceanthrylene	202-33-5	$C_{20}H_{12}$	252.3
Benz[*l*]aceanthrylene	Benz[*l*]aceanthrylene	211-91-6	$C_{20}H_{12}$	252.3
Benz[*a*]anthracene	Benz[*a*]anthracene	56-55-3	$C_{18}H_{12}$	228.3
Benzo[*b*]chrysene	Benzo[*b*]chrysene	214-17-5	$C_{22}H_{14}$	278.4
Benzo[*g*]chrysene	Benzo[*g*]chrysene	196-78-1	$C_{22}H_{14}$	278.4
Benzo[*a*]fluoranthene	Benz[*a*]aceanthrylene	203-33-8	$C_{20}H_{12}$	252.3
Benzo[*b*]fluoranthene	Benz[*e*]acephenanthrylene	205-99-2	$C_{20}H_{12}$	252.3
Benzo[*ghi*]fluoranthene	Benzo[*ghi*]fluoranthene	203-12-3	$C_{18}H_{10}$	226.3
Benzo[*j*]fluoranthene	Benzo[*j*]fluoranthene	205-82-3	$C_{20}H_{12}$	252.3
Benzo[*k*]fluoranthene	Benzo[*k*]fluoranthene	207-08-9	$C_{20}H_{12}$	252.3
Benzo[*a*]fluorene	11*H*-Benzo[*a*]fluorene	238-84-6	$C_{17}H_{12}$	216.3
Benzo[*b*]fluorene	11*H*-Benzo[*b*]fluorene	243-17-4	$C_{17}H_{12}$	216.3
Benzo[*c*]fluorene	7*H*-Benzo[*c*]fluorene	205-12-9	$C_{17}H_{12}$	216.3
Benzo[*ghi*]perylene	Benzo[*ghi*]perylene	191-24-2	$C_{22}H_{12}$	276.3
Benzo[*c*]phenanthrene	Benzo[*c*]phenanthrene	195-19-7	$C_{18}H_{12}$	228.3
Benzo[*a*]pyrene	Benzo[*a*]pyrene	50-32-8	$C_{20}H_{12}$	252.3
Benzo[*e*]pyrene	Benzo[*e*]pyrene	192-97-2	$C_{20}H_{12}$	252.3
Chrysene	Chrysene	218-01-9	$C_{18}H_{12}$	228.3
Coronene	Coronene	191-07-1	$C_{24}H_{12}$	300.4
4*H*-Cyclopenta[*def*]chrysene	4*H*-Cyclopenta[*def*]chrysene	202-98-2	$C_{19}H_{12}$	240.3
Cyclopenta[*cd*]pyrene	Cyclopenta[*cd*]pyrene	27208-37-3	$C_{18}H_{10}$	226.3
5,6-Cyclopenteno-1,2-benzanthracene	2,3-Dihydro-1*H*-benzo[*a*]cyclopent[*h*]anthracene	7099-43-6	$C_{21}H_{16}$	268.4
Dibenz[*a,c*]anthracene	Benzo[*b*]triphenylene	215-58-7	$C_{22}H_{14}$	278.4
Dibenz[*a,h*]anthracene	Dibenz[*a,h*]anthracene	53-70-3	$C_{22}H_{14}$	278.4

Table 1 (Contd)

Common name (name used in this volume)	IUPAC name	CAS Registry No.	Molecular formula	Relative molecular mass
Dibenz[*a,j*]anthracene	Dibenz[*a,j*]anthracene	224-41-9	$C_{22}H_{14}$	278.4
Dibenzo[*a,e*]fluoranthene	Dibenz[*a,e*]aceanthrylene	5385-75-1	$C_{24}H_{14}$	302.4
13*H*-Dibenzo[*a,g*]fluorene	13*H*-Dibenzo[*a,g*]fluorene	207-83-0	$C_{21}H_{14}$	266.3
Dibenzo[*h,rst*]pentaphene	Dibenzo[*h,rst*]pentaphene	192-47-2	$C_{28}H_{16}$	352.4
Dibenzo[*a,e*]pyrene	Naphtho[1,2,3,4-*def*]chrysene	192-65-4	$C_{24}H_{14}$	302.4
Dibenzo[*a,h*]pyrene	Dibenzo[*b,def*]chrysene	189-64-0	$C_{24}H_{14}$	302.4
Dibenzo[*a,i*]pyrene	Benzo[*rst*]pentaphene	189-55-9	$C_{24}H_{14}$	302.4
Dibenzo[*a,l*]pyrene	Dibenzo[*def,p*]chrysene	191-30-0	$C_{24}H_{14}$	302.4
Dibenzo[*e,l*]pyrene	Dibenzo[*fg,op*]naphthacene	192-51-8	$C_{24}H_{14}$	302.4
1,2-Dihydroaceanthrylene	1,2-Dihydroaceanthrylene	641-48-5	$C_{16}H_{12}$	204.3
1,4-Dimethylphenanthrene	1,4-Dimethylphenanthrene	22349-59-3	$C_{16}H_{14}$	206.3
Fluoranthene	Fluoranthene	206-44-0	$C_{16}H_{10}$	202.3
Fluorene	9*H*-Fluorene	86-73-7	$C_{13}H_{10}$	166.2
Indeno[1,2,3-*cd*]pyrene	Indeno[1,2,3-cd]pyrene	193-39-5	$C_{22}H_{12}$	276.3
1-Methylchrysene	1-Methylchrysene	3351-28-8	$C_{19}H_{14}$	242.3
2-Methylchrysene	2-Methylchrysene	3351-32-4	$C_{19}H_{14}$	242.3
3-Methylchrysene	3-Methylchrysene	3351-31-3	$C_{19}H_{14}$	242.3
4-Methylchrysene	4-Methylchrysene	3351-30-2	$C_{19}H_{14}$	242.3
5-Methylchrysene	5-Methylchrysene	3697-24-3	$C_{19}H_{14}$	242.3
6-Methylchrysene	6-Methylchrysene	1705-85-7	$C_{19}H_{14}$	242.3
2-Methylfluoranthene	2-Methylfluoranthene	33543-31-6	$C_{17}H_{12}$	216.3
3-Methylfluoranthene	3-Methylfluoranthene	1706-01-0	$C_{17}H_{12}$	216.3
1-Methylphenanthrene	1-Methylphenanthrene	832-69-9	$C_{15}H_{12}$	192.3
Naphtho[1,2-*b*]fluoranthene	Indeno[1,2,3-*hi*]chrysene	111189-32-3	$C_{24}H_{14}$	302.4
Naphtho[2,1-*a*]fluoranthene	Dibenz[*a,j*]aceanthrylene	203-20-3	$C_{24}H_{14}$	302.4
Naphtho[2,3-*e*]pyrene	Dibenzo[*de,qr*]naphthacene	193-09-9	$C_{24}H_{14}$	302.4
Perylene	Perylene	198-55-0	$C_{20}H_{12}$	252.3

Table 1 (Contd)

Common name (name used in this volume)	IUPAC name	CAS Registry No.	Molecular formula	Relative molecular mass
Phenanthrene	Phenanthrene	85-01-8	$C_{14}H_{10}$	178.2
Picene	Picene	213-46-7	$C_{22}H_{14}$	278.4
Pyrene	Pyrene	129-00-0	$C_{16}H_{10}$	202.3
Triphenylene	Triphenylene	217-59-4	$C_{18}H_{12}$	228.3

Compiled from IUPAC (1979), Chemical Abstract Services (1978)

Table 2. Selected physical and chemical properties of the polycyclic aromatic compounds covered in this monograph[a]

Compound	Melting-point (°C)	Vapour pressure (Pa at 25 °C)	*n*-Octanol:water partition coefficient (log K_{ow})	Solubility in water at 25 °C[d] (mg/L)	Henry's law constant at 25 °C (Pa•m^3/mol)
Acenaphthene	95	0.29	3.92	3.9	18.5
Acepyrene	133–134	–	–	–	–
Anthanthrene	264	–	7.04	–	–
Anthracene	216.4	8.0×10^{-4}	4.5	0.0436	5.64
11*H*-Benz[*b*,*c*]aceanthrylene	123	–	–	–	–
Benz[*j*]aceanthrylene	170–171	–	–	–	–
Benz[*l*]aceanthrylene	157–158	–	–	–	–
Benz[*a*]anthracene	160.7	2.8×10^{-5}	5.91	0.0090	1.22
Benzo[*b*]chrysene	294	–	7.11	–	–
Benzo[*g*]chrysene	116	–	–	–	–
Benzo[*a*]fluoranthene	146.3	–	–	–	–
Benzo[*b*]fluoranthene	168.3	–	5.78	0.0015	0.051
Benzo[ghi]fluoranthene	128.4	–	6.63	–	–
Benzo[*j*]fluoranthene	165.4	–	–	0.0025	–
Benzo[*k*]fluoranthene	215.7	–	6.11	0.0008	0.044
Benzo[*a*]fluorene	189–190	–	5.40	0.045	2.70
Benzo[*b*]fluorene	213.5	–	5.75	0.002	–
Benzo[*c*]fluorene	126.5	–	–	–	–
Benzo[*ghi*]perylene	278.3	–	6.90	0.00026	0.027 (20 °C)
Benzo[*c*]phenanthrene	66.1	–	–	–	–
Benzo[*a*]pyrene	178.1	–	6.35	0.00162; 0.0038	0.034
Benzo[*e*]pyrene	178.7	–	6.44	0.0063	–
Chrysene	253.8	–	5.86	0.00179	0.53
Coronene	439	–	6.50	0.00014	–
4*H*-Cyclopenta[*def*]chrysene	171–173	–	–	–	–
Cyclopenta[*cd*]pyrene	170	–	–	–	–
5,6-Cyclopenteno-1,2-benzanthracene	197–199	–	–	–	–

Table 2 (Contd)

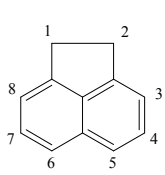
Compound	Melting-point (°C)	Vapour pressure (Pa at 25 °C)	*n*-Octanol:water partition coefficient (log K_{ow})	Solubility in water at 25 °C[d] (mg/L)	Henry's law constant at 25 °C (Pa•m^3/mol)
Dibenz[*a*,*c*]anthracene	205.6	–	6.17	0.0016	–
Dibenz[*a*,*h*]anthracene	266.6	–	6.75	0.00050	–
Dibenz[*a*,*j*]anthracene	197.3	–	7.11	0.012	–
Dibenzo[*a*,*e*]fluoranthene	232	–	–	–	–
13*H*-Dibenzo[*a*,*g*]fluorene	176–178	–	–	–	–
Dibenzo[*h*,*rst*]pentaphene	315	–	–	–	–
Dibenzo[*a*,*e*]pyrene	244.4	–	–	–	–
Dibenzo[*a*,*h*]pyrene	317	–	–	–	–
Dibenzo[*a*,*i*]pyrene	282	–	–	–	–
Dibenzo[*a*,*l*]pyrene	162.4	–	7.71	–	–
Dibenzo[*e*,*l*]pyrene	–	–	–	–	–
1,2-Dihydroaceanthrylene	118–119	–	–	–	–
1,4-Dimethylphenanthrene	50–51	–	–	–	-
Fluoranthene	108.8	0.00123	5.20	0.205; 0.26	1.96
Fluorene	115–116	0.08	4.18	1.68	9.81
Indeno[1,2,3-*cd*]pyrene	163.6	–	–	0.00019	0.029 (20 °C)
1-Methylchrysene	254.4	–	–	–	–
2-Methylchrysene	230.2	–	–	–	–
3-Methylchrysene	171.9	–	–	–	–
4-Methylchrysene	150.6	–	–	–	–
5-Methylchrysene	117.1	–	–	0.062 (27 °C)	–
6-Methylchrysene	160–161	–	–	0.065 (27 °C)	–
2-Methylfluoranthene	79–81	–	–	–	–
3-Methylfluoranthene	65–66	–	–	–	–
1-Methylphenanthrene	123	–	5.08	0.269	5.00
Naphtho[1,2-*b*]fluoranthene	188–189	–	–	–	–

Table 2 (Contd)

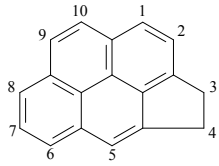
Compound	Melting-point (°C)	Vapour pressure (Pa at 25 °C)	*n*-Octanol:water partition coefficient (log K_{ow})	Solubility in water at 25 °C[d] (mg/L)	Henry's law constant at 25 °C (Pa•m^3/mol)
Naphtho[2,1-*a*]fluoranthene	180	–	–	–	–
Naphtho[2,3-*e*]pyrene	–	–	–	–	–
Perylene	277.5	–	6.25	0.0004	–
Phenanthrene	100.5	0.016	4.52	0.977; 1.18	4.29
Picene	364	–	7.11	0.00431	–
Pyrene	150.4	0.00060	5.00	0.130; 0.135	1.71
Triphenylene	199	–	5.49	0.00632	–

[a] For the original references that contain measurement details, refer to the text.
– , not available

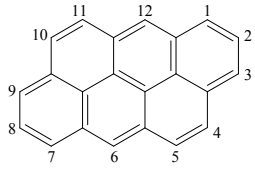
Figure 1. Structural formulae of polycyclic aromatic hydrocarbons covered in this monograph



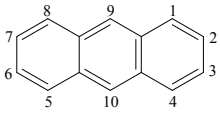
Acenaphthene



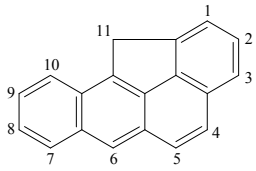
Acepyrene



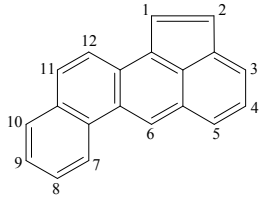
Anthanthrene



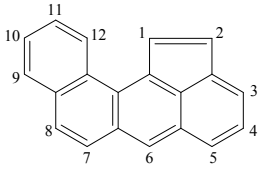
Anthracene



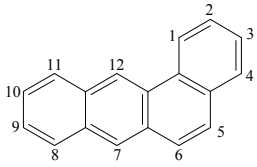
11*H*-Benz[*bc*]aceanthrylene



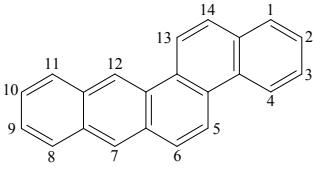
Benz[*j*]aceanthrylene



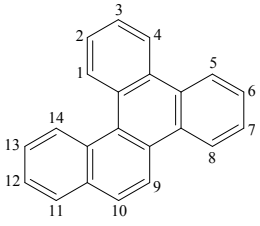
Benz[*l*]aceanthrylene



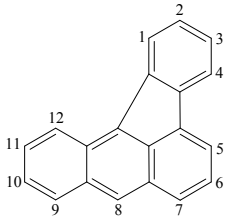
Benz[*a*]anthracene



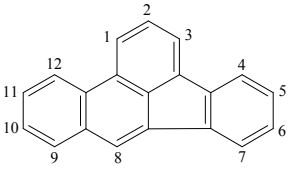
Benzo[*b*]chrysene



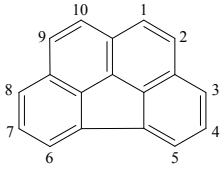
Benzo[*g*]chrysene



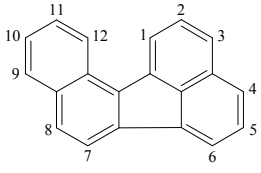
Benzo[*a*]fluoranthene



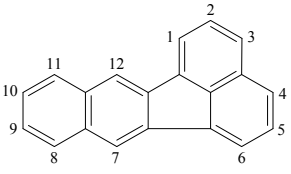
Benzo[*b*]fluoranthene



Benzo[*ghi*]fluoranthene

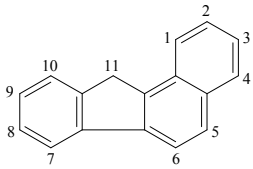


Benzo[*j*]fluoranthene

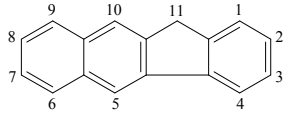


Benzo[*k*]fluoranthene

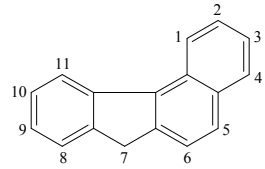
Figure 1 (contd)



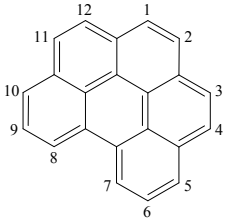
Benzo[*a*]fluorene



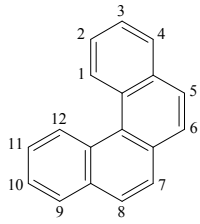
Benzo[*b*]fluorene



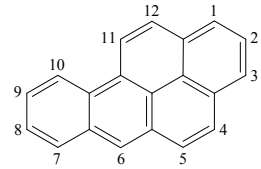
Benzo[*c*]fluorene



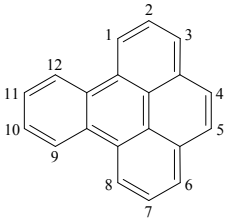
Benzo[*ghi*]perylene



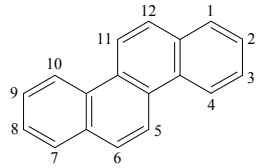
Benzo[*c*]phenanthrene



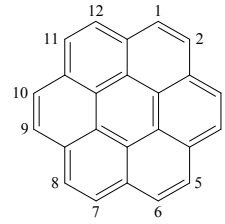
Benzo[*a*]pyrene



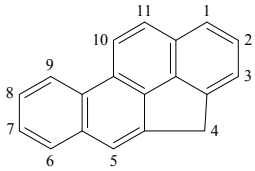
Benzo[*e*]pyrene



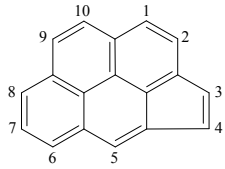
Chrysene



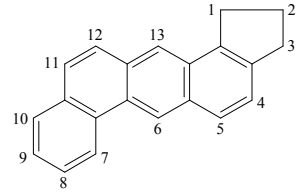
Coronene



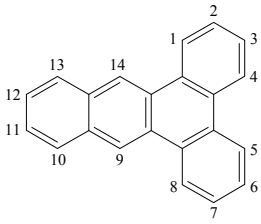
4*H*-Cyclopenta[*def*]chrysene



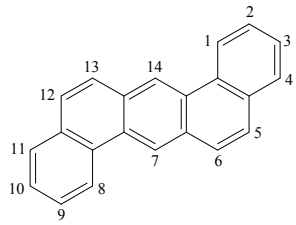
Cyclopenta[*cd*]pyrene



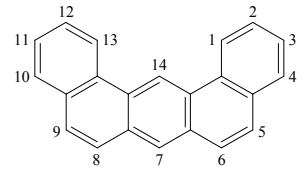
5,6-Cyclopenteno-1,2-benzanthracene



Dibenz[*a*,c]anthracene

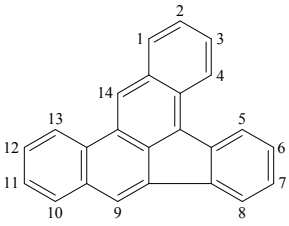


Dibenz[*a,h*]anthracene

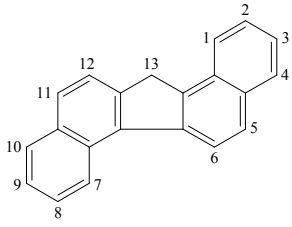


Dibenz[*a,j*]anthracene

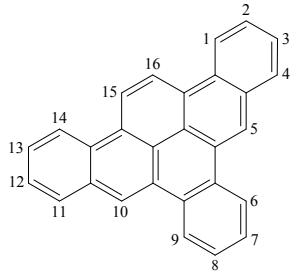
Figure 1 (contd)



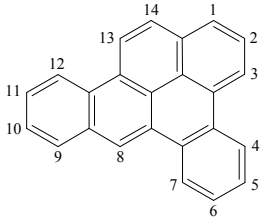
Dibenzo[*a,e*]fluoranthene



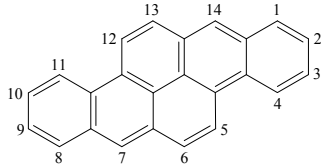
13*H*-Dibenzo[*a,g*]fluorene



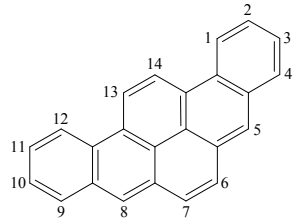
Dibenzo[*h,rst*]pentaphene



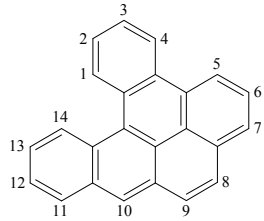
Dibenzo[*a,e*]pyrene



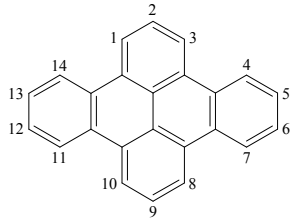
Dibenzo[*a,h*]pyrene



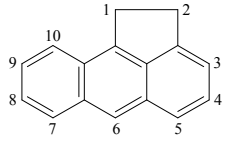
Dibenzo[*a,i*]pyrene



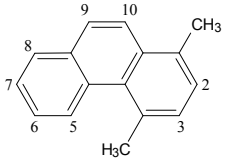
Dibenzo[*a,l*]pyrene



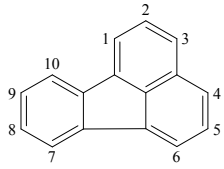
Dibenzo[*e,l*]pyrene



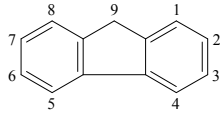
1,2-Dihydroaceanthrylene



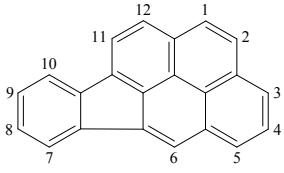
1,4-Dimethylphenanthrene



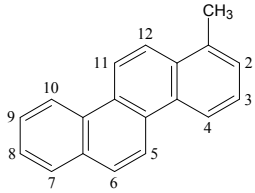
Fluoranthene



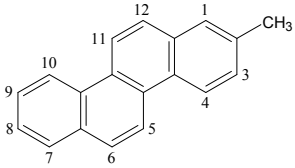
Fluorene



Indeno[1,2,3-*cd*]pyrene

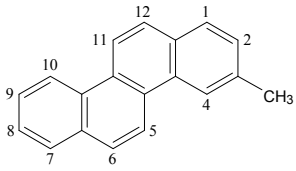


1-Methylchrysene

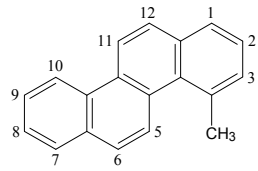


2-Methylchrysene

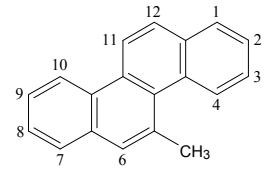
Figure 1 (contd)



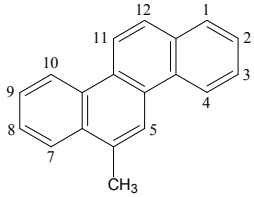
3-Methylchrysene



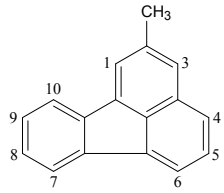
4-Methylchrysene



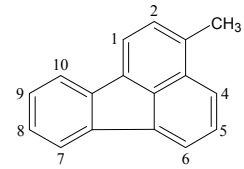
5-Methylchrysene



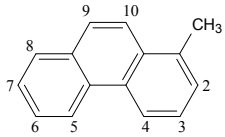
6-Methylchrysene



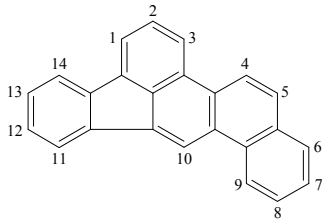
2-Methylfluoranthene



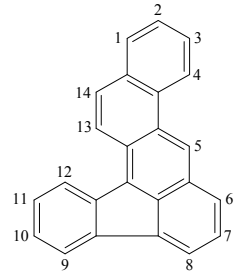
3-Methylfluoranthene



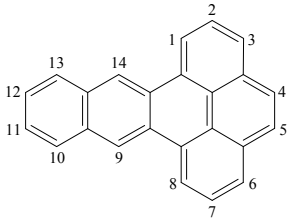
1-Methylphenanthrene



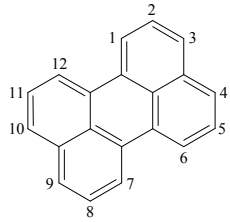
Naphtho[1,2-*b*]fluoranthene



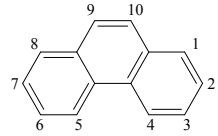
Naphtho[2,1,-*a*]fluoranthene



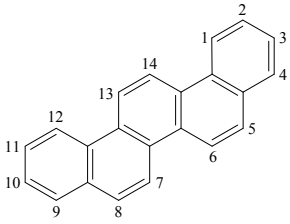
Naphtho[2,3,-*e*]pyrene



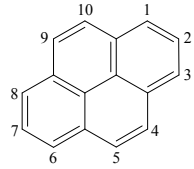
Perylene



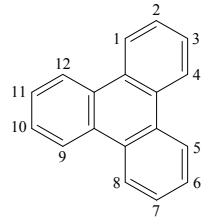
Phenanthrene



Picene



Pyrene



Triphenylene

61. References

Agarwal, S.K., Boyd, D.R. & Jennings, W.B. (1985) Synthesis of benzo[*g*]chrysene, benzo[*g*]chrysene 9,10-oxide and benzo[*g*]chrysene 1,2:9,10-dioxide. *J. chem. Soc. Perkin Trans.*, **1**, 857–860

Bamford, H.A., Poster, D.L. & Baker, J.E. (1999) Temperature dependence of Henry's law constants of thirteen polycylic aromatic hydrocarbons between 4 °C and 31 °C. *Environ. Toxicol. Chem*., **18**, 1905–1912

Becker, H-.D., Hansen, L. & Andersson, K. (1985) Aceanthrylene. *J. org. Chem.*, **50**, 277–279

Blümer, G.-P., Gundermann, K.-D. & Zander, M. (1976) [On the reaction of polycyclic aromatic hydrocarbons with benzene and aluminium chloride.] *Chem. Berichte*, **109**, 1991–2000 (in German)

Buckingham, J. ed. (1996) *Dictionary of Organic Compounds*, 6th Ed., Vols 1, 2 & 5, New York, Chapman & Hall, pp. 617, 1882, 4286

Calvert, J.G., Atkinson, R., Becker, K.H., Kamens, R.M., Seinfeld, J.H., Wallington, T.J. & Yarwood, G. (2002) *The Mechanisms of Atmospheric Oxidation of Aromatic Hydrocarbons*, New York, Oxford University Press, pp. 115, 117–119, 122

Chemical Abstracts Services (1978) *Chemical Abstracts Ninth Collective Index* (9CI) – accessed through SciFinder

Cho, B.P. & Harvey, R.G. (1987a) Polycyclic fluoranthene hydrocarbons. 2. A new general synthesis. *J. org. Chem.*, **52**, 5668–5678

Cho, B.P. & Harvey, R.G. (1987b) Complete ^{1}H and ^{13}C NMR assignment of polycyclic aromatic fluoranthenes by long-range optimized homo- and heteronuclear correlation spectroscopy. *J. org. Chem.*, **52**, 5679–5684

Clar, E., ed. (1964) *Polycylic Hydrocarbons,* Vol. 2, New York, Academic Press, pp. 60–63, 206–208

Cooke, J.W. (1932) The production of cancer by pure hydrocarbons. Part II. *Proc. roy. Soc.*, **B111**, 485–496

Fieser, L.F. & Seligman, A.M. (1935) Cholanthrene and related hydrocarbons. *J. Am. chem. Soc.*, **57**, 2174–2176

Harvey, R.G., Pataki, J., Cortez, C., Di Raddo, P. & Yang, C. (1991) A new general synthesis of polycyclic aromatic compounds based on enamine chemistry. J. org. Chem., 56, 1210–1217

Hopkinson, A.C., Lee-Ruff, E. & Maleki, M. (1986) An efficient synthesis of benzofluorenes via α-alkoxycarbonyldiarylmethyl cations. *Synthesis*, **5**, 366-371

Howard, P.H. & Meylan, W.M., eds (1997) *Handbook of Physical Properties of Organic Chemicals*, Boca Raton, FL, Lewis, pp. 232, 286

ten Hulscher, T.E.M., van der Velde, L.E. & Bruggeman, W.A. (1992) Temperature dependence of Henry's law constants for selected chlorobenzenes, polychlorinated biphenyls and polycyclic aromatic hydrocarbons. *Environ. Toxicol. Chem.*, **11**, 1595–1603

IARC (1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 32, *Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data*, Lyon

Jung, K. & Koreeda, M. (1989) Synthesis of 1,4- 2,4-, and 3,4- dimethylphenanthrenes: A novel deoxygenation of arene 1,4-endoxides with trimethylsilyl iodide. *J. org. Chem.*, **54**, 5667–5675

Karcher, W., Fordham, R.J., Dubois, J.J., Glaude, P.G.J.M. & Ligthart, J.A.M., eds (1985) *Spectral Atlas of Polycyclic Aromatic Compounds*, Dordrecht, D. Reidel

Karcher, W., Ellison, S., Ewald, M., Garrigues, P., Gevers, E. & Jacob, J., eds (1988) *Spectral Atlas of Polycyclic Aromatic Compounds*, Vol. 2, Dordrecht, Kluwer Academic

Karcher, W., Devillers, J., Garrigues, Ph., Jacob, J., Dumler, R., Ellison, S., Florestan, J. & Gevers, E., eds (1991) *Spectral Atlas of Polycyclic Aromatic Compounds*, Vol. 3, Dordrecht, Kluwer Academic

Lee, W., Stevens, P.S. & Hites, R.A. (2003) Rate constants for the gas-phase reactions of methylphenanthrenes with OH as a function of temperature. *J. phys. Chem.*, **A107**, 6603–6608

Lide, D.R., ed. (1992) *CRC Handbook of Chemistry and Physics*, 73rd Ed., Boca Raton, FL, CRC Press

Lide, D.R., ed. (2005) *CRC Handbook of Chemistry and Physics*, 86th Ed., Boca Raton, FL, CRC Press

May, W.E., Wasik, S.P., Miller, M.M., Tewari, Y.B., Brown-Thomas, J.M. & Goldberg, R.N. (1983) Solution thermodynamics of some slightly soluble hydrocarbons in water. *J. chem. Eng. Data*, **28**, 197–200

Mayneord, W.V. & Roe, E.M.F. (1935) The ultra-violet absorption spectra of some complex aromatic hydrocarbons-I. *Proc. roy. Soc.*, **A152**, 299–324

Miller, M.M., Wasik, S.P., Huang, G.-L., Shiu, W.-Y. & Mackay, D. (1985) Relationships between octanol–water partition coefficient and aqueous solubility. *Environ. Sci. Technol.*, **19**, 522–529

Nagel, D.L., Kupper, R., Antonson, K. & Wallcave, L. (1977) Synthesis of alkyl-substituted benzo[*c*]phenanthrenes and chrysenes by photocyclization. *J. org. Chem.*, **42**, 3626–3628

NIST (1998) *NIST/EPA/NIH Mass Spectral Library* (NIST98), Gaithersburg, National Institute of Standards and Technology

NIST (2005) *NIST Standard Reference Database Number 69*, National Institute of Standards and Technology, Gaithersburg. Available at: http://webbook.nist.gov/chemistry/

Olde Boerrigter, J.C., Mulder, P.P.J., van der Gen, A., Mohn, G.R., Cornelisse, J. & Lugtenburg, J. (1989) The use of a double Wolff-Kishner reduction in the preparation of aceanthrene and aceanthrylene. *Rec. Trav. chim. Pays-Bas*, **108**, 79–80

O'Neil M.J., ed. (2006) *The Merck Index,* 14th Ed., Whitehouse Station, NJ, Merck & Co.

Otero-Lobato, M.J., Kaats-Richters, V.E.M., Koper, C., Vlietstra, E.J., Havenith, R.W.A., Jenneskens, L.W. & Seinen, W. (2005) CP-Arene oxides: The ultimate, active mutagenic forms of cyclopenta-fused polycyclic aromatic hydrocarbons (CP-PAHs). *Mutat. Res.*, **581**, 115–132

Papa, D., Perlman, D. & Bogert, M.T. (1938) The synthesis of 1,4-dimethylphenanthrene by cyclodehydration methods. *J. Am. chem. Soc.*, **60**, 319–321

Pearlman, R.S., Yalkowsky, S.H. & Banerjee, S. (1984) Water solubilities of polynuclear aromatic and heteroaromatic compounds. *J. phys. chem. Ref. Data*, **13**, 555–562

Ray, J.K. & Harvey, R.G. (1982) Synthesis of benzo[*a*]fluoranthene and naphtho[2,1-*a*]fluoranthene. *J. org. Chem.*, **47**, 3335–3336

Ray, J.K. & Harvey, R.G. (1983) Synthesis of methylene-bridged polycyclic-hydrocarbons. *J. org. Chem.*, **48**, 1352–1354

Reisen, F. & Arey, J. (2002) Reactions of hydroxyl radicals and ozone with acenaphthene and acenaphthylene. *Environ. Sci. Technol.,* **36**, 4302–4311

Sangaiah, R., Gold, A. & Toney, G.E. (1983) Synthesis of a series of novel polycyclic aromatic systems: Isomers of benz[*a*]anthracene containing a cyclopenta-fused ring. *J. org. Chem.*, **48**, 1632–1638

Sangster Research Laboratories (2005) *LOGKOW© A Databank of Evaluated Octanol–water Partition Coefficients (Log P), ICSU-CODATA*. Available at: http://logkow/cisti.nrc.ca/-logkow/ [last accessed September, 2005]

Schmidt, W., Grimmer, G., Jacob, J., Dettbarn, G. & Naujack, K.W. (1987) Polycyclic aromatic hydrocarbons with mass number 300 and 302 in hard-coal flue gas condensate. Partial identification by spectrometry and independent synthesis. *Fresenius Z. anal. Chem*. **326**, 401–413

Sonnefeld, W.J., Zoller, W.H. & May, W.E. (1983) Dynamic coupled-column liquid chromatographic determination of ambient temperature vapor pressures of polynuclear aromatic hydrocarbons. *Anal. Chem.*, **55**, 275–280

Stubbs, H.W.D. & Tucker, S.H. (1950) Synthesis of fluoranthenes. Part VI. Utilization of the Mannich reaction. *J. chem. Soc.*, 3288–3292

Sukumaran, K.B. & Harvey, R.G. (1981) Novel annelation reaction: Synthesis of polycyclic hydrocarbons from *o*-quinones. *J. org. Chem.*, **46**, 2740–2745

Tintel, C., Cornelisse, J. & Lugtenburg, J. (1983) Convenient synthesis of cyclopenta[*c,d*]pyrene and 3,4-dihydrocyclopenta[*c,d*]pyrene. The reactivity of the pyrene dianion. *Recl. Trav. chim. Pays-Bas*, **102**, 14–20

Tucker, S.H. (1952) Synthesis of fluoranthenes. Part IX. 3-Methylfluoranthene. *J. chem. Soc.*, 803–807

Utermoehlen, C.M., Singh, M. & Lehr, R.E. (1987) Fjord region 3,4-diol 1,2-epoxides and other derivatives in the 1,2,3,4- and 5,6,7,8-benzo rings of the carcinogen benzo[*g*]chrysene. *J. org. Chem.*, **52**, 5574–5582

Weisman, J.L., Mattioda, A., Lee, T.J., Hudgins, D.M., Allamandola, L.J., Bauschlicher, C.W., Jr & Head-Gordon, M. (2005) Electronic transitions in the IR: matrix isolation spectroscopy and electronic structure theory calculations on polyacenes and dibenzopolyacenes. *Phys. Chem. chem. Phys.*, **7**, 109–118

Yu, S. & Campiglia, A.D. (2004) Laser-excited time-resolved Shpol'skii spectroscopy for the direct analysis of dibenzopyrene isomers in liquid chromatography fractions. *Appl. Spectrosc.*, **58**, 1385–1393

LIST OF ABBREVIATIONS

A	adenoma
ABC	adenosine triphosphate binding cassette
ACGIH	American Conference of Governmental Industrial Hygienists
act.	Catalytic activities
AdC	adenocarcinoma
AdSC	adenosquamous carcinoma
AH	aliphatic hydrocarbon
AhR	aryl hydrocarbon receptor
AhRR	aryl hydrocarbon receptor repressor
AKR	aldo-keto reductase
Akt	protein kinase β
ARNT (Arnt)	aryl hydrocarbon receptor nuclear translocator
ASTDR	Agency for Toxic Substances and Disease Registry
ATP	adenosine triphosphate
BaP	benzo[*a*]pyrene
BAX	BCL2-antagonist X protein
BCC	basal-cell carcinoma
BCL2 (Bcl2)	B-cell lymphoma protein 2
BCRP	breast cancer resistance protein
bHLH	basic helix-loop-helix
BPDE	benzo[*a*]pyrene diol epoxide
BPQ	benzo[*a*]pyrene quinone
BSC	British Steel Corporation
bw	body weight
C	carcinoma
CAS	Chemical Abstract Services
CI	confidence interval
COP	coal-oven gas mixed with pyrolysed pitch
COX	cyclooxygenase
CTP	coal-tar paint
CTPV	coal-tar pitch volatiles
CYP	cytochrome P450

DMBA	7,12-dimethylbenz[*a*]anthracene
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DRE	dioxin response element
E	epithelioma
ECD	electrochemical detection
$\Delta E_{deloc}/\beta$	carbonium ion delocalization energies
EGFR	epidermal growth factor receptor
EH	epoxide hydrolase
ELISA	enzyme-linked immunosorbent assay
EPHX	epoxide hydrolase gene
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
excl.	excluding
F	female
GC	gas chromatography
GSH	glutathione
GST	glutathione *S*-transferase
H	hepatoma
HPAH	hydroxyl polychlorinated aromatic hydrocarbon
HPETE	hydroperoxy-5,8,11,13-eicosatranaenoic acid
HPLC	high-performance liquid chromatography
HPRT (hprt)	hypoxanthine(guanine)phosphoribosyl transferase
ICD	International Classification of Diseases
IL	interleukin
IP_3	inositol-1,4,5-triphosphate
IPCS	International Programme on Chemical Safety
ISCO	International Standard Classification of Occupations
ISCP	iron, steel and coke plants
IUPAC	International Union of Pure and Applied Chemistry
JEFCA	FAO/WHO Joint Committee on Food Additives and Contaminants
JNK	Jun N-terminal kinase
K	keratocanthoma
K_d	dissociation constant
K_m	Michaelis constant
LC	liquid chromatography
Log K_{ow}	partition coefficient for *n*-octanol:water
M	male
MAPK	mitogen-activated protein kinase
mEH	microsomal epoxide hydrolase
MMAD	mass median aerodynamic diameter
MPG	manufactured gas plant
MRP	multidrug resistance-associated protein

MS	mass spectrometry
NA	not applied
NAD(P)H	nicotinamide adenine dinucleotide (phosphate)
NAT	*N*-acetyltransferase
NF	nuclear factor
NHANES	National Health and Nutrition Examination Survey
NHBE	normal human bronchial epithelial
NIOSH	National Institute of Occupational Safety and Hygiene
NMR	nuclear magnetic resonance
NPAH	nitrogen-containing polychlorinated aromatic hydrocarbon
NQO1	nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1
NS	not specified
NSF	National Smokeless Fuels
OR	odds ratio
8-oxo-dGuo	8-oxo-7,8-dihydro-2′–deoxyguanosine
P	papilloma
PAH	polychlorinated aromatic hydrocarbon
PAPS	3′-phosphoadenosine-5′-phosphosulfase
PARP	poly(ADP-ribose)polymerase
PAS	period (per)-aryl hydrocarbon receptor nuclear translocator (Arnt)-Single-minded protein (Sim)
PCB	polychlorinated biphenyl
PI3K	phosphatidylinositol-3-kinase
PK	protein kinase
PMR	proportionate mortality rate
PND	postnatal day
prot.	protein
PS	prostaglandin H synthase
PTK	protein tyrosine kinase
QR	quinone reductase
RDD	random-digit dialing
RP	reverse-phase
RR	relative risk
SCC	squamous-cell carcinoma
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SERCA	sarcoplasmic–endoplasmic reticulum Ca^{2+}-ATPase
SGA	sebaceous gland adenoma
SIR	standardized incidence ratio
SMR	standardized mortality ratio

SRC	solvent-refined coal
SRM	standard reference material
SRR	standardized rate ratio
STEL	short-term exposure limit
SULT	sulfotransferase
T	tumour
TBA	tumour-bearing animal
TCDD	2,3,7,8-tetrachlorodibenzo-*para*-dioxin
THF	tetrahydrofuran
TLC	thin-layer chromatography
TLV	threshold limit value
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TWA	time-weighted average
UDP	uridine 5′-diphosphate
UGT	uridine 5′-diphosphate-glucuronosyl transferase
UV	ultraviolet
VIS	visible
V_{max}	maximum velocity
vs	versus
wk	week
XRE	xenobiotic response element

CUMULATIVE CROSS INDEX TO *IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS*

The volume, page and year of publication are given. References to corrigenda are given in parentheses.

B

C

D

E

F

G

H

K

L

M

N

Q

R

S

T

W

X

Y

Z

List of IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*

Volume 1
Some Inorganic Substances, Chlorinated Hydrocarbons, Aromatic Amines, *N*-Nitroso Compounds, and Natural Products
1972; 184 pages (out-of-print)

Volume 2
Some Inorganic and Organometallic Compounds
1973; 181 pages (out-of-print)

Volume 3
Certain Polycyclic Aromatic Hydrocarbons and Heterocyclic Compounds
1973; 271 pages (out-of-print)

Volume 4
Some Aromatic Amines, Hydrazine and Related Substances, *N*-Nitroso Compounds and Miscellaneous Alkylating Agents
1974; 286 pages (out-of-print)

Volume 5
Some Organochlorine Pesticides
1974; 241 pages (out-of-print)

Volume 6
Sex Hormones
1974; 243 pages (out-of-print)

Volume 7
Some Anti-Thyroid and Related Substances, Nitrofurans and Industrial Chemicals
1974; 326 pages (out-of-print)

Volume 8
Some Aromatic Azo Compounds
1975; 357 pages (out-of-print)

Volume 9
Some Aziridines, *N*-, *S*- and *O*-Mustards and Selenium
1975; 268 pages (out-of-print)

Volume 10
Some Naturally Occurring Substances
1976; 353 pages (out-of-print)

Volume 11
Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics
1976; 306 pages (out-of-print)

Volume 12
Some Carbamates, Thiocarbamates and Carbazides
1976; 282 pages (out-of-print)

Volume 13
Some Miscellaneous Pharmaceutical Substances
1977; 255 pages

Volume 14
Asbestos
1977; 106 pages (out-of-print)

Volume 15
Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals
1977; 354 pages (out-of-print)

Volume 16
Some Aromatic Amines and Related Nitro Compounds—Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals
1978; 400 pages

Volume 17
Some *N*-Nitroso Compounds
1978; 365 pages

Volume 18
Polychlorinated Biphenyls and Polybrominated Biphenyls
1978; 140 pages (out-of-print)

Volume 19
Some Monomers, Plastics and Synthetic Elastomers, and Acrolein
1979; 513 pages (out-of-print)

Volume 20
Some Halogenated Hydrocarbons
1979; 609 pages (out-of-print)

Volume 21
Sex Hormones (II)
1979; 583 pages

Volume 22
Some Non-Nutritive Sweetening Agents
1980; 208 pages

Volume 23
Some Metals and Metallic Compounds
1980; 438 pages (out-of-print)

Volume 24
Some Pharmaceutical Drugs
1980; 337 pages

Volume 25
Wood, Leather and Some Associated Industries
1981; 412 pages

Volume 26
Some Antineoplastic and Immunosuppressive Agents
1981; 411 pages (out-of-print)

Volume 27
Some Aromatic Amines, Anthraquinones and Nitroso Compounds, and Inorganic Fluorides Used in Drinking-water and Dental Preparations
1982; 341 pages (out-of-print)

Volume 28
The Rubber Industry
1982; 486 pages (out-of-print)

Volume 29
Some Industrial Chemicals and Dyestuffs
1982; 416 pages (out-of-print)

Volume 30
Miscellaneous Pesticides
1983; 424 pages (out-of-print)

Volume 31
Some Food Additives, Feed Additives and Naturally Occurring Substances
1983; 314 pages (out-of-print)

Volume 32
Polynuclear Aromatic Compounds, Part 1: Chemical, Environmental and Experimental Data
1983; 477 pages (out-of-print)

Volume 33
Polynuclear Aromatic Compounds, Part 2: Carbon Blacks, Mineral Oils and Some Nitroarenes
1984; 245 pages (out-of-print)

Volume 34
Polynuclear Aromatic Compounds, Part 3: Industrial Exposures in Aluminium Production, Coal Gasification, Coke Production, and Iron and Steel Founding
1984; 219 pages (out-of-print)

Volume 35
Polynuclear Aromatic Compounds, Part 4: Bitumens, Coal-tars and Derived Products, Shale-oils and Soots
1985; 271 pages

Volume 36
Allyl Compounds, Aldehydes, Epoxides and Peroxides
1985; 369 pages

Volume 37
Tobacco Habits Other than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines
1985; 291 pages (out-of-print)

Volume 38
Tobacco Smoking
1986; 421 pages

Volume 39
Some Chemicals Used in Plastics and Elastomers
1986; 403 pages (out-of-print)

Volume 40
Some Naturally Occurring and Synthetic Food Components, Furocoumarins and Ultraviolet Radiation
1986; 444 pages (out-of-print)

Volume 41
Some Halogenated Hydrocarbons and Pesticide Exposures
1986; 434 pages (out-of-print)

Volume 42
Silica and Some Silicates
1987; 289 pages

Volume 43
Man-Made Mineral Fibres and Radon
1988; 300 pages (out-of-print)

Volume 44
Alcohol Drinking
1988; 416 pages

Volume 45
Occupational Exposures in Petroleum Refining; Crude Oil and Major Petroleum Fuels
1989; 322 pages

Volume 46
Diesel and Gasoline Engine Exhausts and Some Nitroarenes
1989; 458 pages

Volume 47
Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting
1989; 535 pages (out-of-print)

Volume 48
Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry
1990; 345 pages

Volume 49
Chromium, Nickel and Welding
1990; 677 pages

Volume 50
Pharmaceutical Drugs
1990; 415 pages

Volume 51
Coffee, Tea, Mate, Methylxanthines and Methylglyoxal
1991; 513 pages

Volume 52
Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds
1991; 544 pages

Volume 53
Occupational Exposures in Insecticide Application, and Some Pesticides
1991; 612 pages

Volume 54
Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals
1992; 336 pages

Volume 55
Solar and Ultraviolet Radiation
1992; 316 pages

Volume 56
Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins
1993; 599 pages

Volume 57
Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants; Some Hair Dyes, Cosmetic Colourants, Industrial Dyestuffs and Aromatic Amines
1993; 428 pages

Volume 58
Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry
1993; 444 pages

Volume 59
Hepatitis Viruses
1994; 286 pages

Volume 60
Some Industrial Chemicals
1994; 560 pages

Volume 61
Schistosomes, Liver Flukes and *Helicobacter pylori*
1994; 270 pages

Volume 62
Wood Dust and Formaldehyde
1995; 405 pages

Volume 63
Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals
1995; 551 pages

Volume 64
Human Papillomaviruses
1995; 409 pages

Volume 65
Printing Processes and Printing Inks, Carbon Black and Some Nitro Compounds
1996; 578 pages

Volume 66
Some Pharmaceutical Drugs
1996; 514 pages

Volume 67
Human Immunodeficiency Viruses and Human T-Cell Lymphotropic Viruses
1996; 424 pages

Volume 68
Silica, Some Silicates, Coal Dust and *para*-Aramid Fibrils
1997; 506 pages

Volume 69
Polychlorinated Dibenzo-*para*-Dioxins and Polychlorinated Dibenzofurans
1997; 666 pages

Volume 70
Epstein-Barr Virus and Kaposi's Sarcoma Herpesvirus/Human Herpesvirus 8
1997; 524 pages

Volume 71
Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide
1999; 1586 pages

Volume 72
Hormonal Contraception and Post-menopausal Hormonal Therapy
1999; 660 pages

Volume 73
Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances
1999; 674 pages

Volume 74
Surgical Implants and Other Foreign Bodies
1999; 409 pages

Volume 75
Ionizing Radiation, Part 1, X-Radiation and γ-Radiation, and Neutrons
2000; 492 pages

Volume 76
Some Antiviral and Antineoplastic Drugs, and Other Pharmaceutical Agents
2000; 522 pages

Volume 77
Some Industrial Chemicals
2000; 563 pages

Volume 78
Ionizing Radiation, Part 2, Some Internally Deposited Radionuclides
2001; 595 pages

Volume 79
Some Thyrotropic Agents
2001; 763 pages

Volume 80
Non-Ionizing Radiation, Part 1: Static and Extremely Low-Frequency (ELF) Electric and Magnetic Fields
2002; 429 pages

Volume 81
Man-made Vitreous Fibres
2002; 418 pages

Volume 82
Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene
2002; 590 pages

Volume 83
Tobacco Smoke and Involuntary Smoking
2004; 1452 pages

Volume 84
Some Drinking-Water Disinfectants and Contaminants, including Arsenic
2004; 512 pages

Volume 85
Betel-quid and Areca-nut Chewing and Some Areca-nut-derived Nitrosamines
2004; 334 pages

Volume 86
Cobalt in Hard Metals and Cobalt Sulfate, Gallium Arsenide, Indium Phosphide and Vanadium Pentoxide
2006; 330 pages

Volume 87
Inorganic and Organic Lead Compounds
2006; 506 pages

Volume 88
Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol
2006; 478 pages

Volume 89
Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines
2007; 626 pages

Volume 90
Human Papillomaviruses
2007; 670 pages

Volume 91
Combined Estrogen-Progestogen Contraceptives and Combined Estrogen-Progestogen Menopausal Therapy
2007; 528 pages

Volume 92
Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures
2010; 853 pages

Volume 93
Carbon Black, Titanium Dioxide and Non-Asbestiform Talc
(in preparation)

Volume 94
Ingested Nitrates and Nitrites, and Cyanobacterial Peptide Toxins
(in preparation)

Volume 95
Household Use of Solid Fuels and High-temperature Frying
(in preparation)

Volume 96
Consumption of Alcoholic Beverages and Ethyl Carbamate (Urethane)
(in preparation)

Volume 97
1,3-Butadiene, Ethylene Oxide and Vinyl Halides (Vinyl Fluoride, Vinyl Chloride and Vinyl Bromide)
2008; 510 pages

Supplement No. 1
Chemicals and Industrial Processes Associated with Cancer in Humans (*IARC Monographs*, Volumes 1 to 20)
1979; 71 pages (out-of-print)

Supplement No. 2
Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal
1980; 426 pages (out-of-print)
(updated as IARC Scientific Publications No. 83, 1986)

Supplement No. 3
Cross Index of Synonyms and Trade Names in Volumes 1 to 26 of the *IARC Monographs*
1982; 199 pages (out-of-print)

Supplement No. 4
Chemicals, Industrial Processes and Industries Associated with Cancer in Humans (*IARC Monographs*, Volumes 1 to 29)
1982; 292 pages (out-of-print)

Supplement No. 5
Cross Index of Synonyms and Trade Names in Volumes 1 to 36 of the *IARC Monographs*
1985; 259 pages (out-of-print)

Supplement No. 6
Genetic and Related Effects: An Updating of Selected *IARC Monographs* from Volumes 1 to 42
1987; 729 pages (out-of-print)

Supplement No. 7
Overall Evaluations of Carcinogenicity: An Updating of *IARC Monographs* Volumes 1–42
1987; 440 pages (out-of-print)

Supplement No. 8
Cross Index of Synonyms and Trade Names in Volumes 1 to 46 of the *IARC Monographs*
1990; 346 pages (out-of-print)